meaton 2023
62nd International Meat Industry Conference

SCIENTIFIC JOURNAL

ISSN 2466-2852
UDK 664.9

meat technology

special issue

64 Vol. 02 No. 2023 Belgrade

Founder and Publisher
Institute of Meat Hygiene
and Technology

Meat Technology — Special Issue 64/2

DEDICATED TO

62nd International Meat Industry Conference

„EXPLORING CURRENT CHALLENGES AND FUTURE PERSPECTIVES IN FOOD AND NUTRITION“

Mt. Kopanik, October 1st–4th, 2023.

PROGRAMME COMMITTEE

The Chairperson: Dr Jelena Ćirić – *Serbia*

Vice Chairpersons: Dr Tatjana Baltić – *Serbia*
Dr Radmila Mitrović – *Serbia*
Dr Mirjana Lukić – *Serbia*

Honorary Chairperson: Prof. dr Igor Tomašević – *Serbia*

Members: Dr Vesna Đorđević – *Serbia*
Dr Nenad Parunović – *Serbia*
Dr Ivana Branković Lazić – *Serbia*
Dr Nenad Katanić – *Serbia*
Dr Miloš Petrović – *Serbia*
Prof. dr David Hopkins – *Australia*
Prof. dr Milorad Mirilović – *Serbia*
Prof. dr Vladimir Tomović – *Serbia*
Prof. dr Radivoje Papović – *Serbia*
Dr Branko Velebit – *Serbia*
Dr Brankica Lakićević – *Serbia*
Dr Vesna Janković – *Serbia*
Dr Srđan Stefanović – *Serbia*
Dr Radivoj Petronijević – *Serbia*
Prof. dr Muhamed Smajlović – *Bosnia and Herzegovina*
Prof. dr Lazo Pendovski – *Republic of North Macedonia*
Prof. dr Dean Jankuloski – *Republic of North Macedonia*
Prof. dr Andrej Kirbiš – *Slovenia*
Prof. dr Dušan Živković – *Serbia*
Prof. dr Muhamed Brka – *Bosnia and Herzegovina*
Prof. dr Volker Heinz – *Germany*
Prof. dr Fatih Oz – *Turkey*
Prof. dr Weizheng Sun – *China*
Prof. dr Mohammed Gagaoua – *France*
Prof. dr Angela Trocino – *Italy*
Prof. dr Bartosz Solowiej – *Poland*
Prof. dr Ariane Pietzka – *Austria*
Prof. dr Terjung Nino – *Germany*
Prof. dr Attila Gere – *Hungary*

ORGANISING COMMITTEE

The Chairperson: Dr Lazar Milojević

Vice Chairpersons: Dr Nikola Betić
Dr Stefan Simunović
Čaba Silađi

Honorary Chairperson: Ivan Zeković

Conference Host: Dr Branko Velebit

Technical Secretary: Saša Prečanica

Members: Dr Jelena Jovanović
Dragan Knežević, *PI Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka*
Saša Bošković, *Veterinary Office of Bosnia and Herzegovina*
Dr Aleksandar Bajčić
Sara Simunović
Nikola Borjan
Milenko Babić
Dunja Mišić
Miljana Krstić
Dr Danijela Šarčević
Igor Milošević
Gordana Terzić
Andrea Končar
Marijana Trajković
Tamara Penjić, *Carnex*
Igor Janković, *Carnex*
Nemanja Knežević, *Zlatiborac*

MEAT TECHNOLOGY – SPECIAL ISSUE 64 (2) 2023

CONTENTS

- **What is meat in Serbia?**
  Igor Tomašević, Vladimir Tomović, Nedjeljko Karabasil, Nino Terjung, Ilija Đekić and Đorđević Vesna 1
- **Characteristics of traditional guaranteed meat specialties in Slovakia**
  Miroslav Kročko, Viera Ducková and Marek Bobko 12
- **Odors change visual attention. A case study with stawberry odor and differently flavoured yoghurts**
  Dorina Szakál, Abdul Hannan Bin Zulkarnain, Xu Cao and Attila Gere. 17
- **Use of whole genome sequencing as routine typing method — improvements in the investigation of foodborne outbreaks of *Listeria monocytogenes***
  Ariane Pietzka, Brankica Lakićević, Lazar Milojević and Werner Ruppitsch 25
- **Nutritional and feeding strategies for controlling breast muscle myopathy occurrence in broiler chickens: a survey of the published literature**
  Angela Trocino, Gerolamo Xiccato, Massimiliano Petracci and Marija Bošković Cabrol 30
- **Overview of microplastics in the meat: occurrence, detection methods and health effects**
  Branko Velebit, Vesna Janković, Lazar Milojević, Tatjana Baltić, Jelena Ćirić and Radmila Mitrović 36
- **(Mis)understandings in research methodology and chemometrics in meat science**
  Predrag Putnik, Daniela Šojić Merkulov, Branimir Pavlić, Branko Velebit and Danijela Bursać Kovačević 42
- **Is food oral processing a new meat quality dimension?**
  Ilija Đekić 48
- **Nutritional quality of selected Croatian traditional dry-fermented sausages**
  Ana Vulić, Nina Kudumija, Tina Lešić and Jelka Pleadin 52
- **Essential oils as emerging ingredients in processing of minced meat products**
  Branislav Šojić, Branimir Pavlić, Milo Mujović, Predrag Ikonić and Snežana Škaljac 58
- **Effect of commercial starter culture on physicochemical properties and biogenic amine formation in traditional dry-fermented beef sausage**
  Predrag Ikonić, Tatjana Peulić, Marija Jokanović, Jovana Delić, Snežana Škaljac, Branislav Šojić and Ljubiša Šarić. 63
- **Polycyclic aromatic hydrocarbons (PAHs) in Visočka pečenica, a traditional dry-cured meat product from Bosnia and Herzegovina**
  Munevera Begić, Selma Čorbo, Jasna Đinović-Stojanović and Saša Janković. 69
- **Wooden breast, white striping and spaghetti meat: chemical composition, technological quality, microbiological profile and sensory attributes of broiler breasts**
  Marija Bošković Cabrol, Massimiliano Petracci and Angela Trocino 75
- ***Toxoplasma gondii* infection in pigs in Serbia**
  Aleksandra Uzelac, Nikola Betić, Nedjeljko Karabasil, Vladimir Ćirković, Olgica Đurković-Đaković and Ivana Klun 82

- **Detection of pathogenic Yersinia enterocolitica strains in pre-packed fresh pork minced meat — preliminary data**
  Sead Hadžiabdić, Dörte Haase, Andrea Barać, Stefan Hertwig and Anja Buschulte 89

- **Enhanced biosecurity measures may contribute to the reduction of Campylobacter incidence in slaughterhouses**
  Jelena Maletić, Jasna Kureljušić and Nenad Katanić 93

- **Challenges in agri-food chain: Biosensors in the meat production system**
  Ivan Nastasijević, Ivana Podunavac, Saša Janković, Radmila Mitrović and Vasa Radonić 101

- **Changes in bacterial status and aw values during the maturation of fermented sausages**
  Radmila Mitrović, Jelena Janjić, Vesna Janković, Brankica Lakićević, Lazar Milojević, Branko Velebit and Branislav Baltić 106

- **Major allergens — the big nine**
  Vesna Janković, Branko Velebit, Brankica Lakicević, Radmila Mitrović and Lazar Milojević 111

- **Agricultural waste: a source of bioactive compounds for potential application in meat products**
  Milica Glišić, Marija Bošković Cabrol, Nikola Čobanović, Milan Ž. Baltić, Vladimir Drašković, Stevan Samardžić and Zoran Maksimović 116

- **Zoonotic potential of *Eustrongylides spp.* in freshwater fish meat**
  Dragana Ljubojević Pelić, Miloš Pelić, Nikolina Novakov, Milica Živkov Baloš and Vesna Đorđević 122

- ***Toxoplasma gondii* — control measures for reducing risks in the pork production chain**
  Nikola Betić, Ivana Branković Lazić, Nedjeljko Karabasil, Dragan Vasilev, Aleksandra Uzelac and Ivana Klun . . . 129

- **Sustainable meat production**
  Nedjeljko Karabasil, Tamara Bošković, Nataša Kilibarda, Nikola Čobanović, Ivan Vićić and Mirjana Dimitrijević 133

- **Viruses in shellfish — food safety risks**
  Mirjana Dimitrijević , Nevena Grković, Ivana Milošević and Nedjeljko Karabasil 136

- **Perspectives in fat replacement in sausages**
  Dragan Vasilev, Branko Suvajdžić, Aleksandar Bajčić and Silvana Stajković. 140

- **Acute phase proteins as biomarkers of pre-slaughter stress in pigs**
  Silvana Stajković and Dragan Vasilev 145

- **Non-thermal technologies for milk and dairy processing**
  Tijana Ledina, Jasna Đorđević, Marija Kovandžić and Snežana Bulajić 149

- **Levels and accumulation of selected heavy metals in the One Health approach**
  Nevena Grković, Nikola Čobanović, Branko Suvajdzić , Dragoljub Jovanović and Mirjana Dimitrijević 155

- **Horse carcass and meat quality — current knowledge and research gaps**
  Nikola Čobanović, Nevena Grković, Branko Suvajdžić, Ivan Vićić and Nedjeljko Karabasil 160

- **Production and trade of milk and dairy products in Serbia**
  Jasna Đorđević, Tijana Ledina, Marija Kovandžić and Snežana Bulajić 166

- **The nutritional profile and technological properties of rabbit meat**
  Branko Suvajdžić, Nikola Čobanović, Nevena Grković, Ivan Vićić and Dragan Vasilev 171

- **Meat matters: tackling food loss and waste in the meat sector**
  Nataša Kilibarda, Neđeljko Karabasil and Jelena Stojanović 177

- **Composition and diversity of microbial communities of industrial environment objects**
  Yuliya Yushina, Elena Zaiko, Anzhelika Makhova, Oxana Kuznetsova, Anastasia Semenova, Dagmara Bataeva, Maria Grudistova and Nasarbay Nasyrov 183

- **Nutritional approaches to enhance fatty acid composition of beef: a review**
  Mirjana Lukić, Sara Simunović and Stefan Simunović 189

- **Anticoagulant rodenticides in game meat: a risk to human health**
  Vladimir Drašković, Milica Glišić, Ružica Cvetković, Radislava Teodorović, Ljiljana Janković and Milutin Đorđević 194

- **Reviewing the current situation and opinions of the hepatitis E virus among natural reservoirs and through the food chain**
  Lazar Milojević, Branko Velebit and Nikola Betić 199

- **Meat products and functional food**
  Slaviša Stajić, Nikola Stanišić and Vladimir Kurćubić 206

- **Sensory quality, oxidative stability, textural and fatty acid profile of nitrite-reduced kulen fermented sausage during ripening**
  Stefan Simunović, Vesna Ž. Đorđević, Sara Simunović, Daniel Franco, Slaviša Stajić and Igor Tomašević 212

- **Enrichment of table eggs with selenium through designed feed for laying hens**
  Dragan Šefer, Dejan Perić and Radmila Marković 218

- **Live yeast cells in nutrition of monogastric animals**
  Radmila Marković, Dejan Perić, Svetlana Grdović, Dragoljub Jovanović, Dragan Šefer, Jelena Janjić and Željko Maksimović 222

- **Effects of spent mushroom substrate on growth performance and meat characteristics of animals**
  Svetlana Grdović, Radmila Marković, Dejan Perić and Dragan Šefer 227

- **Entomophagy — a novel option in animal and human nutrition**
  Ksenija Nešić, Radmila Marković and Dragan Šefer 231

- ***Yersinia enterocolitica* and control measures for reducing risks in the pork production chain**
  Miloš Arsić, Ivan Vićić, Miloš Petrović, Marko Dmitrić and Nedjeljko Karabasil 237

- **The One Health concept: a comprehensive approach to the function of a sustainable food system**
  Dragan Milićević, Radman Šelmić and Zoran Petrović 242

- **Porosity and discontinuity of food can protective coatings — simple chemical tests and serious consideration**
  Zoran Petrović, Dragan Milićević, Saša Janković, Nikola Borjan, Danijela Vranić and Dejana Trbović 248

- **Whole genome sequencing as the ultimate genomic subtyping tool for the identification and control of *Listeria monocytogenes* in the RTE food chain**
  Brankica Lakićević, Vesna Janković, Ariane Pietzka and Werner Ruppitsch 252

- **Development of functional meat cutlets with improved nutritional value and antioxidant properties to correct the diet of patients with cardiovascular disease**
  Marietta A. Aslanova, Olga K. Derevitskaya, Andrey S. Dydykin, Anna L. Bero and Nataliya E. Soldatova 256

- **Monitoring of sulfites in kebabs and grilled meat**
  Jasna Kureljušić, Nikola Roković, Marija Pavlović, Aleksandra Tasić, Jelena Maletić, Dragana Ljubojević-Pelić and Tanja Bijelić 263

- **Qualimetric assessment as a tool of the quality and safety management system of meat products with undeclared components**
  Elizaveta Kryuchenko, Yulia Kuzlyakina and Valentina Zamula 267
- **Honeybee pollen as a bioindicator of contamination: an overview**
  Jelena Ćirić, Nils Haneklaus, Tatjana Baltić, Sara Simunović, Nenad Parunović, Dejana Trbović and Boris Mrdović 273
- **Reduction of salt content in meat products**
  Milenko Babić, Danijela Vranić, Branka Borović, Jelena Babić Milijašević, Tamara Gerić and Slobodan Lilić . . 277
- **The influence of different gas mixtures on the shelf life of fresh beef**
  Milan Milijašević, Jelena Babić Milijašević, Ivan Nastasijević and Slavica Vesković Moračanin 281
- **Detection of milk fat in dairy products — an alternative approach**
  Aleksandar Bajčić, Dejana Trbović, Dragan Vasilev, Vesna Đorđević, Ivana Branković Lazić, Čaba Silađi and Radivoj Petronijević 285
- **Fennel (*Foeniculum vulgare*) extracts as potential antioxidants in beef burgers**
  Milo Mujović, Branislav Šojić, Branimir Pavlić, Predrag Ikonić, Snežana Škaljac and Danijela Bursać Kovačević . . . 289
- **Food loss and waste: a global problem**
  Slavica Vesković Moračanin, Milan Milijašević, Branka Borović and Jasna Kureljušić 293
- **Evaluation of sensory characteristics of common carp reared in purified wastewater from a slaughterhouse**
  Miloš Pelić, Milica Živkov Baloš, Nikolina Novakov, Nikola Puvača, Jasna Kureljušić, Ana Gavrilović and Dragana Ljubojević Pelić 298
- **Dietary fibre and carbohydrates in frozen vegetables**
  Dejana Trbović, Danijela Vranić and Vladimir Korićanac 303
- **Evaluation of content and ratio of calcium and phosphorus in commercially available pet food for dogs and cats**
  Danijela Vranić, Vladimir Korićanac, Dejana Trbović, Dragan Milićević, Tamara Gerić, Jasna Đinović-Stojanović and Zoran Petrović 307
- **Polycyclic aromatic hydrocarbons in dry fermented sausages from the Serbian market**
  Jasna Đinović-Stojanović, Danijela Vranić, Munevera Begić, Jelena Babić Milijašević, Milan Milijašević, Srdjan Stefanović and Saša Janković 312
- **Principal component analysis and cluster analysis for fatty acid assessment of backfat in three pig breeds**
  Irina Chernukha, Marina Nikitina, Elena Kotenkova and Liliya Fedulova 317
- **Evaluation of the quality of minced meat and minced formed meat on the market of the Republic of Srpska**
  Biljana Pećanac, Bojan Golić and Dragan Knežević 323
- **Investigation of the volume of fish production and catch in Serbia from 2012 to 2021**
  Branislav Baltić, Aksentijević Ksenija, Danica Bogunović, Marija Starčević, Radmila Mitrović, Boris Mrdović and Jelena Janjić 329
- **The presence of nitrites and nitrates in various type of meat semi-products intended for grilling from the Serbian market**
  Vladimir Korićanac, Danijela Vranić, Dejana Trbović, Stefan Simunović and Sara Simunović 334

- **Prevalence and main factors for Salmonella spreading in wild boars — a risk for food safety**
  Jelena Petrović, Jovan Mirčeta and Jasna Prodanov-Radulović. 339
- **Investigating the influence of rosehip tea marination on lipid oxidation in turkey breast meat**
  Meltem Serdaroğlu, Özlem Yüncü-Boyacı, Merih Karaman and Hülya Serpil Kavuşan. 344
- **Changes of sensory attributes of carp steaks (*Cyprinus carpio*) packaged in vacuum and modified atmosphere**
  Jelena Babić Milijašević, Milan Milijašević, Slobodan Lilić, Branka Borović, Jasna Đinović-Stojanović, Jelena Jovanović and Mirjana Lukić 350
- **Innovative coating approach: vacuum impregnation with chia mucilage and sage infusion for turkey fillets**
  Elnaz Sharefiabada, Hulya Serpil Kavusan and Meltem Serdaroğlu. 354
- **Microbiological parameters and sensory characteristics of sliced meat products packaged in modified atmosphere throughout the shelf life**
  Jelena Vranešević, Suzana Vidaković Knežević, Anja Novaković, Anđela Pavlović Snežana Škaljac, Jasna Kureljušić and Dragan Vasilev 360
- **Bioactive compounds in honey: a literature overview**
  Tatjana Baltić, Jelena Ćirić, Sara Simunović, Ivana Branković Lazić, Vesna Đorđević, Nenad Parunović and Nenad Katanić 365
- **Sodium chloride replacement with other chloride salts in chicken burgers**
  Branka Borović, Slobodan Lilić, Danijela Vranić, Dragan Milićević, Slavica Vesković, Jelena Babić Milijašević and Tamara Gerić 369
- **The effect of sample temperature on sensory quality of caseless sausages — cevap**
  Čaba Silađi, Vesna Đorđević, Volker Heinz, Nino Terjung, Franziska Witte and Igor Tomašević 372
- **Distribution of pyrethroids and piperonyl butoxide in foods and feeds analysed with GC-MS/MS in 2022–2023**
  Nikola Borjan, Stefan Simunović, Srđan Stefanović, Zoran Petrović, Jasna Đinović-Stojanović, Vedrana Jelušić and Saša Janković 377
- **Effect of chokeberry (*Aronia melanocarpa)* extract on the sensory properties of raw cooked meat products (frankfurters)**
  Andrea Mesárošová, Marek Bobko, Lukáš Jurčaga, Alica Bobková, Katarína Poláková, Alžbeta Demianová, Miroslav Kročko, Judita Lidiková, Ondřej Bučko and Andrea Mendelová 382
- **Nutritional strategies to reduce ammonia and carbon dioxide production in intensive livestock production**
  Miljana Krstić, Vesna Đorđević, Jelena Ćirić, Tatjana Baltić, Aleksandar Bajčić, Sara Simunović and Dejan Perić 387
- **Fermented dry Sremska sausages made of pork meat from various breeds — chemical content and sensory properties**
  Nenad Parunović, Jelena Ćirić, Tatjana Baltić, Dejana Trbović, Nikola Betić, Čedomir Radović and Radomir Savić 392
- **Reduction of eggshell microbial load of table eggs by ultra-violet treatment**
  Suzana Vidaković Knežević, Slobodan Knežević, Jelena Vranešević, Marko Pajić, Zoran Ružić, Sava Spiridonović and Mirjana Đukić Stojčić 397
- **Proteomics as an emerging tool in equine meat research: an overview**
  Antonella della Malva, Mohammed Gagaoua, Agostino Sevi and Marzia Albenzio 401

- **The effect of Swiss chard powder and starter cultures on color development and stability in dry cured pork loin**
  Aleksandra Silovska Nikolova, Zlatko Pejkovski, Daniela Belichovska and Katerina Belichovska 407
- **Sensory and chemical characteristics of dry fermented sausages**
  Jelena Jovanović, Ivana Branković Lazić, Aleksandra Nikolić, Mladen Rašeta, Boris Mrdović, Tatjana Baltić and Jelena Babić Milijašević 412
- **Physicochemical and sensory properties of pork liver pâté formulated with sunflower oleogel as fat substituent**
  Miloš Županjac, Predrag Ikonić, Branislav Šojić and Branislava Đermanović 416
- **Influence of feed for horse nutrition on the chemical parameters and fatty acid composition of mare's milk**
  Aleksandra Nikolić, Jelena Jovanović, Vesna Djordjević, Dejana Trbović, Milijana Sinđić, Jelena Babić Milijašević and Dragan Milićević 422
- **Protein oxidation in meat products: exploring the role of natural antioxidants in preservation and quality enhancement**
  Meltem Serdaroğlu 427
- **On-farm killing as a method to minimize pre-slaughter stress: a qualitative analysis from Switzerland**
  Lisa Märcz, Anna Francesca Corradini, Eugenio Demartini and Michael Gibbert 432
- **Effects of replacing chicken meat with chicken liver on some quality characteristics of model system chicken meat emulsions**
  Berker Nacak 438
- **Mitigating the allergenicity of lupin seeds through germination to enhance food safety**
  María López-Pedrouso, Ricard Bou, Roberto Bermúdez, Ruben Domínguez and José Manuel Lorenzo 443
- **Validation of LC-MS/MS for food colors in foodstuffs and household products**
  Danka Spirić, Srđan Stefanović, Čaba Silađi, Radivoj Petronijević, Tamara Gerić, Nikola Borjan and Silvana Stajković 449
- **Ensuring the safety of cooked and smoked sausages of a narrower diameter, in a cellulose casing, by heat treatment**
  Mladen Rašeta, Ivana Branković-Lazić, Boris Mrdović, Stefan Simunović, Sara Simunović, Jelena Jovanović and Zsolt Becskei 453
- **Microbiological status of minced meat at retail in Belgrade district**
  Nikola Betić, Ivana Branković Lazić, Lazar Milojević, Ivan Vićić, Nedjeljko Karabasil, Nenad Parunović and Vesna Đorđević 460
- **Phosphate additives in meat products: analytical determination and interpretation of results**
  Radivoj Petronijević, Čaba Silađi, Danijela Vranić, Srđan Stefanović, Danka Spirić, Jelena Ćirić and Aleksandar Bajčić 465
- **Prevention of mycotoxins' effects — from field to table**
  Dejan Perić, Radmila Marković, Stamen Radulović, Svetlana Grdović, Dragoljub Jovanović and Dragan Šefer 470
- **Performance evaluation of the ISO 18363-4:2021 method for quantitative determination of chloropropanediols and glycidol in fats and oils by GC-MS/MS**
  Srđan Stefanović, Saša Janković, Nikola Borjan, Stefan Simunović, Radivoj Petronijević, Dejana Trbović and Čaba Silađi 475

- **Consumer attitudes and preferences toward traditional meat products in the autonomous province of Vojvodina**
  Tatjana Peulić, Predrag Ikonić, Jovana Delić, Bojana Kalenjuk Pivarski, Nikola Maravić, Aleksandar Marić and Aleksandra Novaković 480
- **Indexing of fatty acids in raw turkey meat and products for their characterization in a healthy diet**
  Aleksandra Tasić, Ivan Pavlović, Tanja Bijelić, Jasna Kureljušić, Marija Pavlović and Milan Baltić 485
- **Effects of different cooking methods of pork and beef on textural properties and sensory quality**
  Sara Simunović, Igor Tomasević, Vesna Đorđević, Tatjana Baltić, Jelena Ćirić, Stefan Simunović and Ilija Đekić 489
- **Flexibility and amendments of the Codex Alimentarius aimed towards small food business entities**
  Boris Mrdovć, Branislav Baltić, Nikola Betić, Mladen Rašeta, Jelena Jovanović, Ivana Branković Lazić and Aleksandar Bajčić 495
- **Examination of the volume of meat production and the value of meat imports to Serbia from 2012 to 2021**
  Ivana Branković Lazić, Milan Ž. Baltić, Stefan Simunović, Jelena Ćirić, Tatjana Baltić, Jelena Jovanović and Vesna Ž. Đorđević 500
- **Magnesium content in chicken meat — share in food intake**
  Saša Janković, Stefan Simunović, Srđan Stefanović, Zoran Petrović, Jasna Đinović-Stojanović, Sanin Tanković and Nikola Borjan 505
- **Microbial biofilms in a meat processing environment**
  Viera Ducková, Miroslav Kročko and Jana Tkáčová 508
- **Tackling African swine fever and highly pathogenic animal diseases for sustainable meat production and food security**
  Budimir Plavšić 513

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5(497.11)

ID: 126075913

https://doi.org/10.18485/meattech.2023.64.2.1

Original scientific paper

What is meat in Serbia?

Igor Tomašević[ab], Vladimir Tomović[c], Nedjeljko Karabasil[d], Nino Terjung[b], Ilija Đekić[a] and Đorđević Vesna[e]*

[a] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia*
[b] *German Institute of Food Technologies (DIL), Quackenbruck, Germany*
[c] *University of Novi Sad, Faculty of Technology, Department of Food Preservation Engineering, Boulevard of Cara Lazara 1, Novi Sad, 21000, Serbia*
[d] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar oslobođenja 18, Belgrade, Serbia*
[e] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, 11040, Serbia*

ARTICLE INFO

Keywords:
Quality
Safety
Welfare
Environment

ABSTRACT

Ensuring meat safety is a significant concern in Serbia, as in any country. To address this issue, the Serbian government has implemented several measures, including regular inspections of slaughterhouses and meat processing facilities and adhering to EU regulations on meat safety. These regulations stipulate that all meat products must meet stringent hygiene, storage, and labeling standards. In addition, consumers are advised to buy meat products only from trustworthy sources to safeguard their health. While the issue of meat safety in Serbia remains a concern, the government and consumers are taking steps to mitigate the risks associated with consuming meat products. Serbia's meat processing industry focuses on developing new, healthier products with "clean label" formulations and innovative packaging films. However, the welfare of animals during the slaughtering process has been a topic of concern among animal rights organizations. Although regulations exist to ensure the humane treatment of animals during the slaughtering process, enforcing these regulations has been criticized as inadequate. Efforts are being made to educate and enforce humane treatment, but much more work is needed to ensure that animals are treated with dignity and respect. From a research perspective, it is evident that the Serbian meat sector significantly impacts natural resources, especially water, and energy. The industry also pollutes the environment through wastewater discharge and contributes to climate change in terms of global warming, acidification, and eutrophication. Future research should focus on finding ways to minimize the environmental impact of the meat value chain.

1. Introduction

Meat has been an essential part of the Serbian diet for centuries. Serbian cuisine is known for its rich and hearty meat dishes, and meat consumption is deeply ingrained in the country's culture and traditions. Meat has been an integral part of Serbian cuisine since ancient times, and the preparation and consumption of meat dishes are deeply embedded in the country's culture and traditions (*Baltic et al.*, 2018). Meat dishes such as "pljeskavica," *"ćevapčići" (Trbovic et al.*, 2021), "Pirotska peglana kobasica" (*Bogdanović et al.*, 2023), "Sjenički sudžuk" (*Ikonić et al.*, 2023) and "Uzice pršuta" (*Tomić et al.*, 2008) are some of the most popular and beloved foods in Serbia. These dishes are often prepared and served during special occasions and family gatherings, reinforcing the cultural signifi-

***Corresponding author:** Igor Tomasevic, *tbigor@agrif.bg.ac.rs*

Paper received April 21st 2023. Paper accepted May 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

cance of meat in the Serbian diet. The importance of meat is also evident in Serbian folklore and literature. Many Serbian folk tales and songs are centered around hunting and consuming meat, highlighting the cultural significance of meat as a source of sustenance and pleasure (*Mijatović*, 2008).

Meat production and consumption play a significant role in the Serbian economy. The country has a long history of livestock farming, and the meat industry is a vital part of the agricultural sector. In addition, producing and selling meat and meat products provide employment opportunities for many people in the country, contributing to the local and national economy. Serbia has a high per capita meat consumption rate, with an average of 85 kg per person per year, according to a report by the Food and Agriculture Organization of the United Nations (*FAO*, 2019). This rate is significantly higher than the global average of 34 kg per person per year. The consumption of red meat, in particular, is widespread, with pork being the most popular, followed by beef and poultry. Serbia is known for its high-quality meat products, which consumers in countries worldwide seek after. As a result, the country's meat exports have grown significantly in recent years, with major markets including Russia, China, and the European Union (*Mitic et al.*, 2018).

Serbia's meat production industry is dominated mainly by small and medium-sized farms, which often use traditional farming methods and raise livestock on pasture (*Karabasil et al.*, 2018). However, larger commercial farms have emerged in recent years, which use more modern farming methods and technologies to increase efficiency and productivity. This has led to concerns about the impact of commercial farming on the environment and the welfare of animals raised for meat production (*Đekić & Tomašević*, 2017; *Djekic et al.*, 2016; *Skunca et al.*, 2018). In response to these concerns, the Serbian government has regulated and monitored the country's meat production industry, focusing on improving animal welfare and reducing the environmental impact of farming practices. In addition, the government has also encouraged the adoption of more sustainable and eco-friendly farming methods, such as organic farming and agroforestry (*Nikolić et al.*, 2017; *Živanović Miljković & Crnčević*, 2019).

Therefore, this narrative review aims to provide a critical overview of previously published research on meat quality, meat safety, animal welfare, and the impact on the environment of the meat produced in the Republic of Serbia.

2. Materials and methods

We conducted a scoping review, performed using different databases named Scopus (www.scopus.com), Web of Science Core Collection –WOS (www.webofscience.com/), Google Scholar (https://scholar.google.com), and Kobson (https://kobson.nb.rs). For grey literature identification, we used databases available at Google search (www.google.com). According to data availability, documents and other related information were surveyed, considering a 15-year gap from 2008 to 2023. Aggregative synthesis was used to analyse papers and documents.

3. Results and discussion

3.1. Quality

Carcass quality (after bleeding and dressing) is regulated by the Regulation on the slaughter of livestock, poultry, and game (*Serbian Regulation*, 1974), the Regulation on the quality of slaughtered pigs and pork categorization (Serbian Regulation, 1985), and Regulation on the quality of poultry meat (*Serbian Regulation*, 1981). These acts also define significant meat cuts from the carcasses and minimum requirements for packaging, labeling, storage, and transporting the meat.

The population of domestic Simmental breed represents a dominant population of cattle in Serbia (more than 70%) as a breed of combined production traits. A particular emphasis has been given to the importance of organizing beef production from male calves in the cow-calf in an intensive system (*Kučević et al.*, 2019). On the other hand, meat produced from small ruminants (sheep and goats) in Serbia is obtained mainly from young lambs, lambs, and kids. Meat sheep and goat farming systems are characterized by rearing autochthonous breeds (Pramenka and Tsigai sheep and Balkan and Serbian White goat) and using natural resources through grazing. Also, most lamb and kid meat come from male animals of dairy breeds (East Friesian sheep, Alpine, Saanen, and German fawn goat), which are kept in modern dairy farms (*Tomović et al.*, 2016). Bearing in mind that genetics (breed) is one of the most critical factors affecting carcass and meat quality, genetic improvement of cattle, sheep, and goats in Serbia, i.e., replacement of traditional breeds by high-performance industrial breeds for meat, is necessary. Pig (pork meat) and poultry (broiler chicken meat) production systems have reached high-performance levels over the last two decades. Production system change refers to developments and innovations in

all aspects of meat production, from breeding, housing, feeding, and pre-slaughter handling to slaughter-line, chilling, and deboning time (*Dokmanović et al.*, 2014) (*Leskovec et al.*, 2019; *Perić et al.*, 2022; *Tomović et al.*, 2013; *Tomović, Petrović, & Džinić*, 2008; *Tomović et al.,* 2011). However, pale, soft, and exudative (PSE) meat was determined predominantly in pork and chicken meat (*Popović et al.*, 2019; *Tomovic et al.*, 2014; *Tomović et al.*, 2013; *Tomović et al.*, 2008). Thus, the main objective for the economic consideration of pork and chicken meat production in Serbia primarily comprises the absence of PSE meat.

Meat product quality is regulated by the Regulation on the quality of minced meat, semi-finished meat products, and meat products (*Serbian Regulation*, 2019). Among other things, this act regulates the following: classification, categorization, and product name; physical, chemical, physicochemical, and sensory properties, as well as the product composition; physical, chemical, physicochemical, and sensory properties of raw materials, as well as the type and amount of raw materials, additives and other substances used in the processing; storage conditions; etc. Raw materials include meat, fat, connective tissue, offal, blood, and blood products, mechanically separated meat, and freeze-dried meat. Processing meat is defined as the skeletal muscle suitable for human consumption with naturally included or adherent tissue of domestic ungulates (Artiodactyla and Perissodactyla), bird and lagomorphs, and game species. This regulation does not apply to traditional meat products and products from fish, shellfish, sea urchins, sea cucumbers, frogs, turtles, and snails. The types and amounts of additives (preservatives, colours, emulsifiers, stabilisers, etc.) are regulated by the Regulation on food additives (*Serbian Regulation*, 2018). This act is fully harmonised with the European Union Regulation (*Regulation (EC) No 1333/2008*). The quality and uses of other ingredients, such as salt, spices, aromas, sugars, starch, non-meat proteins, starter cultures, enzymes, etc., are described by special national regulations. Meat products were classified into 12 main groups. Certain meat products have been accredited with protected status. Any products using this name must comply with the compositional (what it contains) requirements. Some meat products must have a minimum amount of meat proteins, and some can only include certain types of meat and/or specific animal cuts. For example, the requirements for cooked sausages are summarised in Table 1.

Table 1. Requirements for cooked sausages

Sub-group	Product name	Type of meat	MP	TMP	CC in MP or CC in TP	Storage wtemperature
Finely chopped cooked sausages	'Viršla'	Not specified	≥ 11%	≥ 11%	≤ 20% ≤ 10% (poultry)	0–4°C
	Frankfurter	Pork	≥ 11%		≤ 20%	
	Parisian sausage	Not specified	≥ 10%		≤ 20% ≤ 10% (poultry)	
	White sausage 'Weisswurst'	Not specified	≥ 10%		≤ 20% ≤ 10% (poultry)	
	Other products	Not specified	≥ 10%	≥ 10%	≤ 25% ≤ 15% (poultry)	
Roughly chopped cooked sausages	Serbian sausage	Pork	≥ 16%		≤ 15%	
	Tyrol sausage	Not specified	≥ 12%		≤ 20% ≤ 10% (poultry)	
	Mortadella	Not specified	≥ 12%	≥ 12%	≤ 30%	
	Other products	Not specified	≥ 12%	≥ 12%	≤ 25% ≤ 15% (poultry)	
Cooked sausages with large pieces of meat	Ham sausage	Not specified	≥ 14%		≤ 15% ≤ 10% (poultry)	
	Other products	Not specified	≥ 14%	≥ 14%	≤ 20% ≤ 15% (poultry)	
Meatloaf	Meatloaf	Not specified	≥ 10%	≥ 10%	≤ 25% ≤ 15% (poultry)	

Legend: MP – minimum meat protein content; TMP – minimum total meat protein content; CC – maximum collagen content.

Novel technological developments include decreasing salt content, avoiding nitrite addition or partial replacement of nitrite, adding dietary fiber, probiotics, prebiotics, and other functional components, adding natural antioxidants and other bioactive ingredients, the substitution of saturated fats with unsaturated fats, coating meat products, etc. (*Danilović et al.*, 2021; *Jokanović et al.*, 2020; *Novakovic et al.*, 2020; *Novakovic et al.*, 2020; *Sojic et al.*, 2022; *Sojic et al.*, 2020; *Stajić et al.*, 2022; *Stajić et al.*, 2020; *Šojić, Pavlić, et al.*, 2020; *Šojić et al.*, 2020; *Šojić et al.*, 2021; *Tomović et al.*, 2020; *Tomović et al.*, 2022).

3.2. Safety

Food safety management systems (FSMS) are programs designed to prevent food safety hazards and protect consumers from adverse health effects. FSMS also helps food businesses comply with international standards and regulations, such as IFS, ISO 22000, and the Hazard Analysis and Critical Control Points (HACCP). Ensuring due diligence is one of the primary importance of FSMS. Not all food processing companies meet the required regulations and standards set by law, and FSMS ensures that food processing companies take necessary measures to prevent food safety hazards from occurring. The most important of FSMS is that it ensures the production and service of safe food to consumers, protecting them from foodborne illnesses and related injuries. Therefore, food safety management systems are crucial in protecting public health, ensuring compliance with regulations and standards, and maintaining consumer confidence in the food industry.

Out of 77 Serbian meat producers who participated in the survey, 93.5% claimed to have a fully operational and certified HACCP system in place. In contrast, 6.5% implemented HACCP without third-party certification(*Tomašević et al.*, 2013). ISO 22000 was implemented and certified in 9.1% of the companies, and only 1.3% had implemented and certified the IFS standard. The primary motivation for implementing food safety management systems among Serbian meat producers was to increase and improve the safety and quality of meat products. The initial set-up costs, including investment in new equipment, civil work in the plant, and the redesign of production facilities, were the most significant expenses. According to the results, the main difficulty during HACCP implementation and operation was financial, with companies unable to recoup the costs associated with implementing and operating the HACCP system. The most significant identified benefit was the increased safety of food products, with a mean rank score of 6.45. The increased quality of food products and improved working discipline of staff in food processing were also significant benefits of implementing and operating HACCP in the Serbian meat industry. Overall, the study suggests that HACCP implementation, whether as a standalone food safety system or as part of ISO 22000, is high in Serbia and provides widespread and significant benefits to the meat industry (*Tomašević et al.*, 2013).

Over a span of 10 years, a total of 7351 meat preparations and freshly processed meat products were examined from 555 different Serbian meat producers, with 4.5 years prior and 5.5 years after the obligatory implementation of Hazard Analysis and Critical Control Points (HACCP). The results demonstrated that implementing HACCP has improved compliance with legal regulations. Before HACCP implementation, 18.6% of the samples did not comply with legal requirements, which decreased to 8.3% following the implementation of HACCP. The average sulfite concentrations in all meat preparations and freshly processed meat products decreased by 43%, from 33.6 to 19.3 mg kg−1. The misuse and abuse of sulfites were common regardless of the season. HACCP implementation in the Serbian meat industry brought attention to the misuse of sulfites, leading to better control and minimization of sulfite exposure (*Tomasevic et al.*, 2018). Also, over thirteen years, 20,106 cured meat product samples were examined from 268 different Serbian meat processing plants, covering 7.5 years before and 5.5 years after mandatory HACCP implementation. The findings demonstrate that the compulsory introduction of HACCP had a significant positive impact on the use and regulation of nitrites in the Serbian meat industry. All cured meat products' average residual nitrite levels fell by 30.65%. In addition, the period following mandatory HACCP implementation saw a decrease of 52% in products with residual nitrite levels above 80 mg/kg. Although the proportion of non-compliant samples (0.19%) remained unchanged, the use and control of nitrites in the Serbian meat industry increased due to the application of HACCP principles, resulting in reduced exposure. However, further improvements are necessary to reach the standards observed in cured meat products from more advanced meat industries (*Tomasevic et al.*, 2017).

Over seven years, we collected 48,246 microbiological test results from 130 meat processing plants and 220 meat retail facilities. This data was collect-

ed 41 months before and 43 months after implementing mandatory HACCP (Hazard Analysis and Critical Control Points). Our findings demonstrate a significant improvement in process hygiene indicators in meat establishments due to implementing HACCP. We observed decreased hygiene indicator organisms on all surfaces and meat establishments. The improvement in process hygiene was evident with at least a 1.0 log10CFU/cm2 reduction in aerobic colony counts for food contact surfaces and over two log10CFU/cm2 reduction for cooling facilities such as refrigerators, freezers and other meat cooling devices. Although the improvement in hand hygiene of meat handlers was less evident, there was a steady decline of positive Enterobacteriaceae and Staphylococcus samples after the mandatory HACCP implementation. The improvement in process hygiene for meat processing plants and retail meat facilities was similar (*Tomasevic et al.*, 2016).

We also assessed the food safety knowledge of meat handlers at various stages of the meat chain in Serbian meat establishments, including slaughterhouses, meat processing plants, and retail stores. A self-administered questionnaire was used to evaluate the food safety knowledge of 352 meat handlers, with 31% (110) from slaughterhouses, 36% (125) from meat processing plants, and 33% (116) from retail stores. The questionnaire was structured and consisted of questions measuring the knowledge level among the handlers. The findings indicated that the average knowledge score for all participants was 64%. Handlers from slaughterhouses and meat processing plants obtained significantly better scores (65% and 66%, respectively) than handlers from retail stores (60%). The knowledge score among meat handlers was associated with age, education, and previous food safety training. Moreover, the results revealed that 57.9% of meat handlers knew that bacteria multiply rapidly at 25°C. Still, only 5.5% knew that food contaminated with food poisoning bacteria could not be recognized by visual, olfactory, or taste checks (*Smigic et al.*, 2016).

Finally, all Serbian meat-producing companies claimed that meat safety was not compromised during the Covid-19 pandemic. However, less than half of the food companies had documented any emergency plans related to pandemics or health issues. Staff awareness and hygiene emerged as the most crucial factors in combating Covid-19, whereas temperature checks and World Health Organization health protocols were deemed less important. Companies reported implementing more stringent hygiene protocols and increasing their purchase of personal protective equipment during the pandemic. Retailers were identified as the most affected link in the food supply chain, while food storage facilities were the least affected (*Tomasevic et al.*, 2020).

3.3. Welfare

As global meat production and market growth (*FAO*, 2022) and intensive farming systems increase, the impact of/on livestock is a worldwide concern on various issues such as the environment, animal welfare and protection, carcass/meat quality, meat price, and consumption (*Karabasil et al.*, 2019; *Liu et al.*, 2023). Consumers' awareness about the livestock impact depends on the context – is it low/middle or high-income countries (*Liu et al.*, 2023; *Parlasca & Qaim*, 2022; *Salmon et al.*, 2020). In law- and middle-income countries, livestock is often perceived positively as it provides food and essential nutrients (*Salmon et al.*, 2020). On the other hand, in developed countries, there is a continuous increase in meat production and consumption and awareness of animal welfare and protection (*Parlasca & Qaim*, 2022). Welfare is a growing issue of concern worldwide. It is one of the criteria used to decide whether a system is sustainable because the general public will not accept systems that cause poor welfare and the absence of animal protection (*Broom*, 2011). Along the meat chain, in the pre-slaughter and slaughter phases, many activities lead to stress reactions depending on the species, breed, sex, and age of the animal (*Karabasil et al.*, 2019). Factors that compromise animal well-being and influence animal homeostasis include exposure to unfavorable environmental conditions (farm, market, transport, lairage), improper handling and social mixing, food and water deprivation, and slaughter (*Cappellozza & Marques*, 2021). All these factors (psychological, physical, or physiological) vary in duration (acute, intermittent, or chronic), leading to the stress response and having the potential to disrupt homeostasis and the animal's capability to cope and adapt to the stressors (*Nielsen et al.*, 2020). Stress is an inevitable consequence of livestock production (*Kumar et al.*, 2022), and the key goal is to minimise animals' negative experiences towards "a life worthy of living" (*Mellor*, 2016) so that the housing and management systems result in good welfare (*Broom*, 2021). Nowadays, consumers believe that only such systems can provide products of good quality where the welfare of the farm animals is appropriate (*Broom*,

2021; *Kumar et al.*, 2022). So there is a challenge for the meat sector and stakeholders in preslaughter stress mitigation, ensuring good animal welfare, and producing good quality carcass/meat (*Kumar et al.*, 2022). Therefore, evaluating the stress in livestock through the appropriate indicators and eliminating factors that cause stress-induced responses (*Kumar et al.*, 2022). The important issue is how these situations influence products and their acceptability, as poor welfare led to some important defects such as the death of animals, body weight loss, carcass lesions and composition, and meat quality defects, i.e., pale, soft, exudative (PSE) and dark, firm, dry (DFD) meats (*Čobanović et al.*, 2019; *Čobanović et al.,* 2021; *Čobanović et al.,* 2023; *Karabasil et al.*, 2019; *Karabasil et al.*, 2017; *Urrea et al*, 2021; *Vicic et al.*, 2021; *Zappaterra et al.*, 2022). According to (*Čobanović et al.*, 2023), carcass lesions (severe, moderate or low) are connected with alterations in blood measurements in slaughtered pigs and compromised animal welfare, affected by both animal characteristics and pre-slaughter conditions. As pointed out by the same authors, the presence of carcass lesions, severe or moderate, led to the alterations in meat quality with the higher occurrence of DFD or PSE meat and consequently to economic and financial losses. Good communication between stakeholders (slaughterhouses, transporters, and farmers), providing feedback of information among others, e.g., results of carcass lesions in slaughtered pigs from abattoir to previous steps in the meat chain could contribute to identifying critical points and eliminating negative factors with improved animal welfare in each phase (*Čobanović et al.*, 2021; *Čobanović et al.*, 2023; *Karabasil et al.*, 2017). The application of regular, educational and training programs (animal behaviour, good management practice, standard operating procedure) to train operators (on the farm, during transport, at slaughterhouse) for prudent management of livestock is of great importance for good practice and proper handling (*Čobanović et al.*, 2023; *Kjosevski et al.*, 2021; *Zappaterra et al.*, 2022). Good practice should be embodied in appropriate standards and national regulations that are enforced in consistent science-based knowledge and information, cost-effective and applicable, competent and well-trained personnel for livestock husbandry and management, to apply high animal welfare and protection standards and to fulfill public expectations (*de Witte*, 2009; *Sundermann et al.*, 2023). Transport and slaughter of animals is an unavoidable process step in meat production. National regulations and standards in animal welfare and protection are updated in continuity to meet consumer and public expectations. Official controls of Food business operators (FBOs) are in charge of providing clear evidence and confirmation that animals are spared any pain/distress/suffering during the transport, killing, and related operations (*Sundermann et al.*, 2023). FBOs know that a successful manufacturer has an attitude "as an industry that cares," and consumer trust is the foundation.

3.4. Environment

It has been scientifically confirmed that meat production has a severe environmental impact and is identified as a leading polluter among food sectors (*Djekic & Tomasevic*, 2016). Although livestock farms are pointed as the most significant contributors to environmental pollution, other actors in the meat chain continuum also have their eco-roles. Considerable interference with natural spheres — atmosphere, lithosphere, hydrosphere, and biosphere, is mainly observed at the farm level through exploiting and polluting natural resources (*Röös et al.,*2013). Environmental impacts occurring in the following supply chain stages – slaughterhouses and meat processing plants are not as high and dominant but may not be neglected and are the focus of the academia (*Djekic et al.*, 2016). The Serbian meat industry has been analyzed from two perspectives, as depicted by *Djekic & Tomasevic* (2016) – in terms of life-cycle assessment (LCA) of meat and meat products and based on various environmental practices occurring at different actors of the value chain – livestock farms, slaughterhouses, and meat processors.

Life cycle assessment is a scientifically validated method outlined in ISO 14040 (*ISO*, 2006) covering the goals and scope of the LCA, inventory analysis of all inputs, processes, and outputs, impact assessment for selected environmental indicators, and interpretation of the results. Analysis of meat products from a "farm to fork" perspective shows that it consists of five main subsystems – farms, slaughterhouses, meat processing plants, retailers, and consumers (*Djekic & Tomasevic*, 2016). When it comes to environmental impacts associated with meat LCA, four of them prevail (*Djekic & Tomasevic*, 2018). Global warming potential (GWP) measures the emission of greenhouse gasses, represents the damage level expressed in kg CO2 equivalent (*IPCC*, 2013), and is mainly linked with enteric fermentation and manure management on farms as well as feed pro-

duction (*Gerber et al.*, 2015). However, it's important to emphasize that besides the impact of meat production on climate change, there is a vice versa effect of climate change on meat production, mainly at farms through increased depletion of resources for combating heat stress of animals, biodiversity loss, and shifting towards sustainable agriculture associated with feed production (*Djekic & Tomasevic*, 2020). Acidification potential analyzes the impact of various acidifying substances on the ecosystem (soil, water, flora, and fauna) expressed as kg SO2 equivalent (*Čuček et al.*, 2015). This is mainly associated with nitrates and ammonia from manures (*Dalgaard et al.*, 2007). Eutrophication potential calculates excessive levels of macronutrients (from livestock feed production) in air, water, and soil, expressed as kg PO4 equivalents (*Djekic & Tomasevic*, 2018). Finally, ozone depletion potential computes the destructive effects of halogenated hydrocarbons (used in meat cold chains – both refrigeration and deep freezing). It is determined in association with R-11 equivalent (*Hischier et al.*, 2010). Inadequate temperatures in the cold meat supply chains cause an increase in the biological activity of microorganisms and thus increase their growth, causing unsafe meat (*Ren et al.*, 2022). Current logistics innovations in the food sector are shifted towards developing low-carbon and eco-friendly supply chains (*Han et al.*, 2021). LCA study of Serbian pork farms at the farm gate (expressed in kg of livestock weight as a functional unit — FU) shows that this meat sector contributes to the emission of 3.50 kg CO2e / FU, 31.257 g SO2e / FU, 55.030 g PO4e / FU and 0.151 mg R11e / FU (*Djekic et al.*, 2021). The primary indicator of a studied life cycle is a FU, as it represents a benchmark point for comparing different types of food. Its appropriate choice is paramount (*Djekic et al.*, 2019). In meat science, three functional units occur – one kg of livestock (at farms), one kg of the carcass (at slaughterhouses), and one kg of meat/meat products (stages from meat processing plants to consumers) (*Djekic & Tomasevic*, 2016). From the perspective of consumers, a look at the other side of the coin was performed by analyzing pork meat dietary habits in Serbia based on reported weekly consumption. Results show that an average Serbian meat consumer contributes to 4.032 kg CO2e / week, 35.972 g SO2e / week, 63.466 g PO4e / week, and 0.17435 mg R11e / week (*Djekic et al.*, 2021).

The survey that covered 16 slaughterhouses and 14 meat processing plants participating with more than half of national Serbian meat production shows that environmental practices (energy and water consumption, waste management, and wastewater discharge) are implemented on a higher level in large companies, in slaughterhouses and in companies with implemented environmental management system (*Djekic et al.*, 2016). Water is used throughout all meat production steps, from feeding animals to slaughtering and meat processing (*Kupusovic et al.*, 2007). Energy is needed mainly for meat processing and maintaining the meat cold chain (*IPPC*, 2006). Wastewater is rich in various pollutants, both organic (blood, fat, manure, undigested stomach contents, meat, and meat extracts) (*Djekic & Tomasevic*, 2016) and inorganic from cleaning chemicals needed for good hygiene practices (*CAC*, 2020). *Aleksić et al.* (2020) performed an interesting study on water consumption and wastewater characteristics of the Serbian meat slaughtering industry. Their study covered 41 slaughterhouses, revealing that more than half have no wastewater treatment and that wastewater quality in some of the observed objects exceeded prescribed legal limits for chemical and biochemical oxygen demands mainly due to the presence of organic matter such as blood, tallow, and mucosa. The freshwater consumption varied depending on the type and size of the slaughterhouse, but the highest need was associated with good hygiene.

4. Conclusion

Meat safety is an important issue in Serbia, as in any country. The Serbian government has implemented several measures to ensure the safety of meat products, including regular inspections of slaughterhouses and meat processing facilities. The country has also adopted EU regulations on meat safety, meaning that all meat products must meet strict hygiene, storage, and labeling standards. Consumers in Serbia are advised to purchase meat products only from reputable sources and to ensure that the meat is properly cooked before consumption. Additionally, individuals are encouraged to practice good hygiene when handling meat products, such as washing their hands thoroughly before and after handling raw meat. While meat safety is a reasonable concern in Serbia, the government, producers, and consumers are taking steps to mitigate the risks associated with consuming meat products. The current situation and prospects for the meat processing industry in Serbia are based mainly on the development of new healthier products ('clean label') with altered and novel formulations and on the development of new pack-

aging films. The welfare of slaughtering animals in Serbia has been a concern among animal rights activists and organizations. While there are regulations to ensure the humane treatment of animals during the slaughtering process, enforcing these regulations has been criticized as insufficient.

In some cases, animals may be subjected to overcrowding and rough handling before being slaughtered, which can cause distress and pain. Additionally, there have been reports of illegal slaughtering practices that further compromise the welfare of animals. Efforts are being made to improve the situation through education and enforcement, but there is still much work to be done to ensure the humane treatment of animals in Serbia. Regardless of the research perspective (meat-based or meat company-based), it is evident that the entire Serbian meat sector has severe pressure on natural resources (mainly water and energy), pollutes the environment by discharging wastewater, and influences climate change concerning global warming, acidification, and eutrophication potentials. Future research should focus on finding solutions how to decrease the environmental burden of the meat value chain.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Aleksić, N., Nešović, A., Šušteršič, V., Gordić, D. & Milovanović, D. (2020). Slaughterhouse water consumption and wastewater characteristics in the meat processing industry in Serbia. *Desalin Water Treat,* 190, 98–112.

Baltic, M. Z., Janjic, J., Popovic, M., Baltic, T., Jankovic, V., Starcevic, M. & Sarcevic, D. (2018). Meat in traditional Serbian cuisine. *Meat Technology,* 59(1), 54–62.

Bogdanović, S., Stanković, S., Berić, T., Tomasevic, I., Heinz, V., Terjung, N. & Dimkić, I. (2023). Bacteriobiota and Chemical Changes during the Ripening of Traditional Fermented "Pirot 'Ironed' Sausage". *Foods,* 12(3), 664, doi.org/10.3390/foods12030664

Broom, D. (2011). A history of animal welfare science. *Acta Biotheoretica,* 59, 121–137.

Broom, D. (2021). Farm animal welfare: A key component of the sustainability of farming systems. *Veterinarski Glasnik,* 75(2), 145–151.

CAC, (2020). CXC-1 1969, Rev. 5–2020 General principles of food hygiene *Codex Alimentarius Commission — International Food Standards*. Rome. Italy: Food and Agriculture Organization and World Health Organization.

Cappellozza, B. I. & Marques, R. S. (2021). Effects of Pre-Slaughter Stress on Meat Characteristics and Consumer Experience. *Meat and Nutrition*: IntechOpen doi: 10.5772/intechopen.96742

Čobanović, N., Janković, L., Vasilev, D., Dimitrijević, M., Teodorović, V., Kureljušić, B. & Karabasil, N. (2019). Slaughterline records of various postmortem pathological lesions and their influence on carcass and meat quality in slaughtered pigs. *Animal Science Journal,* 90(11), 1475–1483.

Čobanović, N., Novaković, S., Tomašević, I. & Karabasil, N. (2021). Combined effects of weather conditions, transportation time and loading density on carcass damages and meat quality of market-weight pigs. *Archives Animal Breeding,* 64(2), 425–435.

Čobanović, N., Suvajdžić, B., Vićić, I., Vasilev, D. & Karabasil, N. (2023). Prevalence of carcass lesions and their effects on welfare, carcass composition and meat quality in slaughtered pigs. *Annals of Animal Science,* 23(2), 597–609.

Čuček, L., Klemeš, J. J. & Kravanja, Z. (2015). Overview of environmental footprints *Assessing and Measuring Environmental Impact and Sustainability,* pp. 131193: Elsevier.

Dalgaard, R., Halberg, N. & Hermansen, J. E. (2007). Danish pork production — An environmental assessment *DJF Animal Science*, 82, University of Aarhus — Faculty of Agricultural Sciences.

Danilović, B., Đorđević, N., Milićević, B., Šojić, B., Pavlić, B., Tomović, V. & Savić, D. (2021). Application of sage herbal dust essential oils and supercritical fluid extract for the growth control of Escherichia coli in minced pork during storage. *LWT,* 141, 110935.

de Witte, K. (2009). Development of the Australian Animal Welfare standards and guidelines for the land transport of livestock: Process and philosophical considerations. *Journal of Veterinary Behavior,* 4(4), 148–156.

Djekic, I. & Tomašević, I. (2017). *Environmental footprints in the meat chain.* Paper presented at the IOP Conference Series: Earth and Environmental Science, 85 012015, doi :10.1088/1755-1315/85/1/012015

Djekic, I., Blagojevic, B., Antic, D., Cegar, S., Tomasevic, I. & Smigic, N. (2016). Assessment of environmental practices in Serbian meat companies. *Journal of Cleaner Production,* 112, Part 4, 2495–2504.

Djekic, I., Blagojevic, B., Antic, D., Cegar, S., Tomasevic, I. & Smigic, N. (2016). Assessment of environmental practices in Serbian meat companies. *Journal of Cleaner Production,* 112, 2495–2504.

Djekic, I., Bozickovic, I., Djordjevic, V., Smetana, S., Terjung, N., Ilic, J., A. Dorski & Tomasevic, I. (2021). Can we associate environmental footprints with production and consumption using Monte Carlo simulation? Case study with pork meat. *Journal of the Science of Food and Agriculture,* 101(3), 960–969.

Djekic, I., Pojić, M., Tonda, A., Putnik, P., Bursać Kovačević, D., Režek-Jambrak, A. & Tomasevic, I. (2019). Scientific Challenges in Performing Life-Cycle Assessment in the Food Supply Chain. *Foods,* 8(8), 301, doi.org/10.3390/foods8080301

Djekic, I. & Tomasevic, I. (2016). Environmental impacts of the meat chain — Current status and future perspectives. *Trends in Food Science & Technology,* 54, 94–102.

Djekic, I. & Tomasevic, I. (2018). Chapter 3 — Environmental Indicators in the Meat Chain In S. S. Muthu (Ed.), *Quantification of Sustainability Indicators in the Food Sector* (1st ed., pp. 55–82). Singapore: Springer Singapore.

Djekic, I. & Tomasevic, I. (2020). Impact of Animal Origin Food Production on Climate Change and Vice Versa: Analysis from a Meat and Dairy Products Perspective. In W. Leal Filho, J. Luetz & D. Ayal (Eds.), *Handbook of Climate Change Management: Research, Leadership, Transformation* (pp. 1–16). Cham: Springer International Publishing.

Dokmanović, M., Velarde, A., Tomović, V., Glamočlija, N., Marković, R., Janjić, J. & Baltić, M. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. *Meat Science,* 98(2), 220–226.

FAO, (2019). Food Outlook—biannual report on global food markets. *Rome, Food and Agriculture Organization of the United Nations.*

FAO, (2022). Food Outlook-Biannual report on global food markets. *Rome: FAO, June 2020*.

Gerber, P. J., Mottet, A., Opio, C. I., Falcucci, A. & Teillard, F. (2015). Environmental impacts of beef production: Review of challenges and perspectives for durability. *Meat Science,* 109, 2–12.

Han, J.-W., Zuo, M., Zhu, W.-Y., Zuo, J.-H., Lü, E.-L. & Yang, X.-T. (2021). A comprehensive review of cold chain logistics for fresh agricultural products: Current status, challenges, and future trends. *Trends in Food Science & Technology,* 109, 536–551.

Hischier, R., Weidema, B., Althaus, H.-J., Bauer, C., Doka, G., Dones, R., Frischknecht, R., Hellwg, S., Humbert, S., Jungbluth, N., Köllner, T., Loerincik, Y., Margni, M. & Nemecek T., (2010). Implementation of life cycle impact assessment methods. *Final report ecoinvent v2, 2*.

Ikonić, P., Jokanović, M., Ćućević, N., Peulić, T., Šarić, L., Tomičić, Z. & Tomašević, I. (2023). Effect of different ripening conditions on amino acids and biogenic amines evolution in Sjenički sudžuk. *Journal of Food Composition and Analysis,* 115, doi: 10.1016/j.jfca.2022.105009

IPCC, (2013). Climate change 2013: The physical science basis. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P. M. Midgley (Eds.), *Report of the Intergovernmental Panel on Climate Change*: Cambridge University Press Cambridge.

IPPC, (2006). Integrated Pollution Prevention and Control *Reference Document on Best Available Techniques in the Food, Drink and Milk Industries*. Seville, Spain: European Commission.

ISO, (2006). ISO 14040:2006 Environmental management — Life cycle assessment — Principles and framework. Geneva, Switzerland: International Organization for Standardization.

Jokanović, M., Ivić, M., Škaljac, S., Tomović, V., Pavlić, B., Šojić, B. & Ikonić, P. (2020). Essential oil and supercritical extracts of winter savory (Satureja montana L.) as antioxidants in precooked pork chops during chilled storage. *LWT, 134*, 110260.

Karabasil, N., Boskovic, T., Vicic, I., Cobanović, N., Dimitrijevic, M. & Teodorovic, V. (2019). *Meat quality: Impact of various pre-slaughter conditions.* Paper presented at the IOP Conference Series: Earth and Environmental Science 333(1), 012033, DOI: 10.1088/1755-1315/333/1/012033

Karabasil, N., Bošković, T., Tomašević, I., Vasilev, D., Dimitrijević, M., Katanić, N. & Antić, D. (2018). Production of traditional meat products in small and micro establishments in Serbia: Current status and future perspectives. *Acta Veterinaria,* 68(4), 373–390.

Karabasil, N., Čobanović, N., Vučićević, I., Stajković, S., Becskei, Z., Forgách, P. & Aleksić-Kovačević, S. (2017). Association of the severity of lung lesions with carcass and meat quality in slaughter pigs. *Acta Veterinaria Hungarica,* 65(3), 354–365.

Kjosevski, M., Nikolovski, M., Ilievska, K., Pendovski, L. & Ilieski, V. (2021). The three pillars of applied farm animal welfare. *Veterinarski Glasnik,* 75(2), 99–111.

Kučević, D., Papović, T., Tomović, V., Plavšić, M., Jajić, I., Krstović, S. & Stanojević, D. (2019). Influence of Farm Management for Calves on Growth Performance and Meat Quality Traits Duration Fattening of Simmental Bulls and Heifers. *Animals (Basel),* 9(11), 941, doi. org/10.3390/ani9110941

Kumar, P., Abubakar, A. A., Verma, A. K., Umaraw, P., Adewale Ahmed, M., Mehta, N., Hayat, M. N., Kaka, U. & Sazili, A. Q. (2022). New insights in improving sustainability in meat production: opportunities and challenges. *Critical Reviews in Food Science and Nutrition*, 1–29, doi: 10.1080/10408398.2022.2096562

Kumar, P., Ahmed, M. A., Abubakar, A. A., Hayat, M. N., Kaka, U., Ajat, M., Goh, Y. M. & Sazili, A. Q. (2022). Improving animal welfare status and meat quality through assessment of stress biomarkers: A critical review. *Meat Science*, 109048, doi: 10.1016/j.meatsci.2022.109048

Kupusovic, T., Midzic, S., Silajdzic, I. & Bjelavac, J. (2007). Cleaner production measures in small-scale slaughterhouse industry – case study in Bosnia and Herzegovina. *Journal of Cleaner Production,* 15(4), 378–383.

Leskovec, J., Levart, A., Perić, L., Đukić Stojčić, M., Tomović, V., Pirman, T., Salobir, J. & Rezar, V. (2019). Antioxidative effects of supplementing linseed oil-enriched diets with α-tocopherol, ascorbic acid, selenium, or their combination on carcass and meat quality in broilers. *Poultry Science,* 98(12), 6733–6741.

Liu, J., Chriki, S., Kombolo, M., Santinello, M., Pflanzer, S. B., Hocquette, É., Ellies-Oury, M.-P. & Hocquette, J. F. (2023). Consumer perception of the challenges facing livestock production and meat consumption. *Meat Science,* 200, 109144, doi: 10.1016/j.meatsci.2023.109144

Mellor, D. J. (2016). Updating animal welfare thinking: Moving beyond the "Five Freedoms" towards "a Life Worth Living". *Animals,* 6(3), 21.

Mijatović, E. L. (2008). *Serbian folk-lore: Popular Tales*: Isbister.

Mitic, V., Kilibarda, N., Brdar, I., Kostic, M., Sarcevic, D., Karabasil, N. & Mizdrakovic, V. (2018). Measuring competitiveness in the meat industry market: Are there any oligopolies in Serbia? *Meat Technology,* 59(2), 127–136, doi.org/10.18485/meattech.2018.59.2.8

Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Drewe, J. A., Garin-Bastuji, B., Rojas, J. L. G., Schmidt C. G., Michael, V., Chueca, M. A. M., Roberts, H. C., Sihvonen L. H., Spoolder, H., Stahl, K., Verlade, A., Viltrop, A., Candiani, d., Van der Stede, Y. & Schmidt, C. G. (2020). Welfare of cattle at slaughter. *EFSA journal, 18*(11), e06275, doi: 10.2903/j.efsa.2020.6275

Nikolić, S. R., Vuković, P. & Grujić, B. (2017). Measures to support the development of organic farming in the EU and Serbia. *Ekonomika Poljoprivrede*, 64 (1), 323–337.

Novakovic, S., Djekic, I., Klaus, A., Vunduk, J., Dordevic, V. & Tomovic, V. (2020). The potential of horn of plenty mushroom (Craterellus cornucopioides). *Fleischwirtschaft International: Journal for Meat Production and Meat Processing,* (4), 57–63.

Novakovic, S., Djekic, I., Klaus, A., Vunduk, J., Đorđević, V., Tomovic, V., Kocic-Tanackov, S., Lorenzo J. M. &Tomasevic, I. (2020). Application of porcini mushroom (Boletus edulis) to improve the quality of frankfurters. *Journal of Food Processing and Preservation,* 44(8), e14556, DOI: 10.1111/jfpp.14556

Parlasca, M. C. & Qaim, M. (2022). Meat consumption and sustainability. *Annual Review of Resource Economics,* 14, 17–41.

Perić, L., Žikić, D., Stojčić, M. Đ., Tomović, V., Leskovec, J., Levart, A., Salobir, J., Kanački, Z. & Rezar, V. (2022). Effect of Chestnut Tannins and Vitamin E Supplementation to Linseed Oil-Enriched Diets on Growth Performance, Meat Quality, and Intestinal Morphology of Broiler Chickens. *Agriculture,* 12(11), 1772, doi.org/10.3390/agriculture12111772

Popović, S., Puvača, N., Peulić, T., Ikonić, P., Spasevski, N., Kostadinović, L. & Đuragić, O. (2019). The usefulness of dietary essential oils mixture supplementation on quality aspect of poultry meat. *Journal of Agronomy, Technology and Engineering Management,* 2(6), 335–343.

Ren, Q.-S., Fang, K., Yang, X.-T. & Han, J.-W. (2022). Ensuring the quality of meat in cold chain logistics: A comprehensive review. *Trends in Food Science & Technology,* 119, 133–151.

Röös, E., Sundberg, C., Tidåker, P., Strid, I. & Hansson, P.-A. (2013). Can carbon footprint serve as an indicator of the environmental impact of meat production? *Ecological Indicators,* 24, 573–581.

Regulation (EC) No 1333/2008 of the european parliament and of the council of 16 December 2008 on food additives. Official Journal of the European Union, L 354, 16–33.

Serbian Regulation, (1974). Pravilnik o kvalitetu mesa stoke za klanje, peradi i divljači. Službeni list SFRJ, broj 74; Rulebook on slaughter livestock, poultry and game. Official Gazette of the SFRJ, No 34.

Serbian Regulation, (1981). Pravilnik o kvalitetu mesa pernate živine. Službeni list SFRJ, broj 1; Rulebook on quality of poultry meat. Official Gazette of the SFRJ, No 1.

Serbian Regulation, (1985). Pravilnik o kvalitetu zaklanih svinja i kategorizaciji svinjskog mesa. Službeni list SFRJ, broj 2; Rulebook on quality of slaughtered pigs and pork categorization. Official Gazette of the SFRJ, No 2.

Serbian Regulation, (2018). Pravilnik o prehrambenim aditivima. Službeni glasnik Repblike Srbije, broj 53; Rulebook on food additives. Official Gazette of the Republic of Serbia, No 53.

Serbian Regulation, (2019). Pravilnik o kvalitetu usitnjenog mesa, poluproizvoda od mesa i proizvoda od mesa. Službeni glasnik Repblike Srbije, broj 50; Rulebook on quality of minced meat, semi-finished meat products and meat products. Official Gazette of the Republic of Serbia, No 50.

Salmon, G. R., MacLeod, M., Claxton, J., Ciamarra, U. P., Robinson, T., Duncan, A. & Peters, A. (2020). Exploring the landscape of livestock 'Facts'. *Global Food Security,* 25, 100329.

Skunca, D., Tomasevic, I., Nastasijevic, I., Tomovic, V. & Djekic, I. (2018). Life cycle assessment of the chicken meat chain. *Journal of Cleaner Production,* 184, 440–450.

Smigic, N., Antic, D., Blagojevic, B., Tomasevic, I. & Djekic, I. (2016). The level of food safety knowledge among meat handlers. *British Food Journal,* 118(1), 9–25.

Sojic, B., Pavlic, B., Tomovic, V., Danilovic, B., Loncarevic, I., Petrovic, J. & Skaljac, S. (2022). The effect of wild thyme extracts on quality and shelf-life of cooked sausages Possibilities for the application of thyme extract as an antioxidant in sausage manufacturing. *Fleischwirtschaft,* 102(5), 82–87.

Sojic, B., Pavlic, B., Tomovic, V., Kocic-Tanackov, S., Zekovic, Z., Skaljac, S. & Jokanovic, M. (2020). Supercritical fluid extract of winter savory. *Fleischwirtschaft,* 100(11), 92–95.

Stajić, S., Tomasević, I., Tomović, V. & Stanišić, N. (2022). Dietary fibre as phosphate replacement in all-beef model system emulsions with reduced content of sodium chloride. *Journal of Food and Nutrition Research*, 61, 277–285.

Stajić, S., Tomašević, I., Stanišić, N., Tomović, V., Lilić, S., Vranić, D. & Živković, D. (2020). Quality of dry-fermented sausages with backfat replacement. *Fleischwirtschaft,* 100(7), 74–81.

Sundermann, T., Bibbal, D., Holleville, N. & Salines, M. (2023). Moving from routine to risk-based official controls in slaughterhouses: Development of a scoring tool for the risk of non-compliance with animal welfare regulations. *Food Control,* 143, 109321.

Šojić, B., Pavlić, B., Tomović, V., Kocić-Tanackov, S., Đurović, S., Zeković, Z., Belović, M., Torbica, A., Jokanović, M., Urumović, N., Vujadinović, D., Ivić, M. & Škaljac, S. (2020). Tomato pomace extract and organic peppermint essential oil as effective sodium nitrite replacement in cooked pork sausages. *Food Chemistry,* 330, 127202.

Šojić, B., Tomović, V., Kocić-Tanackov, S., Kovačević, D. B., Putnik, P., Mrkonjić, Ž., Đurović, S., Jokanović, M., Ivić, M., Škaljac, S. & Pavlić, V. (2020). Supercritical extracts of wild thyme (Thymus serpyllum L.) by-product as natural antioxidants in ground pork patties. *LWT,* 130, 109661, doi.org/j.lwt.2020.109661

Šojić, B., Tomović, V., Savanović, J., Kocić-Tanackov, S., Pavlić, B., Jokanović, M., Milidrag, A., Martinović, A., Vujadinović, D. & Vukić, M. (2021). Sage (Salvia officinalis L.) essential oil as a potential replacement for sodium nitrite in dry fermented sausages. *Processes,* 9(3), 424, doi.org/10.3390/pr9030424

Tomasevic, I., Dodevska, M., Simić, M., Raicevic, S., Matovic, V. & Djekic, I. (2017). The use and control of nitrites in Serbian meat industry and the influence of mandatory HACCP implementation. *Meat Science,* 134, 76–78.

Tomasevic, I., Dodevska, M., Simić, M., Raicevic, S., Matovic, V. & Djekic, I. (2018). A decade of sulphite control in Serbian meat industry and the effect of HACCP. *Food Additives and Contaminants: Part B Surveillance,* 11(1), 49–53.

Tomasevic, I., Kovačević, D. B., Jambrak, A. R., Szendrő, K., Dalle Zotte, A., Prodanov, M., Sołowiej, B., Subić, A., Roljević, J., Semenova, S., Kročko, A, Duckova, M., Getya, V., Kravchenko O. A. & Djekic, I. (2020). Validation of novel food safety climate components and assessment of their indicators in Central and Eastern European food industry. *Food Control,* 117.

Tomasevic, I., Kuzmanović, J., Andelković, A., Saračević, M., Stojanović, M. M. & Djekic, I. (2016). The effects of mandatory HACCP implementation on microbiological indicators of process hygiene in meat processing and retail establishments in Serbia. *Meat Science,* 114, 54–57.

Tomašević, I., Šmigić, N., Dekić, I., Zarić, V., Tomić, N. & Rajković, A. (2013). Serbian meat industry: A survey on food safety management systems implementation. *Food Control,* 32(1), 25–30.

Tomić, N., Tomašević, I., Radovanović, R. & Rajković, A. (2008). "Uzice beef prshuta": influence of different salting processes on sensory properties. *Journal of Muscle Foods,* 19(3), 237–246.

Tomovic, V. M., Zlender, B. A., Jokanović, M. R., Tomovic, M. S., Sojic, B. V., Skaljac, S. B. & Hromis, N. M. (2014). Technological quality and composition of the M. semimembranosus and M. longissimus dorsi from Large White and Landrace Pigs. *Agricultural and Food Science*, 23(1), 9–18.

Tomović, V., Šojić, B., Savanović, J., Kocić-Tanackov, S., Pavlić, B., Jokanović, M., Đorđević, V., Parunović, N., Martinović, A. & Vujadinović, D. (2020). New formulation towards healthier meat products: Juniperus communis L. essential oil as alternative for sodium nitrite in dry fermented sausages. *Foods,* 9(8), 1066.

Tomović, V., Šojić, B., Savanović, J., Kocić-Tanackov, S., Pavlić, B., Jokanović, M., Đorđević, V., Parunović, N., Martinović, A. & Vujadinović, D. (2022). Caraway (Carum carvi L.) essential oil improves quality of dry-fermented sausages produced with different levels of sodium nitrite. *Journal of Food Processing and Preservation,* 46(10), e15786.

Tomović, V. M., Jokanović, M. R., Petrović, L. S., Tomović, M. S., Tasić, T. A., Ikonić, P. M., Šumić, M. Z., Škaljac S. B. & Šošo, M. M. (2013). Sensory, physical and chemical characteristics of cooked ham manufactured from rapidly chilled and earlier deboned M. semimembranosus. *Meat Science,* 93(1), 46–52.

Tomović, V. M., Jokanović, M. R., Švarc-Gajić, J. V., Vasiljević, I. M., Šojić, B. V., Škaljac, S. B., Pihler, I., Simin, V., Krajinović, M. & Žujović, M. M. (2016). Physical characteristics and proximate and mineral composition of Saanen goat male kids meat from Vojvodina (Northern Serbia) as influenced by muscle. *Small Ruminant Research,* 145, 44–52.

Tomović, V. M., Petrović, L. S. & Džinić, N. R. (2008). Effects of rapid chilling of carcasses and time of deboning on weight loss and technological quality of pork semimembranosus muscle. *Meat Science,* 80(4), 1188–1193.

Tomović, V. M., Petrović, L. S., Tomović, M. S., Kevrešan, Ž. S. & Džinić, N. R. (2011). Determination of mineral contents of semimembranosus muscle and liver from pure and crossbred pigs in Vojvodina (northern Serbia**).** *Food Chemistry,* 124(1), 342–348.

Trbovic, D., Petronijevic, R., Lakicevic, B., Nikolic, A., Bajcic, A., Sefer, M. & Parunovic, N. (2021). *Traditional meat preparations in the Balkans region.* Paper presented at the IOP Conference Series: Earth and Environmental Science, 854 012100, doi:10.1088/1755-1315/854/1/012100

Urrea, V. M., Bridi, A. M., Ceballos, M. C., Paranhos da Costa, M. J. & Faucitano, L. (2021). Behavior, blood stress indicators, skin lesions, and meat quality in pigs transported to slaughter at different loading densities. *Journal of Animal Science,* 99(6), 119.

Vicic, I., Petrovic, M., Stajkovic, S., Cobanović, N. & Karabasil, N. (2021). *Seasonal overview of beef meat quality in a small-scale slaughterhouse.* Paper presented at the IOP Conference Series: Earth and Environmental Science, 854 012103, doi 10.1088/1755-1315/854/1/012103

Zappaterra, M., Padalino, B., Menchetti, L., Arduini, A., Pace, V. & Nanni Costa, L. (2022). Carcass Lesion Severity and Pre-Slaughter Conditions in Heavy Pigs: A Prospective Study at a Commercial Abattoir in Northern Italy. *Applied Sciences,* 12(3), 1078.

Živanović Miljković, J. & Crnčević, T. (2019). Multifunctional urban agriculture and agroforestry for sustainable land use planning in the context of climate change in Serbia. *Climate Change-Resilient Agriculture and Agroforestry: Ecosystem Services and Sustainability*, 197–213.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 658.626:392.8(437.6)

ID: 126078729

https://doi.org/10.18485/meattech.2023.64.2.2

Review paper

Characteristics of traditional guaranteed meat specialties in Slovakia

Miroslav Kročko[a], Viera Ducková[a] and Marek Bobko[a]*

[a] *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Science, Tr. A. Hlinku 2, Nitra, Slovakia*

ARTICLE INFO

Keywords:
Salami
Sausages
Traditional guaranteed specialties

ABSTRACT

An agricultural product intended for human consumption or foodstuff with a traditional composition, or produced according to a traditional production method, can become a traditional speciality guaranteed (TSG). This possibility encourages the diversification of agricultural production and has positive consequences in several areas. The introduction of a TSG boosts farmers' revenues and maintains the population in less favored or remote areas by promoting the rural economy. It also increases the market value of the products of economic operators, by guaranteeing that they are distinguishable from other similar products or foodstuffs. In addition, thanks to the introduction of this designation, consumers will able be to make more informed choices on the basis of clear information on the specific characteristics of the products they buy. Major TSG products containing meat sold in Slovakia include: *špekačky*, lovecký salami, Spiš sausages, and Liptov salami. These products are contained among TSGs of the Slovak and also Czech Republic. The reason is that all these products have a production tradition in both the Czech Republic and Slovakia. However, Spiš sausages and Liptov salami have a primary geographic relation to the Slovak Republic.

1. Introduction

The European Union recognizes three types of trademarks for foodstuffs: protected geographical indication (PGI), protected designation of origin (PDO) and traditional guaranteed specialty (TGS). Slovakia has already registered: 11 products designated as PDO — Bardejov honey, South Slovak wine, Central Slovak wine, Eastern Slovak wine, Carpathian pearl (wine), Little Carpathian wine, Nitra wine, Zitava pepper, Ruby of Skalica, Stupava cabbage, Tokaj wine; 13 registered as PGI — Skalica trdelnik, Bryndza, Parenica, Ostiepok, Tekov salami cheese, Zazriva and Orava korbáčik, Klenovec cheese (syrec), Hrušov lepnik, Levice malt, Liptov droby, Slovak wine, Zazriva vojky. In total, there are only 84 foods in the world registered in the register of guaranteed traditional EU specialties, and among them, Slovakia has registered 7 products — Sheep's smoked cheese, Sheep's lump cheese, Bratislava roll, Liptov salami, Spiš sausages, Lovecký salami, Špekačky. TGS refers to typical ingredients and production process. The TGS must be produced in a traditional way, having a traditional composition and character. Only food that has been used on the same market for at least one human generation, i.e., for at least 25 years, and which differs significantly from similar products can be labeled 'traditional' (*eAmbrosia*, 2023; EU No. 1151/2012).

***Corresponding author:** Miroslav Kročko, *miroslav.krocko@uniag.sk*

Paper received July 30th 2023. Paper accepted August 5th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Liptov salami

The name Liptov salami is associated with a specific type of meat product. It is known and used thanks to its long tradition. The original Liptov salami smells of mace, nutmeg, ginger and smoking. When cut, it has a soft, uniform appearance. It is made from beef and pork, cutlet and bacon. The Slovak Association of Meat Processors and the Czech Association of Meat Processors jointly applied for registration in the register. In 1956, at the meat processing plant in Dubnica nad Váhom, they tried to prepare a product that would differ from other finely ground meat products. Therefore, part of the bacon was replaced with pork cutlet. The mixture was not ground into a mosaic, as was common at the time, but finely. Paprika was not added to it at that time, because it was not customary to add it to meat products in the Liptov region. That is why the new product was named Liptov salami. At the start of the 1970s, the unique recipe for Liptov salami was created at this research department, and the product went on to find great favor. It was gradually put into production at many meat processing enterprises. Industry standard ON 57 6913 was adopted in 1978. This standard has been regularly updated and revised. One of the most recently renewed joint standards is the technical-economic standard (THN) for product number 764421 64, produced on 1 September 1988. The introduction to this standard includes the following note: 'Conforms to ON 57 6913'. In 1978, the Western Slovakia Meat Industry works at Trnava began producing Liptov salami in cooperation with the research department of the GRT. Until 1990 we produced Liptov salami according to the traditional recipe and did not use paprika. Liptov salami allowed pork fat to be processed to an increased level, and around 600 kg was produced at the Trnava factory daily. It was very popular among consumers because of its distinctive taste (EC. No. 509/2006).

3. Traditional Lovecký salami (hunter's salami)

Traditional lovecký salami differs from other long-lasting fermented meat products on the one hand by its characteristic flat prism shape, which the product acquires by shaping during maturation. Furthermore, it is the specific taste of the product defined by the prescribed composition of the main raw materials, spices, but also by the applied fermentation process. The beginning of the production of traditional lovecký salami can be dated back to the beginning of the 20th century. At this time, it was produced mainly in the winter season due to more favorable conditions for the ripening process and also due to the demanding processing of the raw materials by mild freezing, which is a prerequisite for the creation of perfect graining of the raw material. Later, after the improvement of the cooling and the machinery of the smokehouses, its production was focused mainly on supplying the Easter and Christmas markets and the summer tourist season. Today it is a year-round, traditional and popular durable product. The product "Traditional Lovecký Salami" was listed in the publication, Technology of Meat Industry (Part II, 1955, Main Administration of Meat and Fish Industry, Ministry of Food Industry) and was subsequently included in the Technical and Economic Standards for Meat Products (1st part, set valid from January 1, 1977, MP — General Directorate Prague) as a Czechoslovak state standard under the number ČSN 57 7269, which resulted in the expansion of its production according to this standard throughout the territory of the then Czechoslovakia. Gradual changes in production technologies, due to the limited availability of some production raw materials, but also with the aim of increasing the safety of the final product, resulted in the creation of a stable recipe, which is listed in the description of the production method for traditional lovecký salami. To produce traditional lovecký salami, these ingredients are used: beef with a fat content of up to 10%, pork with a fat content of up to 20%, pork cutlet (pork meat with a fat content of up to 30%), pork meat with a fat content of up to 50%, pork bacon, nitrite salting mixture, antioxidant [E 315 or E 316 (max. 500 mg/kg expressed as erythorbic acid)], ground black pepper, sugar, garlic (in the form of flakes, concentrate or powder in an amount corresponding to the standard amount of fresh garlic), ground cloves, starter cultures [combined culture containing strains of lactic acid bac-

teria (genus *Lactobacillus* and/or *Pediococcus*) and coagulase-negative cocci of the family Micrococcaceae and collagen casings (E.C. No. 509/2006).

It is well known that lactic acid bacteria are among the most important microorganisms used in food fermentation. They contribute to the flavor and texture of fermented products and inhibit food spoilage bacteria by producing inhibitory substances and lactic acid (*Morelli et al*., 2012). An important feature of starter cultures used in the production of fermented meat products is their ability to colonize the meat environment and dominate the microbial community of fermented products in competition with autochthonous microbiota (*Barbosa et al*., 2015). The starter culture must compete with the natural microbiota found in the raw material used with regard to metabolic activities, growth rate and survival in adverse conditions during the production of fermented meat products. Low temperatures, pH, and water activity, high salt concentration and low oxygen availability are among the most important preservation effects, creating an unfavorable environment for microorganisms during meat fermentation (*Vignolo, et al*., 2015). *Casquete et al*. (2011a; 2011b) called for the use of autochthonous starter cultures that not only improve the homogeneity and safety of fermented meat products, but also do not change their sensory properties. They further emphasized the importance of selecting combined starter cultures consisting of strains suitable for each maturation procedure. They concluded that the flavor and aroma of fermented meat products is the result of the combined interaction of lactic acid bacteria producing lactic acid, small amounts of acetic acid, ethanol and acetoin with lactic acid bacteria with proteolytic and lipolytic properties as well as Gram-positive catalase-positive cocci (GCC+) strains, which are essential for the overall sensory quality of fermented meat products. Most of the coagulase-negative strains of *Staphylococcus xylosus* and *Staphylococcus carnosus* are characterized by catalase activity and the ability to reduce nitrate to nitrite. Their antioxidant properties prevent yellowing of fats (*Barriere et al*., 2001; *Rosenstein et al*., 2009), and their catabolism of pyruvate into diacetyl and of acetoin is responsible for the aroma of butter (*Sondergaard and Stahnke*, 2002).

The drying time is approximately 14 days in order for the fermentation process to take place sufficiently in the product at a temperature and relative humidity allowing the development of starter cultures and uniform drying of the product (temperature range 16°C to 27°C; relative humidity range 75% to 92%). In general, fiber, non-meat protein and mechanically separated meat cannot be used in fermented meat products in Slovakia. The product is sold in the characteristic shape of a flat rectangular block with a gut casing.

4. Spiš sausages

Spiš sausages were born in Spišské Podhradie at the turn of the 19th and 20th century. There were three butchers at their birth: Michal Blaško, Karol Grieger and Štefan Varsányi. 'Spiš sausages' have a good name not only in the Spiš region, Slovakia as a whole and the Czech Republic, but also in other countries. Among those who regularly enjoyed them were President T.G. Masaryk and Count Albín Csáky, who was speaker of the upper house of the Hungarian Parliament and Minister for Education and Culture. This influential man made sure that these sausages were served at Hungarian cabinet meetings. After the Second World War, a standard was adopted throughout the Czechoslovak Republic, in the context of standardization and maintenance of traditional quality, establishing the composition of the ingredients and defining the production method (Quality standard TP of 8 September 1954, Ministry of the Food Industry). It is clear from historical records that the product recipe gradually changed somewhat, with the addition of a proportion of beef to the recipe; this did not change the nature or use of the product, but on the contrary, this combination of ingredients improved its flavor (ÚNK 57 7260, 1964). The product's defining characteristic features are its succulence after being cooked and the mildly piquant taste that the paprika imparts. This recipe is still used by producers of Spiš sausages today (CSN 57 71 34, dating from 1977, and later STN 57 71 34). Spiš sausages are registered with name reservation in the Register of Traditional Specialties Guaranteed since 2011 (Commission

Regulation (EU) no. 159/2011). The name "Spiš sausages" is specific in itself, because it is well-established and well-known in both Slovakia and the Czech Republic, has a long tradition and a good reputation and relates to *párky'*sausage of a particular type. The specific character of Spiš sausages derives from the composition and proportions of the ingredients and seasonings used, the smoothness of the homogeneous mass, the use of sheep-intestine casings and their physical and chemical and organoleptic properties. Spiš sausages are contained in sheep-intestine casings of up to 24 mm in diameter and are separated by twisting. The individual sausages weigh about 50 g. The surface of the product is smooth or slightly wrinkled, orange-brown in color and glossy to matt. The cut surface is pinkish red, owing to paprika, and small collagen particles are permitted. The product has a pleasant, freshly-smoked aroma. The taste is slightly hot, appropriately salty and succulent to the bite when heated up. Consistency is soft to compact (EC. No. 509/2006).

5. Špekačky

Špekačky was applied by the Czech Association of Meat Processors and by the Slovak Association meat processors. The name "špekačky" expresses the specific character of the agricultural product or foodstuff, which derives from the unevenly distributed pieces of bacon (spek) in a coarse mixture with a small proportion of collagen particles. The basic character of the products is smoked, and heat-processed meat sausage made from a continuous strand several meters long, stuffed into casing made of pork small intestine or beef rounds, and the products are golden-brown in color. The size of each piece is 4.0 to 4.6 cm in diameter and 8 to 9 cm in length. They weigh around 65 to 85 g. In terms of their consumption, ingredients and production processes, špekačky have been known in the territory of the Czech Republic for over 100 years now. They began to be produced on a large-scale scale in the second half of the 19th century, with the development of the smoked meat industry, and came to be regarded as a high-quality meat product in beef round casing. In 1891, they were exhibited. After meat-production and meat-processing businesses were nationalized, the composition of the ingredients, additives, casings and technological processes became subject to technical and economic standards, which continued to improve the quality of this traditional Czech product. Production of špekačky was covered by the technical and economic standards for meat products (Part 1 of the rules applicable from 1 January 1977, meat industry directorate-general, Prague) under No. ČSN 57 7115. As a result, their manufacture according to those standards spread throughout the former Czechoslovakia. As the production technology gradually changed, and owing to the limited availability of certain ingredients or casings (for example beef rounds), a set recipe was created, which is given above in the description of špekačky production methods. Bacon, chopped into pieces up to approximately 8 mm, is then added to the mixture, which is stuffed into beef rounds or pork small intestine casing with a maximum diameter of 4.0 to 4.6 cm. The mixture is then divided off into individual segments with string. The strands of product are tied to a smoking stick, then taken to a smokehouse, where they are dried and smoked in order to achieve their distinctive color and aroma. The smoked product is then heat-processed at 75 to 78°C until the middle of the product reaches 70°C for at least 10 minutes (EU No. 1151/2012).

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Visegrad+Grant (No. 22230075), KEGA 034SPU-4/2021 and VEGA 1/0402/23.

References

Barbosa, M. Z., Todorov, S. D., Ivanova, I., Chobert, J.-M., Haertlé, T. & De Melo Franco, B. D. G. (2015). Improving safety of salami by application of bacteriocins produced by an autochthonous *Lactobacillus curvatus* isolate. *Food Microbiology*, 46, 254–262.

Commission Regulation (EU) no. 159/2011, (2011). https://www.legislation.gov.uk/eur/2011/159

Morelli, L., Calleagri, M. L., Vogensen, F. K. & Wright, A. V. 2012. Genetics of Lactic Acid Bacteria. In: Lactic Acid Bacteria: Microbiological and Functional Aspects, In Lactic Acid Bacteria: Microbiological and Functional Aspects, S. Lahtinen, A. C. Ouwehand, S. Salminen, and A. V. Wright, Eds., pp. 17–37, CRC Press: Taylor Francis Group, New York, US.

Publication of an application for registration pursuant to Article 8(2) of Council Regulation (EC) No 509/2006 (2006) on agricultural products and foodstuffs as traditional specialities guaranteed. 2010/C 96/07. *European Union. 16 April 2010*. Retrieved 31 October 2021.

Publication of an application for registration pursuant to Article 8(2) of Council Regulation (EC) No 509/2006 (2006) on agricultural products and foodstuffs as traditional specialities guaranteed. 2010/C 95/09. *European Union. 15 April 2010*. Retrieved 31 October 2021.

Publication of an application for registration pursuant to Article 8(2) of Council Regulation (EC) No 509/2006 (2006) on agricultural products and foodstuffs as traditional specialities guaranteed 2010/C 103/07. *European Union. 15 April 2010*. Retrieved 31 October 2021.

Publication pursuant to Article 26(2) of Regulation (EU) No 1151/2012 (2012) of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs as regards a name of traditional speciality guaranteed (2016/C 167/13) . *European Union. 11 May 2016*. Retrieved 31 October 2021.

Vignolo, G., Castellano, P. & Fadda, S. 2015. Bioprotective Cultures, In Handbook of Fermented Meat and Poultry, F. Toldrá, Ed., pp. 129–138, John Wiley & Sons, Ltd.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:103:0014:0019:SK:PDF

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:096:0018:0022:SK:PDF

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:094:0018:0022:SK:PDF

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:095:0034:0039:SK:PDF

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:103:0014:0019:SK:PDF

Sondergaard & Stahnke (2002). Growth and aroma production by *Staphylococcus xylosus, S. carnosus and S. equorum*--a comparative study in model systems. *International Journal of Food Microbiology 5*,75(1–2), 99–109.

Rosenstein, R., Nerz, C. H. & Biswas, L. (2009). Genome Analysis of the Meat Starter Culture Bacterium *Staphylococcus carnosus* TM300. *Applied and Environmental Microbiology*, vol. 75.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.146.057:634

ID: 126083849

https://doi.org/10.18485/meattech.2023.64.2.3

Original Scientific Paper

Odors change visual attention. A case study with stawberry odor and differently flavoured yoghurts

Dorina Szakál[ab], Abdul Hannan Bin Zulkarnain[c], Xu Cao[c] and Attila Gere[c]*

[a] *Department of Hospitality, Faculty of Commerce, Hospitality and Tourism, Budapest Business School; Alkotmány utca 9–11., H-1045 Budapest, Hungary*
[b] *Institute of Agribusiness, Hungarian University of Agriculture and Life Sciences; Villányi út 29–31, H-1118 Budapest, Hungary*
[c] *Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Villányi út 29–31, H-1118 Budapest, Hungary*

ARTICLE INFO

Keywords:
Eye-tracking
Odor
Yoghurt
Time to first fixation
Fixation duration

ABSTRACT

Eye-movements provide unique information on the understating of human behavior and food choices. Analysis of gazing behavior has been proven to be a successful tool to predict eye-movements or to analyze the effects of certain factors on purchase behavior, choice or opinions. In the current papers we introduce a case study on the analysis of gazing behavior, more precisely the analysis of time to first fixations and fixations durations, to understand the effects of scents/odors on eye-movements. The presented study was part of a larger set of studies and focuses on a four alternative forced choice task, that included four differently branded and flavored yoghurt products, while strawberry odor was/was not sprayed into the air. The analysis revealed that time to first fixations shortened when strawberry odor was present and the length first fixation increased for the strawberry flavored product when the odor was present. The results support the hypothesis that aroma compounds have significant effect on visual attention, however, they do not show significant effect on food choices, as these are driven by a myriad of other factors.

1. Introduction

Eye-tracking technology involves monitoring and recording an individual's eye movement and focus. It is most commonly used in psychology, market research, user experience design, and human-computer interaction. Eye-tracking can reveal important information about visual attention, gaze patterns, and user engagement (*Motoki et al*., 2021). Eye-tracking technology could be used to study consumer preferences and behavior in the context of meat sciences. Researchers could gain insights into what aspects of presentation, branding, or labelling influence consumer decisions by tracking where individuals' eyes are drawn when presented with various meat products or packaging.

There have been many earlier uses of eye tracking in meat sciences, such as understanding consumer preferences and responses to different visual aspects of meat products. According to (*Ballco et al.,* 2019) research on consumer preferences on nutritional claims of yoghurt product on 100 participants, the presence of nutritional claims on yoghurts' front of pack increases consumer attention and visual attention (fixation count) increases the likelihood of purchase decisions. In addition, *Fraser et al.* (2021) conducted research on the relationship between the extent of visual attention and preference stability in a discrete choice exper-

***Corresponding author:** Attila Gere, *gere.attila@uni-mate.hu*; *gereattilaphd@gmail.com*

Paper received August 12th 2023. Paper accepted August 26th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

iment using eye-tracking to investigate country of origin information for meat on 100 participants and found that visual attention was positively related to preference instability. Researchers could gain insights into what visual cues or attributes influence consumer decisions by tracking where consumers' eyes are drawn when viewing meat products and/or packages.

Labelling and information display represent other applications of eye-tracking in meat sciences. Eye-tracking could be used to evaluate the effectiveness of meat product labels and information displays. As price is important to shoppers, a study conducted on 307 participants to investigate beef consumers' attention paid to price label information discovered that the use of an eye-tracking device was able to assist in improving consumer research by detecting a difference between what the participants said was important and what they focused on when shown packs of beef (*Lombard*, 2022).

Eye-tracking can also be used in food choices and sensory science because it is necessary to assess which factors of a dish affect the visual appeal and influence consumers' overall evaluation, as different dish cues are closely related to people's acceptance and consumption. A study conducted by *Zhou et al.* (2021) investigated 100 older people's evaluations of dishes by incorporating dish attributes: main course (meat and vegie meat), potatoes, vegetables, and dish label, where most older participants' first gaze settled on the main course area, and they had the longest total eye fixation time on the dish valued as the healthiest. This is associated with a study done by *Zhang et al.* (2021) where they investigated the 46 participants influenced by the container color of food (meat and vegetable dishes) on food choices and ratings in virtual reality (VR), and the results showed that container color influences food choices.

As a growing technology, virtual reality (VR) is an immersive technology that simulates a computer-generated environment, typically through the use of a head-mounted display (HMD). VR has found applications in a variety of industries, which may include the meat industry. VR could potentially be used in retail settings for virtual meat tastings or virtual meat counters. Customers could explore different cuts, textures, and cooking methods by creating realistic virtual representations of meat products, potentially improving their shopping experience. A conjoint analysis of eye-tracking and VR has great potential for various applications; however, their specific use in the meat industry may be limited or underutilised. A study of 124 participants was conducted to determine whether providing consumers with more detailed information about animal husbandry systems could influence their product choices at a virtual supermarket. The study discovered that the price of the product was the most important factor in the purchase decision (*Xu et al.,* 2023). However, as technology advances, these technologies may find novel and innovative applications within the meat production and consumption domains.

2. The role of vision and food choices

Consumer's food choices are driven by a complex set of elements, such as feelings, attitudes, and values (*Gere et al.,* 2017*; Mathiesen et al.,* 2022; *Pantoja & Borges*, 2021; *Szakál et al.,* 2023; *Takahashi et al.,* 2018). Vision is one of the crucial factors on construction of feeling. Although food is often linked with various senses, people rely on visual cues to determine essential characteristics of food. Previous research shows that the color or animation significantly influence attention, and the attention is the key factor of decision making (*Chen & Antonelli*, 2020; *Ye et al*., 2021). Bright and vibrant colors in fruits and vegetables are perceived as indicators of freshness and nutritional quality, making them more appealing to consumers (*Pathare et al.,* 2013). Similarly, food packaging and presentation can significantly influence consumer's perception of the food quality, and impact food selection (*Piqueras-Fiszman & Spence*, 2015).

The eye movement is associated with perception and cognitive processes (*Alemdag & Cagiltay*, 2018). Visual attention is linked to perceptual processing, and it affects what information is processed and remembered. The rational models of decision making, where attention is seen as a passive information gathering tool (*Orquin & Mueller Loose*, 2013). However, newer models, such as those derived from neuroscientific research, highlight the active role of attention in the construction of decisions (*Krajbich et al.,* 2010). Research on attention and eye movements has illuminated the cognitive processes before and during fixations, including the integration between attention and working memory. Evidence suggests that attention not only serves to enhance perception, it also limits and controls it (*Droll et al*., 2005). This modulation of perception by attention plays an integral role in decision-making, particularly in situations where an individual select from a variety of stimuli, such as in a grocery store. The enhanced perception provided by fixating on an item can result in a stronger influence on the decision making process (*Ye et al.,* 2021).

Furthermore, the control of visual attention can be influenced by both bottom-up and top-down processes (*Simonetti & Bigné*, 2022). Bottom-up control is driven by the physical characteristics of the stimuli, such as color or shape, which draw our attention. Conversely, top-down control is influenced by our cognitive expectations and perceptions (*Orquin & Mueller Loose*, 2013). For instance, an individual committed to environmental sustainability is likely to choose food products marked with the Rainforest Alliance logo, given that the price is comparable to other options. Besides, the role of working memory in decision making is also noteworthy. The findings on attention and working memory suggest that people often trade-off between fixations and working memory, potentially using fixations as an external memory space to lower working memory demands. For example, in a supermarket scenario, a consumer might remember the location of their favorite product on a shelf and ignore it until they need to make a direct comparison with another product (*Orquin & Mueller Loose*, 2013).

Vision is undoubtedly crucial in food selection, and it is important to note that other sense also impacts food choices. Previous study shows that the sense of smell can significantly influence the food selection, intake and evaluation (*Morquecho-Campos et al.*, 2020; *Proserpio et al.,* 2019). The aroma of a particular food can evoke emotional responses, memories, and physiological responses that can ultimately affect our food choices (*Herz,* 2016; *Köster & Mojet,* 2015; *Shepherd,* 2006). Studies have shown that pleasant aromas can increase the desire to eat, even when we are full (*Yeomans,* 2006). Conversely, unfavorable odors can render food unappetizing (*Parker et al.,* 2022). Additionally, olfaction can also contribute to the perception of flavor, as it combines with taste to form a multisensory experience, guiding us towards nutritious food and away from potentially harmful substances (*Small,* 2012). In conclusion, previous research show that food choices are significantly influenced by the sensory perceptions, particularly by vision. An additional question arises about the effect of fragrances on eye-movements and choice. The aim of the presented paper therefore is to analyze the effects of strawberry odor on food choices.

3. Materials and methods

Location and participants

The measurement was carried out in a quiet, well-lit room at the Buda Campus of the Hungarian University of Agriculture and Life Sciences. The com-

Table 1. Participant demographic datas for the control (odorless) and odor groups (%)

			Control	Odorless
Gender	male		19.4	22.6
	female		30.6	27.4
Place of living	male	capital city	11.3	14.5
		large city	3.2	1.6
		small town	3.2	1.6
		rural	1.6	4.8
	female	capital city	8.1	3.2
		large city	4.8	3.2
		small town	9.7	12.9
		rural	8.1	8.1
Education	male	graduate	3.2	1.6
		undergraduate	16.1	21.0
	female	graduate	8.1	6.5
		undergraduate	22.6	21.0
Visual aid	male	contact lenses	1.6	3.2
		glasses	4.8	6.5
	female	contact lenses	1.6	4.8
		glasses	8.1	3.2

puter was placed on a table in the middle of the room, above which an LED panel provided light (6500 K, 1600 lm). A pleasant strawberry scent was sprayed into the air using MAYAM elements essential oil and a Sencor SHF 920BL (Ricany, Czech Republic) vaporizer.

The participants were recruited at the Buda Campus. A total of 70 people took part in the survey. The participants were divided into two groups: one was the control group, where no strawberry scent was applied in the air, but the other group was. The mean age of the control group (odorless) was 22.85 years (SD = 6.55). The mean age of the odor group was 22.8 years (SD = 2.97). A total of 8 participants were excluded from the measurement due to low (<80%) eye-tracking quality. Detailed demographic data of the participants are shown in Table 1.

The most prevalent visual abnormalities were nearsightedness and farsightedness, and three participants squinted. Six subjects reported having suffered a partial loss of smell, and two participants reported having a partial loss of taste as a result of post-COVID symptoms at the end of the questionnaire. According to the subjects, these post-COVID symptoms have fully subsided, hence the measurement was unaffected.

Eye-tracker and software

According to the guidelines outlined in *Fiedler et al.* (2020), information regarding the eye-tracking process has been added. The measurement was performed using the Tobii Pro X2-60 (Tobii Pro AB, Danderyd, Sweden), a desktop type of eye-tracker. The images were presented to the participants via Tobii Pro Lab v.1.171 (Tobii Pro AB, Danderyd, Sweden) software. The eye-tracker illuminates the eye with a near-infrared pattern before taking high-resolution pictures of it. The 3D eye model algorithm is used by the image processing algorithms to determine the gaze point and location of the user's eyes by looking for distinctive characteristics and reflection patterns in their eyes. This kind of eye-tracker has the benefit of being compact, inconspicuous, and allowing some head movement during the measurement without disturbing the subject. The recommended viewing angle is 65°, and the optimal distance between the eye and the camera is 60 to 65 cm.

Process

The measurement was performed in two groups: control (odorless) and odor group. In both groups, 31 participants took part in the measurement. The control group was measured first, with no strawberry odor in the air. In the second round, the odor group was measured. The measurement procedure for the two groups was identical, the difference being the absence or presence of odor.

First, we asked participants to sit down in front of the computer and make themselves comfortable. They were then briefed on the measurement process and given some important information about the eye-tracker. The software of eye-tracker then performed a 9-point calibration, which, if successful, triggered a series of plots entered into the software.

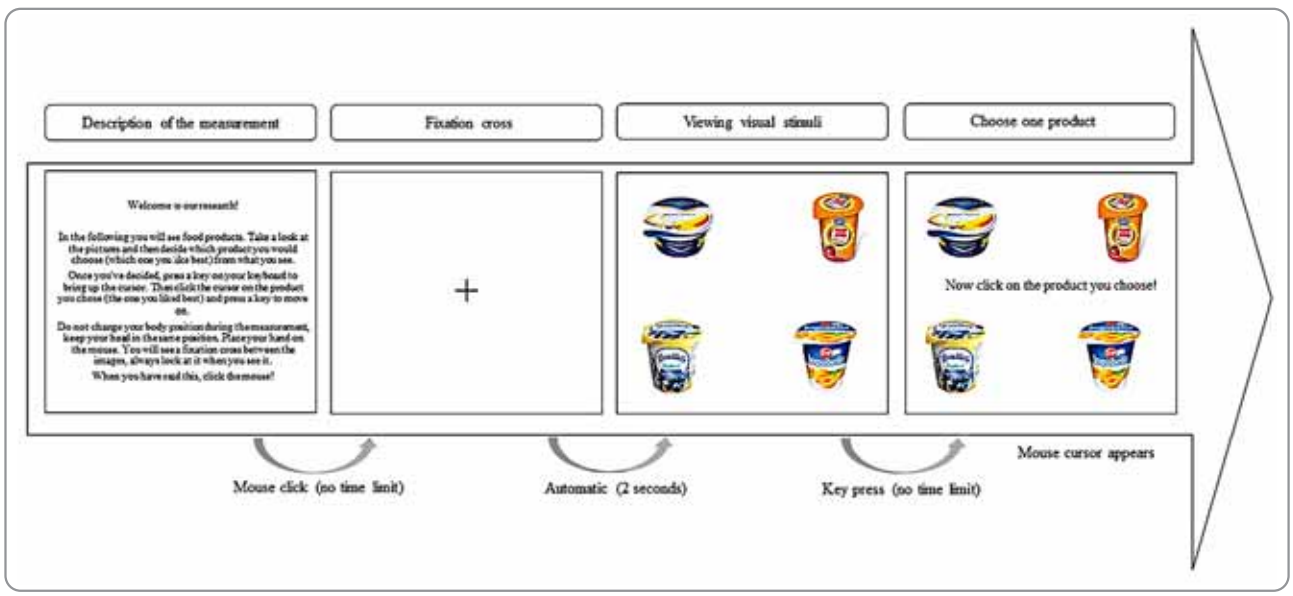


Figure 1. Detail of the timeline presented in the measurement

The first slide of the timeline contained an informative text describing what the participants should do during the measurement. After reading this, participants could jump to the next slide by pressing the left mouse button. At the beginning of the timeline, a trial slide was presented to the participants for practice. The trial slide included four limonades with different flavors (forest fruit, lemon, orange and peach). The trial slide contained products independent of the measurement and these were not included in the data analysis. After seeing the four products, participants were first asked to visualise them and then decide which one they would like to choose. Once they had made their decision, pressing a key on the keyboard brought up the mouse cursor so they could click on the product of their choice. After clicking with the mouse, they had to press a key again to move to the next slide. They had unlimited time to make a decision. A fixation cross appeared before each slide containing new products. The fixation cross was visible in the middle of the screen for 2 seconds. After this time, the software automatically moved on to the next slide. The measurement process is shown in Figure 1.

Visual stimuli

Participants had to choose one product from a total of four series of pictures. The first of the four image series was the trial slide, which was not included in the data analysis. The series of images included yoghurts in different configurations. The products included in the measurement are shown in Figure 2. The visual stimuli were presented on an LG W245VPF 24" Full HD LCD monitor with 1366 x 768 resolution. Areas of interest (AOIs) were defined per product presented, with the distance between AOIs maximized to avoid overlap.

Data analysis

During the data analysis, the following eye-tracking parameters were used:

- Time To First Fixation (TTFF, seconds passed between the introduction of a stimulus and the user focusing their attention on a substitute initially);
- First Fixation Duration (FFD, duration of the first focus on a substitute, in seconds).

The eye-tracking parameters were used as dependent (quantitative) variables, while qualitative variables were the presence of odor (odor vs. no odor) and the chosen products (Mm, De, La and Jb). Two-way multivariate analysis of variance (two-way MANOVA) was used to test the relationship between the two sets of variables.

Tobii Pro Lab v.1.171 (Tobii Pro AB, Danderyd, Sweden) was used to record the data. IBM SPSS STATISTICS (Version 25) (IBM, Armonk, New York, USA) was used for the analysis.

4. Results and discussion

Two-way MANOVA was conducted to determine whether strawberry flavour has an effect on product choice. There was a significant difference in test scores for choice: $F(72.1103) = 6.433$, $p = 0.000$; Wilk's lambda = 0.351; for odor: $F(24.369) = 6.123$, $p = 0.000$; Wilk's lambda = 0.715; and for the choice-odor interaction: $F(72.1103) = 7.239$, $p = 0.000$; Wilk's lambda = 0.315.

When looking at the eye-tracking parameters for each yoghurt product, there was a significant difference for the choice, odor and choice-odor interaction for the TTFF and FFD parameters for the strawberry flavored product.

Figure 3 shows the TTFF results for choice and the effect of scent. For choice, the time to first fixation was significantly reduced when strawberry

Figure 2. Products included in the measurement (name of products from left to right: Mövenpick Mango (Mm), Danone strawberry (De), Landliebe blueberry (Lá) and Jogobella peach (Jb))

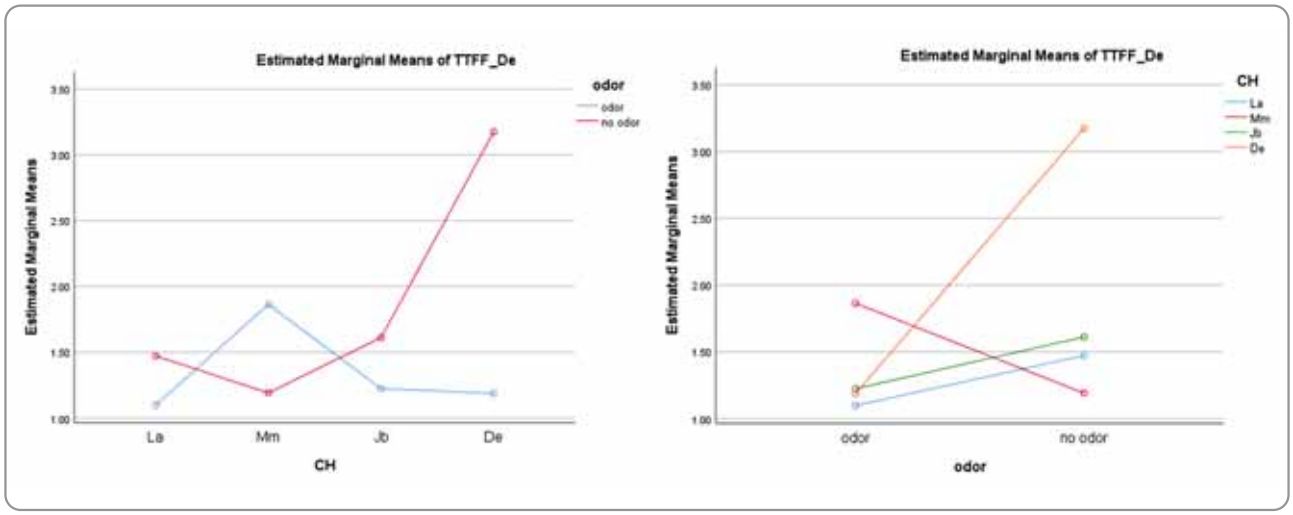


Figure 3. Time to first fixation (TTFF) results of the choice and between the odor and odorless conditions of four yoghurt product alternatives

Indicates: CH = choice, La = Landliebe blueberry, Mm = Mövenpick mango, Jb = Jogobella peach, De = Danone strawberry, TTFF = Time to First Fixation.

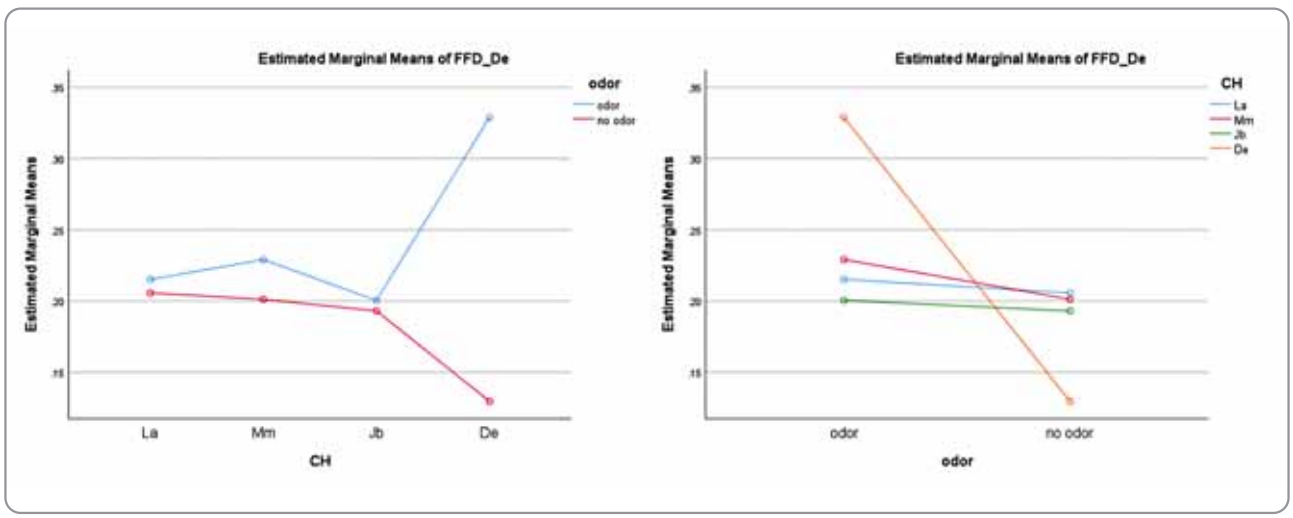


Figure 4. First fixation duration (FFD) results of the choice and between the odor and odorless conditions of four yoghurt product alternatives

Indicates: CH = choice, La = Landliebe blueberry, Mm = Mövenpick mango, Jb = Jogobella peach, De = Danone strawberry, FFD = First Fixation Duration.

odor was present. Thus, the participants perceived the strawberry flavored yoghurt (De) first. Regardless, the choice was based on preference, so it can be said that the choice was not influenced by the odor.

Figure 4 shows the results from the FFD eye — tracking parameter analysis. It can be clearly seen that the first fixation period by odor increased significantly for the strawberry flavored product, while the increase was minimal for the other products. Furthermore, it can be seen that more people chose the strawberry flavored product than the other flavors when exposed to fragrance.

5. Conclusion

The results demonstrated that time to first fixation and first fixation duration parameters are influenced by the presence of strawberry odor. Previous studies reported that first fixation duration might be influenced by odors (Seo & Hummel, 2009), however, recent studies demonstrated that this effect is dependent on the product being evaluatued (Szakál et al., 2022). Odors have been identified as being able to 'grab' participants visual attention and to drive it to conguent images, however, the role of odors in final

decisions is considered minor as there are multiple other factors influencing the final decision (Yang et al., 2023). A possible future direction would be to analyze differences between consumer groups (e.g. clusters), as a mind-set-based segmentation could reveal substantial differences among participants (Gere, 2023).

Disclosure statement: No potential conflict of interest was reported by the authors.

Acknowledgment: D.S. thanks the support of the Doctoral School of Economic and Regional Sciences, Hungarian University of Agriculture and Life Sciences. A.G. thanks the support of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. A.G. thank the support of the National Research, Development, and Innovation Office of Hungary (OTKA, contracts No FK 137577). The authors would like to thank AC Nielsen Ltd.—Nielsen Brandbank (Váci u. 8., H-1056 Budapest, Hungary) for kindly providing the product photos used in the research.

References

Alemdag, E. & Cagiltay, K. (2018). A systematic review of eye tracking research on multimedia learning. *Computers and Education,* 125, 413–428, https://doi.org/10.1016/j.compedu.2018.06.023

Ballco, P., de-Magistris, T. & Caputo, V. (2019). Consumer preferences for nutritional claims: An exploration of attention and choice based on an eye-tracking choice experiment. *Food Research International, 116(August 2018),* 37–48, https://doi.org/10.1016/j.foodres.2018.12.031

Chen, P., & Antonelli, M. (2020). Conceptual Models of Food Choice: Influential Factors Related to Foods. *Foods,* 9(1898), 1–21.

Droll, J. A., Hayhoe, M. M., Triesch, J. & Sullivan, B. T. (2005). Task demands control acquisition and storage of visual information. *Journal of Experimental Psychology: Human Perception and Performance,* 31(6), 1416–1438, https://doi.org/10.1037/0096-1523.31.6.1416

Fiedler, S., Schulte-Mecklenbeck, M., Renkewitz, F. & Orquin, J. L. (2020). Guideline for reporting standards of eye-tracking research in decision sciences. PsyArXiv.

Fraser, I., Balcombe, K., Williams, L. & McSorley, E. (2021). Preference stability in discrete choice experiments. Some evidence using eye-tracking. *Journal of Behavioral and Experimental Economics,* 94(July), 101753, https://doi.org/10.1016/j.socec.2021.101753

Gere, A. (2023). Recommendations for validating hierarchical clustering in consumer sensory projects. *Current Research in Food Science,* 6(February), 100522, https://doi.org/10.1016/j.crfs.2023.100522

Gere, A., Kókai, Z. & Sipos, L. (2017). Influence of mood on gazing behavior: Preliminary evidences from an eye-tracking study. *Food Quality and Preference,* 61(November 2016*),* 1–5. https://doi.org/10.1016/j.foodqual.2017.05.004

Herz, R. S. (2016). The role of odor-evoked memory in psychological and physiological health. *Brain Sciences,* 6(3), https://doi.org/10.3390/brainsci6030022

Köster, E. P. & Mojet, J. (2015). From mood to food and from food to mood: A psychological perspective on the measurement of food-related emotions in consumer research. *Food Research International,* 76(P2), 180–191. https://doi.org/10.1016/j.foodres.2015.04.006

Krajbich, I., Armel, C. & Rangel, A. (2010). Visual fixations and the computation and comparison of value in simple choice. *Nature Neuroscience,* 13(10), 1292–1298, https://doi.org/10.1038/nn.2635

Lombard, W. A. (2022). Comparison of the importance of beef price labelling aspects: An eye-tracking approach. *Heliyon,* 8(7), e09783, https://doi.org/10.1016/j.heliyon.2022.e09783

Mathiesen, S. L., Hopia, A., Ojansivu, P., Byrne, D. V. & Wang, Q. J. (2022). The sound of silence: Presence and absence of sound affects meal duration and hedonic eating experience. *Appetite,* 174, 106011, https://doi.org/https://doi.org/10.1016/j.appet.2022.106011

Morquecho-Campos, P., de Graaf, K. & Boesveldt, S. (2020). Smelling our appetite? The influence of food odors on congruent appetite, food preferences and intake. *Food Quality and Preference,* 85(March), 103959, https://doi.org/10.1016/j.foodqual.2020.103959

Motoki, K., Saito, T. & Onuma, T. (2021). Eye-tracking research on sensory and consumer science: A review, pitfalls and future directions. *Food Research International,* 145(December 2020), 110389, https://doi.org/10.1016/j.foodres.2021.110389

Orquin, J. L. & Mueller Loose, S. (2013). Attention and choice: A review on eye movements in decision making. *Acta Psychologica,* 144(1), 190–206, https://doi.org/10.1016/j.actpsy.2013.06.003

Pantoja, F. & Borges, A. (2021). Background music tempo effects on food evaluations and purchase intentions. *Journal of Retailing and Consumer Services,* 63(November 2020), 102730, https://doi.org/10.1016/j.jretconser.2021.102730

Parker, J., Methven, L., Pellegrino, R., Smith, B., Gane, S. & Kelly, C. (2022). Emerging Pattern of Post-COVID-19 Parosmia and Its Effect on Food Perception. *Foods,* 11, 967, https://doi.org/10.3390/foods11070967

Pathare, P. B., Opara, U. L. & Al-Said, F. A. J. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology,* 6(1), 36–60, https://doi.org/10.1007/s11947-012-0867-9

Piqueras-Fiszman, B. & Spence, C. (2015). Sensory expectations based on product-extrinsic food cues: An interdisciplinary review of the empirical evidence and theoretical accounts. *Food Quality and Preference,* 40(PA), 165–179, https://doi.org/10.1016/j.foodqual.2014.09.013

Proserpio, C., Invitti, C., Boesveldt, S., Pasqualinotto, L., Laureati, M., Cattaneo, C. & Pagliarini, E. (2019). Ambient odor exposure affects food intake and sensory specific appetite in obese women. *Frontiers in Psychology,* 10(JAN), 1–6, https://doi.org/10.3389/fpsyg.2019.00007

Seo, H.-S. & Hummel, T. (2009). Effects of olfactory dysfunction on sensory evaluation and preparation of foods. *Appetite,* 53(3), 314–321, https://doi.org/10.1016/j.appet.2009.07.010

Shepherd, G. M. (2006). Smell images and the flavour system in the human brain. *Nature,* 444(7117), 316–321, https://doi.org/10.1038/nature05405

Simonetti, A. & Bigné, E. (2022). How visual attention to social media cues impacts visit intention and liking expectation for restaurants. *International Journal of Contemporary Hospitality Management,* 34, https://doi.org/10.1108/IJCHM-09-2021-1091

Small, D. M. (2012). Flavor is in the brain. *Physiology and Behavior,* 107(4), 540–552, https://doi.org/10.1016/j.physbeh.2012.04.011

Szakál, D., Cao, X., Fehér, O. & Gere, A. (2023). How do ethnically congruent music and meal drive food choices? *Current Research in Food Science,* 6(February), https://doi.org/10.1016/j.crfs.2023.100508

Szakál, D., Fehér, O., Radványi, D. & Gere, A. (2022). Effect of Scents on Gazing Behavior and Choice. *Applied Sciences,* 16, 6899, https://doi.org/https://doi.org/10.3390/app12146899 Academic

Takahashi, R., Todo, Y. & Funaki, Y. (2018). How Can We Motivate Consumers to Purchase Certified Forest Coffee? Evidence From a Laboratory Randomized Experiment Using Eye-trackers. *Ecological Economics,* 150, 107–121, https://doi.org/https://doi.org/10.1016/j.ecolecon.2018.04.010

Xu, C., Hartmann, C. & Siegrist, M. (2023). The impact of information about animal husbandry systems on consumers' choice of meat products in a virtual supermarket. *Food and Humanity,* 1, 459–470, https://doi.org/https://doi.org/10.1016/j.foohum.2023.06.019

Yang, X., Zandstra, E. H. & Boesveldt, S. (2023). How sweet odors affect healthy food choice: An eye-tracking study. *Food Quality and Preference,* 109, 104922, https://doi.org/https://doi.org/10.1016/j.foodqual.2023.104922

Ye, L., Su, H., Zhao, J. & Hang, Y. (2021). The Impact of Multimedia Effect on Art Learning: Eye Movement Evidence from Traditional Chinese Pattern Learning. *International Journal of Art and Design Education,* 40(2), 342–358, https://doi.org/10.1111/jade.12347

Yeomans, M. R. (2006). Olfactory influences on appetite and satiety in humans. *Physiology and Behavior,* 87(4), 800–804, https://doi.org/10.1016/j.physbeh.2006.01.029

Zhang, W., Wang, C. & Wan, X. (2021). Influence of container color on food ratings and choices: Evidence from a desktop VR study. *Food Quality and Preference,* 96, https://doi.org/10.1016/j.foodqual.2021.104448

Zhou, X., Perez-Cueto, F., Ritz, C. & Bredie, W. (2021). How dish components influence older consumers' evaluation? –A study with application of conjoint analysis and eye tracking technology. *Food Quality and Preference,* 97, 104484, https://doi.org/10.1016/j.foodqual.2021.104484

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664:579.86
579.86:575.111

ID: 126103049

https://doi.org/10.18485/meattech.2023.64.2.4

Review paper

Use of whole genome sequencing as routine typing method — improvements in the investigation of foodborne outbreaks of *Listeria monocytogenes*

Ariane Pietzka[a], Brankica Lakićević[b], Lazar Milojević[b] and Werner Ruppitsch[a]*

[a] *Austrian Agency for Health and Food Safety — AGES, Institute of Medical Microbiology and Hygiene Division for Public Health, Beethovenstraße 6, 8010, Graz, Austria*
[b] *Institute of Meat Hygiene and Technology, Department for Microbiological and Molecular-biological Testing, Belgrade, Kaćanskog 13, 11040, Republic of Serbia*

ARTICLE INFO

Keywords:
Whole genome sequencing
WGS
Listeria monocytogenes
Typing
Outbreak

ABSTRACT

Sequencing technologies have revolutionized the characterization of microorganisms in the recent years to a level that was previously unimaginable. Whole-genome sequencing (WGS) techniques have evolved from an expensive luxury typing method affordable only to a few institutions to a common tool for routine analysis in public health microbiology. In particular, improvements in pathogen source tracking, determination of phylogenetic relationship, antibiotic resistance and virulence-traits have improved outbreak investigation tremendously. In addition, WGS allows the easy establishment of global databases based on standardized nomenclatures facilitating international data exchange, cross-border outbreak investigation strain tracking and source identification.

1. Introduction

International trade in fresh and frozen foods has become a major food — and public health — safety challenge today (*Allerberger et al.,* 2022). The contamination of food and feed with pathogenic microorganisms has become a major global health threat. Therefore, a well-functioning surveillance system is a necessary tool for early detection of microbial threats to prevent or at least stop outbreaks and prevent further transmission and morbidity.

In recent years, whole genome sequencing (WGS) has become the new standard in public health surveillance worldwide, allowing the most accurate and detailed characterization of microorganisms that significantly improved outbreak investigation, surveillance, and infection prevention and control in public health microbiology. For zoonotic foodborne pathogens, *Listeria (L.) monocytogenes, Escherichia (E.) coli* and *Salmonella (S.) enterica* were used as model organisms for a European pilot project with the aim to implement and evaluate WGS for routine analysis. Similar projects have been carried out in the US and elsewhere (*Jackson et al.,* 2016; *Van Walle et al.,* 2018; *ECDC et al.,* 2019).

L. monocytogenes is one of the most important foodborne pathogens because of the severity of certain clinical manifestations, i.e., infections of the central nervous system, septicaemia and abortion and the high case-fatality rate of up to 30% of cases (*Halbedel et al.,* 2020). *L. monocytogenes* causes inva-

***Corresponding author:** Ariane Pietzka, *ariane.pietzka@ages.at*

Paper received April 25th 2023. Paper accepted May 4th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

sive illness mainly in certain well-defined high-risk groups, the so called YOPIs (Young-Old-Pregnant and Immunocompromised). However, listeriosis can also occur in otherwise healthy individuals, especially during an outbreak (*Allerberger et al.,* 2022). Given the strong impact of listeriosis on human health and the challenges of controlling *L. monocytogenes* along the food supply chain, listeriosis has been classified as high priority for molecular surveillance in the European Union/European Economic Area (EU/EEA) over the last two decades (*Gattuso et al.,* 2022).

L. monocytogenes is ubiquitous in the environment and has been isolated from soil, dust, food products for humans (both of animal and vegetable origin), feed, water and sewage, and it can be carried by almost any animal species, including asymptomatic humans. The principal reservoirs of the organism are said to be soil, forage, water, mud, livestock feed and silage (*Heymann,* 2015). Due to this environmental ubiquity, *Listeria* strains are also frequently detected in food products. Strains causing illnesses are mainly found in foods that are packaged and prepared commercially, rather than in home cooked foods. The change in lifestyle with less time for home cooking and more ready-to-eat (RTE) and take-away foods increased the risk of listeriosis (*Carpentier and Cerf,* 2011). Changes in food production and technology have led to the production of foods with a long shelf life that are typical "*Listeria* risk foods," because the bacteria have time to multiply, and the food does not undergo a bactericidal/listericidal process such as cooking before consumption.

Listeriosis outbreaks have historically been difficult to resolve, and only a small proportion of cases could be linked to the food products responsible for an outbreak. This was also due to the fact that previous molecular typing methods, such as pulsed-field gel electrophoresis (PFGE), had limited genetic resolution compared to WGS-based analyses.

2. Whole genome sequencing-based surveillance of *Listeria monocytogenes*

An almost gapless characterization of *L. monocytogenes* isolates is desirable, and the European Centre for Disease Prevention and Control and the European Food Safety Authority (ECDC-EFSA), recommend using WGS-based typing methods (*Van Walle et al.,* 2018; *ECDC et al.,* 2019) along with a European-wide molecular typing database to improve the identification and investigation of multi national outbreaks (*Ruppitsch et al.,* 2015a; *Pietzka et al.*, 2019; *Cabal et al.,* 2022; *Lakicevic et al.,* 2023). There are different analysis pipelines for WGS data, such as analysis of single-nucleotide variants (SNP) or a gene-by-gene allelic profiling using core genome (cgMLST) (*Ruppitsch et al.,* 2015b; *Moura et al.,* 2016) as well as whole-genome multilocus sequence typing (wgMLST) (*Hyden et al.,* 2016a; *Brown et al.* 2019; *Jagadeesan et al.,* 2019; *Lakicevic et al.,* 2023). For backward compatibility with datasets obtained with traditional methods, information on serotype, classical multilocus sequence type (MLST) or MLVA data can be extracted from WGS data (*Hyden et al.,* 2016b).

A multi-country outbreak of *L. monocytogenes* was investigated in a joint ECDC-EFSA rapid outbreak assessment in 2018. The outbreak was ongoing in five EU member states (Austria, Denmark, Finland, Sweden and the United Kingdom) with 32 human cases involved. Six closely related non-human *L. monocytogenes* isolates from frozen corn, frozen vegetables and surfaces on which vegetables had been processed were detected by WGS analysis. WGS analysis provided a strong microbiological link between the human and the non-human isolates. Consumption of frozen corn was later confirmed by several patients in different countries. Contaminated batches of frozen corn and vegetable mixes could be traced back to a company in Poland that packaged frozen vegetables produced and processed in Hungary (*Joint ECDC-EFSA,* 2018).

In Austria, WGS-based surveillance has been successfully implemented and used in combination with analysis of epidemiological data for surveillance and outbreak investigation in recent years. Since 2016, whole genome sequence-based typing is performed at the National Reference Laboratory for all *L. monocytogenes* isolates from different sources such as isolates from patients, foods and food-associated material as well as isolates from the environment and the veterinary sector. This isolate-based surveillance allows successful investigation and confirmation of local and multi-country outbreaks. In 2018, a listeriosis outbreak likely due to contaminated liver pâté was investigated. A group of 32 individuals celebrated at a tavern in Austria, where traditional food was served. Eleven individuals developed gastrointestinal symptoms, including one case with severe sepsis. Human, food and environmental samples taken from the tavern and a local production facility (where some of the served meat products originated) were tested for *L. monocytogenes* and isolates were analyzed. A novel *L. monocytogenes* strain was detected in twelve human, two food and one environmental samples from the meat processing company.

Active case finding identified, from the same region in Austria, two further cases which tested positive for the outbreak strain. These two cases had not visited the tavern, but confirmed regular consumption of locally produced liver pâté. Based on WGS analysis, liver pâté produced by company X was identified as the likely source of the outbreak (*Cabal et al.* 2019).

Lachmann et al. described a listeriosis outbreak in Germany, most likely associated with the consumption of smoked and graved salmon products. In a national surveillance program in Germany, WGS was used for typing and cluster detection of *L. monocytogenes*. In the frame of this programme, twenty-two independent listeriosis outbreaks with 228 cases, occurring between 2010 and 2021, were identified. Listeriosis outbreaks can affect several countries and last for several years, making it difficult to link affected patients without the use of WGS. Systematic WGS-based typing of *L. monocytogenes* isolates from food products enables the identification of outbreak vehicle. Many of these twenty-two outbreaks were cross-border outbreaks with further cases in other countries. WGS analysis revealed closely related *L. monocytogenes* isolates from different salmon products. Interviews on food consumption and shopping behaviour confirmed the WGS results (*Lachmann et al.* 2022).

Advances in outbreak investigation after implementation of WGS based typing have been reported worldwide. Jackson et al. described how WGS has improved the detection and investigation of listeriosis outbreaks in the U.S., and demonstrated significantly more clusters and outbreaks of foodborne listeriosis were identified and resolved thereafter (19 outbreaks) than before the use of WGS (two outbreaks) (*Jackson et al.,* 2016).

The so far biggest reported listeriosis outbreak, with 1060 confirmed cases and 216 confirmed deaths, in South Africa from 2017–2018, was finally successfully terminated with the aid of WGS. Contaminated ready-to-eat meat from a South African producer was identified as the infection source. After withdrawal of contaminated meat products from the market the outbreak ended (*Smith et al.,* 2019). As a consequence, listeriosis has been added to the list of mandatory notifiable diseases in South Africa and surveillance systems have been optimized to improve prevention and early detection of future listeriosis outbreaks.

3. Discussion

WGS enables efficient tracking and distribution of microorganisms on a "farm to fork" principle. Subpopulations of bacterial pathogens can be transferred into food processing facilities from a variety of sources outside processing facilities, including animals, incoming raw materials, soil, dust and water (*Zuber et al.,* 2019; *Lakicevic et al.,* 2023). Within production facilities, microorganisms can persist for long periods of time on various surfaces, equipment, floors and cold rooms (*Stessl et al.,* 2022) and can be transferred to food and ultimately to consumers via aerosols, contaminated contact materials and food processing workflows (*Pightling et al.,* 2018; *Elson et al.,* 2019; *Stessl et al.,* 2019). WGS allows characterization of bacterial subpopulations with the highest discriminatory power at every stage, from the environment to suppliers and food processing facilities, to final products and consumers (*Pightling et al.,* 2018; *Zuber et al.*, 2019). Thus, WGS allows the identification of the responsible source of infection or contamination with a high level of confidence, which has improved correct decision making (*Zuber et al.,*2019; *Stessl et al.,* 2022) and enables authorities and also food companies the timely and selective implementation of appropriate control and preventive measures to stop further transmission and to terminate outbreaks.

4. Conclusion

In conclusion, the use of WGS offers several advantages, namely superior discriminatory power for strain characterization, robustness and stability, which are critical for cluster detection, tracing the source and reservoir of the causative strain (*Ruppitsch et al.,* 2019). In addition, the high resolution of WGS allows public health agencies to take action at a lower level of epidemiologic evidence, which is a critical advantage for reducing disease and resolving outbreaks (*Jackson et al.,* 2016). WGS technologies offer benefits not only to public health and food authorities, but also to the food industry in the context of the farm-to-fork principle and upcoming improvements in technology and bioinformatics, with the perspective of metagenomic sequencing applied directly to samples (*Ruppitsch et al.,* 2019).

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Allerberger, F., Bagó, Z., Huhulescu, S., Pietzka, A. & Pleininger, S. (2022). Listeriosis: The Dark Side of Refrigeration and Ensiling. In: Sing, A. (eds) Zoonoses: Infections Affecting Humans and Animals. Springer, Cham, https://doi.org/10.1007/978-3-030-85877-3_10-1

Brown, E., Dessai, U., McGarry, S. & Gerner-Smidt, P. (2019). Use of Whole-Genome Sequencing for Food Safety and Public Health in the United States. *Foodborne Pathogens and Disease,* 16 (7), 441–450, doi: 10.1089/fpd.2019.2662

Cabal, A., Allerberger, F., Huhulescu, S., Kornschober, C., Springer, B., Schlagenhaufen, C., Wassermann-Neuhold, M., Fötschl, H., Pless, P., Krause, R., Lennkh, A., Murer, A., Ruppitsch, W. & Pietzka, A. (2019). Listeriosis outbreak likely due to contaminated liver pâté consumed in a tavern, Austria, December 2018. Euro surveillance : bulletin Europeen sur les maladies transmissibles = *European Communicable Disease Bulletin*, 24(39), 1900274, https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900274

Cabal, A., Rab, G., Daza-Prieto, B., Stöger, A., Peischl, N., Chakeri, A., Mo, S. S., Bock, H., Fuchs, K., Sucher, J., Rathammer, K., Hasenberger, P., Stadtbauer, S., Caniça, M., Strauß, P., Allerberger, F., Wögerbauer, M. & Ruppitsch, W. (2022). Characterizing Antimicrobial Resistance in Clinically Relevant Bacteria Isolated at the Human/Animal/Environment Interface Using Whole-Genome Sequencing in Austria. *International Journal of Molecular Sciences,* 23 (19), 11276, https://doi.org/10.3390/ijms231911276

Carpentier, B. & Cerf, O. (2011). Review — Resistance of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology,* 145,1–8.

Elson, R., Awofisayo-Okuyelu, A., Greener, T., Swift, C., Painset, A., Amar, C. F. L., Newton, A., Aird, H., Swindlehurst, M., Elviss, N., Foster, K., Dallman, T. J., Ruggles, R. & Grant, K. (2019). Utility of Whole Genome Sequencing To Describe the Persistence and Evolution of *Listeria monocytogenes* Strains within Crabmeat Processing Environments Linked to Two Outbreaks of Listeriosis *Journal of Food Protection,* 82 (1), 30–38. doi: 10.4315/0362-028x.jfp-18-206

European Centre for Disease Control, European Food Safety Authority, Van Walle, I., Guerra, B., Borges, V., Carriço, J. A., Cochane, G., Dallman, T., Franz, E., Karpišková, R., Litrup, E., Mistou, M. -Y., Morabito, S., Mossong, J., Alm, E., Barrucci, F., Bianchi, C., Costa, G., Kotila, S., Mangone, I., Palm, D., Pasinato, L., Revez, J., Struelens, M., Thomas-López, D. & Rizzi, V. (2019). Technical report on the collection and analysis of whole genome sequencing data from food-borne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC–EFSA molecular typing database. *EFSA Support. Publ. 16*:1337E. Available at: https://doi.org/10. 2903/sp.efsa.2019.EN-1337

Gattuso, A., Cella, E., Fillo, S., Ortoffi, M. F., Angeletti, S., Ciccozzi, M., De Medici, D., Lista, F. & Fiore, A. (2022). Phylogenetic and Evolutionary Genomic Analysis of *Listeria monocytogenes* Clinical Strains in the Framework of Foodborne Listeriosis Risk Assessment. *Frontiers in Microbiology,* 13, 816880, doi: 10.3389/fmicb.2022.816880

Halbedel, S., Wilking, H., Holzer, A., Kleta, S., Fischer, M. A., Lüth, S., Pietzka, A., Huhulescu, S., Lachmann, R., Krings, A., Ruppitsch, W., Leclercq, A., Kamphausen, R., Meincke, M., Wagner-Wiening, C., Contzen, M., Kraemer, I. B., Al Dahouk, S., Allerberger, F., Stark, K. & Flieger, A. (2020). Large Nationwide Outbreak of Invasive Listeriosis Associated with Blood Sausage, Germany, 2018-2019. *Emerging Infectious Diseases,* 26 (7)*,* 1456–1464, https://doi.org/10.3201/eid2607.200225

Heymann, D. (ed). (2015). Control of Communicable Diseases Manual, 20th edn. American Public Health Association, Washington, D.C.

Hyden, P., Grim, C., Pietzka, A., Blaschitz, M., Indra, A., Sensen, C. W., Allerberger, F., Rattei, T. & Ruppitsch, W. (2016a). Comparison of SNP based and cgMLST based typing of *Listeria monocytogenes* isolates from Seeliger's historical "Special *Listeria* Culture Collection". Poster; Boston, USA; ASM Microbe.

Hyden, P., Pietzka, A., Lennkh, A., Murer, A., Springer, B., Blaschitz, M., Indra, A., Huhulescu, S., Allerberger, F., Ruppitsch, W. & Sensen, C. W. (2016b). Whole genome sequence-based serogrouping of *Listeria monocytogenes* isolates. *Journal of Biotechnology,* 235*,* 181–186, https://doi.org/10.1016/j.jbiotec.2016.06.005

Jackson, B. R., Tarr, C., Strain, E., Jackson, K. A., Conrad, A., Carleton, H., Katz, L. S., Stroika, S., Gould, L. H., Mody, R. K., Silk, B. J., Beal, J., Chen, Y., Timme, R., Doyle, M., Fields, A., Wise, M., Tillman, G., Defibaugh-Chavez, S., Kucerova, Z., Sabol, A., Roache, K., Trees, E., Simmons, M., Wasilenko, J., Kubota, K., Pouseele, H., Klimke, W., Besser, J., Brown, E., Allard, M. & Gerner-Smidt, P. (2016). Implementation of Nationwide Real-time Whole-genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America,* 63(3), 380–386, https://doi.org/10.1093/cid/ciw242

Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Ching Chai, L., Chapman, J. & Grant, K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. *Food Microbiology, 79,* 96–115, https://doi.org/10.1016/j.fm.2018.11.005

Joint ECDC-EFSA — Rapid outbreak assessment, 2018. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections probably linked to frozen corn. 22 March 2018.

Lachmann, R., Halbedel, S., Lüth, S., Holzer, A., Adler, M., Pietzka, A., Al Dahouk, S., Stark, K., Flieger, A., Kleta, S. & Wilking, H. (2022). Invasive listeriosis outbreaks and salmon products: a genomic, epidemiological study. *Emerging Microbes & Infections*, 11 (1), 1308–1315, https://doi.org/10.1080/22221751.2022.2063075

Lakicevic, B., Jankovic, V., Pietzka, A. & Ruppitsch, W. (2023). Whole genome sequencing as the gold standard approach for control of *Listeria monocytogenes* in the food chain. *Journal of Food Protection,* 86(1)*,* 100003, https://doi.org/10.1016/j.jfp.2022.10.002

Moura, A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C., Björkman, J. T., Dallman, T., Reimer, A., Enouf, V., Larsonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L. S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., Chenal-Francisque, V., Kucerova, Z., Rocha, E. P., Nadon, C., Grant, K., Nielsen, E. M., Pot, B., Gerner-Smidt, P., Lecuit, M. & Brisse, S. (2016). Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nature Microbiology,* 2*,* 16185, https://doi. org/10.1038/nmicrobiol.2016.185

Pietzka, A., Allerberger, F., Murer, A., Lennkh, A., Stöger, A., Cabal Rosel, A., Huhulescu, S., Maritschnik, S., Springer, B., Lepuschitz, S., Ruppitsch, W. & Schmid D. (2019). Whole Genome Sequencing Based Surveillance of L. monocytogenes for Early Detection and Investigations of Listeriosis Outbreaks. *Frontiers in Public Health*, 7, 139, doi: 10.3389/fpubh.2019.00139

Pightling, A. W., Pettengill, J.B., Luo, Y., Baugher, J.D., Rand, H. & Strain, E. (2018). Interpreting Whole-Genome Sequence Analyses of Foodborne Bacteria for Regulatory Applications and Outbreak Investigations. *Frontiers in Microbiology,* 9, 1482, doi: 10.3389/fmicb.2018.01482

Ruppitsch, W., Prager, R., Halbedel, S., Hyden, P., Pietzka, A., Huhulescu, S., Lohr, D., Schönberger, K., Aichinger, E., Hauri, A., Stark, K., Vygen, S., Tietze, E., Allerberger, F. & Wilking, H. (2015a). Ongoing outbreak of invasive listeriosis, Germany, 2012 to 2015. *Euro Surveillance* 20(50) 30094, https://doi.org/10.2807/1560-7917.ES.2015.20.50.30094

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H. L., Allerberger, F. & Mellmann, A. (2015b). Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. *Journal of Clinical Microbiology,* 53*,* 2869–2876, https://doi.org/10.1128/ JCM.01193-15

Ruppitsch, W. , Pietzka, A., Cabal, A., Chakeri, A., Schmid, D., Lakicevic, B., Lepuschitz, S. & Allerberger, F. (2019). Advances in foodborne outbreak investigation and source tracking using whole genome sequencing *IOP Conf. Series: Earth and Environmental Science 333* 012010, doi:10.1088/1755-1315/333/1/012010

Smith, A. M., Tau, N. P., Smouse, S. L., Allam, M., Ismail, A., Ramalwa, N. R., Disenyeng, B., Ngomane, M. & Thomas. J. (2019). Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: Laboratory Activities and Experiences Associated with Whole-Genome Sequencing Analysis of Isolates. *Foodborne Pathology and Disease*, 16 (7), 524–530.

Stessl, B., Ruppitsch, W. & Wagner, M. (2022). *Listeria monocytogenes* post-outbreak management — When could a food production be considered under control again? *International Journal of Food Microbiology,* 379*,* 109844, https://doi.org/10.1016/j.ijfoodmicro.2022.109844

Van Walle, I., Björkman, J. T., Cormican, M., Dallman, T., Mossong, J., Moura, A., Pietzka, A., Ruppitsch, W., Takkinen, J. & European *Listeria* WGS typing group (2018). Retrospective validation of whole genome sequencing-enhanced surveillance of listeriosis in Europe, 2010 to 2015. *Euro Surveillance : bulletin Europeen sur les maladies transmissibles = European Communicable Disease Bulletin,* 23 (33)*,* 1700798, https://doi.org/10.2807/1560-7917.ES.2018.23.33.1700798

Zuber, I., Lakicevic, B., Pietzka, A., Milanov, D., Djordjevic, V., Karabasil, N., Teodorovic, V., Ruppitsch, W. & Dimitrijevic, M. (2019). Molecular characterization of *Listeria monocytogenes* isolates from a small-scale meat processor in Montenegro 2011–2014. *Food Microbiology,* 79*,* 116–122, doi: 10.1016/j.fm.2018.12.005

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.5.083
637.5.053:636.52/.58

ID: 126113289

https://doi.org/10.18485/meattech.2023.64.2.5

Original scientific paper

Nutritional and feeding strategies for controlling breast muscle myopathy occurrence in broiler chickens: a survey of the published literature

Angela Trocino[ab], Gerolamo Xiccato[a], Massimiliano Petracci[c] and Marija Bošković Cabrol[a]*

[a] *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy*
[b] *Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy*
[c] *Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum — University of Bologna, Piazza Goidanich 60, 47522 Cesena, Italy*

ARTICLE INFO

Keywords:
White striping
Wooden Breast
Spaghetti meat
Nutritional manipulation
Feed additives

ABSTRACT

Myopathies of the breast muscle in broiler chickens are a great concern of modern poultry production because of the economic losses associated with the waste of unfit meat, the effects on meat nutritional quality for food and on meat technological quality for processing, and last, but not less important, the effects on animal welfare and health. Largely spread in fast-growing genotypes, these muscle defects have a low hereditability for which genetic selection for their control seems to be a weak strategy or, at least, a weak long-term strategy. On the other hand, several investigations have been performed on the physiological mechanisms triggering the onset and the evolution of myopathies, and different non-genetic strategies have been proposed to control their *in vivo* occurrence. Thus, the present paper aimed to analyse the scientific literature investigating the effects of feeding and nutritional strategies on the occurrence of myopathies to find out the most tested strategies, the most promising ones and, in perspective, strengths and weakness of the same strategies.

1. Introduction

In the last decade, emerging myopathies, such as white striping (WS), wooden breast (WB), and more recently spaghetti meat (SM), have raised great concerns in poultry production because they can affect a high proportion of chicken breasts at slaughtering time (*Che et al.,* 2022; *Bordignon et al.,* 2022) which implies high economic losses; they importantly modify meat's nutritional quality and technological properties (*Petracci et al.,* 2019; *Bošković Cabrol et al.,* 2023), besides raising concerns for animal health and welfare (*Kawasaki et al.,* 2016; *Kieronczyk et al.,* 2017; *Norring et al.,* 2019). The occurrence of breast myopathies has been primary linked to the great hypertrophy of muscle fibres resulting from genetic selection in the fast-growing and high-breast yield commercial genotypes currently used (*Petracci et al.,* 2019; *Soglia et al.,* 2021). On the other hand, based on the review of several studies on the physiological and structural changes combined with the omic insights related to the muscle response, *Soglia et al.* (2021) have identified a sarcoplasmic reticulum stress as the most

***Corresponding author:** Angela Trocino, *angela.trocino@unipd.it*

Paper received August 9th 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

likely responsible of myopathies' onset, and hypoxic conditions as a cause of the conditions activating the muscle response, as for energetic metabolism, inflammation, degeneration, and regeneration. Nevertheless, despite being a condition common to selected commercial genotypes, the hereditability of myopathy occurrence is low to moderate, and the environmental and/or management factors contribute greater than 65% and 95% of the variance in the incidence of WS and WB, respectively (*Bailey et al.*, 2015; *Bailey,* 2023). Accordingly, to reduce myopathy occurrence and degree, several studies have been conducted to date on the non-genetic strategies that can mitigate the onset and development of these defects. Several of these studies focused on different nutritional and feeding strategies, especially intended to manipulate growth trajectory and/or modulate muscle response to oxidative stress and oxygen lack, besides inflammation, and, more widely, to regulate muscle metabolism. Thus, the present paper aimed at analysing the scientific literature investigating the effects of feeding and nutritional strategies on the occurrence of myopathies, to detect the most tested and promising strategies and, when possible, highlight their strengths and weakness.

2. Materials and methods

Bibliographical information and abstracts related to the scientific publications to be used in this paper were obtained by using Scopus (Elsevier B.V., Amsterdam, The Netherlands) on July 17, 2023. Four different searches were set and corresponding databases downloaded, i.e., 1) myopath* AND diet* AND chicken OR broiler*; 2) myopath* AND additive* AND chicken OR broiler*; 3) myopath* AND feed* AND chicken OR broiler*; 4) myopath* AND nutrition* AND chicken OR broiler*. Then, the four data sets were individually checked to exclude reviews, duplicates, and articles out of scope. Articles out of scope included all those articles describing studies not specifically testing the effect of nutritional/feeding strategies on the occurrence of myopathies; not reporting data on myopathy occurrence at a macroscopic examination; investigating nutritional myopathies other than WS, WB, and SM (Table 1). Then, the four data sets were joined and checked for duplicates. A total of 60 articles were finally obtained.

The full articles included in the final data set were retrieved as pdf files from the corresponding collections, and the following items were added to the data set spreadsheet in further columns based on abstracts and full text: effects tested in the study, distinguishing between detailed effects and the nutritional/feeding strategy adopted; and effects (decrease, increase, no effect) on myopathy occurrence, on growth traits of animals, and on breast weight and/or yield.

The PROC FREQ of SAS (SAS, 2013) was used to obtain the distribution of publications according to the publishing year, the investigated myopathies (WS, WB, and SM), the tested nutritional/feeding strategy, and the effects on myopathy occurrence. Finally, the correlation between the effects on myopathy occurrence, animal growth (final live weight and/or daily weight gain), and breast weight and/or yield were studied by the PROC CORR of SAS.

Table 1. Results of the analysis of the publications obtained by the literature search from the data available in Scopus on July 17, 2023

	Search words	All retrieved articles and reviews	Duplicates	Reviews	Out of scope	Final set of selected articles
Set 1	myopath* AND diet* AND chicken OR broiler*	95	0	9	30	56
Set 2	myopath* AND additive* AND chicken OR broiler*	7	0	1	4	2
Set 3	myopath* AND feed* AND chicken OR broiler*	113	3	14	42	54
Set 4	myopath* AND nutrition* AND chicken OR broiler*	75	0	26	25	24
TOTAL		290	3	50	101	136

3. Results and discussion

The first scientific article about the effect of nutritional/feeding strategies to control myopathy occurrence was published in 2012 (Figure 1a), while the first paper on the occurrence of WS myopathy appeared in Scopus in 2009 (*Soglia et al*., 2021). After 9 years and 4 articles (published between 2012 and 2017), results of the studies investigating strategies to control myopathy increased from 2018 onwards, with the number of published articles ranging 6 to 15 per year. Consistently, with the evolution of studies about the occurrence of the different myopathies (*Soglia et al.,* 2021), the first articles focused on WS only, followed by articles including results about WS and WB at the same time (Figure 1b). Then, by 2021, the number of studies addressing WB alone increased, which could be attributed to its higher impact on consumers' acceptance and on technological properties for processing compared to WS (*Petracci et al.*, 2019), and, consequently, the higher economic losses for the industry. Research on nutritional/feeding strategies focusing on SM started to appear in 2018 (Figure 1b), while the first article about the occurrence of SM was published in 2016 *(Soglia et al.,* 2021). Nevertheless, the most recently discovered SM remains the least investigated myopathy (9 papers in the present review; 21 papers in *Soglia et al.,* 2021) compared to WS and WB.

The main nutritional/feeding strategies tested in the articles were identified and papers consistently assigned, as summarized in Table 2. Numerically, most studies investigated the effects of reducing the supply of dietary amino acids (13), and those addressed manipulation of the growth trajectory by nutrient allocation (13 in total), both with a qualitative approach (feeding plans based on decreasing dietary energy and/or protein content) (7 papers) and a quantitative approach (feeding plans based on different feed restriction levels, and different restriction and re-feeding periods) (6 papers). A total of 11 studies used different dietary supplementations, with additives acting as antioxidant or playing a role in inflammation, whereas another 4 studies addressed muscle oxygen homeostasis, using phytase and inositol. Finally, 5 studies were aggregated under the antibiotic/feed additive strategy, and another 5 specifically addressed supplementation with guanidine acetic acid (GAA). Due to the specific action mode of GAA (*Oviedo-Rondón et al.*, 2020), a specific set of articles was defined even if GAA supplementation finally contributed to improve the oxidative status of the muscle. The remaining 9 studies included a variety of approaches not ascribable to the previous ones.

Regardless of the specific myopathies, the data set was analysed to evaluate the most successful within the different nutritional/feeding strategies adopted to reduce the occurrence of defects (Figure 2). The highest success rates (100% and 80%, respectively) were obtained for the studies adopting strategies for controlling oxygen homeostasis and those using GAA, which however, were few in absolute number compared to the other strategies (3 and 5 articles, respectively) and require confirmation. On the other hand, the reduction of die-

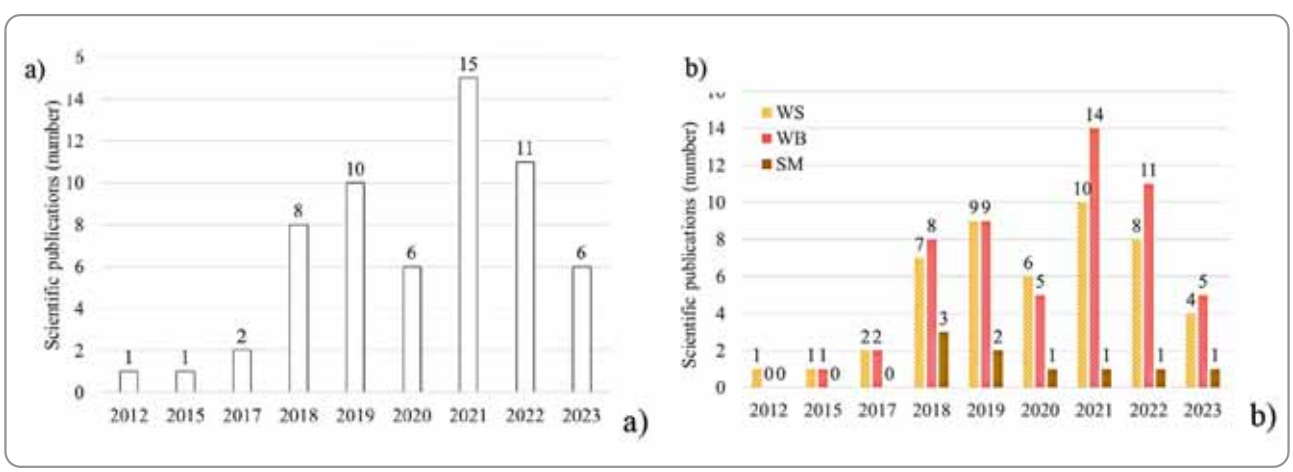


Figure 1. Number of publications testing the effects of nutritional/feeding strategies on the occurrence of myopathies retrieved from Scopus (data available on July 17, 2023) according to the publishing year (a); and number of the publications (same set) per publishing year reporting results about the occurrence of white striping (WS), wooden breast (WB), and spaghetti meat (SM) myopathies. The same article can report results about more than one myopathy.

Table 2. Results of the analysis about the main nutritional and feeding strategies identified based on the final data set of selected articles obtained by the literature search from the data available in Scopus on July 17, 2023

Main nutritional and feeding strategies	Tested strategies	Total number of articles
Reducing dietary amino acids (AA)	Lysine, methionine, glutamine, arginine, valine, leucine, histidine, total sulphur AA, threonine, total amino acids	13
Nutrient allocation (qualitative)	Feeding plans based on diets with different energy and protein contents	7
Nutrient allocation (restriction)	Feeding plans based on different feed restriction levels, and different restriction and refeeding periods	6
Supplementation for controlling oxidation/inflammation	Se, Mg, Zn, Cu, Mn, antioxidants (including vegetal extracts), Vitamin E, alpha lipoic acid, n3 fatty acids (including algae inclusion)	11
Supplementation for improving oxygen homeostasis	Inositol, phytase (one study including a blend with vitamins)	4
Use of antibiotic/feed additives	Antibiotics and feed additives (probiotic, vegetal functional compounds, organic acids)	5
Supplementation with guanidine acetic acid	Guanidino acetic acid (plus nucleotides in one paper)	5
Miscellaneous	Fat source, choline chloride, Ca, P, K, vitamins, inorganic (sulphates) and carbo-aminophospho-chelate, methionine (synthetic vs. natural), *in-ovo* vitamin D, hatching system	9

Table 3. Correlation coefficient and p-value (in italics between parenthesis) between effects on myopathy occurrence (YES, NO), growth performance (YES, NO), and breast weight/yield within the selected articles of the final data set (data available in Scopus on July 17, 2023)

	Effects on myopathy occurrence	Effects on growth	Effects on breast weight/yield
Effects on myopathy occurrence	-	0.283 *(0.034)*	0.143 *(0.325)*
Effects on growth	-	-	0.517 *(<0.001)*
Effects on breast weight/yield	-	-	-

tary amino acids resulted in a reduction of myopathy occurrence in 58% of studies, while contrasting results were reported in a further study within the same strategy. As for nutrient allocation, the modulation of dietary energy and protein was successful in 26.8% of the studies, whereas strategies based on feed restriction were successful in the 50% of cases. The different strategies behind the studies included in the miscellaneous category justify the different results obtained with reference to the effects on myopathy occurrence (22.2% decrease, 44.4% no effect, 33.3% increase).

Based on the results of the selected articles, a significant correlation between the effects on growth (as for final body weight and daily weight gain) and myopathy occurrence (across the three myopathies)

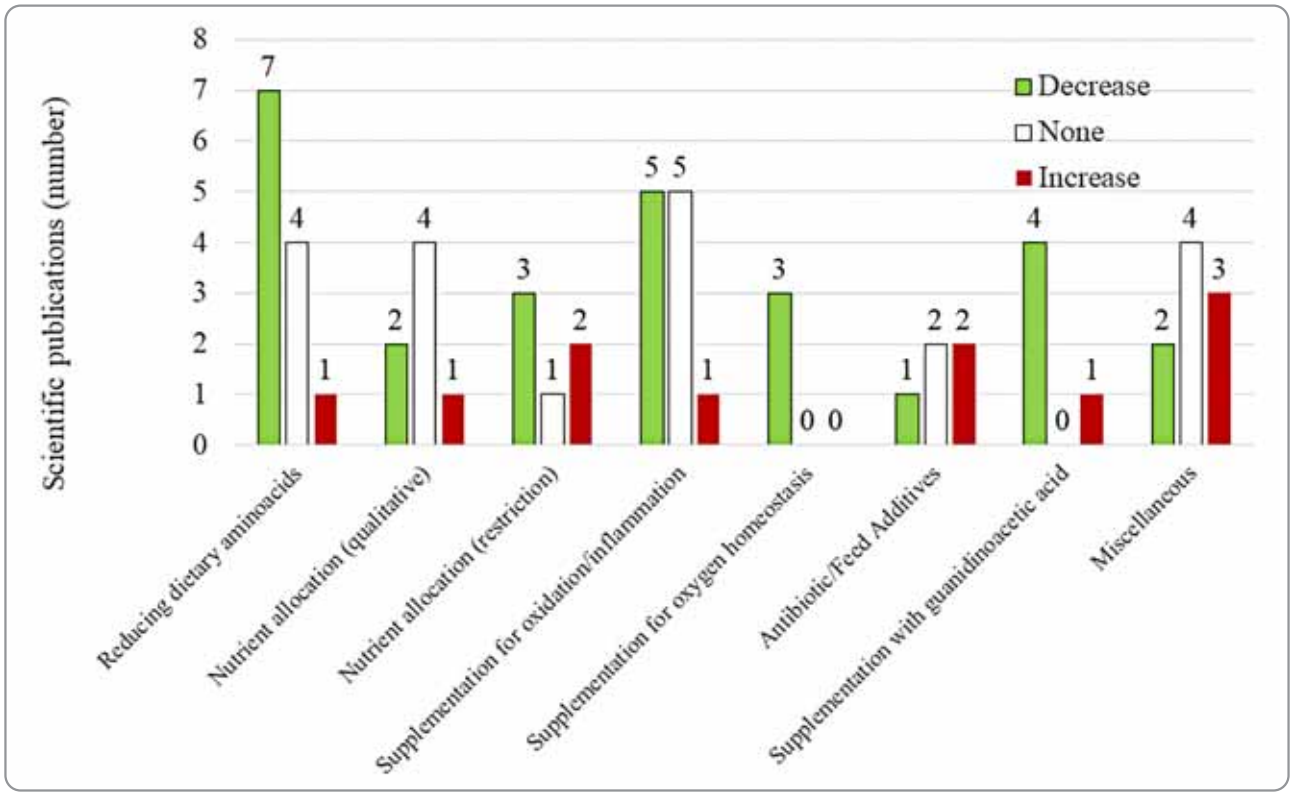


Figure 2. Number of selected articles of the final data set reporting a reduction (green bars, decrease), no change (white bars, none), and an increase of myopathy occurrence (red bars, increase) within nutritional/feeding strategy. Two articles (out of 60 selected ones) with contrasting results on myopathy occurrence were not included.

was recorded ($P<0.05$) (Table 3). Differently, no significant correlation was found between the effects on breast (weight and/or yield) and myopathy occurrence, whereas effects on growth and breast weight/yield were significantly correlated with myopathy occurrence ($P<0.001$) (Table 3).

4. Conclusion

Among nutritional and feeding strategies tested to reduce myopathy occurrence, most studies addressed nutrient manipulation and allocation both qualitatively (as macronutrients, i.e., protein and energy as well as amino acids) and quantitatively (using feed restriction), which firstly impacted on the growth trajectory of chickens. Nevertheless, despite the significant correlation found in the articles in the data set between myopathy occurrence and growth, the success of the strategies was also affected by the stage of growth at which nutrient allocation was applied as well as the duration of the manipulation period, i.e., compensatory growth has a key role. On the other hand, despite being studied less often compared to the other strategies, feeding strategies based on manipulation of nutrients that can play a role on the oxygen homeostasis (i.e. inositol, phytase, GAA) proved to be almost always successful compared to the use of feed additives acting directly as antioxidants or compared to feed additives with other metabolic roles. Further analysis of available data should distinguish between the most effective strategies for the different myopathies and their effects on growth and muscle development.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The research grant of Dr. Marija Bošković Cabrol is funded by the European Union's Horizon 2020 research and innovation programme, Marie Skłodowska-Curie grant agreement No 101063055 (DeMYO project).

References

Bailey, R. A. (2023). Strategies and opportunities to control breast myopathies: An opinion paper. *Frontiers Physiology*, 14, Article 1173564, doi: 10.3389/fphys.2023.1173564SAS

Bailey, R. A., Watson, K. A., Bilgili, S. F. & Avendano, S. (2015). The genetic basis of pectoralis major myopathies in modern broiler chicken lines. *Poultry Science*, 94, 2870–2879, doi:10.3382/PS/PEV304

Bordignon, F., Xiccato, G., Bošković Cabrol, M., Birolo, M. & Trocino, A. (2022). Factors Affecting breast myopathies in broiler chickens and quality of defective meat: A Meta-Analysis. *Frontiers Physiology*, 13, Article 933235, doi: 10.3389/fphys.2022.933235

Bošković Cabrol, M., Petracci, M. & Trocino, A. (2023, October). Wooden breast, white striping and spaghetti meat: chemical composition, technological quality, microbiological status and sensory attributes of broiler breasts. Oral presentation at the 62nd International Meat Industry Conference, Mt. Kopaonik, Serbia.

Che, S., Wang, C., Varga, C., Barbut, S. & Susta, L. (2022). Prevalence of breast muscle myopathies (Spaghetti Meat, Woody Breast, White Striping) and associated risk factors in broiler chickens from Ontario Canada. *PloS One*, 17, Article e0267019, doi:10.1371/journal.pone.0267019

Kawasaki, T., Yoshida, T. & Watanabe, T. (2016). Simple method for screening the affected birds with remarkably hardened pectoralis major muscles among broiler chickens. *Journal of Poultry Science*, 53, 291–297.

Kieronczyk, B., Rawski, M., Józefiak, D. & Swiatkiewicz, S. (2017). Infectious and non-infectious factors associated with leg disorders in poultry–a review. *Annals of Animal Science*, 17, 645–669.

Norring, M., Valros, A., Valaja, J., Sihvo, H. K., Immonen, K., & Puolanne, E. (2019). Wooden breast myopathy links with poorer gait in broiler chickens. *Animal*, 13, 1690–1695.

Oviedo-Rondón, E.O. & Córdova-Noboa, H. A. (2020). The potential of guanidino acetic acid to reduce the occurrence and severity of broiler muscle myopathies. *Frontiers Physiology*, 11, Article 909, doi: 10.3389/fphys.2020.00909

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E. & Estévez, M. (2019). Wooden-Breast, White Striping, and Spaghetti Meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comprehensive Reviews in Food Science and Food Safety*, 18, 565–583, doi:10. 1111/1541-4337.12431

Soglia, F., Petracci, M., Davoli, R. & Zappaterra, M. (2021). A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles. *Poultry Science*, 100, Article 101180, doi: 10.1016/j.psj.2021.101180

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.054

ID: 126129929

https://doi.org/10.18485/meattech.2023.64.2.6

Review paper

Overview of microplastics in the meat: occurrence, detection methods and health effects

Branko Velebit[a], Vesna Janković[a], Lazar Milojević[a], Tatjana Baltić[a], Jelena Ćirić[a] and Radmila Mitrović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Microplastics
Meat
Detection

ABSTRACT

The exponentially increasing annual production of plastic waste at a global level has raised concerns regarding the prospective infiltration of microplastics into terrestrial ecosystems. The transport of microplastics via wind advection and stormwater runoff presents a substantial hazard to food resources. The most recent studies reveal that chicken, pork, and beef samples are contaminated with microplastic particles, encompassing polystyrene, polyethylene terephthalate, and polypropylene. However, the employment of various non-standardized detection methodologies poses a significant obstacle in the interpretation of acquired results. Furthermore, the thorough clarification of the correlation between human exposure to microplastics and various health consequences, such as carcinogenicity, infertility, metabolic disorders and pregnancy complications, remains inadequately explored and necessitates additional investigation to enhance public awareness and propose solutions and integrated strategies for the mitigation of microplastic contamination in meat chain.

1. Introduction

Microplastics (MP) refer to minuscule fragments of plastic material, characterized by dimensions ranging from 1 μm to 5 mm (*Frias and Nash,* 2019). The predominant source of microplastics is attributed to the degradation of macroscopic plastic waste from diverse origins, encompassing plastic objects, synthetic fabrics, and increased industrial activities. As a result of its favourable attributes and cost-effectiveness, coupled with its versatility for various commercial and industrial purposes, the production of plastics experienced a substantial increase of approximately 200-fold over a span of 65 years (*Klöckner et al.,* 2021). Presently, the annual production of plastic waste stands at approximately 2.1 billion metric tons, with projections indicating a surge to 3.4 billion metric tons by the year 2050 (*Khan et al.,* 2022). In conjunction with an increased likelihood of ingestion and absorption by a wide array of species and potential existence of MP within food sources, encompassing meat, the multifaceted nature of this diversity has engendered apprehension regarding the potential risk that microplastics may pose to both humans and the surrounding ecosystem (*Kedzierski et al.,* 2020; *Jin et al.,* 2021; *Koelmans et al.,* 2022; *Patil et al.,* 2022).

The term "microplastics" attained eminence during the beginning of the 21st century, as researchers commenced comprehending the magnitude of plastic contamination within the ecological milieu (*Moore et al.,* 2001; *Thompson et al.,* 2004). The observed phenomenon entailed the progressive fragmentation of plastic objects within marine and aquat-

***Corresponding author:** Branko Velebit, *branko.velebit@inmes.rs*

Paper received August 7th 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ic environments. The initial investigations pertaining to the occurrence of MP in seafood (primarily fish and bivalves) and other marine organisms were documented throughout the 2010s (*Lusher et al.,* 2013; *De Witte et al.,* 2014; *Davidson and Dudas,* 2016; *Li et al.,* 2018; *Slootmaekers et al.,* 2019). These investigations brought attention to the capacity of MP to infiltrate the food chain through ingestion by marine organisms. However, since MP have capacity to be transported through various mechanisms such as wind advection, stormwater runoff, drainage systems and wastewater, they also pose a potential risk of ingestion by terrestrial fauna (*Rillig and Lehmann*, 2020). A number of studies demonstrated the presence of MP in food, such as sugar cane, honey, packaged food and beverages (*Mühlschlegel et al.,* 2017; *Liebezeit and Liebezeit*, 2013; *Oliveri Conti et al.,* 2020; *Karami et al.,* 2018).

2. Occurrence of microplastics in meat

A typical route by which microplastics could enter the meat supply chain is through the utilization of livestock feed. If animals consume contaminated water, feed, or fodder, MP could accumulate in their tissues. Current scientific data on the occurrence of MP in meat are scarce and basically rely on just a dozen or so studies.

Veen et al. (2022) observed that approximately 80% of meat and dairy products derived from farm animals in The Netherlands exhibited the presence of MP. Additionally, it has been postulated by researchers that the potential aetiology could be attributed to the feed of cows and pigs, as evidenced by the presence of plastic in all twelve examined samples of feed pellets and shredded feed. Conversely, no cases of contamination were detected in the freshly procured sustenance. In the subsequent analysis, it was observed that plastic particles were present in seven out of the eight beef samples, whereas five out of the eight pork samples exhibited the presence of at least one variant of plastic. Plastic was detected in 18 out of the 25 milk samples that underwent testing.

Plastic and edible films are integral components in the packaging of meat, as they offer a multitude of advantages that contribute to the preservation of meat products' quality, safety and shelf life. These films serve as a protective barrier, effectively separating the meat from the external environment. By maintaining a controlled atmosphere around the meat, they extend its shelf life. Additionally, they provide protection against physical damage and enhance convenience for both retailers and consumers by offering resealable bags or individually portioned packages. Nevertheless, there are growing concerns that packaging materials have the potential to liberate plastic particles, leading to the subsequent contamination of our food with MP fragments. *Kedzierski et al.* (2020) evaluated migration of plastic particles from extruded polystyrene trays (MP-XPS) used to pack chicken and demonstrated that these type of microplastics are highly adherent to the meat surface, despite the thorough rinsing of the surface of the meat, and are likely to be eaten by consumers. While polystyrene trays are composed of food-grade polystyrene, the presence of MP-XPS on the food surface poses potential concerns due to the toxicity of the microparticles, the possibility of styrene desorption, and the formation of degradation products during the cooking process. Furthermore, some studies have demonstrated that bisphenol A microplastic particles can migrate from the inner layer of plastic packaging into beef and chicken *(Thomson and Grounds,* 2005*; Sajiki et al.,* 2007, *Siddique et al.,* 2021*).* The levels exhibited a range of 4 µg/kg to 10 µg/kg. In a similar vein, *Stojanovic et al*. (2019) conducted an investigation into the levels of bisphenol A present in canned meatballs following the sterilization procedure. The samples were subsequently stored at two distinct temperatures (20 and 40°C) for a duration spanning 15 to 105 days. In their study, the researchers noted a significant increase in the concentration of bisphenol A from 5 to 23.5 µg/kg (at 20°C) and from 20 to 30 µg/kg (at 40°C) during a period of 15 days. Subsequent storage resulted in a gradual and minimal rise in the level of this type of MP.

The recent findings of a study conducted by *Habib et al.* (2022a, 2022b) revealed that plastic cutting boards were identified as the primary origin of polythene microplastic contamination in commercially sold cut meat at both butchers and a supermarket chain in the Middle East. Consequently, these cutting boards serve as a direct contributor to the presence of MP in wastewater. The average size of the microplastic particles found in the raw meat was determined to be 1.2 mm. However, after subsequent heat treatment, the authors observed that the size of these particles decreased due to melting, and this was followed partial recrystallization upon cooling. Washing the meat for a short duration (around 10 seconds) resulted in a negligible reduction in MP contamination. Only when the meat was subjected to a more thorough washing process lasting

3 minutes did a significant decrease in microplastic count to 0.07 MP/g meat occur. The same researchers also demonstrated that the average MP contamination of packed chicken commercially available on the Middle East was 1.19 MP/g, while in fish, the contamination level was 2.60 MP/g. In a study conducted by *Katsara et al*. (2022), it was discovered that low-density polyethylene microplastics (LDPE MPs) were present in bacon, salami and mortadella. The migration of LDPE was seen in the aforementioned samples, commencing after a period of 9 days and persisting throughout the duration of the 28-day investigation. The low-density polyethylene microplastics (LDPE MPs) exhibited migration into the meat samples, even when stored at a temperature of 4°C.

3. Microplastics detection methods

To date, there is a lack of a universally accepted and standardized approach for the sampling, pretreatment, identification and quantification of MP in meat and other food products. This phenomenon results in a lack of coherence in the interpretation and comparison of data derived from different meat products. The second obstacle in accurately measuring the ingestion of MP through dietary intake is in the inherent uncertainty around the levels of MP contamination present in both raw and heat-treated meat. The amounts of MP in meat and meat products are frequently found to be minimal, necessitating laborious pretreatment procedures to isolate the MP. Also, shape and size of MP play critical role in detection. MP particles are often classified as fibres, fragments, pellets, or films. Fibres stand out as the most frequently found and also critical form because they are more easily ingested by humans and animals, retained in their bodies and cause toxic effects at lower doses than spherical particles (*Ziajahromi et al.,* 2017; *Cverenkárová et al*., 2021; *Scopetani et al.,* 2022). Due to their susceptibility to loss during digestion and filtration, fibres necessitate additional precautions for their retrieval from food matrices (*Thiele et al.,* 2019).

Pretreatment of meat and meat products usually includes digestion as a necessary step to remove large amounts of organic impurities from the solid samples. Various digestion processes encompass acid digestion, alkaline digestion, and enzyme digestion. Typically, a mixture consisting of 30% hydrogen peroxide and 65% nitric acid has been employed for the purpose of digesting organic interference. In addition, the digestion of biological tissues usually involves the utilization of nitric acid, perchloric acid, hydrochloric acid, or a combination thereof. Nitric acid (69%) is extensively employed as a reagent in acid digestion processes, particularly in scenarios involving elevated temperatures. Additionally, the process of alkaline digestion is employed to break down more delicate muscle and connective tissues, utilizing a heated solution of 10% potassium hydroxide (KOH) at a temperature of 40°C (*Dowarah et al.,* 2020; *Oliveri Conti et al.,* 2020;). Enzyme digestion is also applicable. Furthermore, the utilization of enzymes, such as proteinase-K, lipase and cellulase, has been employed for the breakdown of organic substances (*Jin et al.,* 2021). This is particularly relevant in cases when the presence of microplastics can be readily destroyed through chemical means.

Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy are frequently employed for the identification of MP, i.e., the determination of their chemical composition. The infrared spectrum of the measured microparticles exhibits distinct peaks that correspond to particular chemical bonds. The spectrum that is acquired can be utilized for the purpose of identifying the chemical compositions by means of comparing it with the reference spectrum derived from a library of spectra. *Huang et al.* (2020) employed attenuated total reflection mid-infrared spectroscopy in conjunction with chemometric methodologies to rapidly identify and quantitatively assess the presence of MP (polystyrene and polyvinyl chloride) that were introduced or contaminated within chicken samples. The Raman spectrum is acquired through the collection of scattered light, which is directly correlated to the unique molecular structure and atomic composition of the food samples. In contrast to the FT-IR technique, Raman spectroscopy exhibits superior spatial resolution capabilities, since it can analyze MP greater than 1 μm in size. Nevertheless, Raman spectroscopy is susceptible to interference triggered by fluorescence resulting from interaction between bacteria and MP (*Cverenkárová et al*., 2021; *Jin et al.,* 2021). Raman spectroscopy is more sensitive to nonpolar symmetric bonds than Fourier-transform infrared (FTIR) spectroscopy (*Lenz et al.,* 2015), whereas FTIR is more sensitive to the identification of polar groups.

MP can also be quantified in the environmental samples, albeit with variable success when testing food. Pyrolysis, in conjunction with gas chro-

matography-mass spectrometry (py-GC-MS), has garnered interest as a quantitative analytical technique for assessing the mass of MP (*Fries et al.,* 2013). While this method is inherently destructive in nature, its efficacy and potential applications have captured the scientific community's attention. The utilization of chemical fingerprints subsequent to pyrolysis enables the concurrent determination of both plastic materials and key additives.

4. Human health risks

Comprehensive understanding of the impact of ingested microplastics on human health remains limited (*Rahman et al.,* 2021; *Brouwer et al.,* 2023). It is established, however, that MP has been detected in the human population. There are primarily two chief mechanisms via which humans might be exposed to MP: inhalation and ingestion. MP are introduced into the human food chain predominantly through the consumption of contaminated food sources, hence posing possible implications for human health (*Patil et al.,* 2022). After entering the gastrointestinal system, over 90% of the total MP (mainly polypropylene, polyethylene terephthalate, and polystyrene) is expelled by defecation (*Schwabl et al.,* 2020). However, the remaining 10% of MP (size < 100 μm) have the potential to be absorbed by enterocytes and a fraction of MP, smaller than 1 μm, translocate to several organs, with the extent of this translocation being influenced by their size and shape (*EFSA*, 2016). The propensity for translocation is contingent upon various parameters, including as the adsorption of particles, the hydrophobicity of the surface, the intercellular space available for particle passage, surface functionalization, and the protein profile (*Peters et al.,* 2022).

Once absorbed by the epithelial cells of the small intestine, MP enter the bloodstream and are easily distributed throughout the body. The liver and gallbladder are primary organs for accumulation but nano-MP can even pass the blood-brain and placental barriers (*Grodzicki et al.,* 2021; *Ragusa et al.,* 2021; *Medley et al.,* 2023). The precise modus operandi by which these entities penetrate the placental barrier remains elusive, concomitant with the yet undetermined ramifications for gestation and embryonic maturation (*Ragusa et al.,* 2021). Clinical manifestations of MP-accumulated organs are primarily driven by generation of reactive oxygen species during the inflammatory response, resulting in the initiation of oxidative stress and subsequent cytotoxic effects (*Patil et al.,* 2022). The symptomatology encompasses carcinogenicity and inflammation in the liver, renal dysfunction and decline due to phthalate accumulation in kidneys, circulatory distress in the heart, thyroid disfunction caused by polybrominated diphenyl ethers, bisphenol A-mediated male infertility and spermatogenesis distress, metabolic disorders and so on (*Lee et al.,* 2023).

5. Conclusion

MP can easily contaminate various environmental compartments, encompassing the entirety of the meat chain. Due to their diminutive size, MP possess a heightened propensity for ingestion, thereby provoking harmful consequences upon the well-being of animals, including humans. While the scientific knowledge pertaining to the prevalence of MP in meat is expanding, lack of standardized methodology and variability in methodological approaches across individual studies necessitates a nuanced evaluation of MP contamination, rendering its interpretation complex and challenging. Furthermore, the correlation between exposure to MP and specific health impacts in humans has not been fully determined. The preliminary findings have substantiated the classification of MP as a growing hazard inside the food chain. Nevertheless, further research should be conducted with the objective of raising the general public's awareness and coming up with strategies for mitigation.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143) and Contract number — Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Brouwer, H., Van Oijen, F. L. N. & Bouwmeester, H. (2023). Potential human health effects following exposure to nano- and microplastics, lessons learned from nanomaterials. *Present Knowledge in Food Safety,* 590–605, https://doi.org/10.1016/b978-0-12-819470-6.00014-7

Cverenkárová, K., Valachovičová, M., Mackuľak, T., Žemlička, L. & Bírošová, L. (2021). Microplastics in the food chain. *Life*, 11(12), 1349, https://doi.org/10.3390/life11121349

Davidson, K., & Dudas, S. E. (2016). Microplastic ingestion by wild and cultured manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Archives of Environmental Contamination and Toxicology*, 71(2), 147–156, https://doi.org/10.1007/s00244-016-0286-4

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K. & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine Pollution Bulletin*, 85(1), 146–155, https://doi.org/10.1016/j.marpolbul.2014.06.006

Dowarah, K., Patchaiyappan, A., Thirunavukkarasu, C., Jayakumar, S. & Devipriya, S. P. (2020). Quantification of microplastics using Nile Red in two bivalve species *Perna viridis* and *Meretrix meretrix* from three estuaries in Pondicherry, India and microplastic uptake by local communities through bivalve diet. *Marine Pollution Bulletin*, 153, 110982, https://doi.org/10.1016/j.marpolbul.2020.110982

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*, 14(6), https://doi.org/10.2903/j.efsa.2016.4501

Frias, J. P.G. L., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine Pollution Bulletin*, 138, 145–147, https://doi.org/10.1016/j.marpolbul.2018.11.022

Fries, E., Dekiff, J. H., Willmeyer, J., Nuelle, M.-T., Ebert, M. & Remy, D. (2013). Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environmental Science: Processes & Impacts*, 15(10), 1949, https://doi.org/10.1039/c3em00214d

Grodzicki, W., Dziendzikowska, K., Gromadzka-Ostrowska, J. & Kruszewski, M. (2021). Nanoplastic impact on the gut-brain axis: Current knowledge and future directions. *International Journal of Molecular Sciences*, 22(23), 12795, https://doi.org/10.3390/ijms222312795

Habib, R. Z., Kindi, R. A., Salem, F. A., Kittaneh, W. F., Poulose, V., Iftikhar, S. H., Mourad, A.-H. I. & Thiemann, T. (2022a). Microplastic contamination of chicken meat and fish through plastic cutting boards. *International Journal of Environmental Research and Public Health,* 19(20), 13442, https://doi.org/10.3390/ijerph192013442

Habib, R. Z., Poulose, V., Alsaidi, R., al Kendi, R., Iftikhar, S. H., Mourad, A.-H. I., Kittaneh, W. F. & Thiemann, T. (2022b). Plastic cutting boards as a source of microplastics in meat. *Food Additives & Contaminants: Part A*, 39(3), 609–619, https://doi.org/10.1080/19440049.2021.2017002

Huang, Y., Chapman, J., Deng, Y. & Cozzolino, D. (2020). Rapid measurement of microplastic contamination in chicken meat by mid infrared spectroscopy and chemometrics: A feasibility study. *Food Control*, 113, 107187, https://doi.org/10.1016/j.foodcont.2020

Jin, M., Wang, X., Ren, T., Wang, J. & Shan, J. (2021). Microplastics contamination in food and beverages: Direct exposure to humans. *Journal of Food Science*, 86(7), 2816–2837, Portico, https://doi.org/10.1111/1750-3841.15802

Karami, A., Golieskardi, A., Choo, C. K., Larat, V., Karbalaei, S. & Salamatinia, B. (2018). Microplastic and mesoplastic contamination in canned sardines and sprats. *Science of the Total Environment,* 612, 1380–1386, https://doi.org/10.1016/j.scitotenv.2017.09.005

Katsara, K., Kenanakis, G., Alissandrakis, E., & Papadakis, V. M. (2022). Low-density polyethylene migration from food packaging on cured meat products detected by micro-Raman spectroscopy. *Microplastics*, 1(3), 428–439, https://doi.org/10.3390/microplastics1030031

Kedzierski, M., Lechat, B., Sire, O., Le Maguer, G., Le Tilly, V. & Bruzaud, S. (2020). Microplastic contamination of packaged meat: Occurrence and associated risks. *Food Packaging and Shelf Life*, 24, 100489, https://doi.org/10.1016/j.fpsl.2020.100489

Klöckner, P., Reemtsma, T. & Wagner, S. (2021). The diverse metal composition of plastic items and its implications. *Science of the Total Environment*, 764, 142870, https://doi.org/10.1016/j.scitotenv.2020.142870

Koelmans, A. A., Redondo-Hasselerharm, P. E., Nor, N. H. M., de Ruijter, V. N., Mintenig, S. M. & Kooi, M. (2022). Risk assessment of microplastic particles. *Nature Reviews Materials*, 7(2), 138–152, https://doi.org/10.1038/s41578-021-00411-y

Lee, Y., Cho, J., Sohn, J. & Kim, C. (2023). Health effects of microplastic exposures: Current issues and perspectives in South Korea. *Yonsei Medical Journal*, 64(5), 301, https://doi.org/10.3349/ymj.2023.0048

Lenz, R., Enders, K., Stedmon, C. A., Mackenzie, D. M. A. & Nielsen, T. G. (2015). A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. *Marine Pollution Bulletin*, 100(1), 82–91, https://doi.org/10.1016/j.marpolbul.2015.09.026

Li, J., Green, C., Reynolds, A., Shi, H. & Rotchell, J. M. (2018). Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environmental Pollution*, 241, 35–44, https://doi.org/10.1016/j.envpol.2018.05.038

Liebezeit, G. & Liebezeit, E. (2013). Non-pollen particulates in honey and sugar. *Food Additives & Contaminants Part A: Chemistry Analysis Control Exposure Risk Assessment*, 30(12), 2136–2140, https://doi.org/10.1080/19440049.2013.843025

Lusher, A. L., McHugh, M. & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin*, 67(1–2), 94–99, https://doi.org/10.1016/j.marpolbul.2012.11.028

Medley, E. A., Spratlen, M. J., Yan, B., Herbstman, J. B. & Deyssenroth, M. A. (2023). A systematic review of the placental translocation of micro- and nanoplastics. *Current Environmental Health Reports*, 10(2), 99–111, https://doi.org/10.1007/s40572-023-00391-x

Moore, C. J., Moore, S. L., Leecaster, M. K. & Weisberg, S. B. (2001). A comparison of plastic and plankton in the North Pacific central gyre. *Marine Pollution Bulletin*, 42(12), 1297–1300, https://doi.org/10.1016/s0025-326x(01)00114-x

Mühlschlegel, P., Hauk, A., Walter, U. & Sieber, R. (2017). Lack of evidence for microplastic contamination in honey. *Food Additives and Contaminants: Part A*, 34(11), 1982–1989, https://doi.org/10.1080/19440049.2017.1347281

Oliveri Conti, G., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M. & Zuccarello, P. (2020). Micro- and nanoplastics in edible fruit and vegetables. The first diet risks assessment for the general population. *Environmental Research*, 187, 109677, https://doi.org/10.1016/j.envres.2020.109677

Patil, P. B., Maity, S. & Sarkar, A. (2022). Potential human health risk assessment of microplastic exposure: current scenario and future perspectives. *Environmental Monitoring and Assessment*, 194(12), https://doi.org/10.1007/s10661-022-10539-1

Peters, R., de Jong, N., de Haan, L., Wright, S. & Bouwmeester, H. (2022). Release and intestinal translocation of chemicals associated with microplastics in an in vitro human gastrointestinal digestion model. *Microplastics and Nanoplastics*, 2(1), 1–21, https://doi.org/10.1186/s43591-021-00022-y

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M. & Giorgini, E. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environment International,* 146, 106274, https://doi.org/10.1016/j.envint.2020.106274

Rahman, A., Sarkar, A., Yadav, O. P., Achari, G. & Slobodnik, J. (2021). Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: A scoping review. *Science of The Total Environment*, 757, 143872, https://doi.org/10.1016/j.scitotenv.2020.143872

Rillig, M. C. & Lehmann, A. (2020). Microplastic in terrestrial ecosystems. *Science*, 368(6498), 1430–1431, https://doi.org/10.1126/science.abb5979

Sajiki, J., Miyamoto, F., Fukata, H., Mori, C., Yonekubo, J. & Hayakawa, K. (2007). Bisphenol A (BPA) and its source in foods in Japanese markets. *Food Additives and Contaminants*, 24(1), 103–112, https://doi.org/10.1080/02652030600936383

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T. & Liebmann, B. (2019). Detection of various microplastics in human stool. *Annals of Internal Medicine*, 171(7), 453–457, https://doi.org/10.7326/m19-0618

Scopetani, C., Martellini, T. & Campos, D. (2022). Editorial for the Special Issue "Microplastics in Aquatic Environments: Occurrence, Distribution and Effects." *Toxics*, 10(7), 407, https://doi.org/10.3390/toxics10070407

Siddique, M. A. Bakar, Harrison, S. M., Monahan, F. J., Cummins, E. & Brunton, N. P. (2021). Bisphenol A and metabolites in meat and meat products: Occurrence, toxicity, and recent development in analytical methods. *Foods*, 10(4), 714, https://doi.org/10.3390/foods10040714

Slootmaekers, B., Carteny, C. C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R. & Bervoets, L. (2019). Microplastic contamination in gudgeons (*Gobio gobio*) from Flemish rivers (Belgium). *Environmental Pollution*, 244, 675–684, https://doi.org/10.1016/j.envpol.2018.09.136

Stojanović, B., Radović, L., Natić, D., Dodevska, M., Vraštanović-Pavičević, G., Balaban, M., Stojanović, Z. & Antić, V. (2019). Migration of bisphenol A into food simulants and meat rations during initial time of storage. *Packaging Technology and Science*, 33(2), 75–82, Portico, https://doi.org/10.1002/pts.2485

Thiele, C. J., Hudson, M. D. & Russell, A. E. (2019). Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine Pollution Bulletin*, 142, 384–393, https://doi.org/10.1016/j.marpolbul.2019.03.003

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D. & Russell, A. E. (2004). Lost at sea: Where is all the plastic? *Science*, 304(5672), 838–838, https://doi.org/10.1126/science.1094559

Thomson, B. M. & Grounds, P. R. (2005). Bisphenol A in canned foods in New Zealand: An exposure assessment. *Food Additives and Contaminants*, 22(1), 65–72, https://doi.org/10.1080/02652030400027920

Veen, I., Mourik, L. M, Velzen, M. J. M., Groenewoud, Q. R. & Leslie, H. A. (2022). Plastic particles in livestock feed, milk, meat and blood. A pilot study. Retrieved from https://www.plasticsoupfoundation.org/wp-content/uploads/2022/07/Final-Report-pilot-study-plastic-particles-in-livestock-feed-milk-meat-and-blood-SIGNED-1.pdf/. Accessed August 19, 2023.

Ziajahromi, S., Neale, P. A., Rintoul, L. & Leusch, F. D. L. (2017). Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. *Water Research*, 112, 93–99, https://doi.org/10.1016/j.watres.2017.01.042

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.053

ID: 126133513

https://doi.org/10.18485/meattech.2023.64.2.7

Review paper

(Mis)understandings in research methodology and chemometrics in meat science

Predrag Putnik[a], Daniela Šojić Merkulov[b], Branimir Pavlić[c], Branko Velebit[d] and Danijela Bursać Kovačević[e]*

[a] *Department of Food Technology, University North, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia*
[b] *University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*
[c] *Faculty of Technology, University of Novi Sad, Blvd. Cara Lazara 1, 21000 Novi Sad, Serbia*
[d] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[e] *Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia*

ARTICLE INFO

Keywords:
Research methodology
Data analysis
Mathematical modeling
Principal component analysis
Hypothesis testing
Chemometrics
Meat production

ABSTRACT

The main purpose of this manuscript is to discuss the most common errors in reporting food science data, with special attention to meat science, while offering suggestions that are common and long known in the regular research methodology of any field. Because the quality of data determines the quality of conclusions that are decisive for subsequent actions and the allocation of (often scarce) resources, low quality data can be a barrier to progress in the field rather than paving the way to a better understanding of the important aspects of food (meat) production. For valid conclusions, it is important to define hypotheses for a particular data collection, to collect data correctly, and to choose the right test for analysis. If a professional in meat production needs to optimize or predict a particular production outcome, mathematical modeling is the right choice. On the other hand, if one is looking for structure within the data, principal component analysis (PCA) is one of the valid options. Both approaches have unlimited applications in meat and food science in general, which can also provide various benefits for industrial purposes, such as getting ahead of competitors in the market (by identifying optimal customers, predicting customer acceptance of a meat product, various aspects of business intelligence such as improving effectiveness and efficiency etc.).

1. Introduction

All branches of science have the same common denominator, namely the generation of data and the need to analyze these data in order to draw valid conclusions (*Green et al*., 2007). This is accentuated by the accelerated development of instrumental analysis in modern wet chemistry, which is capable of generating large datasets from routine analyzes (*Varmuza & Filzmoser*, 2009). For instance, it is now quite common for analytical methods, such as gas chromatography, to yield datasets containing information on hundreds of compounds in meat products (*Sohail et al*., 2022), which can be difficult to understand unless the research team has experienced data analysts.

However, due to necessity and need, the majority of time, statistical methods for data analysis are superficially learned as some side skills by researchers who are originally experts in different fields of

***Corresponding author:** Predrag Putnik, *pputnik@alumni.uconn.edu*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

research. Unfortunately, that outcomes with number of shortcomings in research and questionable usefulness of the data obtained (*Ali & Bhaskar*, 2016). Egregiously, the conclusions drawn then do not best serve their intended purposes. We have reached the point in food science that quite often not even basic notations are done correctly.

Here, the first error that comes to mind is the way of denoting the probability in majority of statistical tests, the well-know and used p-value (i.e., the probability calculated by a statistical test) that is commonly and wrongly reported in food science papers as 'p<0.05' rather than 'p≤0.05', which is the correct way to refer to this statistical metric. The former notation (use of the character 'less-than') symbolizes that 0.05 is not the limit of significance (anything below this value is significant, but not the sole 0.05 value), whereas the later notation ('less than or equal to') includes 0.05 as a value that properly outlines the significance of the statistical test.

Numerous studies do not even contain a correctly written hypothesis, and seriously lack experimental design or examination of the suitability of different statistical tests for different datasets, which will be discussed later in the text. The purpose of this paper is to draw attention to the most common errors in the reporting of food science data, with particular emphasis on meat science, while providing the correct approaches that are a common part of research methodology and have been established for decades, well back into the last century.

2. Materials and methods

The data in this manuscript were obtained from common sources such as Web of Science, PubMed, Scopus, and other platforms that provide scientific references. In addition, various search engines such as Google.com, Ask.com, Bing.com, and other, were used to search for relevant terms. The concept of the paper was designed to include selected examples of research methodology, from common basic (mis)understandings to complex ones with a focus on meat science. It is important to note that this short manuscript is far from sufficient to list all methodological issues encountered in meat science. Rather, it should be seen as the 'tip of the iceberg,' encouraging readers to investigate further and fill in the gaps in their own knowledge of research methodology, so the entire food science field provides information of the highest possible quality.

3. Hypothesis testing and why it is used

Most researchers in food science are familiar with the concepts of stating hypothesis and that all research should include some form of hypotheses. What is less known is that the purpose of hypotheses is to test them with some statistical test with purpose of rejecting or accepting either the null hypothesis or hypothesized alternative. A null hypothesis generally conveys absence of difference, while an alternative hypothesis states the presence of difference in the research (usually among studied groups).

However, it is frequently observed that in doctoral dissertations, research papers and various research proposals, the research hypotheses are listed in an erroneous way. Usually, this is done in a descriptive manner in which the authors explain what they hope to achieve from their research. For example, a commonly stated hypothesis may sound like this:

'*Selected meat products with a high protein content that are commonly present in them will increase the levels of amino acids in the diet*'

In the above example, there are the following issues: first it is not known what kind of hypothesis is being talked about. Second, the hypotheses should be stated at least in pairs (with at least the null hypothesis H_0 paired with an alternative hypothesis or H_1), and third, they should be stated for a particular data analytic test. Another problem with the above hypothesis is the specification of direction. Saying '*…content will increase…*' assuming that the data are normally distributed, means that only half of the normally distributed data will be analyzed. The correct formulation of the hypothesis in meat science for the above example and for normally distributed data to be analyzed with the independent *t*-test should be something like this:

'*H_0: $\mu_1 = \mu_2$ or in plain English: meat products with a high protein content from group 1 will yield* ***equal*** *average levels (means) of amino acids (g/100 g product) in the diet as meat products with a high protein content from group 2'*

and

'H_1: $\mu_1 \neq \mu_2$ or in plain English: meat products with a high protein content from group 1 will yield ***different*** *average levels (means) of amino acids (g/100 g product) in the diet from meat products with a high protein content from group 2'*

In stating hypotheses as the above example, all methodological requirements were met and readers

knows what the authors hypothetically compared (i.e. quantities of amino acids among two different groups of meat products expressed as grams of amino acids per 100 grams of meat product).

4. Use of experimental design and why it is needed

Experimental design is the most important step to obtain valid data and conclusions (*Croarkin*, 2013). It consists of defining important experimental factors that are under the control of the experimenter (also referred to as independent variables) and linking them to response variables, correspondingly referred to as dependent variables, so that they can be modeled for the purpose of extrapolation (prediction). The experimental design is usually given in a table (e.g., Table 1) and is used by analytical chemists as a navigational map when conducting series of experimentations (e.g., runs). In other words, it specifies the order of experimentations and the combination of independent variables expected to alter the dependent variables (Figure 1). Experiments should be in randomized order to prevent any potential biases, i.e., to provide homogeneity of variance in the examined groups.

Table 1. Example of experimental design table

Experimental run	Replications	Pressure	Temperature	Time	Microbial load	Amino acid content	Vit. B_{12} content
		(MPa)	(°C)	(min)	CFU	g/100 g meat	µg/100 g
1	1	10	30.0	15.0			
2	1	100	15.0	5.0			
3	2	10	15.0	15.0			
4	2	100	30.0	5.0			
5	3	100	15.0	15.0			
6	3	10	30.0	5.0			
7	4	10	15.0	5.0			
8	4	100	30.0	15.0			
9	5	10	30.0	15.0			
10	5	100	15.0	5.0			
11	6	10	15.0	15.0			
12	6	100	30.0	5.0			
13	7	100	15.0	15.0			
14	7	10	30.0	5.0			
15	8	10	15.0	5.0			
16	8	100	30.0	15.0			

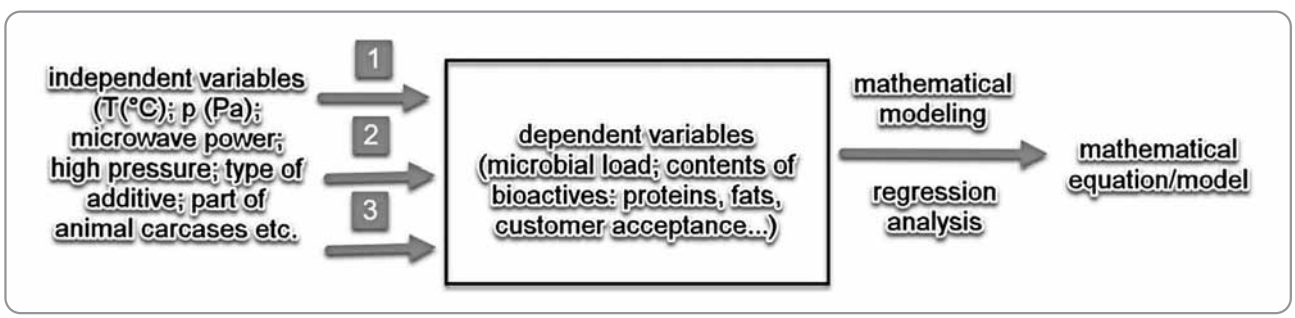


Figure 1. Summary of mathematical modeling.

Dependent and independent variables can be grouped as qualitative or quantitative (*Szymańska et al*., 2015), with the former including nominal variables (e.g., meat types, meat seasonings), dichotomous variables (authentic/adulterated meat products, male/female animals etc.), and ordinal variables (the data have some rankings, as levels of the hedonic scale for sensory evaluation of cured sausage). In contrast, quantitative variables include scales, intervals, and ratios (*Larson-Hall*, 2010). In meat science, examples of scales include the duration of processing in minutes, the temperature of bratwurst, the amount of salt added to the brine, etc. Intervals and ratios are usually similar to terms used in everyday life.

5. Use of mathematical modelling and principal component analysis

In the field of food science, engineers often try to predict or optimize certain aspects of food production (*Croarkin*, 2013). Usually, this means that for a particular process, a particular dependent variable is as high/low as possible. In the meat industry, for example, market pressures dictate that products must have the highest possible microbial safety, while minimizing the use of (unpopular) additives such as nitrates and nitrites (*Šojić et al*., 2022). Therefore, in order to optimize (in this case, minimize) the use of additives for a preservation process (e.g., high-pressure processing), we construct mathematical equations that are capable of describing, to a meaningful (significant) extent, the changes in microbial load upon addition of that particular additive while simultaneously accounting for the changes in high-pressure parameters. If constructed according to valid methodological principles, created mathematical equation should be able to follow (predict) changes across the entire spectrum of variations in high-pressure parameters while simultaneously allowing for altering amounts of the unpopular additive, and perhaps finding the point at which high-pressure processing is sufficient to provide full microbial safety without the use of nitrates/nitrites, even if this is in settings that were not originally included by experimental design. This process is called extrapolation, and the predictions can later be tested in the laboratory to observe the full accuracy of a model.

Here it is important to note that mathematical models are usually built by some type of regression analysis, using potentially relevant factors for testing that were initially chosen by experts in the field of meat science (production technologists, engineers etc.). Clearly, the relevance of each factor should be tested for significance, and all insignificant components of a model must be omitted from the equation as they serve no purpose. This is typically achieved by some multivariate analysis of variance (MANOVA). In practice, optimization/prediction for industrial purposes can provide various benefits, such as an edge over competitors (e.g., efficient production of food, optimal exploitation of the market, identification of customers from the existing customer pool, prediction of customer acceptance, various aspects of business intelligence). Another possibility is to embed models in computer software for commercialization or public access (*Putnik et al*., 2017).

Another data analysis tool commonly used in meat science comes from the group of factor analyses known as principal component analysis (PCA) (*Granato et al*., 2018). This type of analytical approach is also known as ‘dimension reduction’ because the main idea is to reduce a large number of correlated variables from the dataset by using as few uncorrelated factors as possible. The main purposes of PCA are to find underlying correlation patterns within a dataset or to identify structural patterns, i.e., to create indices, otherwise known as principal components or factors (hence the name, PCA).

Often, PCA is erroneously used to group data according to certain criteria, which is not the purpose of this tool; this data grouping should be avoided due to its questionable usefulness. For the purposes of grouping (clustering), there is an entire range of cluster analyses that differ from PCA (*Aldenderfer and Blashfield,* 1984). Moreover, a large number of PCA studies in scientific papers do not even check whether the given data are suitable for this analysis. For example, it is very rare to find statistical metrics for the Kaiser-Meyer-Olkin (KMO) test and the Bartlett’s test for sphericity in meat science reports; these two tests are needed to check that the basic requirements are fulfilled for performing PCA. The KMO test measures the proportion of variance in a dataset that might be caused by the underlying factors, while Bartlett’s test verifies whether the initial variables in a dataset are correlated (*Tabachnick and Fidell*, 2007). Both of these tests should be reported together with complete factor loadings for all factor analyses, including for PCA.

6. Chemometrics and meat production

Because meat is an important source of protein for human diets, it is globally produced and processed, while it is projected that this segment of the food industry will expand in the future (*Gómez et al.*, 2019). This will likely result in widening analytical methods for determining organoleptic, physiochemical and food safety parameters required by the market (or by lawmakers that regulate food markets) to ensure consumer acceptance of such meat products. Accordingly, as mentioned earlier, major improvements in laboratory equipment will result in adding additional information to already large datasets from wet chemistry laboratories. These large datasets need to be analyzed in a practical and meaningful way (*Varmuza and Filzmoser*, 2009).

To address this challenge, data analysts employ statistical concepts and tests known as multivariate statistics (*Hidalgo and Goodman*, 2013). The most important aspect of multivariate statistics is the simultaneous testing of multiple independent variables against one (or more) dependent variable(s) to avoid inflating Type I errors. This decreases the corresponding inclination to misleadingly show effects and significances in the dataset that do not actually exist (*Dumancas et al.*, 2015).

In chemometrics, multivariate statistics and data mining are used to draw valid conclusions from large datasets (*Granato et al*., 2018). Multivariate tests include the aforementioned multivariate analysis of variance (MANOVA), numerous factor analyses (e.g., PCA), mathematical modelling, discriminant analysis, etc. (*Dziurkowska and Wesolowski*, 2015). Recently, chemometrics has been used by government agencies and industry to address the challenges of increasingly prevalent food fraud and public concerns about food safety and quality (*Danezis et al.*, 2016). This is in addition to multivariate methods being suitable for determining optimal processing parameters for different production conditions and raw materials (*Granato et al.*, 2018). Accordingly, the application of chemometrics in meat science is only expected to increase as more applications are added to those already mentioned for food safety (*Jurica et al.*, 2021).

7. Conclusion

In conclusion, there are many misconceptions about data analysis in food and meat science, very few of which have been reported in this manuscript. Improper experimental design and data analysis yield data and conclusions of less than optimal quality and diminish the prosperity of the entire field. Most of the methodological principles discussed in this report have existed for a very long time and are well used and known in different scientific disciplines (medicine, epidemiology, etc.). For valid conclusions, it is important to define research hypotheses for a particular test, while data should be collected in the right way and using an experimental design that not only provides useful data, but also saves time and other resources that are very scarce for most researchers around the world. The most frequent types of chemometric tests include MANOVAs, different kinds of factor analysis (e.g., PCA), and mathematical modeling, along with numerous others. When meat scientists and engineers decide to use PCA or mathematical modeling, it should be kept in mind that modeling is used to predict or optimize by some mathematical equation, while PCA is used to find the structure in the data, making it easier for the analyst to deal with large datasets while drawing meaningful conclusions. Both statistical methods have wide applications in the food industry and elsewhere.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was funded by the Croatian Science Foundation through the funding of the Hurdle Technology and 3D Printing for Sustainable Fruit Juice Processing and Preservation project, IP-2019-04-2105, and by the Republic of Croatia Ministry of Science.

References

Aldenderfer, M. & Blashfield, R. (1984). *Cluster Analysis*, https://doi.org/10.4135/9781412983648

Ali, Z. & Bhaskar, S. (2016). Basic statistical tools in research and data analysis. *Indian Journal of Anaesthesia*, 60(9), https://doi.org/10.4103/0019-5049.190623

Croarkin, C. (2013). *NIST/SEMATECH e-Handbook of Statistical Methods*

Danezis, G. P., Tsagkaris, A. S., Camin, F., Brusic, V. & Georgiou, C. A. (2016). Food authentication: Techniques, trends & emerging approaches. *TrAC Trends in Analytical Chemistry*, 85, 123–132, https://doi.org/10.1016/j.trac.2016.02.026

Dumancas, G. G., Ramasahayam, S., Bello, G., Hughes, J. & Kramer, R. (2015). Chemometric regression techniques as emerging, powerful tools in genetic association studies. *TrAC Trends in Analytical Chemistry*, 74, 79–88, https://doi.org/10.1016/j.trac.2015.05.007

Dziurkowska, E. & Wesolowski, M. (2015). Multivariate statistical analysis as a supplementary tool for interpretation of variations in salivary cortisol level in women with major depressive disorder. *The Scientific World Journal*, 2015, 1–8, https://doi.org/10.1155/2015/987435

Gómez, B., Munekata, P. E. S., Zhu, Z., Barba, F. J., Toldrá, F., Putnik, P., Bursać Kovačević, D. & Lorenzo, J. M. (2019). Challenges and opportunities regarding the use of alternative protein sources: Aquaculture and insects. In, https://doi.org/10.1016/bs.afnr.2019.03.003

Granato, D., Putnik, P., Kovačević, D. B., Santos, J. S., Calado, V. M. d. A., Rocha, R. S., Cruz, A. G., Jarvis, B., Rodionova, O. Y. & Pomerantsev, A. (2018). Trends in chemometrics: food authentication, microbiology, and effects of processing. *Comprehensive Reviews in Food Science and Food Safety*.

Green, J., Willis, K., Hughes, E., Small, R., Welch, N., Gibbs, L. & Daly, J. (2007). Generating best evidence from qualitative research: the role of data analysis. *Australian and New Zealand Journal of Public Health*, 31(6), 545–550, https://doi.org/10.1111/j.1753-6405.2007.00141.x

Hidalgo, B. & Goodman, M. (2013). Multivariate or multivariable regression? *American Journal of Public Health*, 103(1), 39–40, https://doi.org/10.2105/ajph.2012.300897

Jurica, K., Brčić Karačonji, I., Lasić, D., Bursać Kovačević, D. & Putnik, P. (2021). Unauthorized Food Manipulation as a Criminal Offense: Food Authenticity, Legal Frameworks, Analytical Tools and Cases. *Foods*, 10(11), https://doi.org/10.3390/foods10112570

Larson-Hall, J. (2010). 2.1.1 Levels of Measurement of Variables in A Guide to Doing Statistics. In *Second Language Research Using SPSS* (pp. 33–35). Taylor & Francis.

Putnik, P., Roohinejad, S., Greiner, R., Granato, D., Bekhit, A. E.-D. A. & Bursać Kovačević, D. (2017). Prediction and modeling of microbial growth in minimally processed fresh-cut apples packaged in a modified atmosphere: A review. *Food Control*, 80, 411–419, https://doi.org/10.1016/j.foodcont.2017.05.018

Sohail, A., Al-Dalali, S., Wang, J., Xie, J., Shakoor, A., Asimi, S., Shah, H. & Patil, P. (2022). Aroma compounds identified in cooked meat: A review. *Food Research International*, 157, https://doi.org/10.1016/j.foodres.2022.111385

Szymańska, E., Gerretzen, J., Engel, J., Geurts, B., Blanchet, L. & Buydens, L. M. C. (2015). Chemometrics and qualitative analysis have a vibrant relationship. *TrAC Trends in Analytical Chemistry*, 69, 34–51, https://doi.org/10.1016/j.trac.2015.02.015

Šojić, B., Putnik, P., Danilović, B., Teslić, N., Bursać Kovačević, D. & Pavlić, B. (2022). Lipid Extracts Obtained by Supercritical Fluid Extraction and Their Application in Meat Products. *Antioxidants*, 11(4), https://doi.org/10.3390/antiox11040716

Tabachnick, B. G. & Fidell, L. S. (2007). Principal components and factor analysis. In *Using multivariate statistics (5th ed.)* (pp. 607–615). Allyn & Bacon.

Varmuza, K. & Filzmoser, P. (2009). Chemoinformatics-chemometrics — statistics. In *Introduction to multivariate statistical analysis in Chemometrics* (pp. 1–26). CRC Press Taylor and Francis Group.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 612.311:637.5

ID: 126135817

https://doi.org/10.18485/meattech.2023.64.2.8

Original scientific paper

Is food oral processing a new meat quality dimension?

Ilija Đekić[a]*

[a] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Meat oral processing
Meat properties
Mastication indicators
Bibliometric analysis

ABSTRACT

This study gives an overview of food oral processing of meat. Literature review in the last two decades shows three perspectives of research. First, the perspective known as 'ex ante' studies serves to analyse mechanical and textural properties of meat and enable correlation between these instrumental testing and food oral processing parameters. The second is clearly associated with the mastication process revealing the following parameters: number of chews, mastication duration, chewing and eating rates, saliva incorporation, food breakdown, particle number and size throughout the mastication process. The third 'ex post' perspective is associated with the post-swallowing period, focused on digestibility, satiety and energy intake.

The main conclusion of this study is that food oral processing studies pave the way for understating mastication of meat and widen meat science research perspectives in terms of modelling mastication (from the first bite to swallowing) and simulating meat breakage and flavour release.

1. Introduction

Food oral processing is a novel scientific approach that analyses changes that are associated with food from the first bite until swallowing, covering food breakdown, saliva incorporation and in-mouth sensations (*Chen*, 2014). This discipline generates a variety of different indicators such as number of chews needed for mastication, bite size and eating rate (Koç et al., 2014), saliva incorporation (*de Lavergne et al.*, 2015), and particle number and size distribution at pre-defined mastication time (*Rizo et al.*, 2019). A Kano model study on the importance of oral processing indicators has revealed that food breakdown in the mouth, eating rate and bite size are very important quality dimensions (*Djekic et al.*, 2020). The importance of mastication was studied in the perspective of efforts required to masticate a bite of food (*Ilic et al.*, 2021), where authors developed an 'ease of mastication index' that may be considered as a new food characteristic, similar to the total quality index as depicted in works of *Djekic et al.* (2017) and *Režek Jambrak et al.* (2018). The importance of mastication is even pronounced in some types of food, such as meat, where different mastication patterns were identified between humans of good health, the elderly population, people with dysphagia and denture wearers (*Mioche et al.*, 2002).

Wider perspectives of food oral processing have supported sensory analysis through promotion of "temporal dominance of sensations" as a tool that enables researchers to distinguish sensation that dominates throughout the mastication process (*Rizo et al.*, 2019). In parallel, scholars have analysed food intake and acceptance (*Aguayo-Mendoza et al.*, 2019) and the role of food in satiation and satiety (*Campbell et al.*, 2017).

***Corresponding author:** Ilija Djekić, *idjekic@agrif.bg.ac.rs*

Paper received April 26th 2023. Paper accepted May 5th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

The objective of this paper is to give an overview of food oral processing studies performed with meat and to identify potential breakthroughs in future studies.

2. Materials and methods

In order to perform a literature review associating food oral processing and meat, a text mining concept was applied by using VOSViewer tool for the bibliometric analysis. This enables users to understand what are the main research flows among scholars. The input data were captured from academic papers indexed in the Web of Science, with the use of the two keywords: "meat" and "food oral processing". The research revealed 140 articles/review papers that were published in the period 2005 to date. The 'cut-off criteria' was to include keywords mentioned more than five times.

3. Results

Figure 1 shows the created network visualization of titles, abstracts and keywords of the most relevant manuscripts that were published in the last 20 years, covering the selected two keywords. As can be observed, four clusters (depicted in different colours) were revealed.

The green cluster is associated with food oral processing indicators covering the mastication process (bite size, consumption time and sensory sensations). The yellow cluster covers bolus and its characteristics, saliva incorporation and textural properties, while the blue cluster is more focused on digestibility and bolus features after swallowing. Finally, the red cluster is mostly linked with meat intake and potential risks associated with it.

4. Discussion

Djekic et al. (2022) have identified three main evaluation phases in meat oral processing: (i) *ex ante*, consisting of testing physical and mechanical properties of meat prior to mastication, (ii) ongoing, comprising of analysing mastication and food oral processing parameters, and (iii) *ex post,* focused on swallowing, digestibility, satiety and energy intake. As observed in Figure 1, this was mainly confirmed in our current study. In order to understand the complexity of meat oral processing, it is important to understand meat and its physical characteristics. Meat is considered as a postmortem skeletal muscle tissue (*Matarneh et al.*, 2017). After slaughtering, it undergoes various changes, mainly physiological and biochemical (*Bekhit et al.*, 2014). However, its complexity is highly dependent on the species, age, meat part from the carcass and meat cut (*Purslow*, 2005). From basic material science, depending on load, different materials behave differently — as isotropic, orthotropic or anisotropic (*Berthaume,* 2016). Although meat inclines towards being anisotropic, in many studies it has been considered as orthotropic, such as in modelling the first bite of meat (*Djekic et al.*, 2022; *Djekic et al.*, 2021).

In order to understand the oral processing parameters associated with meat, it is necessary to look at its main quality characteristics such as sensory attributes or meat texture from a different perspective. These intrinsic quality cues have been revealed by (*Rajic et al.*, 2022) in their literature review of pork and beef meat. When a sufficient number of panellists is used in food oral processing studies, clear correlations between instrumental texture and some mastication parameters and even saliva incorporation may be revealed (*Ilić et al.*, 2022).

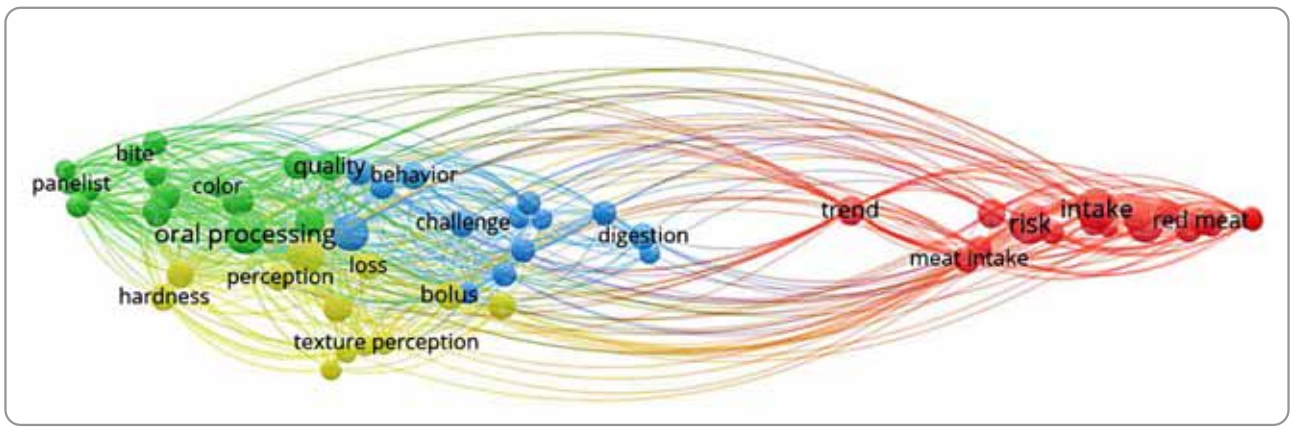


Figure 1. Network visualization of inter-linkage between meat and food oral processing

In addition to abovementioned meat characteristics, culinary methods also play a great role in oral processing characteristics of meat. In the study of pork ham prepared with three culinary methods, number of chews associated with cooked ham was statistically higher contrasted to *sous-vide* or grilled meat (*Djekic et al.*, 2021). Also, the same study revealed the higher number of chews, the more saliva is incorporated. The influence of different culinary techniques affecting food oral processing and dynamic sensory perception of wild boar ham has been studied by *Ilic et al.* (2022). Results revealed that *sous-vide* and grilled meat demanded less effort for mastication and absorbed less saliva, opposed to boiling. An interesting study on food oral processing of meat was performed by *Djekic et al.* (2021), associating grilled meat coated with hot sauces to achieve pungency sensations. There was a slight trend of an increased number of chews and a longer duration of consumption time correlated with pungency intensity. The study showed that after 10 chews, saliva decreases in relation to the pungency intensity while after 25 chews and before swallowing, this trend changes. The role of saliva is that it enables cohesiveness between particles and lubricates the bolus, so aiding swallowing (*Prinz & Lucas*, 1997; *Rizo et al.,* 2019). Also, low eating rates are in correlation with high chew number and long mastication duration (*Aguayo-Mendoza et al.*, 2019). To avoid large discrepancies in-between mastication patterns of human subjects, it is necessary to define characteristics of oral processing panels, similar to strict rules that apply for sensory panels (*Djekic et al.*, 2021).

Particle number and size distribution during mastication (after a pre-defined number of strokes) and just before swallowing depends on mechanical properties of meat and its water content (*Rizo et al.*, 2019). At the middle of mastication process, below 20% of the bolus consists of big particles while at the end of mastication smaller pieces prevail, whereby grilled ham prevailed in number of small particles compared to *sous-vide* (*Djekic et al.*, 2021). Regardless of the type of culinary method applied to meat, the number of large particles decreases while the number of small particles increases during mastication (*Djekic et al.*, 2021).

A new dimension associated with food oral processing is to analyse emotions during mastication. When studying pungency sensations associated with grilled meat, results revealed a clear correlation between increase in non-neutral emotions (angry, happy, sad and surprise) and the increase in pungency intensity (*Djekic et al.*, 2021). Meat samples marinated with pungent spices are being promoted lately in line with their antimicrobial effects (*Vasilijević et al.*, 2019).

Finally, oral processing studies on meat have paved a way for the development of different meat analogues, as some preliminary studies on boluses, particle size distribution and the number of chews before swallowing of plant-based and beef burgers still show a great discrepancy (*Ilić et al.*, 2022).

5. Conclusion

The two main conclusions of the study are that application of food oral processing can help in better understanding mastication of meat and that it brings new research perspectives. Future research should focus on modelling meat mastication (from the first bite to swallowing) to enable simulation of meat breakage and flavour release.

Disclosure statement: No potential conflict of interest was reported by the author.

References

Aguayo-Mendoza, M. G., Ketel, E. C., van der Linden, E., Forde, C. G., Piqueras-Fiszman, B. & Stieger, M. (2019). Oral processing behavior of drinkable, spoonable and chewable foods is primarily determined by rheological and mechanical food properties. *Food Quality and Preference*, 71, 87–95, https://doi.org/https://doi.org/10.1016/j.foodqual.2018.06.006

Bekhit, A. E.-D. A., Carne, A., Ha, M. & Franks, P. (2014). Physical interventions to manipulate texture and tenderness of fresh meat: A review. *International Journal of Food Properties*, 17(2), 433–453, https://doi.org/https://doi.org/10.1080/10942912.2011.642442

Berthaume, M. A. (2016). Food mechanical properties and dietary ecology. *American Journal of Physical Anthropology*, 159, 79–104, https://doi.org/https://doi.org/10.1002/ajpa.22903

Campbell, C. L., Wagoner, T. B. & Foegeding, E. A. (2017). Designing foods for satiety: The roles of food structure and oral processing in satiation and satiety. *Food Structure*, 13, 1–12, https://doi.org/https://doi.org/10.1016/j.foostr.2016.08.002

Chen, J. (2014). Food oral processing: Some important underpinning principles of eating and sensory perception. *Food Structure*, 1(2), 91–105, https://doi.org/https://doi.org/10.1016/j.foostr.2014.03.001

de Lavergne, M. D., Van De Velde, F., Van Boekel, M. & Stieger, M. (2015). Dynamic texture perception and oral processing of semi-solid food gels: Part 2: Impact of breakdown behaviour on bolus properties and dynamic texture perception. *Food Hydrocolloids*, 49, 61–72, https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.02.037

Djekic, I., Ilić, J., Chen, J., Djekic, R., Sołowiej, B. G., Vujadinović, D. & Tomasevic, I. (2021). Analysis of pungency sensation effects from an oral processing, sensorial and emotions detection perspective—case study with grilled pork meat. *Applied Sciences*, 11(21), Article e10459, https://doi.org/https://doi.org/10.3390/app112110459

Djekic, I., Ilic, J., Guiné, R. P. F. & Tomasevic, I. (2020). Can we understand food oral processing using Kano model? Case study with confectionery products. *Journal of Texture Studies*, 51(6), 861–869, https://doi.org/https://doi.org/10.1111/jtxs.12550

Djekic, I., Ilic, J., Lorenzo, J. M. & Tomasevic, I. (2021). How do culinary methods affect quality and oral processing characteristics of pork ham? *Journal of Texture Studies*, 52(1), 36–44, https://doi.org/https://doi.org/10.1111/jtxs.12557

Djekic, I., Ilic, J., Sołowiej, B., Djekić, R. & Tomasevic, I. (2022). Application of food mechanics and oral processing in modelling first bite of grilled meat. *Journal of Food Quality*, 2022, e9176628, https://doi.org/10.1155/2022/9176628

Djekic, I., Lorenzo, J. M., Munekata, P. E. S., Gagaoua, M. & Tomasevic, I. (2021). Review on characteristics of trained sensory panels in food science. *Journal of Texture Studies*, 52(4), 501–509, https://doi.org/https://doi.org/10.1111/jtxs.12616

Djekic, I., Vunduk, J., Tomašević, I., Kozarski, M., Petrovic, P., Niksic, M., Pudja, P. & Klaus, A. (2017). Total quality index of *Agaricus bisporus* mushrooms packed in modified atmosphere. *Journal of the Science of Food and Agriculture*, 97(9), 3013–3021, https://doi.org/10.1002/jsfa.8142

Djekic, R., Ilic, J., Tomasevic, I. & Djekic, I. (2021). Use of engineering tools in modelling first bite–case study with grilled pork meat. IOP Conference Series: Earth and Environmental Science, 854(1), e012022, https://doi.org/https://doi.org/10.1088/1755-1315/854/1/012022

Ilić, J., Djekic, I., Tomasevic, I., Oosterlinck, F. & Berg, M. A. V. D. (2022). Materials properties, oral processing, and sensory analysis of eating meat and meat analogs. *Annual Review of Food Science and Technology*, 13 (1), 193–215, https://doi.org/https://doi.org/10.1146/annurev-food-090821-032332

Ilic, J., Tomasevic, I. & Djekic, I. (2021). Ease of mastication index—Quantification of mastication effort using quality function deployment. *Journal of Texture Studies*, 52(4), 447–460, https://doi.org/https://doi.org/10.1111/jtxs.12621

Ilic, J., Tomasevic, I. & Djekic, I. (2022). Influence of boiling, grilling, and sous-vide on mastication, bolus formation, and dynamic sensory perception of wild boar ham. *Meat Science*, 188, e108805, https://doi.org/https://doi.org/10.1016/j.meatsci.2022.108805

Koç, H., Çakir, E., Vinyard, C., Essick, G., Daubert, C., Drake, M., Osborne, J. & Foegeding, E. (2014). Adaptation of oral processing to the fracture properties of soft solids. *Journal of Texture Studies*, 45(1), 47-61, https://doi.org/https://doi.org/10.1111/jtxs.12051

Matarneh, S. K., England, E. M., Scheffler, T. L. & Gerrard, D. E. (2017). The conversion of muscle to meat. In Lawrie's Meat Science (pp. 159–185). Elsevier.

Mioche, L., Bourdiol, P., Monier, S. & Martin, J.-F. (2002). The relationship between chewing activity and food bolus properties obtained from different meat textures. *Food Quality and Preference*, 13(7–8), 583–588, https://doi.org/https://doi.org/10.1016/S0950-3293(02)00056-3

Prinz, J. & Lucas, P. (1997). Mastication and swallowing: an optimisation model. *Proceedings of the Royal Society B: Biological Sciences*, 264, 1715–1721, https://doi.org/10.1098/rspb.1997.0238

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, 70(3), 435–447, https://doi.org/https://doi.org/10.1016/j.meatsci.2004.06.028

Rajic, S., Simunovic, S., Djordjevic, V., Raseta, M., Tomasevic, I. & Djekic, I. (2022). Quality Multiverse of Beef and Pork Meat in a Single Score. *Foods*, 11(8), e1154, https://doi.org/https://doi.org/10.3390/foods11081154

Režek Jambrak, A., Šimunek, M. & Djekic, I. (2018). Total quality index of ultrasound-treated blueberry and cranberry juices and nectars. *Food Science and Technology International*, 24(5), 434–446, https://doi.org/10.1177/1082013218764962

Rizo, A., Peña, E., Alarcon-Rojo, A. D., Fiszman, S. & Tárrega, A. (2019). Relating texture perception of cooked ham to the bolus evolution in the mouth. *Food Research International*, 118, 4–12, https://doi.org/https://doi.org/10.1016/j.foodres.2018.02.073

Vasilijević, B., Mitić-Ćulafić, D., Djekic, I., Marković, T., Knežević-Vukčević, J., Tomasevic, I., Velebit, B. & Nikolić, B. (2019). Antibacterial effect of *Juniperus communis* and *Satureja montana* essential oils against *Listeria monocytogenes* in vitro and in wine marinated beef. *Food Control*, 100, 247–256, https://doi.org/https://doi.org/10.1016/j.foodcont.2019.01.025

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523(497.5)

ID: 126140425

https://doi.org/10.18485/meattech.2023.64.2.9

Original scientific paper

Nutritional quality of selected Croatian traditional dry-fermented sausages

Ana Vulić[a], Nina Kudumija[a], Tina Lešić[a] and Jelka Pleadin[a]*

[a] Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska Cesta 143, 10 000 Zagreb, Croatia

ARTICLE INFO

Keywords:
Traditional sausages
Physiochemical properties
Fatty acid profile
Mineral content
Households

ABSTRACT

The production of dry-fermented meat products, including dry-fermented sausages, has a long tradition in Croatian households. These sausages differ greatly depending on the production region. The aim of the study was to characterize the best-known types of dry-fermented Croatian sausages from Eastern Croatia, including Kulen, Kulenova Seka and Slavonian sausage. The results uncovered significant differences ($p<0.05$) in chemical properties of the above sausages in terms of not only fat, water and ash, but also mineral (sodium and calcium) content. Fatty acid profiles of the sausages under study did not significantly differ, except for the share of total saturated fatty acids and stearic acid. These findings indicate that different recipes and production processes applied to these fermented pork meat sausages affect their physicochemical properties and mineral content, but not their fatty acid profile.

1. Introduction

The production of dry-fermented meat products has a long tradition in Croatian households. These products are characterized by excellent sensory and good nutritional properties, which make them appealing to consumers. Nowadays, the demand for these types of products is rising globally. Traditional recipes used for the production of traditional dry-fermented sausages greatly vary not only across production regions, but across producing households as well (*Lešić et al.*, 2020). In Croatia, the best-known types of dry-fermented sausages are Kulen (Slavonski and Baranjski), Kulenova Seka, Istrian sausage and Slavonian sausage. Their basic ingredients are pork meat, with or without the addition of beef, as well as fat, which are then mixed with different kinds of spices (salt, black pepper, dried red pepper, etc.). The stuffing is then filled into the intestine, pork appendix for Kulen and small intestine for Kulenova Seka/Slavonian sausage in, and left to dry, ferment and cure (*Kovačević et al.*, 2010; *Kovačević*, 2014). Both the ingredients and the chosen production processes are responsible for the differences in physiochemical properties of the final products. Slavonski/Baranjski Kulen and Slavonian sausage are traditional dry-fermented sausages labelled as Protected Geographical Indications (PGI) (*Pleadin et al.*, 2021), while Kulenova Seka is prepared in the same manner, but using narrower casings. All of the above are prepared from pork meat and fat tissue, and supplemented with spices, but differ in their ripening duration (*Ministry of Agriculture*, 2018; *Ministry of Agriculture*, 2019). The production of these sausages can also differ between households, each of them tending to introduce their own special process modifications.

***Corresponding author:** Ana Vulić, *vulic@veinst.hr*

Paper received July 24th 2023. Paper accepted May 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

The study aimed to characterize the nutritional quality of the selected Croatian traditional dry-fermented sausages Kulen, Kulenova Seka and Slavonian sausage, collected from different households situated in Eastern Croatia.

2. Materials and methods

2.1. Dry-fermented sausage samples

Dry-fermented sausages Kulen (n=17), Kulenova Seka (n=17) and Slavonian sausage (n=17) were collected from households situated in Eastern Croatia, which embraces Vukovar-Srijem, Osijek-Baranja, Virovitica-Podravina, and Požega-Slavonia districts. The sampling was carried out during spring, summer, and autumn of 2021 and 2022, the sampling season thereby depending on the sausage type. During both sampling years, the samples were taken from the same localities and in more or less equal quantities (around 1.5 kilos depending on the product size). Samples were ground using a Grindomix GM 200 laboratory mill (Retsch, Germany); the water content was analysed immediately, while the rest of the sample was stored in a refrigerator at 4 °C pending analysis.

2.2. Chemicals and standards

Ultrapure water was obtained from a Direct-Q 3 UV device (Merck, Darmstadt, Germany). High purity chemicals were obtained from Honeywell (Charlotte, NC, USA) and p.a. chemicals from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure nitric acid was obtained from Merck (Darmstadt, Germany). Standard solution of fatty acid methyl esters (FAME) was prepared by dissolving 100 mg of standard SupelcoTM 37 Component FAME Mix (Pennsylvania, USA) in 10 mL of hexane. For the determination of each mineral, the 1,000 µg/mL in 5% nitric acid standard solution (Agilent Technologies, USA) was used for calibration.

2.3. Determination of chemical properties

Compositional analysis was performed using standard and validated internal analytical methods. Determination of water (*ISO 1442*, 1997) and ash (*ISO 936*, 1998) content was performed using gravimetric methods, which made use of a thermostat (UF75 plus, Memmert, Germany) and muffler burning furnace (Program Controller LV 9/11/P320, Nabertherm, Germany). Crude protein content was determined by the Kjeldahl method (*ISO 937*,1978), which involves block digestion of organic matter (Unit 8 Basic, Foss, Denmark) and titration/distillation (Vapodest 50s, Gerhardt, Germany). The Soxhlet method (*ISO 1443*,1973) was employed for crude fat determination (Soxtherm 2000, Gerhardt, Germany). Sugar content was determined by means of enzymatic method using a commercial enzyme kite (Sucrose/D-glucose/D-fructose, R-Biopharm, Germany), while the content of carbohydrates was calculated.

2.4. Determination of minerals

Approximately 0.2 g of each sample was submitted to microwave (Ethos easy, Milestone, Italy) acidic digestion supported by hydrogen peroxide (7 mL of 60 % nitric acid and 3 mL of hydrogen peroxide). The digested samples were transferred into volumetric flasks and diluted with ultrapure water. Sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe) were analysed by means of flame atomic absorption spectroscopy (200 Series A4 equipped with SPS 4 Autosampler, Agilent Technologies, USA) with wavelengths set at ʎ=589.0 nm for Na, ʎ=422.7 nm for Ca, ʎ=766.5 nm for K, ʎ=285.2 nm for Mg, ʎ=324.8 nm for Cu, ʎ=213.9 nm for Zn and ʎ=248.3 nm for Fe. A HC coded lamp specific for each mineral (Agilent Technologies, SAD) was used.

2.5. Fatty acid methyl esters (FAME) analysis

Extracted fat was used for fatty acid methyl esters preparation according to *ISO 12966-2* (2015) with some modifications, as described by *Vulić et al.* (2021). Briefly, the extracted fat was dissolved in isooctane, following which a methanolic transesterification was performed. Afterwards, saturated sodium chloride solution was added. After the separation of layers, the upper isooctane layer was transferred into another tube and anhydrous sodium hydrogen sulphate was added. The aliquot of each sample was filtered through a PTFE filter (0.2 µm pore size) into vials. Methyl esters of fatty acids were analysed by GC-FID (gas chromatography with flame ionisation detector) method using a 7890 A gas chromatograph (Agilent Technologies, USA) equipped with a DB-23 capillary column (60 m length, internal capillary diameter 0.25 mm and stationary phase thickness 0.20 µm (Agilent Technologies, USA). The carrier gas was helium. A split/splitless injector was

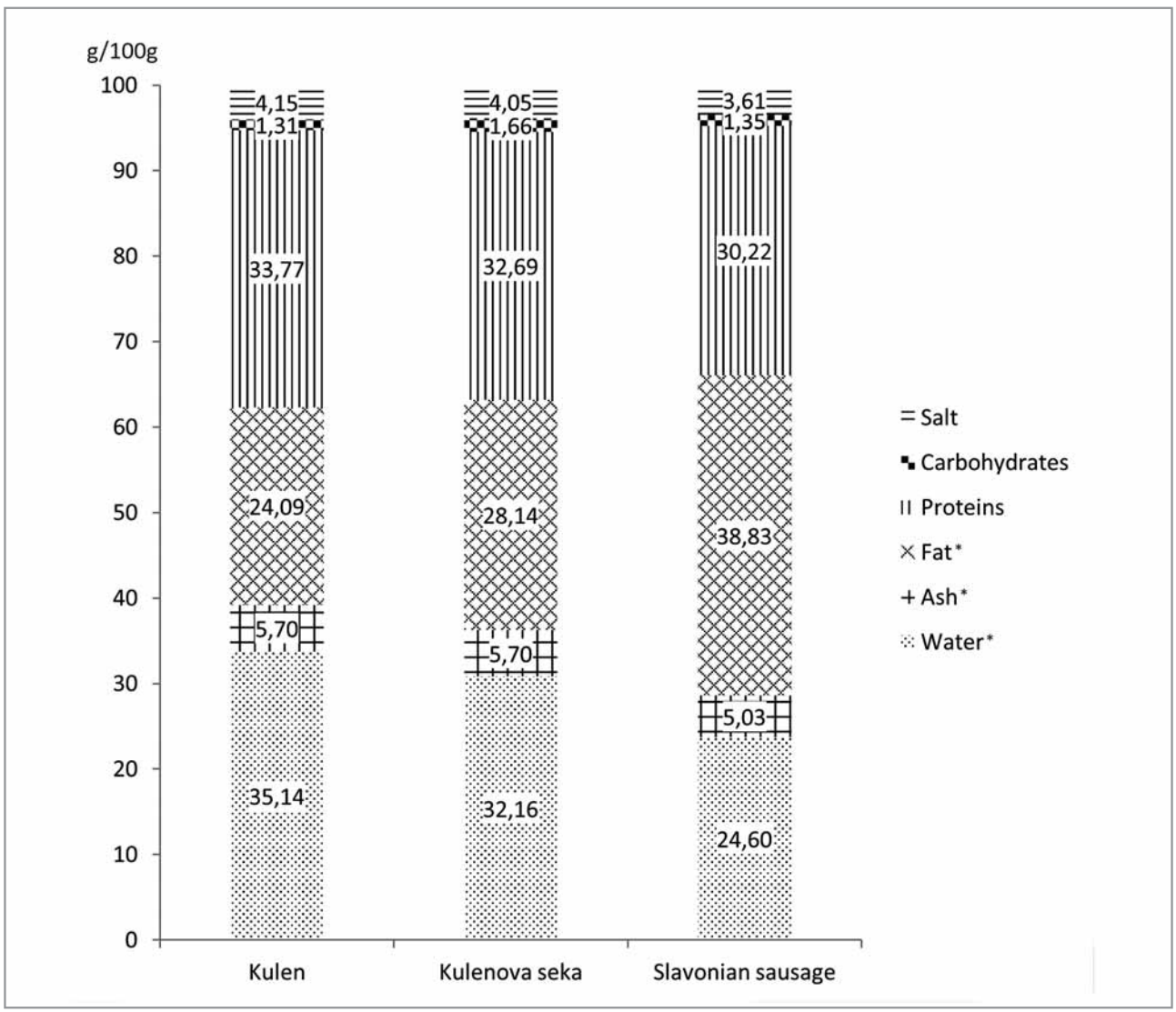
used to inject one microliter of each sample in split ratio of 1:50. FAME identification was performed by comparing the sample FAME retention time to standard solution mixture FAME. Verification of the method was described earlier by *Pleadin et al.* (2014). The results were expressed as the percentage (%) of a given fatty acid in the total fatty acid share.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013, Sankt Ingbert, Germany). The differences between the sample groups were established using the analysis of variance (ANOVA) at 95% significance level ($p<0.05$).

3. Results and discussion

The results of the analysis of chemical properties of traditional dry-fermented sausages carried out in this study are presented in Figure 1. Earlier studies, conducted on dry-fermented sausages from different Croatian regions, have shown that chemical composition of this type of sausages varies greatly depending on the recipes and production processes, which are region-specific (*Lešić et al.*, 2020). This study was focused on dry-fermented sausages from the Eastern Croatia, where recipes and production process used are fairly similar. The results of compositional analysis of Kulen, Kulenova Seka and Slavonian sausage showed significant differences in water, ash and fat content, and no difference in protein, carbohydrate and salt content ($p<0.05$).



*statistical significant difference ($p<0.05$)

Figure 1. The average water, ash, fat, protein, carbohydrate and salt content (g/100 g ± SD) found in Kulen, Kulenova Seka and Slavonian sausage

Water content is a parameter that decreases over the production period, especially during drying, and is important from the food spoilage aspect. The lowest water content determined in the tested sausages was that of Slavonian sausage (24.60 g/100g). Product drying and decrease in water content consequently increase ash, protein and fat content. As compared to Kulen and Kulenova Seka, Slavonian sausage had the highest fat content (38.83 g/100 g as compared to 24.09 and 28.14 g/100 g, respectively). Kulen, as mentioned earlier, was the first Croatian dry-fermented sausage designated as protected by geographic indication (PGI), so that its composition in regard to fat, protein and water content is strictly specified. Among the tested sausages, Kulen had the highest protein content (33.77 g/100 g), which is in accordance with the specification and studies published earlier (*Kovačević et al*., 2014; *Bogdanović et al*., 2016). The low amount of carbohydrates determined in the tested sausages could be attributed to the added sugars, which are sometimes used as fermentation enhancers. Salt is an important ingredient of dry-fermented sausages as it enhances organoleptic properties, but also preserves the product from microbiological spoilage. Salt content did not significantly differ among the tested sausages and was around 4.0 g/100 g. As compared to another group of Croatian traditional meat products — dry-cured meat products — these products can be classified as less salty (*Bogdanović et al*., 2016).

The content of sodium, calcium, potassium, magnesium, copper, zinc and iron determined in Croatian dry-fermented sausages is presented in Table 1. A significant difference between sausages under study in regard to the tested minerals was found only for sodium and calcium content. The highest amount of sodium was found in Kulen due to it having the highest salt content. Similarly, the lowest sodium content was found in Slavonian sausage, the salt content of which was the lowest. Differences in sodium content can be linked to the amount of salt added to the stuffing according to different recipes applied to each type of the tested sausages. Calcium content also varied significantly between the tested sausages, with the highest amount determined in Kulen (377.22 mg/kg) and the lowest in Kulenova Seka (281.11 mg/kg). The main source of calcium in these sausages is meat, while spices are added to the stuffing in small amounts. The recipe and the specification of Kulen and Kulenova Seka are the same when it comes to the pork meat category utilised, so that the difference in calcium content is to be attributed solely to the pork origin.

Fatty acid profiles of Kulen, Kulenova Seka and Slavonian sausage are presented in Table 2. The predominating fatty acid in all analysed sausages was oleic acid, with the average values of 42.40 %, 41.71 % and 40.54 % in Kulen, Kulenova Seka and Slavonian sausage, respectively. The highest share of oleic acid is typical of meat (*Dinh et al*., 2021), but can vary across animal species. The only significant differences between the tested sausages were observed for stearic fatty acid (C18:0) and the share of saturated fatty acids on the whole. Fatty acid profile is mainly related to the meat and fat tissue used in sausage production, so that these findings confirm that the same raw materials (i.e., the same pork meat category), were used for the production of the tested sausages.

Table 1. Mineral content (mg/kg) found in dry-fermented sausages from Croatian households

Sausage	Sodium (mg/kg)	Calcium (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)	Iron (mg/kg)
Kulen	16560.97 ± 2479.57*	372.77 ± 142.08*	5673.59 ± 672.28	319.65 ± 55.76	1.63 ± 0.51	42.57 ± 53.68	17.98 ± 5.53
Kulenova Seka	16179.27 ± 2801.34*	281.11 ± 62.47*	5712.30 ± 1490.31	366.97 ± 71.44	1.83 ± 0.41	39.11 ± 8.86	20.80 ± 6.16
Slavonian sausage	14080.21 ± 2775.30*	318.32 ± 65.18*	5582.62 ± 1168.98	364.80 ± 79.61	1.77 ± 0.47	36.06 ± 8.70	19.90 ± 5.17

The results are expressed as the mean value ± standard deviation; *statistical significant difference ($p<0.05$)

Table 2. Fatty acid profile of the selected Croatian dry-fermented sausages

	Kulen	Kulenova Seka	Slavonian sausage
C10:0	0.07±0.03	0.07±0.04	0.07±0.03
C12:0	0.08±0.01	0.08±0.01	0.08±0.01
C14:0	1.31±0.05	1.33±0.16	1.45±0.45
C15:0	0.05±0.02	0.06±0.04	0.07±0.07
C16:0	24.35±0.93	24.38±1.05	25.07±1.30
C16:1n7t	0.39±0.06	0.42±0.08	0.37±0.08
C16:1n7c	2.59±0.32	2.48±0.37	2.57±0.82
C17:0	0.48±0.10	0.54±0.18	0.54±0.17
C17:1	0.28±0.09	0.28±0.10	0.29±.014
C18:0	12.70±1.07*	13.37±1.34*	14.11±1.82*
C18:1n9t	0.15±0.09	0.19±0.14	0.14±0.13
C18:1n9c	42.40±2.56	41.71±1.67	40.54±2.49
C18:1n7	3.17±0.46	3.00±0.29	2.82±0.41
C18:2n6t	0.12±0.05	0.10±0.07	0.14±0.09
C18:2n6c	9.41±2.63	9.53±1.80	9.32±2.58
C18:3n3 (ALA)	0.40±0.17	0.45±0.16	0.44±0.14
C18:4n3	0.05±0.06	0.06±0.05	0.06±0.08
C20:0	0.22±0.02	0.21±0.02	0.22±0.04
C20:1n9	0.96±0.15	0.90±0.14	0.85±0.19
C20:2n6	0.46±0.10	0.45±0.07	0.44±0.13
C20:3n6	0.05±0.05	0.06±0.05	0.06±0.05
C20:4n6	0.21±0.06	0.24±0.06	0.21±0.08
C20:3n3	0.05±0.07	0.07±0.06	0.05±0.06
C23:0	0.07±0.07	ND	ND
SFA	39.30±1.86*	40.06±2.09*	41.63±3.04*
MUFA	49.93±3.32	48.97±1.96	47.64±3.27
PUFA	10.74±2.95	10.92±2.07	10.72±2.85

The results are expressed as the mean value of the % of total fatty acids ± standard deviation; ND – not detected; LOD – limit of detection = 0.05%; SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; *statistical significant difference ($p<0.05$)

4. Conclusion

Traditional dry-fermented sausages produced in Eastern Croatia differ in water, ash and fat contents, which can be attributed to the different production processes, especially ripening duration differences. Fatty acid profiles of Kulen, Kulenova Seka and Slavonian sausage are comparable since fatty acids are fat constituents, and the same pork meat categories and fat tissue were used in the production. The differences in sodium content can be attributed to the amount of salt added to the stuffing, while calcium content depends on the pork meat origin. The nutritional quality of Kulen, Kulenova Seka and Slavonian sausage is constant and in agreement with the product specifications.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was funded by the Croatian Science Foundation within the frame of the project "Mycotoxins in traditional Croatian meat products: molecular identification of mycotoxin-producing moulds and consumer exposure assessment" (No. IP-2018-01-9017).

References

Bogdanović, T., Pleadin, J., Vahčić, N. & Petričević, S. (2016). Chemical and sensorial properties of fermented meat products. In Zdolec, N. (Ed), *Fermented Meat Products: Health Aspects,* (pp. 359–388), CRC Press.

Dinh, T. T., To, K. V. & Schilling, M. W. (2021). Fatty Acid Composition of Meat Animals as Flavor Precursors. *Meat and Muscle Biology* 5(1), 34, 1–16, https://doi.org/10.22175/mmb.12251

ISO Standard 1442:1997, (1997). Meat and meat products — Determination of moisture content. International Organization for Standardization: Genève, Switzerland, 1997.

ISO Standard 936:1998, (1998). Meat and meat products — Determination of total ash. International Organization for Standardization: Genève, Switzerland, 1998.

ISO Standard 1443:1973, (1973). Meat and meat products — Determination of total fat content. International Organization for Standardization: Genève, Switzerland, 1973.

ISO Standard 937:1978, (1978). Meat and meat products — Determination of nitrogen content. International Organization for Standardization: Genève, Switzerland, 1978.

ISO Standard 12966-2:2015, (2015). Animal and vegetables fats and oils — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids. International Organization for Standardization: Genève, Switzerland, 2015.

Kovačević, D., Mastanjević, K., Šubarić, D., Jerković, I. & Marijanović, Z. (2010). Physico-chemical, colour and textural properties of Croatian traditional dry sausage (Slavonian Kulen). *Meso*, 12, 270–275.

Kovačević, D. (2014). Technology of Kulen and Other Fermented Sausages; Faculty of Food Technology, University of J. J. Strossmayer: Osijek, Croatia.

Lešić, T., Vahčić, N., Kos, I., Zadravec, M., Sinčić Pulić, B., Bogdanović, T., Petričević, S., Listeš, E., Škrivanko, M. & Pleadin, J. (2020). Characterization of Traditional Croatian Household-Produced Dry-Fermented Sausages. *Foods*, 9, 990, https://doi.org/10.3390/foods9080990

Ministry of Agriculture, (2018). Regulation on Meat Products. Official Gazette 62/18. Available online: https://narodnenovine.nn.hr/clanci/sluzbeni/2018_07_62_1292.htm

Ministry of Agriculture, (2019). *Slavonian Sausage–Product Specification*; Ministry of Agriculture: Osijek, Croatia, 2019.

Pleadin, J., Krešić, G., Barbir, T., Petrović, M., Milinović, I. & Kovačević, D. (2014). Changes in basic nutrition and fatty acid composition during production of „Slavonski kulen". *Meso,* 16, 487–492.

Pleadin, J., Lešić, T., Kos, I., Krešić, G., Kudumija, N., Dergestin Bačun, L., Vulić, A., Dujić, T. & Vahčić, N. (2021). Sukladnost fizikalno-kemijskih svojstava sa specifikacijama zaštićenih hrvatskih autohtonih mesnih proizvoda. *Meso*, 23(4), 336–344.

Vulić, A., Kudumija, N., Lešić, T., Tanković, S., Jelušić, V., Frizbegović, J. & Pleadin, J. (2021). Chemical composition and fatty acid profile of Alpine and Saanen goat milk from Bosnia and Herzegovina. *Veterinarska stanica*, 52(1), 0-013-21, https://doi.org/10.46419/vs.52.1.12

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.052
665.528

ID: 126142473

https://doi.org/10.18485/meattech.2023.64.2.10

Review paper

Essential oils as emerging ingredients in processing of minced meat products

Branislav Šojić[a], Branimir Pavlić[a], Milo Mujović[a,b], Predrag Ikonić[c] and Snežana Škaljac[a]

[a] *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[b] *GOMBIT d.o.o. Kralja Petra I 194c, Inđija, Serbia*
[c] *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Essential oil
Supercritical fluid extracts
Natural antioxidants
Natural antimicrobials
Minced meat

ABSTRACT

Essential oils (EOs) are volatile complex molecules of different aromatic and medicinal herbs which contain organic compounds (e.g., phenolics, aldehydes, terpenoids, carotenoids) with strong antioxidative and antimicrobial capacity. EOs can be isolated using different methods, including conventional hydrodistillation (HD) and non-conventional supercritical fluid extraction (SFE). Minced meat products are one of the most dominant products from the meat industry worldwide. These products are susceptible to chemical and microbial deterioration due to their high water, protein, and fat content. In order to enhance the quality and shelf-life of minced meat products, using EOs isolated from different herbs as natural ingredients could be a good solution. Therefore, this review aimed to illustrate the positive aspects of EOs with preservative roles in meat products while, at the same time, underlining the prospective risks influenced by these compounds.

1. Introduction

Burgers, meatballs, and fresh sausages are among the most abundant minced meat products worldwide. These meat products have a substantial nutritive value, extraordinary sensory quality, high availability, and relatively low cost (*Salter*, 2018). Minced meat products are manufactured by grinding and mixing meat (e.g., pork, beef, poultry) and fatty tissue with table salt, spices, or spice mixtures (*Regulation RS 50*, 2019). Also, it should be noted that minced meat products in Serbia are manufactured without the usage of preservatives and synthetic antioxidants, or any thermal treatments. Hence, these products are very susceptible to chemical and microbial deterioration, which leads to a relatively short shelf-life of final products (*Schilling et al*., 2018; *Bantawa et al*., 2018). Lipid and protein oxidation are the chief reasons for chemical deterioration and reduced shelf-life for minced meat products (*Šojić et al*., 2014; *Domínguez et al*., 2019; *Lorenzo et al*., 2018). In order to enhance the quality and safety of different types of fresh or processed meat products, different natural ingredients can be used (*Lorenzo et al*., 2018; *Munekata et al*., 2020). Regarding their recognised levels of phenolics, terpenoids, carotenoids, and other bioactive compounds with significant antioxidative and antimicrobial potential, essential oils (EOs) isolated from different herbs could be applied as natural ingredients and quality enhancers for minced meat products (*Šuput et al*., 2012; *da Silveira et al*., 2014; *Kocić-Tanackov et al*., 2017; *Tomović et al*., 2017; *Araujo et al*., 2018; *Falowo et al*., 2019; *Danilović et al*., 2021; *Pateiro et al*., 2021; *Šojić et al*., 2023a).

***Corresponding author:** Branislav Šojić, *sojic@tf.uns.ac.rs*

Paper received May 26th 2023. Paper accepted Jun 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2.1. Recovery and characterization of EOs

EOs are volatile complex molecules of aromatic plants obtained by hydrodistillation methods or by cold pressing from citrus fruit peel. Terpenoids, aldehydes, ketones, esters, alcohol, and acids are the main constituents of EOs (85%), while trace elements constitute the rest (15%). The chemical profile of EOs is related to climate, geographical origin, development stage, plant cultivar, post-harvest processing and methods used for EO extraction (*Oliveira et al*., 2006; *Pavlić et al*., 2015).

Terpenoids (monoterpenes, sesquiterpenes, and diterpenes) are the principal organic compounds contained in EOs. Carvacrol, linalool, thymol and menthol, as the main terpenoids in EOs, manifest their antioxidative and antimicrobial activities owing to an aromatic structure with extremely potent functional groups. Thymol and carvacrol, as the most frequently phenolic-terpenoids, possess important radical scavenging activity towards hydroxyl radicals. The antibacterial potential of EOs depends on their chemical shape. It is well-known that terpenoids, thymol and carvacrol change cell-membrane permeability, decreasing synthesis of adenosine triphosphate and, lastly, decreasing the viability of microbial cells (Šojić et al., 2023b).

Moreover, terpenoids can affect the aroma and flavour of beverages (e.g., from juniper in gin) and of food aromas. Also, EOs and their constituents are considered as GRAS compounds (generally recognized as safe) in the US, and are indicated as flavouring agents by their food associations, according to the US Food and Drug Administration. Also, they can be applied as quality enhancers in food packaging in the EU. The separation methods for EOs might affect their chemical profile, so finding the optimum extraction methods to deliver enhanced quality and satisfactory yields is crucial (*Becerril et al*., 2020).

Hydrodistillation (HD) is a simple method used to extract pure EO comprised only of volatile compounds. HD is based on interactions among dry herbs, boiling water and steam (*Šojić et al*., 2023b). The vapor given off is additionally chilled, and EO and water are generally collected as a two-phase liquid system, which can be separated. HD is considered as a simple and economical method for EOs recovery. However, it is well known that high temperatures during HD allow hydrolysis and thermal degradation of bioactive compounds (e.g., terpenoids, phenolics, carotenoids), which could reduce the quality of EOs. Furthermore, HD is a highly energy-consuming procedure, including heating and cooling down the vapour. These drawbacks were the core causes for an innovative approach in the progress of novel eco-friendly methods which could diminish solvent, processing times and energy used, and enhance the quality of the obtained EOs (*Burger et al*., 2019).

SFE (Supercritical fluid extraction) is an eco-friendly method for recovering lipophilic extracts, including EOs, from different parts of herbs. In comparison with HD, SFE reduces solvent consumption and extraction time, advances selectivity and extraction yield, saves energy, and preserves target compounds (e.g., terpenoids, phenolics, carotenoids) from decomposition generated by high temperature. The main principle of SFE is the usage of supercritical fluids, which leads to better dispersion into plant material pores and quicker release of target compounds. Carbon dioxide (CO_2) has been primarily used as the solvent for SFEs, because its possesses several attributes required for the extraction: inert and GRAS gas, non-flammable, available, economical, and non-explosive in a high-purity state (*Essien et al*., 2020). Our previous studies determined that using SFE with CO_2 (100 bar at 40°C; 350 bar at 40°C) leads to the isolation of high-quality lipid extracts from sage and winter savory with strong preservative effects in ground meat products (*Šojić et al*., 2018; *Šojić et al*., 2019).

SFE has wide-reaching usage, particularly in producing food ingredients (vitamin-rich extracts and aromas, colorants, etc.) and nutraceuticals. The primary limiting factor for SFE usage in the food sector is the equipment cost, which is above $US 1,150,000 for a high-pressure processing plant with two extractors of 500 L (*Prado et al*., 2012).

2.2. Application of EOs in minced meat products

EOs isolated from different herbs (*Ocimum basilicum* L., *Coriandrum sativum* L., *Allium sativum* L., *Salvia officinalis* L., *Origanum vulgare* L., *Satureja hortensis* L., *Satureja montana* L., *Origanum majorana* L. *Laurus nobilis, Satureja montana* L.) were used as natural ingredients with preservative potential in minced meat products processing (Table 1).

Falowo et al. (2019) determined that EO isolated from *Ocimum basilicum* L. and used at the level of 2% and 4% enhanced the color and reduced lipid

oxidation in minced beef subjected to aerobic conditions and stored (4 ± 1°C) for seven days. Also, a group of researchers (*González-Alonso et al*., 2020) examined the impact of EO isolated from *Coriandrum sativum* L. addition (0.02%) on the microbiological profile of minced beef subjected to cold storage. *González-Alonso et al*. (2020) noted that this EO reduced the growth of *Enterobacteriaceae* in fresh meat products and prolonged their shelf-life. The protective effects of diverse antimicrobial compounds in EO isolated from *Allium sativum* L. on the quality and safety of fresh sausages inoculated with *Escherichia coli* were evaluated by *Araújo et al*. (2018). These scientists indicated that this EO successfully decreased the growth of *E. coli*, as one of the main pathogenic bacteria, and conserved the red colour of final products during cold storage.

In the case of *Salvia officinalis* L., it was noted that EO isolated from this aromatic plant efficiently preserved the quality and prolonged the shelf-life of fresh sausages and minced beef subjected to cold storage (*Šojić et al*., 2018; *Danilović et al*., 2021). Moreover, *Origanum vulgare* L. EO showed a similar protective effect and reduced lipid oxidation and microbiological growth in black wildebeest meat (*Shange et al*., 2019). Our previous study determined that EOs obtained from *Origanum majorana* L., *Satureja hortensis* L., and *Satureja montana* L. possessed strong antimicrobial effects in fresh turkey sausages (*Šojić et al*., 2023a). Also, these EOs, alone and in combinations, reduced biogenic amine formation in final products (*Šojić et al*., 2023a). EO obtained from *Satureja montana* L. also prolongs the shelf-life of pre-cooked pork chops (*Jokanović et al*., 2020). Finally, *da Silveira et al*. (2014) determined that EO obtained from *Laurus nobilis* provides a powerful antimicrobial effect in fresh Tuscan sausages subjected to cold storage.

Also, it should be noted that several studies confirmed the better preservative effect for EOs

Table 1. Application of EOs in minced meat products subjected to cold storage

Herb	Type of extraction	Dose of extract	Minced meat product	Effect	Reference
Ocimum basilicum L.	HD	2–6%	Minced beef	RLO	*Falowo et al*. (2019)
Coriandrum sativum L.	HD	0.02%	Minced chicken	DMG	*González-Alonso et al*. (2020)
Allium sativum L.	HD	125 µL/kg	Fresh sausages	DMG	*Araújo et al*. (2018)
Salvia officinalis L.	HD, SFE	0.05–0.1 µL/g	Fresh sausage	RLO, DMG	*Šojić et al*. (2018)
	HD, SFE	0.2–0.6 µL/mL	Minced pork	DMG	*Danilović et al*. (2021)
Origanum vulgare L.	HD	1% (v/v)	Black wildebeest meat	DMG	*Shange et al*. (2019)
Satureja hortensis L.	HD	0.150 µL/g	Fresh turkey sausages	DMG	*Šojić et al*. (2023a)
Satureja montana L.	HD	0.150 µL/g	Fresh turkey sausages	DMG	
Origanum majorana L.	HD	0.150 µL/g	Fresh turkey sausages	DMG	
Laurus nobilis	HD	0.05 g/100 or 0.10 g/100g	Fresh Tuscan sausage	DMG	*da Silveira et al*. (2014)
Satureja montana L.	HD, SFE	0.075–0.150 µL/g	Fresh sausage	RLO, DMG	*Šojić et al*. (2019)
	HD, SFE	0.200 µL/g	Precooked pork chops	RLO	*Jokanović et al*. (2020)

Legend: HD – hydrodistillation; SFE – supercritical fluid extract; RLO – reduction of lipid oxidation; DMG – decrease of microbiological growth

obtained by SFE than EOs obtained by conventional HD. This could be the result of higher selectivity and solubility of supercritical fluids, which promote isolation of coextracted lipids, which possess a solid antioxidative and antimicrobial activity in meat products (*Šojić et al*., 2018; *Šojić et al*., 2019; *Jokanović et al*., 2020).

Although EOs obtained by HD and SFE had a strong preservative potential, there are a few restrictions concerning the usage of these natural compounds in the meat industry. Primarily, it is well known that some herbs and their EOs might not be harmless to humans (e.g., thujones should not be ≥ 0.5 mg/kg). Therefore, in our prior investigation (*Šojić et al*., 2018), we restricted the addition of sage EOs to under 0.15 μL/g in fresh pork sausages. Also, the high levels of EOs, provide atypical sensory properties of final meat products.

Therefore, it is critical to find a balance among effective doses of EOs and the safety and sensory tolerability of the flavoured meat products. Hence, in our earlier investigations (*Šojić et al*., 2018; *Šojić et al*., 2019), we determined the borderline for sensory appropriateness of sage and winter savory EOs (≤0.150 μL/g) in fresh pork sausages.

3. Conclusion

This review reveals that the use of EOs isolated from diverse herbs (*Ocimum basilicum* L., *Coriandrum sativum* L., *Allium sativum* L., *Salvia officinalis* L., *Origanum vulgare* L., *Satureja hortensis* L., *Satureja montana* L., *Origanum majorana* L. *Laurus nobilis, Satureja montana* L.) can prolong the shelf life of minced meat products and enhanced the quality of final products, in some cases. Also, EOs comprise various bioactive compounds with substantial health benefits for humans. HD, as the conventional method for EO recovery, can negatively affect the thermosensitive compounds (e.g., cause decomposition of phenolics, terpenoids, carotenoids) and can have harmful environmental implications. Therefore, the requirements to discover more sustainable extraction methods for EOs have led scientists to advance novel methods allowing "green" extraction, including SFE.

It should be concluded that before adding EOs to meat batters, it is necessary to define the optimum EO doses and estimate the harmfulness of EOs and their bioavailability for the consumers. Finally, applying these extracts in active packaging is advisable and might find huge interest in the future.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The research in this paper was financed by the Science Fund of Republic Serbia: "Novel extracts and bioactive compounds from under-utilized resources for high-value applications" — BioUtilize, No: 7750168.

References

Araujo, M. K., Gumiela, A. M., Bordin, K., Luciano, F. B. & Macedo, R. E. F. (2018). Combination of garlic essential oil, allyl isothiocyanate, and nisin Z as bio-preservatives in fresh sausage. *Meat Science*,143, 177–183, https://doi:10.1016/J.MEATSCI.2018.05.002

Bantawa, K., Rai, K., Subba Limbu, D. & Khanal, H. (2018). Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. *BMC Research Notes*, 11, 1–5, https://doi.org/10.1186/s13104-018-3722-x

Becerril, R., Nerín, C. & Silva, F. (2020). Encapsulation systems for antimicrobial food packaging components: An update. *Molecules*, 25, 1134, https:// doi:10.3390/MOLECULES25051134

Burger, P., Plainfossé, H., Brochet, X., Chemat, F. & Fernandez, X. (2019). Extraction of natural fragrance ingredients: History overview and future trends. *Chemistry & Biodiversity*, 16, e1900424, https://doi:10.1002/CBDV.201900424

da Silveira, S. M., Luciano, F. B., Fronza, N., Cunha Jr, A., Scheuermann, G. N. & Vieira, C. R. W. (2014). Chemical composition and antibacterial activity of *Laurus nobilis* essential oil towards foodborne pathogens and its application in fresh Tuscan sausage stored at 7°C. *LWT-Food Science and Technology*, 59, 86–93, https://doi.org/10.1016/j.lwt.2014.05.032

Danilović, B., Đorđević, N., Milićević, B., Šojić, B., Pavlić, B., Tomović, V. & Savić, D. (2021). Application of sage herbal dust essential oils and supercritical fluid extract for the growth control of *Escherichia coli* in minced pork during storage. *LWT*, 141, 110935, https://doi.org/10.1016/j.lwt.2021.110935

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W. & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, 8, 429.

Essien, S. O., Young, B. & Baroutian, S. (2020). Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials. *Trends in Food Science & Technology*, 97, 156–169,https://doi:10.1016/j.tifs.2020.01.014

Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Afolayan, A. J. & Muchenje, V. (2019). Phytochemical constituents and antioxidant activity of sweet basil (*Ocimum basilicum* L.) essential oil on ground beef from boran and nguni cattle. *International Journal of Food Science*, 2019, 2628747, https://doi.org/10.1155/2019/2628747

González-Alonso, V., Cappelletti, M., Bertolini, F. M., Lomolino, G., Zambon, A. & Spilimbergo, S. (2020). Research Note: Microbial inactivation of raw chicken meat by supercritical carbon dioxide treatment alone and in combination with fresh culinary herbs. *Poultry Science*, 99, 536–545, https://doi.org/10.3382/ps/pez563

Jokanović, M., Ivić, M., Škaljac, S., Tomović, V., Pavlić, B., Šojić, B., Zeković, Z., Peulić, T. & Ikonić, P. (2020). Essential oil and supercritical extracts of winter savory (*Satureja montana* L.) as antioxidants in precooked pork chops during chilled storage. *LWT*, 134, 110260.

Kocić-Tanackov, S., Dimić, G., Mojović, L., Gvozdanović-Varga, J., Djukić-Vuković, A., Tomović, V. & Pejin, J. (2017). Antifungal activity of the onion (*Allium cepa* L.) essential oil against Aspergillus, Fusarium and Penicillium species isolated from food. *Journal of Food Processing and Preservation*, 41, e13050, https://doi.org/10.1111/jfpp.13050

Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B. & Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, 106, 1095–1104.

Munekata, P. E. S., Rocchetti, G., Pateiro, M., Lucini, L., Domínguez, R. & Lorenzo, J. M. (2020). Addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes: An overview. *Current Opinion in Food Science*, 31, 81–87.

Oliveira, W. P., Bott, R. F. & Souza, C. R. F. (2006). Manufacture of standardized dried extracts from medicinal Brazilian plants. *Drying Technology*, 24, 523–533, https://doi:10.1080/07373930600612073

Pateiro, M., Munekata, P. E., Sant'Ana, A. S., Domínguez, R., Rodríguez-Lázaro, D. & Lorenzo, J. M. (2021). Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. *International Journal of Food Microbiology*, 337, 108966.

Pavlić, B., Vidović, S., Vladić, J., Radosavljević, R. & Zeković, Z. (2015). Isolation of coriander (*Coriandrum sativum* L.) essential oil by green extractions versus traditional techniques. *The Journal of Supercritical Fluids*, 99, 23–28, https://doi.org/10.1016/j.supflu.2015.01.029

Prado, J. M., Dalmolin, I., Carareto, N. D. D., Basso, R. C., Meirelles, A. J. A., Oliveira, J. V., Batista, E. A. C. & Meireles, M. A. A. (2012). Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economic evaluation. *Journal of Food Engineering* , 109, 249–257, https://doi:10.1016/J.JFOODENG.2011.10.007

Regulation on the Quality of Ground Meat, Meat Preparations and Meat Products. UNEP Law and Environment assistance platform. Available online: https://leap.unep.org/countries/rs/national-legislation/regulation-quality-ground-meat-meat-preparations-and-meat

Salter, A. M. (2018). The effects of meat consumption on global health. *Revue Scientifique et Technique* (International Office of Epizootics), 37, 47–55, https://doi: 10.20506/rst.37.1.2739

Schilling, M. W., Pham, A. J., Williams, J. B., Xiong, Y. L., Dhowlaghar, N., Tolentino, A. C. & Kin, S. (2018). Changes in the physiochemical, microbial, and sensory characteristics of fresh pork sausage containing rosemary and green tea extracts during retail display. *Meat Science*, 143, 199–209, https://doi.org/10.1016/j.meatsci.2018.05.009

Shange, N., Makasi, T., Gouws, P. & Hoffman, L. C. (2019). Preservation of previously frozen black wildebeest meat (*Connochaetes gnou*) using oregano (*Oreganum vulgare*) essential oil. *Meat Science*, 148, 88–95, https://doi:10.1016/J.MEATSCI.2018.10.012

Šojić, B. V., Petrović, L. S., Mandić, A. I., Sedej, I. J., Džinić, N. R., Tomović, V. M. & Ikonić, P. M. (2014). Lipid oxidative changes in traditional dry fermented sausage *Petrovská klobása* during storage. *Hemijska industrija*, 68, 27–34.

Šojić, B., Ikonić, P., Kocić-Tanackov, S., Peulić, T., Teslić, N., Županjac, M. & Pavlić, B. (2023a). Antibacterial activity of selected essential oils against foodborne pathogens and their Application in fresh turkey sausages. *Antibiotics*, 12, 182, https://doi.org/10.3390/antibiotics12010182

Šojić, B., Milošević, S., Savanović, D., Zeković, Z., Tomović, V. & Pavlić, B. (2023b). Isolation, bioactive potential, and application of essential oils and Terpenoid-rich extracts as effective antioxidant and antimicrobial agents in meat and meat products. *Molecules*, 28, 2293, https://doi.org/10.3390/molecules28052293

Šojić, B., Pavlić, B., Tomović, V., Ikonić, P., Zeković, Z., Kocić-Tanackov, S. & Ivić, M. (2019). Essential oil versus supercritical fluid extracts of winter savory (*Satureja montana* L.) –Assessment of the oxidative, microbiological and sensory quality of fresh pork sausages. *Food Chemistry*, 287, 280–286, https://doi.org/10.1016/j.foodchem.2018.12.137

Šojić, B., Pavlić, B., Tomović, V., Ikonić, P., Zeković, Z., Kocić-Tanackov, S., Đurović, S., Škaljac, S., Jokanović, M. & Ivić, M. (2019). Essential oil versus supercritical fluid extracts of winter savory (*Satureja montana* L.) — Assessment of the oxidative, microbiological and sensory quality of fresh pork sausages. *Food Chemistry*, 287, 280–286, https://doi:10.1016/j.foodchem.2018.12.137

Šojić, B., Pavlić, B., Zeković, Z., Tomović, V., Ikonić, P., Kocić-Tanackov, S. & Džinić, N. (2018). The effect of essential oil and extract from sage (*Salvia officinalis* L.) herbal dust (food industry by-product) on the oxidative and microbiological stability of fresh pork sausages. *LWT*, 89, 749–755, https://doi.org/10.1016/j.lwt.2017.11.055

Šojić, B., Pavlić, B., Zeković, Z., Tomović, V., Ikonić, P., Kocić-Tanackov, S. & Džinić, N. (2018). The effect of essential oil and extract from sage (Salvia officinalis L.) herbal dust (food industry by-product) on the oxidative and microbiological stability of fresh pork sausages. *LWT*, 89, 749–755, https://doi:10.1016/j.lwt.2017.11.055

Šuput, D., Petrović, L., Šojić, B., Savatić, S., Lazić, V. & Krkić, N. (2012). Application of chitosan coating with oregano essential oil on dry fermented sausage. *Journal of Food and Nutrition Research*, 51, 60–68.

Tomović, V., Jokanović, M., Šojić, B., Škaljac, S. & Ivić, M. (2017). Plants as natural antioxidants for meat products. In IOP conference series: Earth and Environmental Science (Vol. 85, No. 1, p. 012030). IOP Publishing.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523(497.11)

ID: 126472457

https://doi.org/10.18485/meattech.2023.64.2.11

Original scientific paper

Effect of commercial starter culture on physicochemical properties and biogenic amine formation in traditional dry-fermented beef sausage

Predrag Ikonić[a], Tatjana Peulić[a], Marija Jokanović[b], Jovana Delić[a], Snežana Škaljac[b], Branislav Šojić[b] and Ljubiša Šarić[a]

[a] *University of Novi Sad, Institute for Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[b] *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Sjenički sudžuk
Dry-fermented sausage
Starter culture
Biogenic amines

ABSTRACT

The present work aimed to investigate the effect of selected commercial starter culture on the physicochemical characteristics and formation and accumulation of biogenic amines (BAs) in dry-fermented beef sausage (of *Sjenički sudžuk* type) processed in controlled conditions during the summer production season. The results indicated a more intensive decrease in pH value in starter inoculated sausages (SC) compared to control (CO) ones, amounting 4.89 and 5.05 after 40 days of ripening, respectively. On the contrary, water activity (a_w) was found to be higher ($P < 0.05$) in SC sausages at the end of ripening. Two out of six analysed BAs (tryptamine, phenylethylamine) were not detected at any stage of the processing, while tyramine was the predominant amine in final products (CO – 101 mg/kg; SC – 123 mg/kg), regardless of the starter culture inoculation. Putrescine was the second most common amine in SC sausages (111 mg/kg), while cadaverine (58.1 mg/kg) and histamine (78.6 mg/kg) were detected only in CO sausages. This finding largely contributed to the fact that the total BA concentration was significantly higher ($P < 0.05$) in the control sausages (CO) than in inoculated sausages at the end of the production process (314 vs. 235 mg/kg). Based on the obtained results, it can be concluded that using selected starter culture in the production of dry-fermented beef sausage positively reduced the formation and accumulation of total BAs, being especially important when it comes to histamine.

1. Introduction

Traditional dry-fermented sausage, *Sjenički sudžuk*, is a meat product typical for an area around the small town of Sjenica (southwestern Serbia), and the peculiar characteristics of which arise from the combination of environmental/climatic conditions, usage of local ingredients, and manufacturing techniques specific for the Pešter plateau (approximately 1,000 m above sea level). Usually, this sausage is produced during the winter period (air temperature of approximately –1.30°C and relative humidity approximately 81%) following the original procedure and using just beef, sea salt, and spices (*Ikonić et al.*, 2019; 2023; *RHMZ*, 2023). Nevertheless, as a result of the high consumer demand, sudžuk is often produced out of the standard production season, when climate conditions are less proper for this type of manufacturing, i.e. during the summer period. Thus, the quality and safety of the "summer"

***Corresponding author:** Predrag Ikonić, *predrag.ikonic@fins.uns.ac.rs*

Paper received Jun 19th 2023. Paper accepted July 2nd 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

sausages produced are questionable, primarily due to high levels of histamine and total biogenic amines (BAs) (*Ikonić et al.*, 2023; *Ruiz-Capillas & Herrero*, 2019). These anti-nutritional organic bases are very often found in fermented sausages due to the abundance of free amino acids (precursors of BAs) and decarboxylase-positive bacteria and are considered to have multiple adverse effects on human health (*Dominguez et al*., 2016; *Kononiuk & Karwowska*, 2020). Therefore, the summertime production of *Sjenički sudžuk* in small/micro processing facilities should be carried out using decarboxylase-negative starter cultures, as well as appropriate thermo-hygrometric conditions (*Ikonić et al*., 2023).

Accordingly, the aim of this research was to investigate the effect of selected commercial starter culture on the formation and accumulation of BAs in dry-fermented beef sausage (of the *Sjenički sudžuk* type) processed in controlled conditions during the summer period. Physicochemical characteristics were also followed up in order to help with the interpretation of the obtained results.

2. Materials and methods

2.1. Preparation and sampling of sausages

Sausage samples were made in the meat processing pilot plant at the Institute of Food Technology in Novi Sad according to the traditional procedure, with minor modifications, in June 2021. Fresh boneless beef (approximately 85% l ean) was salted using 32 g/kg of a mixture containing sea salt and nitrite salt (1:1) and maintained at 4°C for 7 days (pre-ripening phase). Afterward, salted beef was minced to an end particle size of approx. 4 mm and mixed with the seasonings (raw garlic paste — 4 g/kg, black pepper — 3 g/kg, red sweet paprika powder — 2 g/kg), until a homogeneous composition was achieved. Half of the obtained mixture was inoculated with 20 mg/100 g of starter culture (≥ 1.2×10^{10} cfu/g; M-CULTURE® FA 103 SSP-100, M FOOD GROUP GmbH, DE) containing *Lactobacillus curvatus, Staphylococcus carnosus,* and *Lactobacillus sakei*. Furthermore, prepared mixtures, control (CO), and starter culture inoculated (SC) were stuffed into natural casings (diameter of ~40 mm and a length of ~50 cm), forming a horseshoe shape. Raw sausages were subjected to controlled thermo-hygrometric conditions (Fig. 1) in a smoking, drying, and ripening chamber for 40 days until the required moisture content (<35%) was achieved (*Serbian Regulation*, 2023).

Samples were taken before stuffing (0) and during processing (after 3, 7, 15, 23, and 40 days). Three randomly selected sausages from each batch were taken for physicochemical analyses on each sampling day. The remaining sausages were homogenized, vacuum packed and stored at –20°C for further analyses. Analyses for all samples were carried out in duplicate.

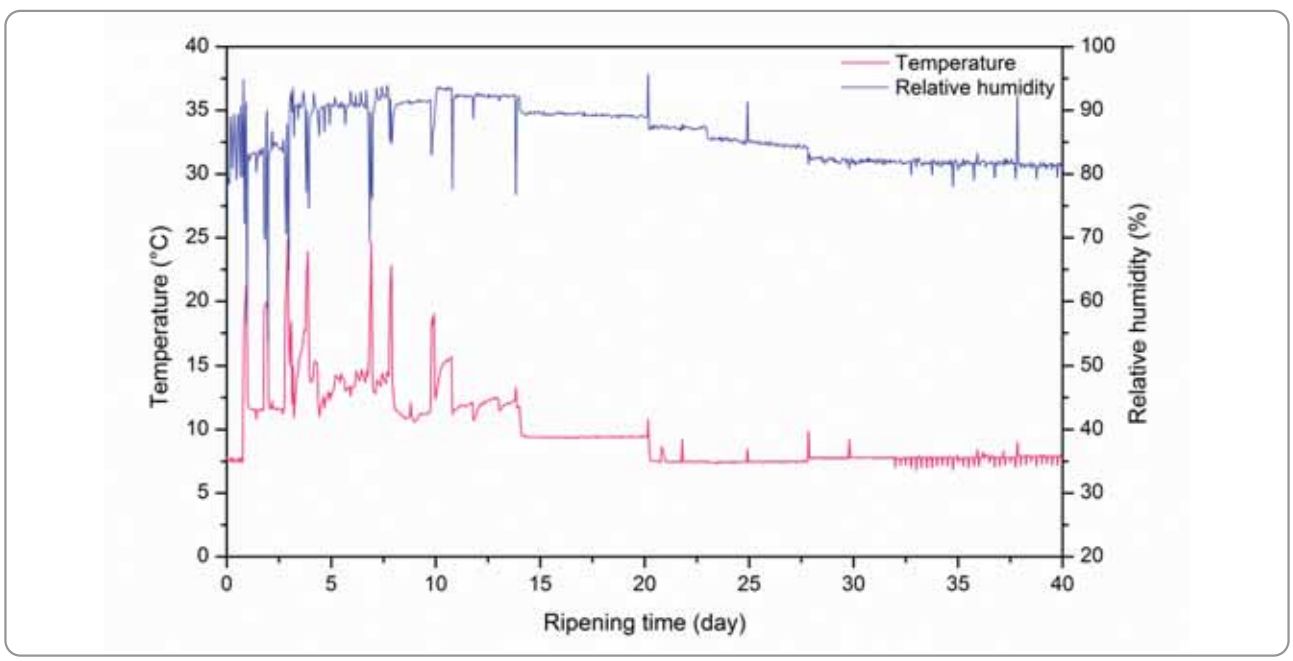


Figure 1. Thermo-hygrometric conditions recorded throughout the manufacturing of dry-fermented beef sausage

2.2. Physicochemical analyses

Sausage pH was measured using a digital pH meter Testo 205 (Testo SE & Co. KGaA, Titisee-Neustadt, DE), equipped with a combined penetration tip with the temperature probe. Water activity (a_w) determination was carried out using a LabSwift-aw measuring instrument (Novasina AG, Lachen, CH).

2.3. Biogenic amines (BAs) analysis

Determination of six dietary BAs, i.e. tryptamine, phenylethylamine, putrescine, cadaverine, histamine, and tyramine, was done according to the procedure described by *Tasić et al.* (2012). BA concentrations are expressed as mg/kg of sample.

2.4. Statistical analyses

Factorial (two-way) analysis of variance (Statistica 14.0, TIBCO Software Inc., Palo Alto, CA, USA) was used to examine the effects of starter culture inoculation and ripening time on the detected variables. Duncan's post-hoc test was run to compare mean values. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of the starter culture on pH and water activity (a_w) values

The effect of starter culture on pH and a_w is presented in Fig. 2. After 7 days of ripening, the SC batch showed a pH value significantly ($P < 0.05$) lower than the one registered in CO, indicating a faster fermentation process and more intensive pH decline in starter inoculated sausage. However, the minimal pH values reached after 15 (SC – 4.81) and 23 days (CO – 4.84) were almost the same ($P > 0.05$). Similar results were reported by *Van Ba et al.* (2016) and *Ren et al.* (2022), who detected faster pH drops and lower pH values in all inoculated sausages. Consequent to proteolytic reactions, i.e., generation of several low molecular weight compounds (peptides, amino acids, ammonia, amines, etc.), final pH values in both batches were significantly higher ($P < 0.05$) than previously detected minimal values (*Ikonić et al.*, 2023; Rocchetti *et al.*, 2021).

Due to the drying process, the a_w value decreased continuously up to the end of the ripening (40 days) in both CO and SC batches, finally amounting 0.86 and 0.89, respectively. Hence, the

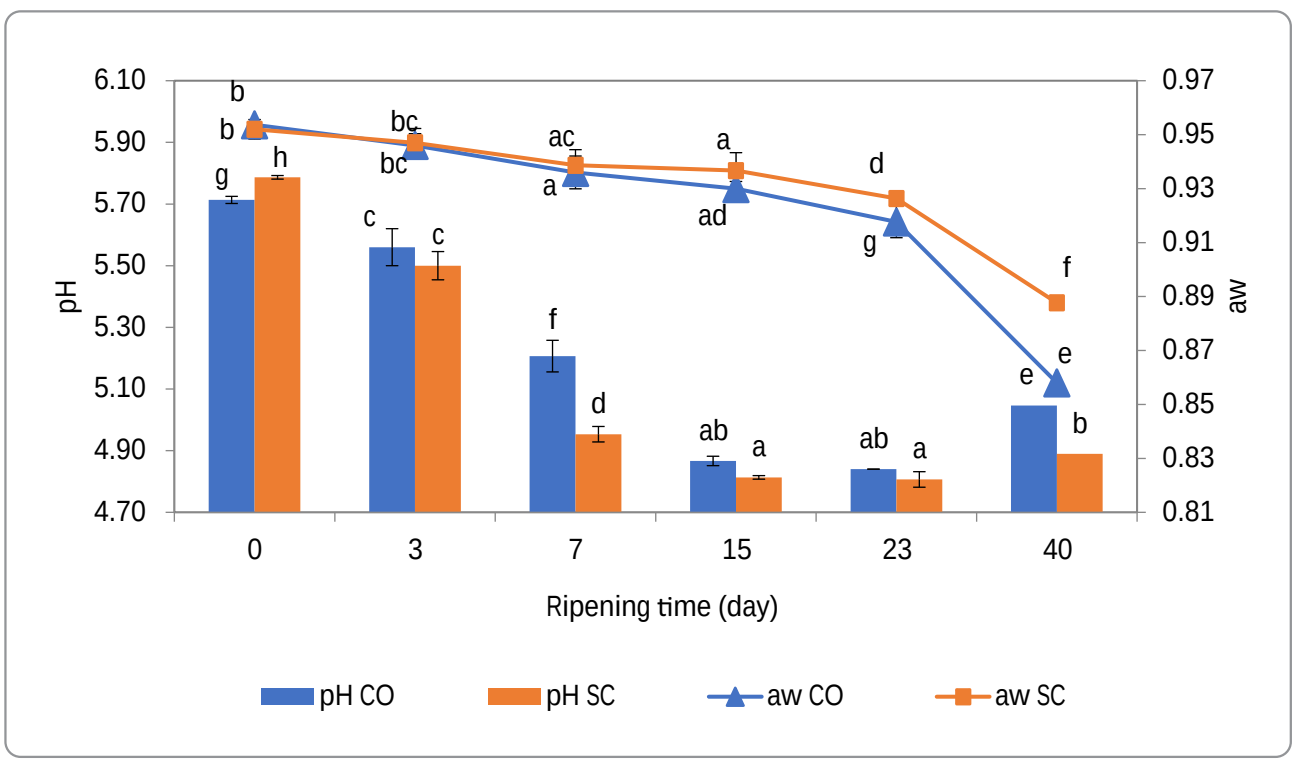


Figure 2. Evolution of pH and water activity (a_w) in control (CO) and starter inoculated (SC) dry-fermented beef sausage during ripening (mean ± standard deviation). Mean values for both physicochemical indicators marked by diverse letters differ significantly ($P < 0.05$).

final a_w in the CO sample was significantly lower than that of the SC sample, which agrees well with *Van Ba et al.* (2016). This finding could be the consequence of the pH in CO sausage being closer to the isoelectric point of actomyosin (≈5.0), thus resulting in lower water holding capacity than in the higher pH SC sausages (*Huff-Lonergan*, 2010).

3.2. Effect of the starter culture on biogenic amine formation

In the present study, four dietary BAs, i.e. putrescine, cadaverine, histamine, and tyramine, were detected in the sausage samples, and their concentrations are presented in Table 1. Tryptamine and phenylethylamine were not detected in any sample. On the contrary, tyramine was the prevailing amine found in both batches starting from day 15 until the end of the ripening period. The ripening time significantly influenced ($P < 0.05$) the concentration of this BA in both batches, as did starter culture inoculation, which resulted in the more intensive accumulation of tyramine in SC samples in the second half of the ripening period. This finding is in accordance with reports of several authors who previously found a highly positive correlation between the concentration of tyramine and the lactic acid bacteria counts in fermented sausages (*Dominguez et al.*, 2016; *Ikonić et al.*, 2023; Šojić et al., 2023).

Putrescine was the second amine found in both batches following the third day of ripening. Its concentration was affected ($P < 0.05$) by both ripening time and starter culture inoculation, resulting in a significant increase until the end of ripening time (when putrescine was 75.6 mg/kg – CO; 111 mg/kg – SC). This result is in concordance with the report of *Dominguez et al*. (2016), who found higher putrescine concentrations in two starter-inoculated batches compared to the non-inoculated control batch. On the other hand, several other authors suggested that starter culture inoculation is beneficial for putrescine reduction (*Ren et al*., 2022; *Van Ba et al.*, 2016). Conversely, in our study, cadaverine was found only in CO sausages at a later ripening stage, i.e. after 23 days. According to others (*Ikonić et al.*, 2023; *Ren et al.*, 2022; *Sun et al.*, 2018), the accumulation of putrescine and cadaverine in meat products could indicate lower processing hygiene and usage of low-quality raw materials, since formation and accumulation of these Bas is related to the growth of contaminant bacteria, such as *Enterobacteriaceae*.

Table 1. Evolution of biogenic amine (BA) concentrations (mg/kg) in control (CO) and starter inoculated (SC) dry-fermented beef sausage during ripening (mean ± standard deviation).

Ripening time (day)	Batch	Putrescine	Cadaverine	Histamine	Tyramine	Total BAs
0	CO	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
	SC	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
3	CO	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
	SC	ND^{a}	ND^{a}	ND^{a}	37.2 ± 7.2^{b}	37.2 ± 7.2^{e}
7	CO	48.8 ± 6.4^{bc}	ND^{a}	68.8 ± 8.3^{b}	54.6 ± 6.7^{f}	172 ± 17^{b}
	SC	43.6 ± 6.7^{c}	ND^{a}	ND^{a}	38.1 ± 4.1^{b}	81.7 ± 8.4^{f}
15	CO	60.6 ± 9.3^{b}	ND^{a}	66.7 ± 4.5^{b}	69.8 ± 12^{c}	197 ± 19^{c}
	SC	80.1 ± 9.1^{d}	ND^{a}	ND^{a}	83.8 ± 14^{d}	164 ± 6.3^{b}
23	CO	59.1 ± 11^{b}	32.2 ± 8.2^{b}	69.3 ± 5.8^{b}	74.2 ± 5.3^{cd}	235 ± 15^{d}
	SC	94.4 ± 7.9^{e}	ND^{a}	ND^{a}	114 ± 7.8^{e}	209 ± 9.1^{c}
40	CO	75.6 ± 5.5^{d}	58.1 ± 6.4^{c}	78.6 ± 1.2^{c}	101 ± 7.9^{g}	314 ± 7.5^{g}
	SC	111 ± 8.5^{f}	ND^{a}	ND^{a}	123 ± 6.3^{e}	235 ± 4.9^{d}
Ripening time		*	*	*	*	*
Batch		*	*	*	*	*
Ripening time x Batch		*	*	*	*	*

$^{a-g}$ Mean values within the same column marked by diverse letters differ significantly ($P < 0.05$); ND – not detected; * $P < 0.05$

Formation and accumulation of histamine, the most dangerous amine for human health, was detected only in control (non-inoculated) sausages after 7 days of ripening, amounting to 68.8 mg/kg. After the following two sampling periods (15 and 23 days), its concentration practically remained constant ($P > 0.05$), but by the end of the ripening period (40 days), it increased significantly to 78.6 mg/kg. Thus, the detected histamine concentration, even in sausages of the CO batch, was lower than its allowable limit (100 mg/kg) (*Dominguez et al.*, 2016; *Ikonić et al.*, 2023).

Regarding the total BA concentration, it ranged from 0 to 314 mg/kg in the CO batch and from 0 to 235 mg/kg in batch SC. It increased significantly ($P < 0.05$) as ripening time elapsed. Also, the influence of added starter culture on the total BA concentration was significant ($P < 0.05$). Nevertheless, both values registered after 40 days of ripening were much lower than the maximum threshold of 1000 mg/kg of total biogenic amines, which is considered dangerous for human health. According to the obtained results, this study confirmed previously published findings regarding the positive effect of starter culture on the reduction of BA formation during the production of fermented sausages (*Dominguez et al.*, 2016; *Ren et al.*, 2022; *Van Ba et al.*, 2016).

4. Conclusion

Putrescine and tyramine were detected in both analysed batches, having significantly higher concentrations in starter-inoculated sausages (SC). Cadaverine and histamine were detected exclusively in CO sausages. However, histamine concentration always remained within the allowable limit (100 mg/kg), above which it is considered dangerous for human health. Also, the total BA concentrations in both batches of sausages (314 mg/kg – CO; 235 mg/kg – SC) were much lower than the maximum threshold of 1000 mg/kg. Thus, the summertime production of dry-fermented beef sausage (of *Sjenički sudžuk* type) in small/micro processing plants is possible if appropriate control and regulation of thermo-hygrometric conditions are applied. Additionally, the results showed that starter culture inoculation is conducive to decreasing the accumulation of BAs, especially histamine.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement number: 451-03-47/2023-01/200222), and by the Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (Project number: 142-451-3149/2022-03).

Acknowledgements: The authors would like to thank the Meat Cracks Balkans d.o.o. Sremska Mitrovica, Serbia, for kindly providing the sample of the starter culture used in this study.

References

Dominguez, R., Munekata, P. E., Agregan, R. & Lorenzo, J. M. (2016). Effect of commercial starter cultures on free amino acid, biogenic amine and free fatty acid contents in dry-cured foal sausage. *LWT*, 71, 47–53.

Huff-Lonergan, E. (2010). Chemistry and Biochemistry of Meat. In F. Toldrá (Ed.), Handbook of Meat Processing (pp. 5–24). Blackwell Publishing Ltd.

Ikonić, P., Jokanović, M., Ćućević, N., Peulić, T., Šarić, Lj., Tomičić, Z., Škaljac, S., Delić, J., Lakićević, B. & Tomašević, I. (2023). Effect of different ripening conditions on amino acids and biogenic amines evolution in *Sjenički sudžuk. Journal of Food Composition and Analysis*, 115, 105009.

Ikonić, P., Jokanović, M., Peulić, T., Ćućević, N., Tomičić, Z., Škaljac, S. & Ivić, M. (2019). Evolution of amino acids and biogenic amines in traditional dry-fermented sausage *Sjenički sudžuk* during processing. Oral presentation at 60th International Meat Industry Conference MEATCON2019, Kopaonik, Serbia, IOP Conf. Series: Earth and Environmental Science 333 (2019) 012021, 1–6.

Kononiuk, A. D. & Karwowska, M. (2020). Comparison of selected parameters related to food safety of fallow deer and beef uncured fermented sausages with freeze-dried acid whey addition. *Meat Science*, 161, 108015.

Ren, H., Deng, Y. & Wang, X. (2022). Effect of a compound starter cultures inoculation on bacterial profile and biogenic amine accumulation in Chinese Sichuan sausages. *Food Science and Human Wellness*, 11(2), 341–348.

RHMZ — Republic Hydrometeorological Service of Serbia. (2022). Meteorology-Climatology. Retrieved from https://www.hidmet.gov.rs/ciril/meteorologija/stanica_sr_sjenica.php. Accessed May 11, 2023

Rocchetti, G., Rebecchi, A., Dallolio, M., Braceschi, G., Domínguez, R., Dallolio, G., Trevisan, M., Lorenzo, J. M. & Lucini, L. (2021). Changes in the chemical and sensory profile of ripened Italian salami following the addition of different microbial starters. *Meat Science*, 180, 108584.

Ruiz-Capillas, C. & Herrero, A. M. (2019). Impact of biogenic amines on food quality and safety. *Foods*, 8(2), 62.

Serbian Regulation. (2019). Rulebook on Quality of Meat Products. *Official Gazette of the Republic of Serbia*, 50.

Šojić, B., Ikonić, P., Kocić-Tanackov, S., Peulić, T., Teslić, N., Županjac, M., Lončarević, I., Zeković, Z., Popović, M., Vidaković, S. & Pavlić, B. (2023). Antibacterial Activity of Selected Essential Oils against Foodborne Pathogens and Their Application in Fresh Turkey Sausages. *Antibiotics*, 12(1), 182.

Sun, Q., Zhao, X., Chen, H., Zhang, C. & Kong, B. (2018). Impact of spice extracts on the formation of biogenic amines and the physicochemical, microbiological and sensory quality of dry sausage. *Food Control*, 92, 190–200.

Tasić T., Ikonić P., Mandić A., Jokanović M., Tomović V., Savatić S. & Petrović Lj. (2012). Biogenic amines content in traditional dry fermented sausage Petrovská klobása as possible indicator of good manufacturing practice. *Food Control*, 23, 107–112.

Van Ba, H., Seo, H. W., Kim, J. H., Cho, S. H., Kim, Y. S., Ham, J. S. & Seong P. N. (2016). The effects of starter culture types on the technological quality, lipid oxidation and biogenic amines in fermented sausages. *LWT*, 74, 191–198.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.52(497.6)

ID: 126481673

https://doi.org/10.18485/meattech.2023.64.2.12

Original scientific paper

Polycyclic aromatic hydrocarbons (PAHs) in Visočka pečenica, a traditional dry-cured meat product from Bosnia and Herzegovina

Munevera Begić[a], Selma Čorbo[a], Jasna Đinović-Stojanović[b] and Saša Janković[b]*

[a] *University of Sarajevo, Faculty of Agriculture and Food Science, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina*
[b] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Polycyclic aromatic hydrocarbons
Traditional meat product
Production technology

ABSTRACT

Visočka pečenica is a traditional dry-cured beef meat product from Bosnia and Herzegovina, and is protected by a geographical indication label at the national level. In this research, the content of 16 EU priority polycyclic aromatic hydrocarbons (PAHs) in Visočka pečenica from traditional and industrial production was examined. The content of PAH compounds has not been analysed in Visočka pečenica to date. Determination and quantification of PAHs in Visočka pečenica were performed by a GC/MS method. The content of all individual PAH compounds was higher in *Musculus longissimus dorsi* than in beef round in both production methods, and their contents increased with the longer smoking process. In addition, higher contents of PAHs were found in Visočka pečenica from traditional than in industrial production, with some exceptions. In Visočka pečenica from traditional production, higher contents of individual PAH compounds and their sums were determined when they were smoked at a higher shelf level in the smokehouse, both halfway through and at the end of the smoking process. Contrarily, in Visočka pečenica from industrial production, PAH contents were higher at the lower rather than the higher shelf level, with some exceptions. The most common PAH compound in the Visočka pečenica from both production methods was chrysene (CHR) (3.00–14.08 µg/kg).

1. Introduction

Visočka pečenica is a traditional dry-cured beef meat product from Bosnia and Herzegovina that has been produced in the municipality of Visoko for many years. It is produced from the highest quality parts of beef carcasses, dry salted only with kitchen salt and cold smoked and dried. The product is characterized by the special production technology characteristic of the aforementioned location, is top quality and is especially valued for its aroma, taste and smoke aroma. Smoking is one of the oldest technologies for preserving meat and meat products and is defined as the process of penetration of meat products by volatiles resulting from thermal destruction of wood (*Andrée et al.,* 2010). Smoking gives special colour, taste and aroma to food, and enhances preservation due to its dehydrating, bactericidal and antioxidant properties (Škaljac *et al.,* 2019; *Roseiro et al.,* 2011; *Puljić et al.,* 2019). However, in addition to the positive effect of the smoking process and a large number of useful compounds that are created by burning wood, harmful components are also creat-

***Corresponding author:** Munevera Begić, *m.begic@ppf.nsa.ba*

Paper received May 31th 2023. Paper accepted Jun 12th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ed, which include polycyclic aromatic hydrocarbons (PAHs). PAHs comprise the largest class of chemical compounds, containing two or more fused aromatic rings made up of carbon and hydrogen atoms, known to be genotoxic agents (*Ciecierska and Obiedzinski,* 2007). About 660 different compounds belong to the PAH group (*Jira et al.,* 2013). Human exposure to PAHs occurs in three ways, i.e., inhalation, dermal contact and consumption of contaminated foods, which accounts for 88–98% of such contamination; in other words, diet is the major source of human exposure to these contaminants (*Tareq et al.,* 2020). Due to the lipophilic and hydrophobic characteristics of PAHs, they tend to accumulate in the food chain (*Bansal and Kim,* 2015). The PAH content in smoked food is determined by various technological factors, such as the species of wood and its humidity, the fat content of the food, the temperature of combustion and oxidation, the method of smoke flow through the chamber and the duration and smoking technique, as well as the humidity and size of the product surface (*García-Falcón and Simal-Gándara,* 2005; *Zachara et al.,* 2017; *Sojinu et al.,* 2019; *Zdolec et al.,* 2019; *Kafouris et al.,* 2020). The European Food Safety Authority (EFSA) identified several PAHs (the content of benzo[a]pyrene (BaP) and the sum of contents of four PAHs, BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)) that would be considered as a reference for the determination of PAHs in food *(EFSA, 2008)*. According to European Commission (EU) regulation (*European Commission*, 2006; 2011; 2014), the maximum permissible content of BaP in meat products is 2 μg/kg, and the sum of the PAH4 content should not exceed 12 μg/kg. The International Agency for Research on Cancer (IARC) classified one PAH of 16 examined PAHs in this study (BaP) as carcinogenic to humans (Group 1), three (cyclopenta[cd]pyrene (CPP), dibenzo[a,l]pyrene (DlP), and dibenz[a,h]anthracene (DhA)) (Group 2A) as probably carcinogenic and nine (BaA, CHR, BbF, 5-methylchrysene (5MC), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), indeno[1,2,3-cd]pyrene (IcP), dibenzo[a,i] pyrene (DiP) and dibenzo[a,i]pyrene (DhP)) as possibly carcinogenic to humans (Group 2B), while three (benzo[ghi]perylene (BgP), dibenzo[a,e]pyrene (DeP) and benzo[c]fluorene (BcF)) were not classifiable according to their carcinogenicity to humans (*IARC,* 2010). The aims of the research were to determine the influence of production technology on the content of PAH compounds in traditional Visočka pečenica. In addition, the research examined the influence of fat content, smoking height and smoking duration on the content of PAH compounds.

2. Materials and methods

2.1. Production and sampling of Visočka pečenica

Experimental production of Visočka pečenica was performed in traditional and industrial conditions. Forty samples were sampled twice for research needs, halfway through and at the end of the smoking process. Accordingly, the total number of analysed samples was 80. Samples were taken from different cuts on the carcass (round — *Musculus gluteobiceps*, *Musculus gluteus medius*, *Musculus semitendinosus* and back musculature — *Musculus longissimus dorsi; MLD*). During the smoking phase, the meat pieces were spread over shelves at two levels. The first level was in relation to the fireplace, at a height of two meters. The height of the second level was four meters. Looking at the phases, the experimental set-up meant that during smoking, five samples of both anatomical regions were placed on both levels of the smokehouse. The set-up of the experiment, from the choice of raw material to the technological processing to the method of sampling, was identical for both productions. In the comparison of traditional and industrial production, the differences were related to the amount of added salt, the length of smoking, and the parameters of the smoking process (temperature and relative humidity inside the smokehouse, outside and the temperature of the stokehole).

2.2. Methods

The content of PAH compounds in the samples of Visočka pečenica was determined by an internal method in the laboratory of the Institute of Meat Hygiene and Technology in Belgrade. Samples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. Determination of 16 EU PAH compounds (BaA, CHR, BbF, BaP, BcL, CPP, 5MC, BjF, BkF, IcP, DhA, BgP, DlP, DeP, DiP, DhP) was performed using gas chromatography with a triple quadrupole mass detector. Briefly, 2.5 g of homogenized sample was weighed into a 50 mL centrifuge tube; 10 mL of acetonitrile were added and the mixture was shaken vigorously for 1 min; after that 1 g NaCl and 4 g $MgSO_4$ were added, with the tube being shaken immediately after addition of the salt. Then each tube was shaken and centrifuged. Superna-

Table 2. Contents (μg/kg) of the examined PAHs in Visočka pečenica

Sample	B*a*A	CHR	B*b*F	B*a*P	B*c*L	CPP	5MC	B*j*F	B*k*F	I*c*P	D*h*A	B*g*P	D*l*P	D*e*P	D*i*P	D*h*P
TRHH	2,47^{a} ^{AxX}±0 ,49	3,00^{a} ^{AxX}±0 ,31	0,80^{a} ^{AxX}±0 ,10	1,36^{a} ^{AxX}±0 ,39	2,25^{a} ^{AxX}±0 ,57	2,10^{a} ^{AxX}±0 ,77	0,13^{a} ^{AxX}±0 ,03	0,35^{a} ^{AxX}±0 ,04	0,65^{a} ^{AxX}±0 ,08	0,94^{a} ^{AxX}±0 ,13	0,16^{a} ^{AxX}±0 ,04	0,82^{a} ^{AxX}±0 ,12	0,20^{a} ^{AxX}±0 ,06	0,13^{aAx} ^{X}±0,01	< 0.1	< 0.1
TMHH	5,42^{b} ^{AyX}±0 ,85	6,14^{b} ^{AyX}±0 ,81	1,56^{b} ^{AyX}±0 ,21	3,04^{b} ^{AyX}±0 ,41	4,80^{b} ^{AyX}±0 ,89	5,30^{b} ^{AyX}±1 ,05	0,28^{b} ^{AyX}±0 ,05	0,66^{b} ^{AyX}±0 ,10	1,16^{b} ^{AyX}±0 ,12	1,78^{b} ^{AyX}±0 ,21	0,33^{b} ^{AyX}±0 ,05	1,54^{b} ^{AyX}±0 ,21	0,41^{b} ^{AyX}±0 ,06	0,23^{bAy} ^{X}±0,06	< 0.1	< 0.1
TRLH	2,95^{a} ^{AxX}±0 ,73	3,77^{a} ^{AxX}±0 ,45	0,94^{a} ^{AxX}±0 ,13	1,65^{a} ^{AxX}±0 ,57	2,33^{a} ^{AxX}±0 ,71	2,54^{a} ^{AxX}±1 ,07	0,16^{a} ^{AxX}±0 ,03	0,40^{a} ^{AxX}±0 ,06	0,76^{a} ^{AxX}±0 ,10	1,13^{a} ^{AxX}±0 ,17	0,18^{a} ^{AxX}±0 ,05	1,02^{a} ^{AxX}±0 ,17	0,23^{a} ^{AxX}±0 ,05	0,13^{aAx} ^{X}±0,03	< 0.1	< 0.1
TMLH	4,43^{b} ^{AyX}±1 ,19	4,89^{b} ^{AyX}±1 ,28	1,27^{b} ^{AyX}±0 ,36	2,48^{b} ^{AyX}±0 ,61	3,99^{b} ^{AyX}±1 ,10	4,51^{b} ^{AyX}±1 ,28	0,25^{b} ^{AyX}±0 ,08	0,55^{b} ^{AyX}±0 ,15	0,96^{b} ^{AyX}±0 ,26	1,42^{b} ^{AyX}±0 ,34	0,25^{b} ^{AyX}±0 ,07	1,24^{b} ^{AxX}±0 ,25	0,32^{b} ^{AyX}±0 ,08	0,16^{bAy} ^{X}±0,04	< 0.1	< 0.1
TRHE	7,82^{a} ^{AxY}±1 ,45	9,20^{a} ^{AxY}±1 ,56	2,20^{a} ^{AxY}±0 ,39	4,25^{a} ^{AxY}±0 ,69	6,96^{a} ^{AxY}±1 ,25	6,93^{a} ^{AxY}±1 ,24	0,42^{a} ^{AxY}±0 ,09	0,94^{a} ^{AxY}±0 ,17	1,71^{a} ^{AxY}±0 ,27	2,62^{a} ^{AxY}±0 ,38	0,45^{a} ^{AxY}±0 ,06	2,25^{a} ^{AxY}±0 ,31	0,57^{a} ^{AxY}±0 ,10	0,32^{aAx} ^{Y}±0,08	< 0.1	< 0.1
TMHE	12,39 ^{bAyY}± 2,12	14,08 ^{bAyY}± 2,19	3,36^{b} ^{AyY}±0 ,57	6,62^{b} ^{AyY}±1 ,17	11,80 ^{bAyY}± 1,66	11,79 ^{bAyY}± 1,92	0,64^{b} ^{AyY}±0 ,09	1,44^{b} ^{AyY}±0 ,24	2,56^{b} ^{AyY}±0 ,47	3,90^{b} ^{AyY}±0 ,65	0,66^{b} ^{AyY}±0 ,08	3,41^{b} ^{AyY}±0 ,70	0,84^{b} ^{AyY}±0 ,16	0,45^{bAy} ^{Y}±0,13	< 0.1	< 0.1
TRLE	6,20^{a} ^{AxY}±0 ,69	7,46^{a} ^{AxY}±0 ,68	1,64^{a} ^{BxY}±0 ,19	3,16^{a} ^{BxY}±0 ,39	5,75^{a} ^{AxY}±0 ,75	4,97^{a} ^{AxY}±0 ,98	0,31^{a} ^{AxY}±0 ,03	0,69^{a} ^{BxY}±0 ,07	1,38^{a} ^{BxY}±0 ,13	1,95^{a} ^{BxY}±0 ,20	0,31^{a} ^{BxY}±0 ,03	1,74^{a} ^{BxY}±0 ,23	0,34^{a} ^{BxY}±0 ,05	0,15^{aBx} ^{Y}±0,04	< 0.1	< 0.1
TMLE	10,97 ^{bAyY}± 3,03	12,06 ^{bAyY}± 3,43	2,64^{b} ^{ByY}±0 ,69	5,03^{b} ^{ByY}±1 ,38	12,27 ^{bAyY}± 3,37	10,78 ^{bAyY}± 3,14	0,55^{b} ^{AyY}±0 ,19	1,13^{b} ^{ByY}±0 ,31	1,94^{b} ^{ByY}±0 ,51	2,74^{b} ^{ByY}±0 ,79	0,49^{b} ^{ByY}±0 ,11	2,20^{b} ^{BxY}±0 ,68	0,58^{b} ^{ByY}±0 ,19	0,32^{bBy} ^{Y}±0,12	< 0.1	< 0.1
IRHH	2,81^{a} ^{AxX}±1 ,00	3,21^{a} ^{AxX}±1 ,09	0,78^{a} ^{AxX}±0 ,28	1,59^{a} ^{AxX}±0 ,57	1,97^{a} ^{AxX}±0 ,82	2,72^{a} ^{AxX}±0 ,99	0,15^{a} ^{AxX}±0 ,06	0,33^{a} ^{AxX}±0 ,12	0,74^{a} ^{AxX}±0 ,25	0,87^{a} ^{AxX}±0 ,37	0,15^{a} ^{AxX}±0 ,06	0,85^{a} ^{AxX}±0 ,26	0,12^{a} ^{AxX}±0 ,07	0,14^{aAx} ^{X}±0,04	< 0.1	< 0.1
IMHH	4,18^{b} ^{AyX}±1 ,47	4,61^{b} ^{AyX}±1 ,50	1,16^{b} ^{AyX}±0 ,38	2,23^{b} ^{AyX}±0 ,70	3,17^{a} ^{AyX}±1 ,24	4,01^{b} ^{AyX}±1 ,43	0,22^{b} ^{AyX}±0 ,08	0,50^{b} ^{AyX}±0 ,17	1,01^{b} ^{AyX}±0 ,30	1,32^{b} ^{AyX}±0 ,44	0,24^{b} ^{AyX}±0 ,09	1,29^{b} ^{AyX}±0 ,32	0,19^{b} ^{AyX}±0 ,07	0,20^{bAy} ^{X}±0,05	< 0.1	< 0.1
IRLH	3,51^{a} ^{AxX}±0 ,64	3,98^{a} ^{AxX}±0 ,66	0,98^{a} ^{AxX}±0 ,17	1,96^{a} ^{AxX}±0 ,30	2,60^{a} ^{AxX}±0 ,62	3,39^{a} ^{AxX}±0 ,68	0,20^{a} ^{AxX}±0 ,04	0,42^{a} ^{AxX}±0 ,07	0,89^{a} ^{AxX}±0 ,12	1,14^{a} ^{AxX}±0 ,18	0,20^{a} ^{AxX}±0 ,04	1,18^{a} ^{AxX}±0 ,15	0,17^{a} ^{AxX}±0 ,04	0,15^{aAx} ^{X}±0,03	< 0.1	< 0.1
IMLH	4,23^{b} ^{AyX}±0 ,84	4,76^{b} ^{AyX}±0 ,92	1,19^{b} ^{AyX}±0 ,25	2,40^{b} ^{AyX}±0 ,54	3,00^{a} ^{AyX}±0 ,65	4,06^{b} ^{AyX}±0 ,79	0,24^{b} ^{AyX}±0 ,06	0,51^{b} ^{AyX}±0 ,11	1,07^{b} ^{AyX}±0 ,23	1,46^{b} ^{AyX}±0 ,39	0,25^{b} ^{AyX}±0 ,07	1,49^{b} ^{AyX}±0 ,46	0,22^{b} ^{AyX}±0 ,07	0,17^{bAx} ^{X}±0,02	< 0.1	< 0.1
IRHE	3,99^{a} ^{AxY}±0 ,76	4,40^{a} ^{AxY}±0 ,75	1,11^{a} ^{AxY}±0 ,19	2,26^{a} ^{AxY}±0 ,36	2,94^{a} ^{AxY}±0 ,90	4,04^{a} ^{AxY}±0 ,90	0,22^{a} ^{AxY}±0 ,04	0,48^{a} ^{AxY}±0 ,08	1,02^{a} ^{AxY}±0 ,14	1,37^{a} ^{AxY}±0 ,20	0,23^{a} ^{AxY}±0 ,03	1,39^{a} ^{AxY}±0 ,19	0,20^{a} ^{AxY}±0 ,04	0,16^{aAx} ^{Y}±0,03	< 0.1	< 0.1
IMHE	6,79^{b} ^{AyY}±1 ,61	7,15^{b} ^{AyY}±1 ,64	1,84^{b} ^{AyY}±0 ,41	3,58^{b} ^{AyY}±0 ,74	5,71^{b} ^{AyY}±1 ,58	6,90^{b} ^{AyY}±1 ,69	0,34^{b} ^{AyY}±0 ,10	0,79^{b} ^{AyY}±0 ,18	1,62^{b} ^{AyY}±0 ,30	2,18^{b} ^{AyY}±0 ,46	0,38^{b} ^{AyY}±0 ,08	2,09^{b} ^{AyY}±0 ,49	0,36^{b} ^{AyY}±0 ,10	0,31^{bAy} ^{Y}±0,08	< 0.1	< 0.1
IRLE	4,36^{a} ^{AxY}±0 ,92	4,64^{a} ^{AxY}±0 ,91	1,17^{a} ^{AxY}±0 ,26	2,45^{a} ^{AxY}±0 ,52	3,65^{a} ^{AxY}±0 ,76	4,68^{a} ^{AxY}±1 ,00	0,22^{a} ^{AxY}±0 ,05	0,50^{a} ^{AxY}±0 ,11	1,08^{a} ^{AxY}±0 ,23	1,53^{a} ^{AxY}±0 ,38	0,25^{a} ^{AxY}±0 ,06	1,49^{a} ^{AxY}±0 ,30	0,24^{a} ^{AxY}±0 ,08	0,19^{aAx} ^{Y}±0,09	< 0.1	< 0.1
IMLE	6,94^{b} ^{AyY}±2 ,43	7,33^{b} ^{AyY}±2 ,43	1,85^{b} ^{AyY}±0 ,64	3,70^{b} ^{AyY}±1 ,29	6,03^{b} ^{AyY}±2 ,35	7,28^{b} ^{AyY}±2 ,68	0,38^{b} ^{AyY}±0 ,12	0,78^{b} ^{AyY}±0 ,26	1,58^{b} ^{AyY}±0 ,50	2,20^{b} ^{AyY}±0 ,78	0,38^{b} ^{AyY}±0 ,11	2,22^{b} ^{AyY}±0 ,88	0,39^{b} ^{AyY}±0 ,16	0,32^{bAx} ^{Y}±0,13	< 0.1	< 0.1

a-b Different lowercase letters in the columns indicate statistically significant differences between samples of "Visočka pečenica" produced from round musculature and MLD at different heights at the halfway through and end of the smoking process; A-B Different capital letters in the columns indicate statistically significant differences between the samples of "Visočka pečenica" at higher and lower smoking heights at the halfway through and end of the smoking process; x-y Different lowercase letters in the columns indicate statistically significant differences between samples of "Visočka pečenica" produced from round musculature and MLD at different lengths of smoking at higher and lower smoking heights; X-Y Different uppercase letters in the columns show statistically significant differences between the "Visočka pečenica" samples at the halfway through and end of the smoking process at higher and lower smoking heights; (T-traditional, I-industrial production; M-MLD, R-round samples; H-higher, L-lower smoking heights; H-halfway through, E-end of the smoking process)

tant was cleaned up by SPE (transferring into a 15 mL centrifuge tube containing 150 mg PSA, 150 mg C18 and 900 mg $MgSO_4$) with the aim of eliminating the possible interfering compounds from the sample extract. After centrifugation, extracts were evaporated under a stream of N_2 at 40°C to dryness and then dissolved in 1 mL of hexane. The extracts were then analysed by GC–MS/MS. A two-factor analysis of variance was used to test the differences between the means and the Tukey test was used as a post hoc test. Statistical analyses were performed using Past software 3.15 *(Hammer et al., 2001)*.

3. Results and discussion

The contents of the analysed PAHs in the samples of Visočka pečenica are shown in Table 2. In the tested samples, chrysene (CHR) was the most abundant of all tested PAH compounds in Visočka pečenica made by both production processes halfway through (4.45 μg/kg-traditional and 4.14 μg/kg-industrial) and at the end of the smoking process (10.70 μg/kg-traditional and 5.88 μg/kg-industrial). The content of all individual PAHs in all samples of Visočka pečenica from both anatomical regions

smoked on both shelf levels in both production processes increased with longer smoking, so there were statistically significantly higher contents of all PAH compounds at the end compared to the halfway through the smoking process. The higher content of PAH compounds in the Visočka pečenica at the end of the smoking process is a consequence of longer exposure of the meat to smoke, so these compounds accumulated to a greater extent. The trend where the content of PAH compounds during smoking mainly increased in smoked meat products was established by *et al.* (2008), *et al.* (2008a), *Fraqueza et al.* (2020), *Hokkanen et al.* (2018) and *Ledesma et al.* (2014) in their research. In the research by *et al.* (2008), the content of BaP in beef ham was 0.02 µg/kg at the beginning of the smoking process, while at the end of the smoking process, the content was 1.09 µg/kg. The content of all individual 16 EU PAH compounds except DiP and DhP in beef ham smoked in a traditional and industrial smokehouse established by *et al.* (2008a) was lower compared to the content in the Visočka pečenica in this work.

In the comparison of both smoking heights, Visočka pečenica from traditional production had a higher content of PAH compounds at a higher height, while Visočka pečenica from industrial production at a lower height contained more PAHs, with some exceptions. *Purcaro et al.* (2013) state that greater distance from the heat source leads to a decrease in the content of PAH compounds in the final product. Of course, it was to be expected that the content of PAH compounds in Visočka pečenica would be higher at a lower height, which was not the case with traditional production. Namely, this difference in the content of PAH compounds in terms of smoking heights is probably a consequence of the different temperature regimes during smoking, the duration of smoking, the position of the products, the smoke source, the structure of the smokehouse as well as the part of the product actually tested (in our study, the entire sample was used for the test without separating the inner and outer parts). Research on the penetration of PAH compounds into the interior of smoked meat products shows that about 99% of all PAH compounds are found in the outer part, which makes up 22% of the total mass of the examined product (*Jira et al.,* 2006). In their research, *Toroman et al.* (2013) recorded the highest content of BaP in Bosnian prosciutto which was located in the closest point under the hearth, with amounts of 5.1 µg/kg (uncontrolled process) and 3.6 µg/kg (controlled process). *Hokkanen et al.* (2018) and *Mastanjević et al.* (2019) also found a higher content of PAH compounds in smoked meat products that were closer to the smoke source in their research.

A statistically significantly higher content of all individual PAH compounds was found in MLD samples compared to round samples in both production methods smoked on both shelf levels and both at halfway through and at the end of the smoking process. The MLD samples were characterized by a higher fat content compared to the round samples, confirmed by their chemical analysis. PAHs are highly lipophilic (*Puljić et al.,* 2019; *Reinik et al.,* 2007; *Martorell et al.,* 2010; *Đinović-Stojanović et al.,* 2016; *Kartalović et al.,* 2015), and generally become more lipophilic, less soluble and less volatile with increasing molecular weight (*Đinović et al.,* 2008a). Despite PAHs accumulating mainly on product surfaces, due to their lipophilic nature, some diffusion of these compounds can take place to inner layers (*Gomes et al.,* 2013), where water activity and fat content have a determinant role (*Hokkanen et al.,* 2018). The higher content of all PAH compounds in MLD samples is a consequence of their higher fat content and their lipophilic nature. In the comparison of both production methods, the content of individual PAH compounds was generally higher in the samples of Visočka pečenica from traditional production compared to industrial production. The higher content of PAH compounds in the samples of Visočka pečenica from traditional production is probably a consequence of longer smoking (for two days), higher temperature of the stokehole during each smoking day, the construction of the smokehouse, the position of the stokehole and the position of the meats inside the smokehouse. Šuvalija (2002) found a higher content of BaP in samples of Bosnian prosciutto from traditional compared to industrial production. A higher content of PAH compounds in smoked meat products from traditional compared to industrial production was established by Đinović *et al.* (2008a), *Puljić et al.* (2019), *Zachara et al.* (2017), *Kubiak and Polak-Śliwińska* (2015), *Mastanjević et al.* (2020) and *Roseiro et al.* (2011) in their research.

The maximum permitted amount (MPA) for smoked meat and smoked meat products according to the *Rulebook on Maximum Permitted Amounts for Food Contaminants* (*Official Gazette of BiH No. 68/14*) for BaP is 2.0 µg/kg. Based on the results presented in the samples of Visočka pečenica, the BaP content ranged from 1.36 µg/kg to 6.62 µg/kg in both production methods. According to our results,

four groups of Visočka pečenica in this research met the requirements of the *Rulebook* regarding BaP content because they contained BaP at <2.0 μg/kg.

4. Conclusion

The conducted research presents the first results of the content of 16 EU priority PAH compounds in Visočka pečenica. The production technology for this product has retained all the characteristics of centuries-old tradition, regardless of the type of production. In the end, such an approach resulted in some high quality products, and the conducted tests showed that some Visočka pečenica was an acceptable product from the health and safety aspect as well. Still, the research found a higher content of PAH compounds with longer smoking, as well as in Visočka pečenica that had a higher fat content. A lower content of PAH compounds was found in Visočka pečenica from industrial production where lower combustion chamber temperatures were recorded. In Visočka pečenica from traditional production, the higher content of PAH compounds occurred in products smoked a higher shelf level, but in industrial production, smoking at a lower shelf level produced Visočka pečenica with higher PAH contents. The content of BaP ranged from 1.36 to 6.62 μg/kg in Visočka pečenica from traditional production and from 1.59 to 3.70 μg/kg in Visočka pečenica from industrial production. In the future, it is recommended that research is continued regarding the qualitative and quantitative presence of PAH compounds in Visočka pečenica and other cured meat products in Bosnia and Herzegovina.

Disclosure statement: No potential conflict of interest was reported by authors.

References

Andrée, S., Jira, W., Schwind, K.-H., Wagner, H. & Schwägele, F. (2010). Chemical safety of meat and meat products. *Meat Science*, 86(1), 38–48, https://doi.org/10.1016/j.meatsci.2010.04.020

Bansal, V. & Kim, K.-H. (2015). Review of PAH contamination in food products and their health hazards. *Environment International* 84, 26–38, http://dx.doi.org/10.1016/j.envint.2015.06.016

Ciecierska, M. & Obiedziński, M. (2007). Influence of smoking process on polycyclic aromatic hydrocarbons content in meat products. *Acta Scientiarum Polonorum, Technologia Alimentaria* 6(4), 17–28.

Đinović, J., Popović, A. & Jira, W. (2008). Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. *Meat Science*, 80(2), 449–456, doi: 10.1016/j.meatsci.2008.01.008.

Đinović, J., Popović, A. & Jira, W. (2008a). Polycyclic aromatic hydrocarbons (PAHs) in traditional and industrial smoked beef and pork ham from Serbia. *European Food Research and Technology*, 227(4), 1191–1198, doi: 10.1007/s00217-008-0836-8.

Đinović-Stojanović, J. M., Stišović, J. M., Popović, A. R., Nikolić, D. M. & Janković S. D. (2016). Benzo[a]piren, benzo[a]antracen, benzo[b]fluoranten i hrizen u dimljenom mesu i dimljenim proizvodima od mesa — Validacija metode. *Hemijska Industrija*, 70(3), 299–305, doi:10.2298/HEMIND150331035D

EFSA, (2008). European Food Safety Authority, Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain, *The EFSA Journal*, 724, 1–114.

European Commission, (2006). Commission Regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L 364, 5–24.

European Commission, (2011). Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, L 215, 4–8.

European Commission, (2014). Commission Regulation (EU) No 1327/2014 of 12 December 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of polycyclic aromatic hydrocarbons (PAHs) in traditionally smoked meat and meat products and traditionally smoked fish and fishery products. *Official Journal of the European Union*, L 358, 13–14.

Fraqueza, M. J., Laranjo, M., Alves, S., Fernandes, M. H., Agulheiro-Santos, A. C., Fernandes, M. J., Potes, M. E. & Elias, M. (2020). Dry-Cured Meat Products According to the Smoking Regime: Process Optimization to Control Polycyclic Aromatic Hydrocarbons. *Foods*, 9(1), 91, 1–11, doi: 10.1016/j.meatsci.2008.01.008

García-Falcón, M. S. & Simal-Gándara, J. (2005). Polycyclic aromatic hydrocarbons in smoke from different woods and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings. *Food Additives and Contaminants*, 22(1), 1–8, doi:10.3390/molecules18043927

Gomes, A., Santos, C., Almeida, J., Elias, M. & Roseiro, L. C. (2013). Effect of fat content, casing type and smoking procedures on PAHs contents of Portuguese traditional dry fermented sausages. *Food and Chemical Toxicology*, 58, 369–374, doi: 10.1016/j.fct.2013.05.015

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1–9.

Hokkanen, M., Luhtasela, U., Kostamo, P., Ritvanen, T., Peltonen, K., Jestoi & M. (2018). Critical Effects of Smoking Parameters on the Levels of Polycyclic Aromatic Hydrocarbons in Traditionally Smoked Fish and Meat Products in Finland. *Journal of Chemistry*, 1–14, https://doi.org/10.1155/2018/2160958

IARC, (2010). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, Vol. 92, Lyon, France.

Jira, W., Pöhlmann, M., Hitzel A. & Schwägele, F. (2013). Smoked meat products — Innovative strategies for reduction of polycyclic aromatic hydrocarbons by optimization of the smoking process. Proceedings of the International 57th Meat Industry Conference, 24–32.

Jira, W., Ziegenhals, K. & Speer, K. (2006). PAH in smoked meat products according to EU standards. *Fleischwirtschaft*, 4, 11–17.

Kafouris, D., Koukkidou, A., Christou, E., Hadjigeorgiou, M. & Yiannopoulos, S. (2020). Determination of polycyclic aromatic hydrocarbons in traditionally smoked meat products and charcoal grilled meat in Cyprus. *Meat Science*, 164, 1–6, https://doi.org/10.1016/j.meatsci.2020.108088

Kartalović, B., Okanović, Đ., Babić, J., Đorđević, **V., Janković, S. & Cirković, M. (2015).** Polycyclic aromatic hydrocarbons in smoked ham. *Procedia Food Science*, 5, 144–147, doi: 10.1016/j.profoo.2015.09.042

Kubiak, M. S. & Polak-Śliwińska, M. (2015). The level of chosen polycyclic aromatic hydrocarbons (PAHs) in meat products smoked by using an industrial and a traditional method. *Polish Journal of Natural Scien*ces, 30(2), 137–147.

Ledesma, E., Rendueles, M. & Díaz, M. (2014). Benzo(a)pyrene penetration on a smoked meat product during smoking time. *Food Additives & Contaminants: Part A*, 31(10), 1688–1698, http://dx.doi.org/10.1080/19440049.2014.949875

Martorell, I., Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M. & Domingo, J. L. (2010). Polycyclic aromatic hydrocarbons (PAH) in foods and estimated PAH intake by the population of Catalonia, Spain: Temporal trend. *Environment International*, 36(5), 424–432, doi: 10.1016/j.envint.2010.03.003

Mastanjević, K., Kartalović, B., Petrović, J., Novakov, N., Puljić, L., Kovačević, D., Jukić, M., Lukinac, J. & Mastanjević, K. (2019). Polycyclic aromatic hydrocarbons in the traditional smoked sausage Slavonska kobasica. *Journal of Food Composition and Analysis*, 83, 1–7, https://doi.org/10.1016/j.jfca.2019.103282

Mastanjević, K., Kartalović, B., Puljić, L., Kovačević, D. & Habschied, K. (2020). Influence of Different Smoking Procedures on Polycyclic Aromatic Hydrocarbons Formation in Traditional Dry Sausage Hercegovačka kobasica. *Processes*, 8(8), 918, 1–7, doi:10.3390/pr8080918

Puljić, L., Mastanjević, K., Kartalović, B., Kovačević, D., Vranešević, J. & Mastanjević, K. (2019). The Influence of Different Smoking Procedures on the Content of 16 PAHs in Traditional Dry Cured Smoked Meat "Hercegovačka Pečenica." *Foods*, 8(12), 690, 1–9, doi: 10.3390/foods8120690

Purcaro, G., Moret, S. & Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. *Talanta*, 105, 292–305, https://doi.org/10.1016/j.talanta.2012.10.041

Reinik, M., Tamme, T., Roasto, M., Juhkam, K., Tenno, T. & Kiis, A. (2007). Polycyclic aromatic hydrocarbons (PAHs) in meat products and estimated PAH intake by children and the general population in Estonia. *Food Additives and Contaminants*, 24(4), 429–437, doi: 10.1080/02652030601182862

Roseiro, L. C., Gomes, A. & Santos, C. (2011). Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. *Food and Chemical Toxicology*, 49(6), 1340–1345, https://doi.org/10.1016/j.fct.2011.03.017

Roseiro, L. C., Gomes, A. & Santos, C. (2011). Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. Food and Chemical Toxicology, 49(6), 1340–1345, https://doi.org/10.1016/j.fct.2011.03.017

Rulebook, (2014). Rulebook on Maximum Permitted Amounts for Food Contaminants (Official Gazette of Bosnia and Herzegovina No. 68/14.

Sojinu, O. S., Olofinyokun, L., Idowu, A. O., Mosaku, A. M. & Oguntuase, B. J. (2019). Determination of Polycyclic Aromatic Hydrocarbons (PAHs) In Smoked Fish and Meat Samples In Abeokuta. *Journal of Chemical Society of Nigeria*, 44(1), 96–106, doi: 10.1016/s0378-4347(01)00438-8

Škaljac, B. S., Jokanović, R. M., Tomović, M. V., Peulić, A. T., Ikonić, M. P., Šojić, **V. B., Ivić, Đ. M., Petrović, S. Lj., Babić, M. J. & Hromiš, M. N. (2019).** Uticaj dimljenja na formiranje boje i sadržaj policikličnih aromatičnih jedinjenja u tradicionalnoj fermentisanoj kobasici. *Glasnik hemičara, tehnologa i ekologa Republike Srpske*, 15, 25–32, doi: 10.7251/GHTE1915025S

Šuvalija, B. (2002). Proizvodnja i kvalitet bosanskog pršuta. Magistarski rad. Poljoprivredni fakultet Univerziteta u Sarajevu. BiH.

Tareq, A. R. M., Afrin, S., Hossen, M. S., Hashi, A. S., Quraishi, S. B., Nahar, Q., Begum, R. & Ullah, A. K. M. A. (2020). Gas Chromatography-Mass Spectrometric (GC-MS) Determination of Polycyclic Aromatic Hydrocarbons in Smoked Meat and Fish Ingested by Bangladeshi People and Human Health Risk Assessment. Polycyclic Aromatic Compounds, 1–11, https://doi.org/10.1080/10406638.2020.1790017

Toroman, A., Karahmet, E., Vileš, A., Đulančić, N. & Jašić, M. (2013). Kontrola procesa dimljenja i nivo benzo(a) pirena u proizvodnji bosanskog pršuta na tradicionalan način pri implementaciji HACCP-a. *Veterinaria,* 62(1–2), 85–92.

Zachara, A., Gałkowska, D. & Juszczak, L. (2017). Contamination of smoked meat and fish products from Polish market with polycyclic aromatic hydrocarbons. *Food Control*, 80, 45–51, http://dx.doi.org/10.1016/j.foodcont.2017.04.024

Zdolec, N., Mežnarić, V., Martinec, N., Čižmak**, A. & Pažin, V. (2019).** Nalaz policikličkih aromatskih ugljikovodika i kemijski sastav dimljenih i nedimljenih kuhanih sireva. *Hrvatski veterinarski vjesnik*, 27(3–4), 38–41.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.54

ID: 126489353

https://doi.org/10.18485/meattech.2023.64.2.13

Original scientific paper

Wooden breast, white striping and spaghetti meat: chemical composition, technological quality, microbiological profile and sensory attributes of broiler breasts

Marija Bošković Cabrol[a], Massimiliano Petracci[b] and Angela Trocino[a]*

[a] *University of Padova, Department of Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), Viale dell'Università 16, 35020 Legnaro, Padova, Italy*

[b] *Alma Mater Studiorum — University of Bologna, Department of Agricultural and Food Sciences, Cesena, Italy*

ARTICLE INFO

Keywords:
Myopathies
P. major
Fatty acids
Texture
Cooking loss
Sensory profile

ABSTRACT

Poultry meat is consumed worldwide and its production is expected to increase in the upcoming years. Genetic selection in poultry focused on growth rate, feed conversion, and breast yield, resulting in the occurrence of white striping and wooden breast abnormalities and, most recently, spaghetti meat. These myopathies affect the quality traits of raw meat, including pH, color, water holding capacity, and cooking loss, which limit its further processing and decrease consumer acceptance. Additionally, the effects of myopathies on the chemical composition, i.e., reduced protein and essential amino acid content and increased fat contents, impair to some extent the nutritional value of the meat.

1. Introduction

Fast growth of poultry production with estimated increase of 16% by 2029 (FAO, 2020) is owed to the higher need for protein following rapid population growth (*UN*, 2019). The selection for fast weight gain puts excessive pressure on the *Pectoralis major* (*P. major*) muscle, resulting in high breast yield but also in a high occurrence of myopathies such as white striping (WS), wooden breast (WB) and spaghetti meat (SM) (*Baldi et al.*, 2021; *Petracci et al.,* 2019; *Trocino et al.,* 2015; *Kuttappan et al.,* 2013). On WS muscles, the presence of the white lines of intramuscular fat deposits can be seen (*Kuttappan et al.*, 2013), whereas WB is characterized by hardening of the muscle, and in severe cases, prominent ridge-like bulge, edema and/or scattered petechiae on the breast surface (*Sihvo et al.*, 2014). *P. major* muscle with SM shows an overall impaired integrity with the separation of the fiber bundles composing the muscle tissue itself, resembling the appearance of spaghetti (*Baldi et al.,* 2018). Although myopathies do not pose a threat to meat shelf life and microbiological safety (*Gratta et al.*, 2019), they impair meat quality to some extent, affecting technological characteristics of raw meat and limiting their further processing (*Gratta et al.*, 2019; *Baldi et al.*, 2018; *Mudalal et al.*, 2015; *Trocino et al.*, 2015; Petracci et al., 2013). Despite carcasses affected by myopathies meeting the market quality standards (*Baéza et*

***Corresponding author:** Marija Bošković Cabrol, *marija.boskoviccabrol@unipd.it*

Paper received May 1st 2023. Paper accepted May 12th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

al., 2022) for weight and yield, muscle abnormalities impair the visual aspect of the meat, preventing the commercialization of whole breasts or, in cases of severe structural changes, requiring the breasts to be discarded as a waste (*Zanetti et al*., 2018).

2. Chemical composition of abnormal breast meat

Pathological alterations related to myopathies result in changes in the chemical composition of muscle tissue. Higher water and fat contents and lower ash and protein contents were reported in breasts affected by WS, WB, WB/WS and SM compared to their normal counterpart (*Dalle Zotte et al.*, 2020; *Gratta et al*., 2019; *Cai et al.*, 2018; *Soglia et al*., 2016 a, b; *Zambonelli et al*., 2016). The greater water content of WB and WS breasts is likely a consequence of inflammatory processes leading to fluid accumulation (*Thanatsang et al.*, 2020; *Sihvo et al.*, 2014). Indeed, *Gratta et al.*, (2019) found higher moisture content in WB than in normal meat during the first 24 h *post-mortem*, while the differences among groups disappeared due to protein denaturation during 7 and 11 days of storage.

The reduction of protein content in WS, WB, and WB/WS meats due to fibrosis has been attributed to the replacement of muscle fibers with connective tissue and the aforementioned accumulation of adipose tissue (*Dalle Zotte et al.*, 2020; *Baldi et al.*, 2018; *Soglia et al.*, 2016 a,b; *Zambonelli et al.*, 2016), where a relationship between protein reduction and the degree of WS (*Petracci et al.*, 2015; *Kuttappan et al.*, 2013) has been also outlined. The protein decrease compared to normal breasts is followed by a reduction in essential amino acids in WS/WB and WB breast (*Dalle Zotte et al.*, 2020; *Thanatsang et al.*, 2020), which could seriously impair the meat nutritional value. The few studies about SM breasts found a reduction of protein content up to 10% compared to unaffected muscles (*Baldi et al.*, 2018; 2019). Some authors found meat affected by SM, WB, WS/WB and WS/SM to display an increased collagen content compared to normal muscles, while others did not observe any difference (*Baldi et al.*, 2018; *Cai et al.*, 2018; *Soglia et al.*, 2016 a,b). The degradation in the nutritional value of WS meat is also due to a lower digestibility and quality of amino acids in collagen compared to myofibrillar proteins (*Huang and Ahn*, 2018; *Mudalal et al*., 2014).

Compared with normal meat, the decrease in ash content in breast muscles associated with WS (*Soglia et al.*, 2018b; *Mudalal et al.*, 2014), WB (*Gratta et al.*, 2019; *Cai et al.*, 2018; *Soglia et al.*, 2016 a,b), and WS/WB (*Dalle Zotte et al.*, 2020; *Zambonelli et al.*, 2016) is probably due to pathological changes at cellular levels. After the initial degeneration, the damage to the sarcoplasmic reticulum surrounding muscle fibers increases Ca influx and activates Ca-dependent protease, triggering myofibers necrosis (*Huang and Ahn*, 2018). Increased Fe levels in WB could be due to hemorrhagic lesions (*Thanatsang et al.,* 2020). In fact, *Thanatsang et al.*, (2020) reported lower P and K and higher contents of Al, Ca, Fe, Na, and S in WB, and elevated Ca and Na content in SM compared to unaffected breasts. Zambonelli et al. (2016) found increased Ca and Na and decreased Mg and P, whereas *Tasoniero et al.*, (2016) reported increased Fe and Na and reduced K and P contents in WS breasts.

Higher lipid content in WS and WB in comparison to normal meat are attributed to myodegeneration and replacement of degenerated muscle fibers by adipose tissue (*Soglia et al.,* 2016a), often accompanied by changes in fatty acid profile. *Baldi et al.* (2018, 2019) reported a decrease in EPA and DHA in meat affected by SM compared to normal breasts, likely due to a different expression of the genes encoding for Δ5 and Δ6 desaturases (Soglia et al., 2016a). *Gratta et al.* (2019) reported chicken breasts affected by WB and WS display lower SFA and MUFA and higher PUFA due to an increase of both n-3 and n-6 FA compared to normal breasts. Lower SFA in WS and WB meat compared to the normal breasts was also reported in other studies (*Soglia et al.*, 2016a; *Kuttappan et al.*, 2012a). The mechanism behind changes in the FA profile of chicken meat affected by abnormalities is not completely clear. Possible explanations are related to the presence of inflammatory cells with a relatively high proportion of n-6 arachidonic acid in their membrane phospholipids (*Calder*, 2012) or the tendency of fiber type switching from fast, glycolytic IIB fibers, in which lipids are generally more saturated, to those within slow and oxidative fibers (*Realini et al.*, 2013). Contrarily, *Traffano-Schiffo et al.* (2017) found that superficial meat layers affected by WS had higher SFA and MUFA and lower PUFA compared to normal meat. In fact, while most of the aforementioned studies reported changes in the overall *P. major*, some research found proximate composition changes only within the surface of the breast muscle *(Baldi et al.* 2019; *Traffano-Schiffo et al.*, 2017). The chemical composition likely depends on the degree of structural changes: severe myopa-

thies will alter breast chemical composition at more profound parts of the muscle, whereas mild changes could only affect superficial meat layers.

Regarding oxidative stability, *Soglia et al.* (2016b) reported higher protein and lipid oxidation in WB and WS/WB compared to normal breasts, although no differences in total SFA, and PUFA were found between the former and the latter. The higher susceptibility of WB meat to protein oxidation was confirmed by *Thanatsang et al.* (2020). Despite higher lipid content, WS breasts do not seem to be more susceptible to lipid oxidation than unaffected ones (*Alnahhas et al.*, 2016; *Soglia et al.*, 2016b).

3. Technological quality of abnormal meat

In the presence of myopathies, reduction in protein content and degeneration of muscle fibers and myofibrils that usually entrap the majority of intracellular water are responsible for lower water holding capacity (WHC), marinade uptake and retention, emulsifying and gelling properties compared to normal breasts (*Petracci et al.*, 2019; *Xing et al.,* 2017; *Bowker and Zhuang*, 2016; *Soglia et al.*, 2016b). Whereas most of the findings on how WB affects WHC and drip and cooking losses are consistent (*Gratta et al.,* 2019; *Dalle Zotte et al.,* 2017; *Kuttappan et al.*, 2017a; *Trocino et al.*, 2015), the results on WS impact vary between studies. *Kuttapan et al.* (2017a) reported a decrease in drip loss in severe WB and WS/WB, but without differences between breasts with severe WS and normal ones as observed by *Kuttappan et al.* (2013) for cooking losses. Differently, *Petracci et al.* (2013) and *Tijare et al.* (2016) found an increase in cooking losses in raw breasts with severe WS. Because of the decreased total protein content but increased collagen and fat contents in WS breasts, their WHC, protein solubility and marinade uptake are lower than the normal muscles (*Sihvo et al.*, 2014). Regarding SM, this myopathy alone, or in combination with WS/WB, significantly increases drip losses (*Wang et al.*, 2023; *Tasoniero et al.*, 2020; *Baldi et al.*, 2018).

In terms of pH, some studies did not find changes in ultimate pH of muscle affected by myopathies (*Pascual et al.*, 2021; *Baldi et al.* 2018; *Trocino et al.,* 2015), while others reported elevated pH in WS, WB and SM breasts compared to their normal counterpart (*Wang et al.*, 2023; *Bordignon et al.*, 2022; *Tasoniero et al.*, 2016; *Mudalal et al.*, 2015; *Dalle Zotte et al.*, 2014; *Petracci et al.*, 2013). A proteomic study (*Kuttappan et al.*, 2017b) suggested down-regulation of carbohydrate metabolic pathways related to reduced glycolysis, gluconeogenesis, TCA (tricarboxylic acid) cycle, glycogen degradation and pyruvate fermentation to lactate to be responsible for the reduced glycolytic potential and higher ultimate pH in affected breasts.

Regarding the effect of myopathies on breast color, inconsistent results have been reported. The meta-study of *Bordignon et al.* (2022) did not report an impact of WS, WB, or SM presence on breast color indices, in agreement with *Zambonelli et al.*, (2016) on WB and WS/WB breasts. Contrarily, *Mazzoni et al.* (2015) found a decrease in red and yellow indexes of WS breasts, whereas *Kuttapan et al.* (2017) reported an increase in yellowness in both WB and WS breasts when compared to control ones. Further, *Dalle Zotte et al.* (2017) noted elevated lightness in WB meat. Some authors suggested higher alteration in breast color as myopathy evolves to a severe degree (*Campo et al.*, 2020; *Tasoniero et al.*, 2016). *Campo et al.* (2020) observed a tendency to increase in lightness and yellowness in severe WB compared to unaffected meat, whereas the presence of WS also resulted in lighter, less red, and more yellow breasts. The presence of moderate SM resulted in higher yellowness. The higher yellowness of defective breasts arises from the elevated fat content (*Kuttappan et al.*, 2012a; 2013; *Petracci et al.*, 2015) and likely a high accumulation of dietary liposoluble pigments (*Campo et al*., 2020).

Although the changes in texture of breasts affected by myopathies are documented, results differ between studies due to sample preparation (raw vs. cooked, surface layers vs. whole muscle), methods and parameters used (*Pascual et al.,* 2021; *Baldi et al.*, 2019; *Soglia et al.,* 2016a). The WB are often characterized by increased hardness of the muscle likely due to the higher collagen content of WB fillets (*Baldi et al.,* 2019). However, the fibrosis in WB and WS/WB may not necessarily affect the texture properties of the breast, which was confirmed in several studies (*Xing et al.,* 2020; *Maxwell et al.*, 2018; *Sihvo et al.*, 2017; *Mudalal et al.*, 2015). Contrarily, SM and WS breasts usually display lower shear force than normal breasts (*Bordignon et al.*, 2022; *Baldi et al.*, 2019; *Tasoniero et al.*, 2016). Compared to normal breasts, lower hardness can be explained due to the higher fat content in WS breasts (*Radaelli et al.*, 2017; *Soglia et al.*, 2016a; *Mudalal et al.*, 2015) and to the reduced collagen cross-linking degree in SM ones (*Baldi et al.*, 2019, 2021). However, *Baldi et al.* (2019) did not find differences in the texture

between raw SM and normal samples, whereas cooking decreased hardness in SM. *Pascual et al.* (2021) reported only the Meullenet-Owens razor blade test to be able to detect the difference between SM and normal cooked breasts.

4. Microbiological quality of abnormal meat

To date, only a few studies investigated the microbiological status of meat with myopathies, suggesting it to be safe for human consumption. *Gratta et al.* (2019) reported higher initial total viable counts (TVC) and *Pseudomonas* spp. counts in normal breasts compared to WS and WB ones. Furthermore, normal breasts had the shortest TVC and *Pseudomonas* spp. Lag phase compared to WS and WB meat, resulting in the later microbial spoilage of breasts-affected abnormalities (5 vs. 6 days). *Enterobacteriaceae* spp. and lactic acid bacteria counts were also highest in unaffected breasts, followed by WS, and lowest in WB meat. *Pereira et al.* (2022) found lower *Staphylococcus* spp. and higher coliform counts in WS breasts, although bacterial counts neither in normal nor defective meat exceeded permitted limits after 12 months of freezing.

5. Sensory attributes of breasts with myopathies

As aforementioned, the impaired visual appearance and quality results in lower consumer acceptance and purchase intention of meat affected by myopathies (De Carvalho et al*.,* 2020; *Petracci et al.,* 2015). Consumers' acceptance of meat with myopathies strongly depends on the degree of muscle tissue changes. *Kuttappan et al.* (2012b) reported a decrease in consumer acceptance along with the severity of white striping, while *Xing et al.* (2020) found moderate and severe raw WB fillets to affect sensory acceptance due to their undesirable appearance, texture and drip loss, but found no differences between normal and mild WB. Indeed, the visual appearance of raw meat leads to meat rejection, which is why some sensory studies are performed on cooked meat. Still, *Tasoniero et al.* (2016) reported more intensive off-odors in cooked WS fillets than in the normal ones, while *López et al.* (2022) observed a decrease in the cohesiveness and increase in the juiciness of severe WB. Although, in most of the studies, a deterioration of eating parameters is evident, *de Almeida Assunção et al.* (2020) found an increase in succulence in WB, probably due to a higher water content, to be well accepted by untrained panelists and increased final satisfaction with this meat. Furthermore, *De Carvalho et al.* (2020) reported lower acceptability of raw WS breast than unaffected counterparts, while upon cooking, consumers did not note differences between WS and normal chicken breasts for color, flavor, and overall acceptability but WS meat achieved higher scores for odor and texture compared to the normal breasts. Some efforts are made to eliminate or decrease the changes related to myopathies by marinating meat. However, *Maxwell et al.* (2018) reported differences in cooked meat sensory texture attributes related to WB to be lessened but not eliminated by vacuum-tumbling marination. Similarly, *Jarvis et al.* (2020) found neither traditional nor clean-label marinades to mask eating characteristics of severe WB, but, to some extent, a positive effect of marination was observed on mild WB. As far as SM goes, the effect of this muscle abnormality on sensory characteristics of breast meat has not yet been investigated.

6. Conclusion

Although the results between studies are sometimes inconsistent regarding how the different myopathies affect *P. major* in broilers, all studies reported at least one or multiple quality traits to be changed compared to unaffected breasts. Some underlying mechanisms behind changes in chemical compositions and quality of breasts affected by myopathies, especially less known SM, are not completely clear, emphasizing the need for further investigation on a molecular level.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: Dr. Marija Bošković Cabrol acknowledges the European Union's Horizon 2020 research and innovation programme for the research grant she received under the Marie Skłodowska-Curie grant agreement No 101063055 (DeMYO project).

References

Alnahhas, N., Berri, C., Chabault, M., Chartrin, P., Boulay, M., Bourin, M. C. & Bihan-Duval, L. (2016). Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. *BMC genetics*, 17, 1–9, https://doi.org/10.1186/s12863-016-0369-2

Baéza, E., Guillier, L. & Petracci, M. (2022). Production factors affecting poultry carcass and meat quality attributes. *Animal*, 16, e100331, https://doi.org/10.1016/j.animal.2021.100331

Baldi, G., Soglia, F. & Petracci, M. (2021). Spaghetti meat abnormality in broilers: current understanding and future research directions. *Frontiers in Physiology*, 12, 684497, https://doi.org/10.3389/fphys.2021.684497

Baldi, G., Soglia, F., Laghi, L., Tappi, S., Rocculi, P., Tavaniello, S., Prioriello, D., Mucci, R., Maiorano, G. & Petracci, M. (2019). Comparison of quality traits among breast meat affected by current muscle abnormalities. *Food Research International*, 115, 369–376, https://doi.org/10.1016/j.foodres.2018.11.020

Baldi, G., Soglia, F., Mazzoni, M., Sirri, F., Canonico, L., Babini, E., Laghi, L., Cavani, C. & Petracci, M. (2018). Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. *Animal*, 12, 164–173, https://doi.org/10.1017/S1751731117001069

Bordignon, F., Xiccato, G., Boskovic Cabrol, M., Birolo, M. & Trocino, A. (2022). Factors affecting breast myopathies in broiler chickens and quality of defective meat: A meta-analysis. *Frontiers in Physiology*, e1322, https://doi.org/10.3389/fphys.2022.933235

Bowker, B. & Zhuang, H. (2016). Impact of white striping on functionality attributes of broiler breast meat. *Poultry Science*, 95, 1957–1965, https://doi.org/10.3382/ps/pew115

Cai, K., Shao, W., Chen, X., Campbell, Y. L., Nair, M. N., Suman, S. P., Beach, C. M., Guyton, M. C. & Schilling, M. W. (2018). Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. *Poultry Science*, 97, 337–346, https://doi.org/10.3382/ps/pex284

Calder, P. C. (2012). The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Molecular Nutrition & Food Research*, 56, 1073–1080, https://doi.org/10.1002/mnfr.201100710

Campo, M. D. M., Mur, L., Guerrero, A., Barahona, M., Resconi, V. C., Magalhaes, D. R., Lisbinski, E., Boito, B., de Oliveira I. M. & Olleta, J. L. (2020). Differentiating breast myopathies through color and texture analyses in broiler. *Foods*, 9, 824, https://doi.org/10.3390/foods9060824

Dalle Zotte, A., Cecchinato, M., Quartesan, A., Bradanovic, J., Tasoniero, G. & Puolanne, E. (2014). How does "wooden breast" myodegeneration affect poultry meat quality. In 60th International Congress of Meat, pp. 476–479.

Dalle Zotte, A., Ricci, R., Cullere, M., Serva, L., Tenti, S. & Marchesini, G. (2020). Research Note: Effect of chicken genotype and white striping–wooden breast condition on breast meat proximate composition and amino acid profile. *Poultry Science*, 99, 1797–1803, https://doi.org/10.1016/j.psj.2019.10.066

Dalle Zotte, A., Tasoniero, G., Puolanne, E., Remignon, H., Cecchinato, M., Catelli, E. & Cullere, M. (2017). Effect of "wooden breast" appearance on poultry meat quality, histological traits, and lesions characterization. *Czech Journal of Animal Science*, 62, 51–57, https://doi.org/10.17221/54/2016-CJAS

de Almeida Assunção, A. S., Garcia, R. G., Komiyama, C. M., de Sena Gandra, É. R., de Souza, J. R., Dos Santos, W., Caldara, F.R. & Martins, R. A. (2020). Wooden breast myopathy on broiler breast fillets affects quality and consumer preference. *Tropical Animal Health and Production*, 52, 3555–3565, https://doi.org/10.1007/s11250-020-02392-6

De Carvalho, L. M., Ventanas, S., Olegario, L. S., Madruga, M. S. & Estévez, M. (2020). Consumers awareness of white-striping as a chicken breast myopathy affects their purchasing decision and emotional responses. *LWT*, 131, e109809, https://doi.org/10.1016/j.lwt.2020.109809

FAO (2020). Dairy and dairy products. *OECD-FAO* Agricultural Outlook, 2029, 174–183.

Gratta, F., Fasolato, L., Birolo, M., Zomeño, C., Novelli, E., Petracci, M., Pascual, A., Xiccato G. & Trocino, A. (2019). Effect of breast myopathies on quality and microbial shelf life of broiler meat. Poultry Science, 98, 2641–2651, https://doi.org/10.3382/ps/pez001

Huang, X. & Ahn, D. U. (2018). The incidence of muscle abnormalities in broiler breast meat–a review. *Korean Journal for Food Science of Animal Resources*, 38, 835, https://doi.org/10.5851/kosfa.2018.e2

Jarvis, T., Byron, M., Von Staden, M., Crist, C., Zhang, X., Rowe, C., Smith, B. & Schilling, M. W. (2020). Quality differences in wooden and normal broiler breast meat marinated with traditional and clean label marinades. *Meat and Muscle Biology*, 4, 1–13, https://doi.org/10.22175/mmb.9458

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W. & Owens, C. M. (2012a). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, 91, 2677–2685, https://doi.org/10.3382/ps.2012-02259

Kuttappan, V. A., Lee, Y. S., Erf, G. F., Meullenet, J. F., McKee, S. R. & Owens, C. M. (2012b). Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. *Poultry Science*, 91, 1240–1247, https://doi.org/10.3382/ps.2011-01947

Kuttappan, V. A., Shivaprasad, H. L., Shaw, D. P., Valentine, B. A., Hargis, B. M., Clark, F. D., McKee, R.S. & Owens, C. M. (2013). Pathological changes associated with white striping in broiler breast muscles. *Poultry Science*, 92, 331–338, https://doi.org/10.3382/ps.2012-02646

Kuttappan, V. A., Owens, C. M., Coon, C., Hargis, B. M. & Vazquez-Anon, M. (2017a). Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. *Poultry Science*, 96, 3005–3009, https://doi.org/10.3382/ps/pex072

Kuttappan, V. A., Bottje, W., Ramnathan, R., Hartson, S. D., Coon, C. N., Kong, B. W., Owens, M.C., Vazquez-Añon,

M. & Hargis, B. M. (2017b). Proteomic analysis reveals changes in carbohydrate and protein metabolism associated with broiler breast myopathy. *Poultry Science*, 96, 2992–2999, https://doi.org/10.3382/ps/pex069

Maxwell, A. D., Bowker, B. C., Zhuang, H., Chatterjee, D. & Adhikari, K. (2018). Descriptive sensory analysis of marinated and non-marinated wooden breast fillet portions. *Poultry Science*, 97, 2971–2978, https://doi.org/10.3382/ps/pey145

Mazzoni, M., Petracci, M., Meluzzi, A., Cavani, C., Clavenzani, P. & Sirri, F. (2015). Relationship between pectoralis major muscle histology and quality traits of chicken meat. *Poultry Science*, 94, 123–130, https://doi.org/10.3382/ps/peu043

Mudalal, S., Babini, E., Cavani, C. & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, 93, 2108–2116, https://doi.org/10.3382/ps.2014-03911

Mudalal, S., Lorenzi, M., Soglia, F., Cavani, C. & Petracci, M. (2015). Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Animal*, 9, 728–734, https://doi.org/10.1017/S175173111400295X

Pascual, A. G., Trocino, A., Susta, L. & Barbut, S. (2021). Comparing three textural measurements of chicken breast fillets affected by severe wooden breast and spaghetti meat. *Italian Journal of Animal Science,* 20, 465–471, https://doi.org/10.1080/1828051X.2021.1893134

Pereira, M. R., Mello, J. L., Oliveira, R. F., Villegas-Cayllahua, E. A., Cavalcanti, E. N., Fidelis, H.XA., Ferrari, B. F., Giampietro-Ganeco, A., Souza, A. P. & Borba, H. (2022). Effect of freezing on the quality of breast meat from broilers affected by White Striping myopathy. *Poultry Science*, 101, 101607, https://doi.org/10.1016/j.psj.2021.101607

Petracci, M., Mudalal, S., Bonfiglio, A. & Cavani, C. (2013). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, 92, 1670–1675, https://doi.org/10.3382/ps.2012-03001

Petracci, M., Mudalal, S., Soglia, F. & Cavani, C. (2015). Meat quality in fast-growing broiler chickens. *World's Poultry Science Journal*, 71, 363–374, https://doi.org/10.1017/S0043933915000367

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E. & Estévez, M. (2019). Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comprehensive Reviews in Food Science and Food Safety*, 18, 565–583, https://doi.org/10.1111/1541-4337.12431

Radaelli, G., Piccirillo, A., Birolo, M., Bertotto, D., Gratta, F., Ballarin, C., Vascellari, M., Xiccato, G. & Trocino, A. (2017). Effect of age on the occurrence of muscle fiber degeneration associated with myopathies in broiler chickens submitted to feed restriction. *Poultry Science*, 96, 309–319, https://doi.org/10.3382/ps/pew270

Realini, C. E., Vénien, A., Gou, P., Gatellier, P., Perez-Juan, M., Danon, J. & Astruc, T. (2013). Characterization of *Longissimus thoracis, Semitendinosus* and *Masseter* muscles and relationships with technological quality in pigs. 1. Microscopic analysis of muscles. *Meat Science*, 94, 408–416, https://doi.org/10.1016/j.meatsci.2013.03.009

Sihvo, H. K., Immonen, K. & Puolanne, E. (2014). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology*, 51, 619–623, https://doi.org/10.1177/0300985813497488

Sihvo, H. K., Lindén, J., Airas, N., Immonen, K., Valaja, J. & Puolanne, E. (2017). Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. *Veterinary Pathology*, 54, 119–128, https://doi.org/10.1177/03009858166580

Soglia, F., Laghi, L., Canonico, L., Cavani, C. & Petracci, M. (2016b). Functional property issues in broiler breast meat related to emerging muscle abnormalities. *Food Research International*, 89, 1071–1076, https://doi.org/10.1016/j.foodres.2016.04.042

Soglia, F., Mudalal, S., Babini, E., Di Nunzio, M., Mazzoni, M., Sirri, F., Cavani, C. & Petracci, M. (2016a). Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. *Poultry Science*, 95, 651–659, https://doi.org/10.3382/ps/pev353

Tasoniero, G., Cullere, M., Cecchinato, M., Puolanne, E. & Dalle Zotte, A. (2016). Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by White Striping and Wooden Breast myopathies. *Poultry Science*, 95, 2707–2714, https://doi.org/10.3382/ps/pew215

Thanatsang, K. V., Malila, Y., Arayamethakorn, S., Srimarut, Y., Tatiyaborworntham, N., Uengwetwanit, T., Panya, A., Rungrassamee, W. & Visessanguan, W. (2020). Nutritional properties and oxidative indices of broiler breast meat affected by wooden breast abnormality. *Animals*, 10, e2272, https://doi.org/10.3390/ani10122272

Tijare, V. V., Yang, F. L., Kuttappan, V. A., Alvarado, C. Z., Coon, C. N. & Owens, C. M. (2016). Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. *Poultry Science*, 95, 2167–2173, https://doi.org/10.3382/ps/pew129

Traffano-Schiffo, M. V., Castro-Giraldez, M., Colom, R. J. & Fito, P. J. (2017). Development of a spectrophotometric system to detect white striping physiopathy in whole chicken carcasses. *Sensors*, 17, e1024, https://doi.org/10.3390/s17051024

Trocino, A., Piccirillo, A., Birolo, M., Radaelli, G., Bertotto, D., Filiou, E., Petracci, M. & Xiccato, G. (2015). Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. *Poultry Science*, 94, e2996–3004, https://doi.org/10.3382/ps/pev296

UN (United Nations), (2019). World population prospects 2019 — highlights. New York, pp 46. (https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html)

Wang, C., Che, S., Susta, L. & Barbut, S. (2023). Textural and physical properties of breast fillets with myopathies (wooden breast, white striping, spaghetti meat) in Canadian fast-growing broiler chickens. *Poultry Science*, 102, 102309, https://doi.org/10.1016/j.psj.2022.102309

Xing, T., Zhao, X., Cai, L., Guanghong, Z. & Xu, X. (2017). Effect of salt content on gelation of normal and wooden breast myopathy chicken pectoralis major meat batters. *International Journal of Food Science & Technology*, 52, 2068–2077, https://doi.org/10.1111/ijfs.13485

Xing, T., Zhao, X., Zhang, L., Li, J. L., Zhou, G. H., Xu, X. L. & Gao, F. (2020). Characteristics and incidence of broiler chicken wooden breast meat under commercial conditions in China. *Poultry Science*, 99, 620–628, https://doi.org/10.3382/ps/pez560

Zambonelli, P., Zappaterra, M., Soglia, F., Petracci, M., Sirri, F., Cavani, C. & Davoli, R. (2016). Detection of differentially expressed genes in broiler pectoralis major muscle affected by White Striping–Wooden Breast myopathies. *Poultry Science*, 95, 2771–2785, https://doi.org/10.3382/ps/pew268

Zanetti, M. A., Tedesco, D. C., Schneider, T., Teixeira, S. T. F., Daroit, L., Pilotto, F., Dickel, E.L., Santos, P.S. & dos Santos, L. R. (2018). Economic losses associated with wooden breast and white striping in broilers. *Semina: Ciências Agrárias*, 39, 887–891, https://doi.org/10.5433/1679-0359.2018v39n2p887

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.4.09:616.993.1

ID: 126529801

https://doi.org/10.18485/meattech.2023.64.2.14

Review paper

Toxoplasma gondii infection in pigs in Serbia

Aleksandra Uzelac[a], Nikola Betić[b], Nedjeljko Karabasil[c], Vladimir Ćirković[a], Olgica Đurković-Đaković[a] and Ivana Klun[a]*

[a] *University of Belgrade, Institute for Medical Research, Centre of Excellence for Food- and Vector-borne Zoonoses, Dr. Subotića 4, 11129 Belgrade, Serbia;*
[b] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia;*
[c] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar oslobođenja 18, 11000 Belgrade, Serbia.*

ARTICLE INFO

Keywords:
Toxoplasma gondii
Pigs
Genotypes
Virulence
Infection
Seroprevalence

ABSTRACT

Toxoplasma gondii is a common zoonotic intracellular parasite in livestock raised for human consumption and is a public health concern. The mode of transmission is ingestion, and meat is considered to be a major vehicle for human and animal infection. As *T. gondii* is environmentally transmissible, other important vehicles in particular for animals include vegetation, soil and water. The seroprevalence of *T. gondii* infection in pigs is Serbia has been determined in several studies over the past two decades. It has been established that it varies considerably, primarily based on husbandry, with strictly to mostly indoor animals having a lower prevalence (below 20%) than animals raised outdoors, where prevalence exceeds 60%. Experimental data suggests that different genotypes of the parasite vary in virulence, but the significance of virulence in terms of pathology and disease manifestations is still being investigated. Genotypes of *T. gondii* isolated from pig tissues in Serbia to date are ToxoDB#1 (archetype II) and ToxoDB#2 (archetype III). Archetype II is predominant and, based on historical reports and recent findings, low to intermediately virulent. The virulence phenotype and mechanisms of archetype III, however, have not been extensively studied, but recent data suggests that its virulence may vary considerably. This review will also summarize the current knowledge regarding the virulence of archetypes II and III and evaluate it in the context of the pig host.

1. Introduction

Toxoplasma gondii is a food and waterborne protozoan parasite of notable significance to public health, as it can infect all warm-blooded species and cause chronic disease, ranging from mild in immunocompetent hosts, up to severe in hosts with inadequate (newborns), or suppressed immunity (*Djurković-Djaković*, 1998; *Montoya and Liesenfeld*, 2004; *Bouwknegt et al.*, 2018). Host immunity alone, however, is not sufficient to predict disease severity, as pathology depends significantly on the genotype of the parasite, which is defined most commonly by multilocus (11 loci) restriction fragment length polymorphism analysis (PCR-RFLP), multi-locus sequence typing (MLST) (10–11 loci) and microsatellite typing (15 loci) (*Ajzenberg et al.*, 2010; *Su et al.*, 2010). Aside from these defined loci, other undefined and unique single nucleotide polymorphisms (SNPs) scattered throughout the genome

***Corresponding author:** Ivana Klun, *iklun@imi.bg.ac.rs*

Paper received August 5th 2023. Paper accepted August 20th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

can endow individual strains with slightly different biological characteristics which modify their virulence for particular hosts. Domestic animals raised for meat are of paramount importance for the transmission of the parasite to humans and are, thus, often the focus of epidemiological studies to determine *T. gondii* infection prevalence and if possible, isolate strains and identify the genotype. The lion's share of the data on genotypes from domestic animals in Serbia are based on isolates from pigs (15/70), which are only two genotypes, ToxoDB#1 (archetype II) and ToxoDB#2 (archetype III) which may have varying degrees of virulence. Symptoms of the disease are generally non-specific and can occur only during the short acute phase of infection, while the chronic phase is clinically inapparent. Aside from known outcomes of infection in most animal species, such as abortion and/or stillbirth which can result from a primary infection in early gravidity, chronic disease in pigs is thought to be essentially without sequelae. This review will summarize our current knowledge regarding *T. gondii* infection with ToxoDB#1 and ToxoDB#2 in pigs.

1.1. Life cycle of T. gondii

The life cycle is complex and involves intermediate and definitive hosts (Felidae only) and progression through three infective life stages, the tachyzoite, the bradyzoite and the sporozoite (*Hutchison et al*., 1969, *Tenter et al*., 2001). Tachyzoites, which are essential for infecting host cells and propagation through tissues, can only be naturally transmitted vertically (from the mother to the foetus), while bradyzoites and sporozoites are transmitted by food and water. In all hosts, tachyzoites convert to bradyzoites after several rounds of asexual division and form cysts, most often located in neurons and myocytes. Tissue cysts increase in size slowly as the bradyzoites divide and persist essentially for the lifetime of the host. Oocysts form after sexual reproduction of the parasite in the gut of the definitive hosts and are shed in faeces, while maturation (sporulation) of oocysts, which facilitates the development of sporocysts and sporozoites, occurs naturally in the environment (*Frenkel et al.* 1970, F*erguson et al*., 1974). Definitive hosts are solely responsible for the contamination of the environment with oocysts, while the robustness of the oocyst wall ensures their maturation and survival after various physical and chemical stresses (*Yilmaz and Hopkins*, 1972, *Dubey*, 1998).

1.2. Transmission modes and vehicles

T. gondii is naturally transmitted by ingestion only or is transmitted vertically from the mother to the foetus (*Tenter et al.,* 2001). Other ways of direct host-to-host transmission include organ transplantation and transfusions. Meat (muscle tissue) containing tissue cysts has repeatedly been shown to be the major source of human infection in Europe, while vegetables and greens contaminated with oocysts are secondary and water is an exceedingly rare vehicle (*Bobić et al.*, 1998, *Deng et al.*, 2021). As bradyzoites can be killed by heat, freezing and salting (dehydration), human infection is due to improper preparation of meat and/or meat products or culinary habits, such as consumption of raw meat dishes, or the inadequate washing/cleaning of vegetables and greens, that fail to remove oocysts. In animals, meat is a primary vehicle for strict carnivores, while strict grazers acquire infection from vegetation and/or soil and omnivores and birds may be infected via either. Water is a common vehicle for all animals. Commercial diagnostic kits for the detection of specific anti-*T. gondii* antibodies in sera are not capable of distinguishing whether infection has occurred due to ingestion of bradyzoites or sporozoites, and thus, the vehicle and source of infection are mainly inferred through epidemiological questionnaires and evidence from scientific studies on the presence of the parasite in the environment. Determining transmission routes and attributing the source of infection have been shown to be important in explaining uncharacteristically severe disease and pathology in several human cases around the globe (*Carme et al*., 2009; *Schumacher et al*., 2021).

1.3. Population structure and genotype virulence

Populations of genotypes of *T. gondii* which circulate in the domestic and sylvatic environment are distinct, and sylvatic genotypes appear to be more virulent (*Dardé*, 2008; *Galal et al*., 2019). Although the genus *Toxoplasma* consists of a single species, the global population structure is complex, with over three hundred genotypes derived from multiple lineages, grouped into six clades (*Shwab et al*., 2014). The three globally distributed lineages (types) are I, II and III represented by reference genotypes ToxoDB#10, ToxoDB#1 and ToxoDB#2, respectively, also known as the archetypes. Archetype II (ToxoDB#1) is the most frequent genotype isolated from humans and animals in Europe,

followed by archetype III (ToxoDB#2), while archetype I (ToxoDB#10) is extremely rare. In Serbia, archetype II (ToxoDB#1) represents 33% of the genotype population in intermediate hosts, while archetype III (ToxoDB#2) represents 9% (*Uzelac et al.*, 2021). The three archetypes differ in virulence, and their pathology phenotype, lethal dose (LD) and mortality in laboratory mice has historically served to experimentally define the three virulence phenotypes: low (archetype III)-$LD10^5$, up to 30% mortality; intermediate (archetype II)-$LD10^3$, 30–99% mortality; and acute (archetype I)-$LD10^1$, 99–100% mortality (*Su et al.*, 2002). Although the phenotype definition is based on a single host species, the archetypes' virulence can be replicated in other species, but not the LD or mortality. However, recent findings suggest that perhaps only the acute phenotype is well defined, as the virulence phenotype and parameters of all of the global archetype I isolates are nearly identical in most laboratory mouse strains, while the virulence phenotype and parameters of the other two archetypes can differ from the definition.

1.4. Virulence of ToxoDB#1 and ToxoDB#2

Recently, the virulence phenotype of a ToxoDB#1 isolate from human fluids in Serbia was shown to be low, without mortality in Swiss-Webster mice, while an isolate of ToxoDB#2 from a free-ranging Iberian pig in Spain (TgPigSp5) was shown to have an intermediately virulent phenotype with nearly 90% mouse mortality, which is exceedingly high (*Fernández-Escobar et al.,* 2020; *Uzelac et al.,* 2020). Some of the differences can be attributed to adaptations induced by laboratory conditions like in vitro culture in rapidly growing cell lines and repeated freezing and thawing of parasite stocks over many years, all of which can change the replication rate, an important factor which contributes to virulence (*Saeij et al*., 2005). This could be the most plausible explanation for this Serbian isolate, given that it was tested against a laboratory-adapted strain, the reference strain ME49, which has a much higher proliferation rate than most other ToxoDB#1 isolates and thus may cause greater mortality. Interestingly, however, the virulence of archetype III may be naturally variable, as both the highly virulent TgPigSp5 and another isolate from a sheep in Spain (TgShSp24), which killed over 20% of mice, were low-passage isolates with different growth rates, thus excluding laboratory adaptation as a possible confounding factor (*Fernández-Escobar et al.*, 2020; *Fernández-Escobar et al.*, 2021).

1.5 Virulence in the pig host

Archetype III (ToxoDB#2) is less frequently isolated from human fluids and tissues but seems to be globally frequent in animals. In Serbia, archetype III (ToxoDB#2) has been detected in pigs, golden jackals and foxes, while archetype II has been found in all thus far examined host species, wild and domestic, including humans. Archetype II (ToxoDB#1) is predominant in pigs, which is expected given the population structure of *T. gondii* in Serbia (Table 1). Pigs are an important link in the transmission of *T. gondii* to humans, as they are

Table 1. *Toxoplasma gondii* seroprevalence and population structure in pigs in Serbia

Sample origin	N of pigs	Seroprevalence (%)	N of isolates	Genotype	References
Farms; Abattoirs	605	28.9	Not stated	-	*Klun et al.,* 2006
Abattoirs	488	9.2	Not stated	-	*Klun et al.,* 2011
Private slaughterhouse	18	66.7	3	ToxoDB#1 (n=3)	*Kuruca et al.,* 2016; 2019
Abattoirs	182	17	6	ToxoDB#1 (n=4) ToxoDB#2 (n=2)	*Kuruca et al.,* 2017; 2019
Abattoirs	825	16.5	6	ToxoDB#1 (n=6)	*Betić et al.,* 2022; *Uzelac et al.,* 2023
TOTAL	**2118**	**18.8**	**15**	**ToxoDB#1 (n=13)** **ToxoDB#2 (n=2)**	

omnivorous and, thus, can ingest both tissue cysts and oocysts, and depending on husbandry, may traverse between the domestic and sylvatic environments (*Klun et al.*, 2011; *Kuruca et al.*, 2017; *Uzelac et al.,* 2023).

2. Materials and methods

All published papers on *Toxoplasma gondii* infection in pigs in Serbia in the last 17 years have been included in this review and the data on seroprevalence and isolated parasite strains have been re-analysed in the context of interpreting *T. gondii* archetype II and III virulence in the pig host.

3. Results and discussion

The cumulative seroprevalence of *T. gondii* infection in Serbian pigs is 18.8%. The lowest seroprevalence of 9.2% was observed in market-weight pigs slaughtered in the Belgrade area abattoirs (*Klun et al.,* 2011), and the highest, of 66.7%, in an outdoor herd of indigenous Mangulitsa breed pigs butchered at a private slaughterhouse (*Kuruca et al.,* 2016). To date, 15 isolates have been obtained from pig tissues, and only two genotypes, archetype II (ToxoDB#1) and archetype III (ToxoDB#2), have been identified (*Kuruca et al.,* 2019; *Uzelac et al.,* 2023).

All sampled pigs were visibly healthy, i.e., in addition to being accompanied with a valid transport manifest and veterinary health certificate, they had been examined for the presence of clinical signs prior to slaughter. The fact that a certain number of animals harboured latent *T. gondii* infection is not surprising, since a number of research studies have shown that adult pigs in the majority of cases do not develop overt clinical signs of toxoplasmosis, thus appearing healthy at routine health check-up. Globally, pigs, especially adults, are considered fairly resistant to toxoplasmosis (*Stelzer et al*., 2019), with the exception of several recent reports from China, and one from Italy, where clinical disease was observed, with dyspnoea being the most common symptom (*Dubey et al*., 2020). Suckling piglets are more susceptible, and in pregnant sows infection can cause congenital disease and piglet mortality.

There are, however, sporadic reports on clinical toxoplasmosis in pigs from Europe. Severe cases of toxoplasmosis were reported nearly 30 years ago on fattening farms in Italy, where pigs exhibited fever, depression and cyanosis, followed by death 2–4 days later (*Gelmetti et al.,* 1999). Another more recent case was recorded in Germany, with dyspnoea and sudden death in a young pig, which was, however, concurrently infected with porcine circovirus-2 (*Klein et al.,* 2010). Unfortunately, as parasite isolation and genotyping was not performed, it is impossible to know whether these were caused by the two archetypes, II and III. All other reported clinical cases in pigs originate from China or South America, where atypical and/or highly virulent *T. gondii* strains are known to circulate (*Galal et al.,* 2019; *Dubey et al.,* 2020). Circulation of atypical, recombinant and variant genotypes, possibly virulent to certain hosts, in wildlife in Europe has been reported (*Fernández-Escobar et al.,* 2022). As *T. gondii* is a generalist parasite (i.e., of low host specificity), it is expected that infected intermediate hosts of any species may transmit the infection to another, provided the chance. Pigs arguably may be particularly susceptible to wildlife strains as outdoor rearing and semi-feral husbandry is practiced in many countries in Europe, thus allowing for strain transfer between ecosystems. A recent report on *T. gondii* infection in wild boars in Italy identified the presence of atypical genotypes, thus possibly explaining some of the deaths in earlier years (*Sgroi et al*., 2020).

Genotyping of parasite strains or their DNA in asymptomatic slaughter animals was performed in several studies. In Portugal, as in Serbia, type II and III were detected (*de Sousa et al.*, 2006). In France (*Djokic et al.*, 2016), Czech Republic (*Slany et al*, 2016) and Belgium (*Gisbert Algaba et al.*, 2020), only archetype II was detected; while in Poland, only partial genotyping was accomplished (*Sroka et al*., 2020). Interestingly, several studies in Italy, including both those with complete and incomplete typing, showed or indicated the presence of more abundant genetic diversity, with DNA from all global lineages (I, II, III) detected in pigs from organic as well from intensive type farms (*Bacci et al*., 2015; *Papini et al*., 2017; *Santoro et al*., 2017; *Pipia et al.,* 2018; *Vergara et al*., 2018).

Of note, in cases of abortion, stillbirth, or premature birth of piglets, the aetiological agent is sometimes unidentified. However, even if *T. gondii* infection had been detected by serology or at necropsy in these cases, parasite isolation is not routinely performed, and therefore, there is no possibility to identify potential genotypes. Genotyping is a technique still associated primarily with scientific research and is not a part of a diagnostic and/or pathological workup, even in human cases, while exper-

imental protocols for virulence determination have only been standardized a few years ago (*Saraf et al.*, 2017). In Serbia, there are no records of *T. gondii*-induced clinical or congenital disease in pigs to date, despite a rather high prevalence particularly in some herds. However, parasite isolation, genotyping and experimental determination of virulence would need to be performed in order to confirm disease aetiology and ascertain the virulence of certain genotypes for pigs. Thus, major conceptual and practical changes in the current standards in pig rearing and veterinary check-ups are necessary in order to raise farmer awareness of *T. gondii* infection and communicate the importance of diagnostics in cases of abortion and mortality among piglets and adults, in order to gain a better understanding of the true cost of toxoplasmosis in pigs. Moreover, once again, the importance of an interdisciplinary approach and cooperation between scientists, veterinarians, policy makers and pig farmers is emphasized.

All *T. gondii* genotypes cause infection that can be clinically severe in the immunologically incompetent, including the foetus (congenital toxoplasmosis). As viable tissue cysts have been isolated from practically all muscles and organs of experimentally infected pigs (*Dubey*, 1988), and up to day 875 post infection, which is much longer than the age at which market-weight pigs are brought to slaughter (at 6–8 months), these data point to a realistic and very present risk of infection for the consumers.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The work was supported by grants from the Ministry of Education, Science and Technological Development of the Republic of Serbia to the Institute for Medical Research (project III 41019; contracts 451-03-68/2022-14/200015 and 451-03-47/2023-01/200015), to the Institute of Meat Hygiene and Technology (contracts 451-03-68/2022-14/200050 and 451-03-47/2023-01/200050), and to the Faculty of Veterinary Medicine, Belgrade (contracts 451-03-68/2022-14/200143 and 451-03-47/2023-01/200143).

References

Ajzenberg, D., Collinet, F., Mercier, A., Vignoles, P. & Dardé, M. L. (2010). Genotyping of *Toxoplasma gondii* isolates with 15 microsatellite markers in a single multiplex PCR assay. *Journal of Clinical Microbiology*, 48, 4641–4645, https://doi.org/10.1128/JCM.01152-10

Bacci, C., Vismarra, A., Mangia, C., Bonardi, S., Bruini, I., Genchi, M., Kramer, L. & Brindani, F. (2015). Detection of *Toxoplasma gondii* in free-range, organic pigs in Italy using serological and molecular methods. *International Journal of Food Microbiology*, 202, 54–56, https://doi.org/10.1016/j.ijfoodmicro.2015.03.002

Betić, N., Karabasil, N., Djurković-Djaković, O., Ćirković, V., Bobić, B., Branković Lazić, I., Djordjević, V. & Klun, I. (2022). Seroprevalence, direct detection and risk factors for *Toxoplasma gondii* infection in pigs in Serbia, and influence of biosecurity measures. *Microorganisms*, 10, 1069, https://doi.org/10.3390/microorganisms10051069

Bobić, B., Klun, I., Nikolić, A. & Djurković-Djaković, O. (2012). *Toxoplasma gondii* infection in South-East Europe: Epidemiology and epizootiology. In O. Djurković-Djaković (Ed.), Toxoplasmosis – Recent Advances (pp. 37–54). InTech, Rijeka, Croatia. ISBN: 978-953-51-0746-0

Bouwknegt, M., Devleesschauwer, B., Graham, H., Robertson, L. J., van der Giessen, J. W. & Euro-FBP workshop participants. (2018). Prioritisation of food-borne parasites in Europe, 2016. *Eurosurveillance*, 23, 17-00161, https://doi.org/10.2807/1560-7917.ES.2018.23.9.17-00161

Carme, B., Demar, M., Ajzenberg, D. & Dardé, M. L. (2009). Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. *Emerging Infectious Diseases*, 15, 656–658, https://doi.org/10.3201/eid1504.081306

Dardé, M. L. (2008). *Toxoplasma gondii*, "new" genotypes and virulence. *Parasite*, 15, 366–371, https://doi.org/10.1051/parasite/2008153366

Deng, H., Cummins, R., Schares, G., Trevisan, C., Enemark, H., Waap, H., Srbljanovic, J., Djurkovic-Djakovic, O., Pires, S.M., van der Giessen, J. W. B. & Opsteegh, M. (1998). Mathematical modelling of *Toxoplasma gondii* transmission: A systematic review. *Food and Waterborne Parasitology*, 22, e00102, doi: 10.1016/j.fawpar.2020.e00102

Dubey, J. P. (1988). Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *T. gondii* oocysts and effect of freezing on viability of tissue cysts in pork. *American Journal of Veterinary Research*, 49, 910–913.

Dubey, J. P. (1998). *Toxoplasma gondii* oocyst survival under defined temperatures. *Journal of Parasitology*, 84, 862–865.

Dubey, J. P., Cerqueira-Cézar, C. K., Murata, F. H. A., Kwok, O. C. H., Hill, D., Yang, Y. & Su, C. (2020). All about *Toxoplasma gondii* infections in pigs: 2009–2020. *Veterinary Parasitology*, 288, 109185, https://doi.org/10.1016/j.vetpar.2020.109185

Djokic, V., Fablet, C., Blaga, R., Rose, N., Perret, C., Djurkovic-Djakovic, O., Boireau, P. & Durand, B. (2016). Factors associated with *Toxoplasma gondii* infection in confined farrow-to-finish pig herds in western France: an exploratory study in 60 herds. *Parasites & Vectors*, 9, 466, https://doi.org/10.1186/s13071-016-1753-5

Djurković-Djaković, O. (1998). Toksoplazmoza i imunosupresija [Toxoplasmosis and immunosuppression]. *Srpski Arhiv Celokupnog Lekarstva*, 126, 197–203.

Fernández-Escobar, M., Calero-Bernal, R., Regidor-Cerrillo, J., Vallejo, R., Benavides, J., Collantes-Fernández, E. & Ortega-Mora, L. M. (2020). Isolation, genotyping, and mouse virulence characterization of *Toxoplasma gondii* from free ranging Iberian pigs. *Frontiers in Veterinary Science*, 7, 604782, https://doi.org/10.3389/fvets.2020.604782

Fernández-Escobar, M., Calero-Bernal, R., Regidor-Cerrillo, J., Vallejo, R., Benavides, J., Collantes-Fernández, E. & Ortega-Mora, L. M. (2021). In vivo and in vitro models show unexpected degrees of virulence among *Toxoplasma gondii* type II and III isolates from sheep. *Veterinary Research*, 52, 82, https://doi.org/10.1186/s13567-021-00953-7

Fernández-Escobar, M., Schares, G., Maksimov, P., Joeres, M., Ortega-Mora, L.M. & Calero-Bernal, R. (2022). *Toxoplasma gondii* genotyping: A closer look into Europe. *Frontiers in Cell Infection and Microbiology*, 12, 842595, https://doi.org/10.3389/fcimb.2022.842595

Ferguson, D. J., Hutchison, W. M., Dunachie, J. F. & Siim, J. C. (1974). Ultrastructural study of early stages of asexual multiplication and microgametogony of *Toxoplasma gondii* in the small intestine of the cat. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica,* 82, 167–181, https://doi.org/10.1111/j.1699-0463.1974.tb02309.x

Frenkel, J. K., Dubey, J. P. & Miller, N. L. (1970). *Toxoplasma gondii* in cats: Fecal stages identified as coccidian oocysts. *Science,* 167, 893–896, https://doi.org/10.1126/science.167.3919.893

Galal, L., Hamidovic, A., Dardé, M. L. & Mercier, A. (2019). Diversity of *Toxoplasma gondii* strains at the global level and its determinants. *Food and Waterborne Parasitology*, 15, e00052, https://doi.org/10.1016/j.fawpar.2019.e00052

Gelmetti, D., Sironi, G., Finazzi, M., Gelmini, L., Rosignoli, C., Cordioli, P. & Lavazza, A. (1999). Diagnostic investigations of toxoplasmosis in four swine herds. *Journal of Veterinary Diagnostic Investigation*, 11, 87–90, https://doi.org/10.1177/104063879901100114

Hutchison, W. M., Dunachie, J. F., Siim, J. C. & Work, K. (1969). Life cycle of *Toxoplasma gondii. British Medical Journal,* 4, 806, https://doi.org/10.1136/bmj.4.5686.806-b

Klein, S., Wendt, M., Baumgärtner, W. & Wohlsein, P. (2010). Systemic toxoplasmosis and concurrent porcine circovirus-2 infection in a pig. *The Journal of Comparative Pathology*, 142, 228–234, https://doi.org/10.1016/j.jcpa.2009.08.155

Klun, I., Djurković-Djaković, O., Katić-Radivojević, S. & Nikolić, A. (2006). Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Veterinary Parasitology*, 135, 121–131, https://doi.org/10.1016/j.vetpar.2005.08.010

Klun, I., Vujanić, M., Yera, H., Nikolić, A., Ivović, V., Bobić, B., Bradonjić, S., Dupouy-Camet, J. & Djurković-Djaković, O. (2011). *Toxoplasma gondii* infection in slaughter pigs in Serbia: Seroprevalence and demonstration of parasites in blood. *Veterinary Research*, 42(1), 17, https://doi.org/10.1186/1297-9716-42-17

Kuruca, Lj., Klun, I., Uzelac, A., Nikolić, A., Bobić, B., Simin, S., Djurković-Djaković, O. & Lalošević, V. (2016). Detection of viable *Toxoplasma gondii* in free-range pigs from the special nature reserve of Zasavica. *Contemporary Agriculture*, 65, 1–6, https://doi.org/10.1515/contagri-2016-0010

Kuruca, Lj., Klun, I., Uzelac, A., Nikolić, A., Bobić, B., Simin, S., Lalošević, V., Lalošević, D. & Djurković-Djaković O. (2017). Detection of *Toxoplasma gondii* in naturally infected domestic pigs in Northern Serbia. *Parasitology Research*, 116, 3117–3123, https://doi.org/10.1007/s00436-017-5623-7

Kuruca, Lj., Uzelac, A., Klun, I., Lalošević, V. & Djurković-Djaković, O. (2019). *Toxoplasma gondii* genotypes circulating in domestic pigs in Serbia. *Acta Veterianria Hungarica*, 67, 204–211, https://doi.org/10.1556/004.2019.022

Montoya, J. G. & Liesenfeld, O. (2004). Toxoplasmosis. *Lancet*, 363, 1965–1976, 10.1016/S0140-6736(04)16412-X

Papini, R., di Ciccio, P., Marangi, M., Ghidini, S., Zanardi, E., Vergara, A., Giangaspero, A., Nardoni, S., Rocchigiani, G., Mancianti, F. & Ianieri A. (2017). Occurrence of *Toxoplasma gondii* in carcasses of pigs reared in intensive systems in Northern Italy. *Journal of Food Protection.* 80, 515–522, https://doi.org/10.4315/0362-028X.JFP-16-314

Pipia, A. P., Varcasia, A., Dessì, G., Panzalis, R., Gai, C., Nonnis, F., Veronesi, F., Tamponi, C. & Scala, A. (2018). Seroepidemiological and biomolecular survey on *Toxoplasma gondii* infection on organic pig farms. *Parasitology Research* 117, 1637–1641, https://doi.org/10.1007/s00436-018-5823-9

Saeij, J. P. J., Boyle, J. P. & Boothroyd, J.C. (2005). Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. *Trends in Parasitology*, 21, 476–481, https://doi.org/10.1016/j.pt.2005.08.001

Santoro, A., Veronesi, F., Milardi, G. L., Ranucci, D., Branciari, R., Diaferia, M. & Gabrielli, S. (2017). Sequence variation in the B1 gene among *Toxoplasma gondii* isolates from swine and cats in Italy. *Research in Veterinary Science,* 115, 353–355, https://doi.org/10.1016/j.rvsc.2017.06.025

Saraf, P., Shwab, E. K., Dubey, J. P. & Su C. (2017). On the determination of *Toxoplasma gondii* virulence in mice. *Experimental Parasitology*, 174, 25–30, https://doi.org/10.1016/j.exppara.2017.01.009

Schumacher, A. C., Elbadawi, L. I., DeSalvo, T., Straily, A., Ajzenberg, D., Letzer, D., Moldenhauer, E., Handly, T. L., Hill, D., Dardé, M. L., Pomares, C., Passebosc-Faure, K., Bisgard, K., Gomez, C.A., Press, C., Smiley, S., Montoya, J. G. & Kazmierczak, J. J. (2021). Toxoplasmosis outbreak associated with *Toxoplasma gondii*-contaminated venison – high attack rate, unusual clinical presentation, and atypical genotype. *Clinical Infectious Diseases*, 72, 1557–1565, https://doi.org/10.1093/cid/ciaa285

Sgroi, G., Viscardi, M., Santoro, M., Borriello, G., D'Alessio, N., Boccia, F., Pacifico, L., Fioretti, A., Veneziano V. & Fusco, G. (2020). Genotyping of *Toxoplasma gondii* in wild boar (*Sus scrofa*) in southern Italy: Epidemiological survey and associated risk for consumers. *Zoonoses and Public Health*, 67(7), 80513, https://doi.org/10.1111/zph.12762

Shwab, E., Zhu, X., Majumdar, D., Pena, H., Gennari, S., Dubey, J. & Su, C. (2014). Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. *Parasitology*, 141, 453–461, https://doi.org/10.1017/S0031182013001844

Slany, M, Reslova, N, Babak, V. & Lorencova, A. (2016). Molecular characterization of *Toxoplasma gondii* in pork meat from different production systems in the Czech Republic. *International Journal of Food Microbiology,* 238, 252–255, https://doi.org/10.1016/j.ijfoodmicro.2016.09.020

de Sousa, S., Ajzenberg, D., Canada, N., Freire, L., Correia da Costa, J. M., Dardé, M. L., Thulliez, P. & Dubey, J. P. (2006). Biologic and molecular characterization of *Toxoplasma gondii* isolates from pigs from Portugal. *Veterinary Parasitology*, 135, 133–136, https://doi.org/10.1016/j.vetpar.2005.08.012

Sroka, J., Karamon, J., Wójcik-Fatla, A., Piotrowska, W., Dutkiewicz, J., Bilska-Zając, E., Zając, V., Kochanowski M., Dąbrowska J. & Cencek T. (2020). *Toxoplasma gondii* infection in slaughtered pigs and cattle in Poland: seroprevalence, molecular detection and characterization of parasites in meat. *Parasites & Vectors*, 13, 223, https://doi.org/10.1186/s13071-020-04106-1

Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L. M., Maksimov, P., Gethmann, J., Conraths, F. J. & Schares, G. (2019). *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. *Food and Waterborne Parasitology*, 15, e00037, https://doi.org/10.1016/j.fawpar.2019.e00037

Su, C., Howe, D. K., Dubey, J. P., Ajioka, J. W. & Sibley, L. D. (2002). Identification of quantitative trait loci controlling acute virulence in *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 10753–10758, https://doi.org/10.1073/pnas.172117099

Su, C., Shwab, E. K., Zhou, P., Zhu, X. Q. & Dubey, J. P. (2010). Moving towards and integrated approach to molecular detection and identification of *Toxoplasma gondii*. *Parasitology*, 137, 1–11, https://doi.org/10.1017/S0031182009991065

Tenter, A. M., Heckeroth, A. R. & Weiss, L. M. (2001). *Toxoplasma gondii*: From animals to humans. *International Journal of Parasitology*, 30, 1217–1258, https://doi.org/10.1016/s0020-7519(00)00124-7. Erratum in: *International Journal of Parasitology*, 31, 217–220.

Uzelac, A., Klun, I., Ćirković, V. & Djurković-Djaković, O. (2020). In vivo and in vitro virulence analysis of four genetically distinct *Toxoplasma gondii* lineage III isolates. *Microorganism*s, 8, 1702, https://doi.org/10.3390/microorganisms8111702

Uzelac, A., Klun, I., Ćirković, V., Bauman, N., Bobić, B., Štajner**, T., Srbljanović, J., Lijeskić, O. & Djurković-Djaković, O. (2021).** *Toxoplasma gondii* genotypes circulating in Serbia – insight into the population structure and diversity of the species in Southeastern Europe, a region of intercontinental strain exchange. *Microorganisms*, 9, 2526, https://doi.org/10.3390/microorganisms9122526

Uzelac, A., Betić, N., Karabasil, N., Ćirković, V., Djurković-Djaković, O. & Klun, I. (2023). A pork industry in the backyard: an analysis of *Toxoplasma gondii* infection in Serbia's pigs. *Microorganisms*, 11, 1857, https://doi.org/10.3390/microorganisms11071857

Vergara, A., Marangi, M., Caradonna, T., Pennisi, L., Paludi, D., Papini, R., Ianieri, A., Giangaspero, A. & Normanno, G. (2018). *Toxoplasma gondii* lineages circulating in slaughtered industrial pigs and potential risk for consumers. *Journal of Food Protection.* 81, 1373–1378, https://doi.org/10.4315/0362-028X.JFP-17-496

Yilmaz, S. M. & Hopkins, S. H. (1972). Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. *Journal of Parasitology*, 58, 938–939.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.4.09:[616.98:579.8

ID: 126541321

https://doi.org/10.18485/meattech.2023.64.2.15

Original scientific paper

Detection of pathogenic Yersinia enterocolitica strains in pre-packed fresh pork minced meat — preliminary data

Sead Hadžiabdić[a], Dörte Haase[a], Andrea Barać[a], Stefan Hertwig[a] and Anja Buschulte[a]*

[a] German Federal Institute for Risk Assessment, Department Biological Safety, Unit Food Hygiene and Technologies, Supply Chains, Food Defense, Diedersdorfer Weg 1, 12277, Berlin, Germany

ARTICLE INFO

Keywords:
Yersinia enterocolitica
Pork minced meat
NGS analysis

ABSTRACT

Yersinia plays an important role as the third most common zoonotic agent of human gastrointestinal diseases in the European Union (EU). Most frequently, an infection in humans is caused by *Yersinia (Y.) enterocolitica*, whereas *Y. pseudotuberculosis* is mainly pathogenic for animals and less frequently associated as a causative agent of foodborne yersiniosis. Pigs are common carriers of *Y. enterocolitica*, which can lead to contamination of pork meat during the slaughter process. In our study, we investigated the occurrence of pathogenic *Y. enterocolitica* strains in pre-packed fresh pork minced meat from supermarkets in Southern Berlin and Brandenburg. In 17 of 104 samples *Y. enterocolitica* was detected by applying two ISO methods (ISO 10273:2017 and CEN ISO/TS 18867). With Illumina short read sequencing technology, an initial insight into genomic properties of selected *Y. enterocolitica* strains was obtained. Further characterisation and sequencing of additional *Y. enterocolitica* strains obtained within this study is planned.

1. Introduction

According to European Food Safety Authority (EFSA), yersiniosis was the third most commonly reported bacterial zoonosis, with 6,789 confirmed human cases, reported by 26 member states (MS) in 2021. The EU notification rate was 1.9 cases per 100,000 population, with an increase of 11.8% compared to 2020 (1.7 per 100,000 population) (*EFSA and ECDC*, 2022). As for Germany, 1,873 cases were reported in 2020. This corresponded to an incidence of 2.3 cases per 100,000 population. The majority of these cases (99%) were caused by *Yersinia (Y.) enterocolitica*, predominantly by the bio/serotype 4/ O:3 (82%) (RKI, 2020).

Pigs are commonly hosts of *Y. enterocolitica*, and this can lead to the contamination of raw pork meat during the slaughter and processing process. A study from Germany in 2018 revealed that among 253 samples of raw minced pork meat and pork meat preparations, *Y. enterocolitica* was detected in approximately one of ten samples (9.5%) (*Verbraucherschutz Sachsen-Anhalt*, 2018). On the other hand, *Y. pseudotuberculosis* is frequently associated with its reservoirs, birds and wild animals (*BfR*, 2013). Outbreaks with *Y. pseudotuberculosis* are mostly linked to raw vegetables and ready-to-eat vegetable products, such as lettuce and carrots, with long periods of cold storage. Although *Y. enterocolitica* is often associated with pork meat, an increasing number of outbreaks are also sporadically linked to vegetables (*ECDC*, 2022). Each of these two *Yersinia* species plays an important role as the causative

***Corresponding author:** Anja Buschulte, *anja.buschulte@bfr.bund.de*

Paper received Jun 22nd 2023. Paper accepted July 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

agent of gastrointestinal yersiniosis. Since *Yersinia* can multiply even at 4°C, it is important to reduce the food contamination significantly. The bacteria are mainly found in the tonsils, lymph nodes and intestines of pigs. As pigs are frequently asymptomatic carriers, contamination during the slaughter process must be avoided as much as possible (*Fredriksson-Ahomaa*, 2012; *BfR*, 2013). In humans, symptoms such as fever and abdominal pain in the right lower abdomen can appear after an incubation period of 4–10 days. In children, bloody diarrhoea can occur as an additional symptom (*EFSA and ECDC*, 2022).

Within the species *Y. enterocolitica*, six biovars are distinguished (1A, 1B, 2, 3, 4, 5). Strains belonging to biovar 1B to 5 possess a virulence plasmid (pYV) and virulence factors, such as enterotoxin YstA, and are considered pathogenic (obligate human pathogen). The biovar 1A strains lack the virulence plasmid; however, they possess the heat-stable enterotoxin (YstB), relevant to the development of diarrhoea (*RKI*, 2021). Although it is assumed that biovar 1A is non-pathogenic, there are indications that certain 1A strains can cause gastrointestinal symptoms as observed in pYV-bearing strains (*Tennant et al*., 2003). Biovar 4 (serotype O:3) and biovar 2 (serotype O:9) are most frequently associated with human yersiniosis in Europe.

In our study, we investigated the occurrence of pathogenic *Y. enterocolitica* strains in 104 samples of pre-packed fresh pork minced meat by applying two ISO methods (ISO 10273:2017 (*ISO*, 2017) and CEN ISO/TS 18867 (*ISO*, 2015). Using Illumina NGS sequencing, we gained initial insight into the genomic properties (such as multilocus sequence typing (MLST) types, antimicrobial resistance (AMR) and virulence genes) in a selection of *Y. enterocolitica* strains. Further serovar and genomic characterisation of current and additional *Y. enterocolitica* strains obtained within this study will follow.

2. Materials and methods

From May to December 2021, in total, 104 pre-packed fresh pork minced meat packages, ranging in weight from 250 grams (g) to 1 kilogram (kg), were purchased in 11 different supermarkets in Southern Berlin and Brandenburg. The samples were transported chilled and submitted to microbiological analysis within the same day. Isolated *Y. enterocolitica* strains were preserved in cryoprotective medium at –80°C for later molecular analysis.

2.1 Isolation and biotyping of Y. enterocolitica strains

The isolation and biotyping of pathogenic *Y. enterocolitica* strains was conducted according to *ISO* (2017). Briefly, 25 g of sample was tenfold diluted in PSB-Bouillon (Peptone Sorbitol Bile Broth) and 1 (mL) was plated directly onto 4 CIN (Cefsulodin-Irgasan-Novobiocin) agar plates and incubated at 30°C for 24 ± 2 hours (h). Additionally, 10 mL were added to 90 mL of ITC (Irgasan-Ticarcillin-Chlorat-Bouillon) as a second enrichment medium. Both enrichment broths were incubated at 25°C for 44 h ± 4 h. From these two enrichment media, 0.5 mL were added to 4.5 mL of 0.5% potassium hydroxide (KOH) for 20 ± *5* seconds (sec.). After the treatment, the CIN agar plates were inoculated and incubated as previously described. The CIN agar plates were investigated under a magnifier to detect characteristic bull's eye colonies of *Y. enterocolitica*. Characteristic colonies were biotyped by testing their reaction to aesculin/salicin, xylose, pyrazinamidase, tween-esterase/lipase, trehalose and indole.

For further confirmation, the MALDI-TOF system was used by transferring a small proportion of the suspect colony from TSA (Tryptic Soy Agar) directly onto a MALDI-TOF target plate and adding 1 microliter (μL) of HCCA matrix. Further steps and the use software were conducted according to the manufacturer's instructions.

To investigate the pathogenic potential of the isolated *Y. enterocolitica* strains, thermal lysis of the selected strains followed by real time PCR, targeting a sequence of the *ail* gen according to CEN ISO/TS 18867 (*ISO*, 2015), was conducted. For specific details on real time PCR, please refer to CEN ISO/TS 18867.

2.2 Illumina whole genome sequencing

One colony of presumptive Y. *enterocolitica* strain was inoculated into 4 mL of Luria Bertani Broth and incubated overnight while gently shaking at 37°C. The following day, the suspension was centrifuged at 13,000 rpm for 5 minutes, and the bacterial pellet was stored at −20°C for the later DNA isolation.

The genomic DNA was extracted using Purelink Genomic DNA Kit according to the manufacturer's instructions. The ILMN DNA LP (M) Tagmentation Kit (96Samples) Beads + Buffers were used for preparing the Illumina sequencing library

Table 1. MLST, AMR and virulence genes of selected *Y. enterocolitica* strains

Strain ID	Total length (bp)	MLST	AMR genes	Some virulence genes
LM00140	4,480,285	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*
LM00141	4,474,096	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*
LM00150	4,437,788	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*
LM00196	4,480,780	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*
LM00921	4,457,321	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*
LM00930	4,475,386	135	*bla*A,*vat*(F)	*yst*A, *vir*F, *ysc*, *yop*

with Nextera™ DNA CD Indexes (96 Indexes, 96 Samples). Sequencing was carried out in 2×151 bp cycles on an Illumina MiSeq system using MiSeq Reagent Kit v3 (600-cycle).

The assembly and quality assessment of microbial isolate sequencing experiments were conducted by AQUAMIS (*Deneke et al*., 2021). Further characterisation of the bacterial genomes was conducted by BakCharak, yielding insight AMR genes, plasmids and virulence factors.

3. Results

Pathogenic *Y. enterocolitica* strains were detected in 17 out of 104 pre-packed fresh pork minced meat samples. The typical bull's eye colonies on CIN agar plates were submitted to thermal lysis, and the DNA was tested by real time PCR (according to *ISO*, (2015), i.e., CEN ISO/TS 18867) and yielded a positive signal, indicating potential pathogenic properties of these *Y. enterocolitica* strains. Two positive minced meat samples had an identical batch number and were sampled on two consecutive days in the same supermarket. In three samples, the typical bull's eye colonies could be quantified (10–20 CFU/g sample) after direct plating on CIN agar. The remaining strains could only be isolated after enrichment in PSB and/or ITC according to *ISO* (2017). Strains selected for further sequencing by Illumina short read technology are shown in Table 1, along with certain outputs of the Aquamis and BakCharak pipelines. For further details on more virulence genes and genes associated with metal resistance, please refer to the authors.

The biotyping according to *ISO* (2017) revealed that the six tested *Y. enterocolitica* strains belong to biovar 4. Serotyping of the obtained isolates was not conducted.

4. Discussion

As in previous years, yersiniosis still plays an important role as a human gastrointestinal disease in the European Union (EU). Similar to other foodborne associated zoonotic bacteria, it is especially important to prevent infections in children, older and immunocompromised people. Children under five years of age are most commonly affected by yersiniosis. According to *RKI* (2020), in Germany, the majority of cases (about 98%) occurs as sporadic cases. A study by *Rosner et al.* (2012) observed that the consumption of raw minced pork, which is also frequently consumed by young children in Germany, was the main risk factor for the disease in Germany. Another risk factor was the preparation of minced pork in the household. These findings confirm that the common route of infection is the consumption of raw or undercooked pork, e.g. as ground pork or minced pork, and hygiene deficiencies in the preparation of ground pork in the household.

Our study showed that pre-packed fresh pork minced meat can be contaminated with *Y. enterocolitica.* It is, therefore, particularly important to avoid the consumption of raw or undercooked pork and to apply appropriate hygienic measures when handling raw pork meat. Proper heating of meat for at least 2 minutes at 70°C is deemed sufficient to kill *Yersinia*, provided that that this temperature is also reached in the core of the food (*BfR*, 2012).

Further characterisation and sequencing of the remaining *Y. enterocolitica* strains obtained in this study will continue for genome characterisation and to determine the degree of their genetic diversity. The detection of particular AMR genes should be supported by respective phenotypical tests to investigate phenotypical antimicrobial resistance in these *Y*. enterocolitica strains.

5. Conclusion

Since pre-packed fresh pork minced meat can be contaminated with pathogenic *Y. enterocolitica* strains, it is critical to avoid eating raw or undercooked pork, e.g., in the form of ground pork or minced pork. Furthermore, appropriate hygiene measures when handling raw pork are crucial to prevent cross-contamination with other foods and to minimise the potential risk of infection for consumers. This applies all the more to households with small children or otherwise vulnerable groups of people.

Disclosure statement: No potential conflict of interest was reported by the authors.

Acknowledgements: We thank Andrea Barac from the Consuliary Laboratory for Yersinia for her skilful assistance in different phases of this study.

References

BfR — Bundesinstitut für Risikobewertun, (2013). Yersinien in Lebensmitteln: Empfehlungen zum Schutz vor Infektionen. Stellungnahme Nr. 002/2013 des BfR vom 18. Januar 2013.

BfR — Bundesinstitut für Risikobewertung, (2012). Hackepeter und rohes Mett sind nichts für kleine Kinder. Pressemitteilung 11/2012, 12.03.2012.

Deneke, C., Brendebach, H., Uelze, L., Borowiak, M., Malorny, B. & Tausch, S. H. (2021). Species-specific quality control, assembly and contamination detection in microbial isolate sequences with AQUAMIS. *Genes* (Basel). 2021 Apr 26;12(5), 644. doi: 10.3390/genes12050644

EFSA and ECDC — European Food Safety Authority and European Centre for Disease Prevention and Control, (2022). The European Union One Health 2021 zoonoses report. *EFSA Journal*, 20(12), 7666, 273 https://doi.org/10.2903/j.efsa.2022.7666

ECDC — European Centre for Disease Prevention and Control, (2022). Yersiniosis. In: ECDC. Annual epidemiological report for 2021. Stockholm: ECDC, 2022.

Fredriksson-Ahomaa, M. (2012). Isolation of Enteropathogenic Yersinia from Non-human Sources. In: de Almeida, A., Leal, N. (eds) Advances in Yersinia Research. Advances in Experimental Medicine and Biology, vol 954. Springer, New York, NY, https://doi.org/10.1007/978-1-4614-3561-7_12.

ISO, (2017), 10273:2017. Microbiology of the food chain — Horizontal method for the detection of pathogenic *Yersinia enterocolitica*.

ISO, (2015). ISO/TS 18867:2015(en) Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*.

RKI — Robert Koch-Institut, (2020). Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2020, Berlin 2021.

Rosner, B. M., Stark, K., Höhle, M. & Werber, D. (2012). Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009–2010. *Epidemiology & Infection*, 140(10), 1738–1747, doi: 10.1017/S0950268811002664

Tennant, S. M., Grant, T. H. & Robins-Browne, R. M. (2003). Pathogenicity of *Yersinia enterocolitica* biotype 1A. *FEMS Immunology and Medical Microbiology*, 22, 38(2), 127 – 137, doi: 10.1016/S0928-8244(03)00180-9

Verbraucherschutz Sachsen-Anhalt, (2018). Rohes Schweinehackfleisch häufig mit *Yersinia enterocolitica* kontaminiert, https://verbraucherschutz.sachsen-anhalt.de/wir-ueber-uns-service/die-pressestelle/presseinformationen-2019/yersinia-enterocolitica-kontaminiertes-schweinehack/page. Accessed May 5, 2023.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.5.09

ID: 126548489

https://doi.org/10.18485/meattech.2023.64.2.16

Original scientific paper

Enhanced biosecurity measures may contribute to the reduction of Campylobacter incidence in slaughterhouses

Jelena Maletić[a], Jasna Kureljušić[a] and Nenad Katanić[b]*

[a] *Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, 11000 Belgrade, Serbia*
[b] *Ministry of Agriculture, Forestry and Water Management, Nemanjina 22–26, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Biosecurity
Assessment
Broilers
Campylobacter

ABSTRACT

As a preventive measure, biosecurity is the first line of defense against many pathogens. Applied biosecurity measures can reduce the prevalence of *Campylobacter* infection in commercial broiler populations. A systematic evaluation, encompassing at least annual monitoring of applied biosecurity measures and on-farm prevalence of *Campylobacter* infection is highly recommended.

This study was performed on three broiler farms with the aim to assess the effectiveness of the biosecurity measures applied. Broiler farms included in the study previously had problems with *Campylobacter* infections, and therefore, after the intervention through a risk-based scoring system and bacteriological testing of samples from the farm and the corresponding carcasses in the slaughterhouse, several biosecurity measures were implemented. Obtained results showed that after the intervention, farms increased their external biosecurity by 16.34%, internal biosecurity by 22%, and overall biosecurity by 18.34%. The major interventions concerned the removal of manure and carcasses, all improved measures taken for feed and drinking water, and measures in the subcategory of cleaning and disinfection protocols carried out between two production cycles. After the improvements, during the screening process on the farms, *Campylobacter* was not isolated from pooled fecal samples in any of the broiler houses. This indicates that at least six houses (two houses per farm) were *Campylobacter*-negative at broiler slaughter age. In pooled neck skin samples originating from studied farms, *Campylobacter* was not isolated after the improved measures were implemented.

The results showed that the assessment of biosecurity protocols on broiler farms is a useful tool, and *Campylobacter* can serve as a biomarker for the efficiency of the implemented biosecurity protocols.

1. Introduction

Biosecurity in animal production implies the sum of management and physical measures that will extenuate the risk of introduction (external biosecurity), development, and spread (internal biosecurity) of diseases between and within farms (*Regulation (EU) 2016/429*, 2022). It is the cornerstone of preventive medicine aimed at preserving public and animal health, plants, and the environment. In such a way, biosecurity is part of the One Health concept (*Renault et al.,* 2022). In broiler production, good biosecurity protocols are very important to reduce the risk of introducing and spreading pathogens and to reduce the use of antimicrobials and consequent-

***Corresponding author:** Jelena Maletić, *jelena.maletic@nivs.rs*

Paper received April 28th 2023. Paper accepted May 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ly the development of antimicrobial resistance in veterinary and human medicine. Applying stringent biosecurity measures may reduce flock infection, but sustaining such measures on the farms seems extremely difficult. Therefore, it is very important to regularly monitor and evaluate the current biosecurity status on the farms (*Newell et al.* 2011; *Gelaude et al.,* 2014; *Dewulf et al.*, 2018; *Caekebeke et al.*, 2021).

One of the major causes of foodborne bacterial gastroenteritis in Serbia is *Campylobacter*. It is the second most common intestinal infection with a high incidence in the last three years: 11.3 (2019), 6.4 (2020), and 6.4 (2021) in 100,000 people (*Institute of Public Health of Serbia "Dr Milan Jovanovic Batut"*, 2022). The most frequently identified source of human infection is fresh broiler meat contaminated during processing. A high incidence of *Campylobacter* infection in broiler flocks is widespread and the infections are age-dependent (*Evans and Sayers*, 2000; *Mullner et al.*, 2009; *EFSA*, 2020). When broilers become infected with *Campylobacter*, the infection is spread from the house, through the anteroom to the areas surrounding the broiler house. Vectors for transmission of the pathogen potentially could be farmers (boots, clothes, and equipment), rodents, and insects (*Newell and Fearnley*, 2003; *Battersby et al.*, 2016; *Royden et al.*, 2021). Studies showed that multiple interventions, hygiene, biosecurity measures, and additional and complementary interventions (probiotics, vaccination) help in controlling the incidence of *Campylobacter* infection in poultry broiler flocks (*Gibbens et al.*, 2001; *Newell et al.*, 2011; *Horvat et al*, 2022). A linear relationship between the flock prevalence and incidence of campylobacteriosis in humans is confirmed (*Rosenquist et al.*, 2003), so reducing the prevalence of positive flocks should contribute to the reduction of disease in humans (*Newell and Fearnley*, 2003; *EFSA*, 2011). The main goal should be to produce chicks free from infection at slaughter, and in this way, the possibility of creating a potential source for human infection would be reduced (*Evans and Sayers*, 2000; *Sibanda et al.*, 2018).

The aim of this study was to assess the effectiveness of the biosecurity measures on the broiler farms that historically had a problem with *Campylobacter* infections, being implemented after the intervention through the risk-based scoring system and bacteriology testing of samples from the farm and the corresponding carcasses in the slaughterhouse.

2. Materials and methods

The study was performed on three broiler farms (labeled from 1 to 3) in Serbia during 2022–2023. The participating broiler farms were 18, 15, and 2 years old. The capacity of the farms was 100000 broilers (large-scale commercial producers), placed in 2–4 houses. Houses were in a good state of repair. Participating farms were visited two times for biosecurity data collection and three times for sample collection.

During the first visit, data were collected regarding biosecurity levels and farm characteristics. In the face-to-face interview with the farmers, it was found that farms had problems with overall broilers' health, mainly related to intestinal disorders, poor weight gain, and high feed conversion rates in the previous period. The farms cooperated with one slaughterhouse for further processing. Slaughterhouse internal control quality results indicated that flocks' neck skin samples were colonized with *Campylobacter* at the final slaughter age, especially during the summer season (chicken carcass neck samples with more than 1000 CFU/g of *Campylobacter*).

Assessment of biosecurity measures on the farms was carried out based on the application of the appropriate questionnaire where farmers on a voluntary basis answered several questions regarding the implemented biosecurity measures. The checklists for the broiler farm comprised of 79 questions divided into 11 categories. External biosecurity was assessed within 8 subcategories: purchase of one-day-old chicks, depopulation of broilers (slaughterhouses, traders, and individuals), feed and water, removal of manure and carcasses, visitors and farm workers, material supply, infrastructure, and biological vectors, and location of the farm. Internal biosecurity was assessed with questions from three categories: disease management, cleaning and disinfection, and materials and measures between compartments. Every category was scored from 0 (absolute lack of biosecurity on the farm) to 100 (when the measures were fully implemented). The study described biosecurity assessment in broiler farms using the risk-based Biocheck.UGent scoring system (http://www.biocheck.ugent.be/). Overall biosecurity was computed as the average of external and internal biosecurity scores. The final scores for each biosecurity category were obtained for every farm separately.

For every non-compliance, correction measures that should be done at the points of attention were suggested to the farmers. Attention was made to external (purchase of one-day-old chick, practice related to the removal of manure and all measures taken for the

feed and drinking water), as well as internal biosecurity measures (monitoring of flock health and practice related to cleaning and disinfection).

The second farm visit was done 10 months later. During the visit, farmers on a voluntary basis answered several questions regarding the implemented biosecurity measures, and data were collected to assess the efficacy of the corrective measures they took. Also, flocks were screened for *Campylobacter* presence over one production cycle, with two flocks included from each farm, about two weeks before slaughtering. In agreement with the farm owner and with respect to all ethical principles, a total of 60 chicken fecal samples were collected. Fecal samples from 10 birds were pooled by mixing and placed into sterile fecal containers. Each container consisted of pooled feces from five different broiler chickens on the same farm.

Fecal samples were transported at 3°C and cultured within an hour of collection onto a *Campylobacter* selective media for isolation. Approximately 0.2Xg of feces was added to 1 ml of the enrichment medium Preston broth and incubated at 41.5°C for 24 h ± 2 h. After incubation, the culture was transferred with a sterile loop of 10 µl on the surface of the isolation medium, mCCD agar, and plates incubate at 41.5°C in a microaerobic atmosphere for 44 h ± 4 h. Suspect colonies were subcultured in *Campylobacter* blood-free selective agar base to get pure cultures according to ISO 10272–1 (*SRPS EN ISO 10272-1:2017*, 2017a**)**.

In addition, neck skin samples were collected after washing with chilled water and tested for concentrations of *Campylobacter*. From each flock, five pooled samples of neck skin (10 g of skin samples were taken) were obtained by blending three neck skins. In total 180 neck skin samples were tested. To obtain *Campylobacter* from the neck skin samples, detection and enumeration of *Campylobacter* (*SRPS EN ISO 10272-2:2017*, 2017b) was according to the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and circulation (*Official Gazette of RS no. 72/10*, 2010) and Rulebook on amendments to the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and traffic (*Official Gazette of RS no. 62/18*, 2018). We prepared an appropriate decimal dilution of the samples and transferred 0.1 ml of the initial suspension to an mCCD agar plate and incubated at 41.5°C in a microaerobic atmosphere for 44 h ± 4 h. The test results were interpreted as satisfactory for the skin-neck samples that had less than 1000 CFU/g.

Differences between obtained scores for subcategories of external and internal biosecurity were analyzed using parametric independent samples t-test for the normally distributed data. The alpha level for significance was 0.05. Statistical analyses were performed using Graph Pad Prims v 9.4.1 software.

3. Results

The results of the biosecurity assessment obtained during the first and the second visits are presented in Table 1. A graphical illustration of the biosecurity score in visit 1 and its post-intervention score are given in Figure 1.

During the initial visit, the average overall biosecurity score ranged from 60% to 63%, with an average score of 61.66 ± 1.25%. Results showed that external biosecurity scores ranged from 62% to 65%, averaging 63.33 ± 1.25%. Internal biosecurity score ranged from 56% to 64%, averaging 58.68 ± 3.77%.

The scores for subcategories varied between the farms. Noteworthily, before the intervention of removing manure and carcasses, the subcategory within the category of external biosecurity had the lowest mean score (a score of 5%). The farms left the manure for some time near the farm, and after 15–20 days, they brought it to the nearest fields. The carcass storage was placed inside the houses, being never disinfected after use, only washed. Also, no gloves were used during the manipulation of dead birds. This point is very important as dead birds have been identified as potential sources for various pathogens. Compared to the world scores (WS) obtained from the Biochek.UGent online survey database, lower overall scores were also obtained for steps of broiler depopulation (51%) and for the measures used for the feed and drinking water (45%). On all farms, there was no clear separation between clean and dirty sections of the premises, especially separation in the anteroom. Also, drinking water quality was never tested on a regular basis.

Concerning internal biosecurity, results obtained on the initial visit showed that the lowest score was obtained for the subcategory cleaning and disinfection protocols carried out between the two production cycles (43%).

Obtained data for biosecurity showed that the overall external, internal, and total biosecurity scores significantly differed between the first and the second visits (Figure 1.). Results were better on the second visit compared to the first visit. Biosecurity scores for subcategories feed and water and the removal of manure and carcasses were improved on all farms. Within internal biosecurity, the biggest

Table 1. The average biosecurity scores (%) of the farms for the different categories of the Biocheck. UGentTM survey in the first and second visits for biosecurity compliance. *p* values are provided for a comparison between the farms in the first and the second visits

	Visit 1 (%)	Visit 2 (%)	*p*-value	World score (%)
External biosecurity				
Purchase of one-day-old chicks	59.00	80.67	0.0266*	63.00
Depopulation of broilers (slaughterhouses, traders, individuals)	51.00	55.67	0.3735	58.00
Feed and water	45.00	81.00	0.0298*	57.00
Removal of manure and carcasses	5.00	77.33	0.0003*	56.00
Visitors and farmworkers	80.00	89.33	0.1161	69.00
Material supply	100.00	100.00	-	69.00
Infrastructure and biological vectors	96.67	95.00	0.4226	76.00
Location of the farm	52.00	57.00	0.7696	64.00
External biosecurity score	63.33	79.67	0.0490*	65.00
Internal biosecurity				
Disease management	63.67	98.00	0.0464*	76.00
Cleaning and disinfection	43.00	67.67	0.0234*	66.00
Materials and measures between compartments	82.00	82.00	-	73.00
Internal biosecurity score	58.67	80.67	0.0027*	71.00
Overall biosecurity score	61.66	80.00	0.0288*	66.00

* *p* values below 0.05 were considered statistically significant

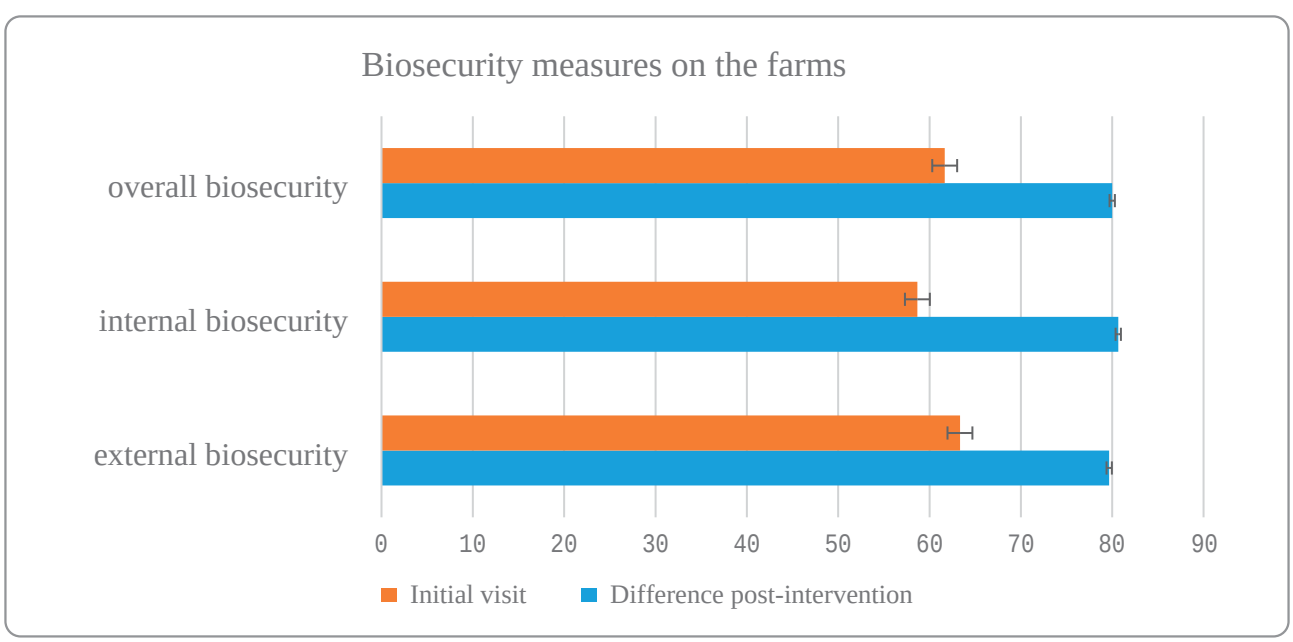


Figure 1. Comparison of the overall, external, and internal biosecurity scores (%) in the first and second visits

improvement was seen in the cleaning and disinfection protocols, with an increased score of 24.67%.

During the screening process of the second visit, *Campylobacter* was not isolated from pooled fecal samples in any of the broiler houses. This indicates that at least six houses (two houses per farm) were *Campylobacter*-negative when the broilers were of slaughter age. In pooled neck skin samples originating from the studied farms, 100% (180/180), i.e. all samples had less than 10 CFU/g *Campylobacter*.

4. Discussion

In order to identify the effectiveness of the biosecurity measures implemented after the intervention at all points of attention on the broiler farms, the investigation covered biosecurity assessment and *Campylobacter* analysis of the samples originating from farms and on the slaughterhouse level. The costs of the intervention were not high as farms had their own biosecurity policy that had to be slightly changed, since there were challenges for farm workers to persevere and consistently perform.

Major differences were observed concerning external biosecurity among all three farms. External biosecurity is fundamental to prevent the entry of the pathogens, and data obtained during the initial visit showed that external biosecurity had lower scores than internal on all farms.

The depopulation of broilers aims to optimize the use of the farm space. On the studied farms, the flocks were partially depopulated in two to three steps. The risk of partial depopulation for *Campylobacter* introduction and transmission has been proven, and this happens due to the contamination of harvesting equipment and materials used by the catching crew. As it is financially challenging to stop partial depopulation practices, it is suggested that the focus should be on external biosecurity to avoid the introduction of pathogens. Therefore, improved hygiene practices and sustainable biosecurity programs are important points of action (*Hertogs et al.*, 2021; *Sarnino et al.*, 2022).

Special attention was put on the removal of manure and carcass management as it was the cause of the low external biosecurity scores. Farms had practiced storing the manure prior to use, and then unprocessed poultry manure was spread as organic fertilizer on the land that surrounds the farms. That manure could be contaminated with pathogenic microorganisms, antibiotics, pathogenic microorganisms with antibiotic-resistant genes, heavy metals, growth and sex hormones, and pesticides. As shown, chicken litter can be a source of *Campylobacter jejuni* (*Wilkinson et al.*, 2011; *Kyakuwaire et al.*, 2019). Managing poultry manure requires a complex approach covering transportation, storage, and further handling and/or processing (*Dróżdż et al.*, 2020). In our study, after our visits, the farmers signed contracts with a bioenergy plant to accept the used poultry litter. Also, the farms increased the carcass storage hygiene and the frequency of carcass elimination from the farm by trucks. With those interventions, the subcategory removal of manure and carcass achieved better scores.

Although rodents were not a problem on the farms due to effective vermin-control programs, insects, including flies, were potential problems. Flies can serve as vectors for *Campylobacter* in broilers. These insects can transmit *Campylobacter* from outside farm livestock into broiler houses. Preventing fly entry during summer leads to a decrease in the prevalence of *Campylobacter* positive flocks at slaughter (*Hald et al.*, 2007). Generally, insect control interventions reduce the peak percentage of contaminated chickens and neck samples of chicken carcasses (*Horvat et al.*, 2022). The problem can be solved by regular removal of carcasses, repairing all leaking water lines and keeping the manure dry, and relocating used litter from the farm.

According to the risk-based scoring system, farms had low scores for feed and water management. They did not conduct microbiological analyses of water samples. The drinking water should be kept at a microbiologically safe level as contaminated feed and water can easily serve as a transfer medium for pathogenic bacteria. The addition of disinfectants to drinking water gave promising results for *Campylobacter* control in chicken flocks (*Maharjan et al.*, 2016; *EFSA*, 2020; *Scollo et al*., 2023).

Sometimes farms made a change of feed supplier, but not during one production cycle. The feed silos are completely sealed against water, birds, and vermin. The feed suppliers did not have access to the houses where direct contact with the poultry would be possible. The major problem was a lack of clear separation between the clean and the dirty areas of the farm premises. The traffic that serves different companies uses the same road as the vehicles for internal movements at the farm. There were no protocols for fully cleaning and disinfecting the trucks at the farm entrances.

Concerning internal biosecurity measures, attention was made to cleaning and disinfection. Farms owned special protocols for cleaning and disinfection between flocks, but they did not perform routine controls, such as bacteriological testing, to verify the efficacy of their applied protocols. Visual inspection of cleaning alone is not reliable to assess the hygiene status of broiler houses (*Luyckx et al.*, 2015). In Ser-

bia, there is no official requirement for periodic control of the general hygiene status of broiler houses after cleaning and disinfection. The downtime was short (approximately seven days) after each production cycle. *Agunos et al.* (2014) reported that inadequate cleaning and disinfection and short downtime between flocks can be a potential source of *Campylobacter* colonization due to the carryover of strains from one flock to the next. Longer periods (over 10 days) between sequential flocks reduce residual bacterial contamination in or around a previously positive house from spreading to a new flock in the same house (*EFSA*, 2020). Every poultry house had an anteroom and a hygiene lock present, where farm workers can wash and disinfect their hands, but this does not necessarily imply their implementation. Farmers and visitors can also transmit *Campylobacter* to chickens, bringing the bacteria through contaminated shoes or boots, contaminated tools, or through contaminated clothes and hands (*Horvat et al*., 2022). A standard hygiene protocol followed by all staff who entered into populated broiler farms could reduce the risk of a flock getting infected with *Campylobacter* by 50%. Hygiene protocols should include a strict procedure with boot dips before entering the farm and houses and specific clothing (*Gibbens et al.*, 2001). As was noted by *Van Limbergen et al.* (2018), better education of the staff could help to improve the overall biosecurity on broiler farms. Continuous training and motivating the staff in combination with the results of monitoring of farm-specific critical points could help to increase efficiency and prevent staff from becoming inattentive due to routine (*Scollo et al.*, 2023).

Studies showed that the transportation crates can be a reservoir of *Campylobacter* if the washing and disinfection processes of crates in the slaughterhouse are not sufficient to eliminate all bacteria (*Horvat et al.*, 2022). The studied farms received transport crates that were disinfected by the slaughterhouse.

Campylobacter is often detected in broiler flocks at 3 to 4 weeks of age. In this study, during the screening process of the broiler houses, *Campylobacter* was not isolated in any of the poultry. This is probably the result of a stronger commitment to biosecurity practices by breeding farms (*Hertogs et al.* 2021). Additionally, farms started with the acidification of drinking water with a buffered organic acid basis, which is proven to reduce the risk of *Campylobacter*-positive flocks (*EFSA*, 2020).

Flocks that are infected with *Campylobacter* can be a source of the bacteria in the corresponding carcasses. Cross-contamination during the transportation and slaughter process is also important, as is the cross-contamination rate between carcasses and processing equipment (*Zhang et al.*, 2018; *Perez-Arnedo and Gonzales Fandos*, 2019). On-farm control of *Campylobacter* in poultry would reduce the risk of human exposure to this pathogen and have a significant impact on food safety. The aim should be to decrease environmental exposure through improved biosecurity measures (*Lin*, 2009).

This study indicated that improved on-farm biosecurity measures can play an important role in reducing the prevalence of *Campylobacter* infection in poultry broiler flocks. The results further indicated the importance of good biosecurity protocols applied during broiler production. Each intervention can be quantified with the continuous assessment of implemented biosecurity programs. On-farm testing of birds can also help to predict the exposure risk of the hazard *Campylobacter* in resultant food.

5. Conclusion

As a preventive measure, biosecurity is the first line of defense against many pathogens. A systematic evaluation, encompassing at least annual biosecurity monitoring and on-farm prevalence of *Campylobacter* infection is recommended. The risk of spreading the pathogen to humans in close contact with broilers, means moving attention to the healthcare of farm workers and slaughterhouse staff as a part of the One-health approach. Our results show that the assessment of biosecurity protocols on broiler farms is useful and that *Campylobacter* can serve as a biomarker for the efficiency of the implemented biosecurity protocols. It is very important that farmers consistently follow the biosecurity rules in their daily work. This study also had some limitations, as it was focused only on one slaughterhouse and three farms, so future studies should include an increased sample size from the farms and slaughterhouses for analysis.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was funded by the Serbian Ministry of Science, Technological Development and Innovation (Contract 451-03-47/2023-01/200030).

Acknowledgments: The authors would like to thank the survey respondents for generously giving their time and sharing their experiences.

References

Agunos, A., Waddell, L., Léger, D. & Taboada, E. (2014). A systematic review characterizing on-farm sources of Campylobacter spp. for broiler chickens. *PloS One*, 9(8), e104905, https://doi.org/10.1371/journal.pone.0104905

Battersby, T., Whyte, P. & Bolton, D. J. (2016). The pattern of Campylobacter contamination on broiler farms; external and internal sources. *Journal of Applied Microbiology*, 120(4), 1108–1118, https://doi.org/10.1111/jam.13066

Biocheck. UGent: https://biocheckgent.com/en/questionnaires/broilers

Caekebeke, N., Ringenier, M., Jonquiere, F. J., Tobias, T. J., Postma, M., van den Hoogen, A., Houben, M. A. M., Velkers, F. C., Sleeckx, N., Stegeman, A., Dewulf, J. & On Behalf Of The I-Health Study Group (2021). Coaching Belgian and Dutch Broiler Farmers Aimed at Antimicrobial Stewardship and Disease Prevention. *Antibiotics* (Basel, Switzerland), 10(5), 590, https://doi.org/10.3390/antibiotics1005059

Dewulf, J., Immerseel, F. V., Luyckx, K., Postma, M. & Vabeselaere, B. (2018). How to measure biosecurity and the hygiene status of farms. Page 117. Pages 3000. in Biosecurity in Animal Production and Veterinary Medicine: From Principles to Practice. J. Dewulf and F. V. Immerseel eds, Uitgeverij Acco, Blijde Inkomststraat, 22. Belgie, Leuven.

Dróżdż, D., Wystalska, K., Malińska, K., Grosser, A., Grobelak, A. & Kacprzak, M. (2020). Management of poultry manure in Poland — Current state and futures perspectives. *Journal of Environmental Management*, 264, 110327, https://doi.org/10.1016/j.jenvman.2020.110327

EFSA Panel on Biological Hazards (BIOHAZ), (2011). Scientific opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal,* 9, 2105.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Alter, T., Crotta, M., Ellis-Iversen, J., … & Chemaly, M. (2020). Update and review of control options for Campylobacter in broilers at primary production. *EFSA Journal — European Food Safety Authority*, 18(4), e06090, https://doi.org/10.2903/j.efsa.2020.6090

Evans, S. J. & Sayers, A. R. (2000). A longitudinal study of campylobacter infection of broiler flocks in Great Britain. Preventive Veterinary Medicine, 46(3), 209–223, https://doi.org/10.1016/s0167-5877(00)00143

Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M. & Dewulf, J. (2014). Biocheck.UGent: a quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. *Poultry Science*, 93(11), 2740–2751, https://doi.org/10.3382/ps.2014-04002

Gibbens, J. C., Pascoe, S. J., Evans, S. J., Davies, R. H. & Sayers, A. R. (2001). A trial of biosecurity as a means to control Campylobacter infection of broiler chickens. Preventive Veterinary Medicine, 48(2), 85–99, https://doi.org/10.1016/s0167-5877(00)00189-6

Hald, B., Sommer, H. M. & Skovgård, H. (2007). Use of fly screens to reduce Campylobacter spp. introduction in broiler houses. *Emerging Infectious Diseases*, 13(12), 1951–1953, https://doi.org/10.3201/eid1312.070488

Hertogs, K., Heyndrickx, M., Gelaude, P., De Zutter, L., Dewulf, J. & Rasschaert, G. (2021). The effect of partial depopulation on Campylobacter introduction in broiler houses. *Poultry Science*, 100(2), 1076–1082, https://doi.org/10.1016/j.psj.2020.11.017

Horvat, A., Luning, P. A., DiGennaro, C., Rommens, E., van Daalen, E., Koene, M. & S. Jalali, M. S. (2022). The impacts of biosecurity measures on Campylobacter contamination in broiler houses and slaughterhouses in the Netherlands: A simulation modeling approach. *Food Control*, Volume 141, https://doi.org/10.1016/j.foodcont.2022.109151

Institute of Public Health of Serbia "Dr. Milan Jovanovic Batut", Centers for Disease Control and Prevention, (2022). The report on infectious diseases in 2021 the territory of the Republic of Serbia.

Kyakuwaire, M., Olupot. G., Amoding. A., Nkedi-Kizza. P. & Basamba T. A. (2019). How Safe is Chicken Litter for Land Application as an Organic Fertilizer? A Review. International *Journal of Environmental Research and Public Health*, 16(19), 3521, https://doi : 10.3390/ijerph16193521

Lin, J. (2009). Novel approaches for Campylobacter control in poultry. *Foodborne Pathogens and Disease*, 6(7), 755–765, https://doi: 10.1089/fpd.2008.0247

Luyckx, K., Dewulf, J., Van Weyenberg, S., Herman, L., Zoons, J., Vervaet, E., Heyndrickx, M. & De Reu, K. (2015). Comparison of sampling procedures and microbiological and non-microbiological parameters to evaluate cleaning and disinfection in broiler houses. *Poultry Science*, 94(4), 740–749, https://doi.org/10.3382/ps/pev019

Maharjan, P., Clark, T., Kuenzel, C., Foy, M. K. & Watkins, S. (2016). On-farm monitoring of the impact of water system sanitation on microbial levels in broiler house water supplies, *Journal of Applied Poultry Research*, 25(2), 266–271, https://doi.org/10.3382/japr/pfw010

Mullner, P., Spencer, S. E., Wilson, D. J., Jones, G., Noble, A. D., Midwinter, A. C., Collins-Emerson, J. M., Carter, P., Hathaway, S. & French, N. P. (2009). Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. *Infection, Genetics, and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 9(6), 1311–1319, https://doi.org/10.1016/j.meegid.2009.09.003

Newell, D. G. & Fearnley, C. (2003). Sources of Campylobacter colonization in broiler chickens. *Applied and Environmental Microbiology*, 69(8), 4343–4351, https://doi.org/10.1128/AEM.69.8.4343-4351.2003

Newell, D. G., Elvers, K. T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N. J., Davies, R., Connerton, I., Pearson, D., Salvat, G. & Allen, V. M. (2011). Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. *Applied and Environmental Microbiology*, 77(24), 8605–8614, https://doi.org/10.1128/AEM.01090-10

Perez-Arnedo, I. & Gonzalez-Fandos, E. (2019). Prevalence of Campylobacter spp. in Poultry in Three Spanish Farms, A Slaughterhouse and A Further Processing Plant. *Foods*, 8(3), 111, https://doi.org/10.3390/foods8030111

Regulation (EU) 2016/429, **(2016)**. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on Transmissible Animal Diseases and Amending and Repealing Certain Acts in the Area of Animal Health ('Animal Health Law'). Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2016.084.01.0001.01.ENG (accessed on 26 April 2022)

Renault, V., Humblet, M. F. & Saegerman, C. (2022). Biosecurity Concept: Origins, Evolution, and Perspectives. *Animals,* 2(1), 63, https://doi.org/10.3390/ani12010063

Rosenquist, H., Nielsen, N. L., Sommer, H. M., Nørrung, B. & Christensen, B. B. (2003). Quantitative risk assessment of human campylobacteriosis associated with thermophilic Campylobacter species in chickens. *International Journal of Food Microbiology*, 83(1), 87–103, https://doi.org/10.1016/s0168-1605(02)00317-3

Royden, A., Christley, R., Prendiville, A. & Williams, N. J. (2021). The Role of Biosecurity in the Control of *Campylobacter*: A Qualitative Study of the Attitudes and Perceptions of UK Broiler Farm Workers. *Frontiers in Veterinary Science*, 8, 751699, https://doi.org/10.3389/fvets.2021.751699

Official Gazette of RS no. 72/10, (2010). Rulebook on general and special conditions of food hygiene at any stage of production, processing, and circulation.

Official Gazette of RS no. 62/18, (2018). Rulebook on amendments to the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and traffic.

SRPS EN ISO 10272-1:2017, (2017a). Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method.

SRPS EN ISO 10272-2:2017, (2017b). Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique.

Sarnino, N., Berge, A. C., Chantziaras, I. & Dewulf, J. (2022). Estimation of the Production Economic Consequences of Stopping Partial Depopulation in Broiler Production. *Animals*, 12(12), 1521, https://doi.org/10.3390/ani12121521

Scollo, A., Perrucci, A., Stella, M. C., Ferrari, P., Robino, P. & Nebbia, P. (2023). Biosecurity and Hygiene Procedures in Pig Farms: Effects of a Tailor-Made Approach as Monitored by Environmental Samples. *Animals*, 13(7), 1262, https://doi.org/10.3390/ani13071262

Sibanda, N., McKenna, A., Richmond, A., Ricke, S. C., Callaway, T., Stratakos, A. C., Gundogdu, O. & Corcionivoschi, N. (2018). A Review of the Effect of Management Practices on *Campylobacter* Prevalence in Poultry Farms. *Frontiers in Microbiology*, 9, https://doi.org/10.3389/fmicb.2018.02002

Van Limbergen, T., Dewulf, J., Klinkenberg, M., Ducatelle, R., Gelaude, P., Méndez, J., Heinola, K., Papasolomontos, S., Szeleszczuk, P., Maes, D. & PROHEALTH consortium (2018). Scoring biosecurity in European conventional broiler production. *Poultry Science*, 97(1), 74–83, https://doi.org/10.3382/ps/pex296

Wilkinson, K. G., Tee, E., Tomkins, R. B., Hepworth, G., Premier, R. (2011). Effect of heating and aging of poultry litter on the persistence of enteric bacteria. *Poultry Science*, 90, 10–18, https://doi.org/10.3382/ps.2010-01023

Zhang, X., Tang, M., Zhou, Q., Zhang, J., Yang, X. & Gao, Y. (2018). Prevalence and characteristics of *Campylobacter* throughout the slaughter process of different broiler batches. *Frontiers in Microbiology*, 9, 2092, https://doi: 10.3389/fmicb.2018.02092

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5

ID: 126556937

https://doi.org/10.18485/meattech.2023.64.2.17

Review paper

Challenges in agri-food chain: biosensors in the meat production system

Ivan Nastasijević[a], Ivana Podunavac[b], Saša Janković[a], Radmila Mitrović[a] and Vasa Radonić[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *BioSense Institute, University of Novi Sad, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Agri-food chain
Meat production
Animal health
Animal welfare
Food safety
Biosensors

ABSTRACT

The complexity of the meat chain is well-known, beginning with the Pre-harvest (feed, farm biosecurity, herd/flock health status, animal welfare, transportation, livestock market/abattoir lairage), followed by Harvest (slaughter, dressing, chilling) and Post-harvest (deboning, meat processing, packaging, distribution, retail, consumer) modules.

Over the previous decade, consumer awareness increased globally towards animal health, animal welfare and food safety issues, including food quality, food fraud, sustainability and climate change impact on meat production. Therefore, consumers demand proper and accurate information on the aforementioned issues in real time for making informed choices when buying their preferred meat/meat products. The transformation of traditional meat value chains towards sustainability needs reliable and affordable tools to optimize such transformation and achieve higher levels of food safety. Sensing systems (biosensors) and their regular use within an integrated meat production chain, from farm-to-fork, can play an important role and be a part of the solution for climate-smart and sustainable agri-food chain considering biosensor function in early and accurate detection of food(meat)-borne pathogens and other food contaminants (residues). The application of biosensors can provide accurate and concentrated data on animal health and welfare, including food borne hazards, to support food safety risk assessment in both, `traditional` and `novel` (cell-based meat) meat value chains for the benefit of the global population.

1. Introduction

The meat production chain is a highly complex system that involves various stages and stakeholders, beginning with Pre-harvest (feed, farm biosecurity, herd/flock health status, animal welfare, transportation, livestock market/abattoir lairage), followed by Harvest (slaughter, dressing, chilling) and Post-harvest (deboning, meat processing, packaging, distribution, retail, consumer) modules. Over the previous decade, consumer awareness increased globally towards animal health, animal welfare and food safety issues and consumers demand proper and accurate information on the aforementioned issues in real-time for making informed choices when buying their preferred meat/meat products. The meat production system is also facing climate change impacts, recognized as the change of trends of global temperatures, precipitations and wind patterns, that are attributed directly or indirectly to human activity (*UNFCCC*, 1992), with extreme events becoming more frequent, severe and unpredictable. These events may jeopardize food security by influenc-

***Corresponding author:** Ivan Nastasijević, *ivan.nastasijevic@inmes.rs*

Paper received May 16th 2023. Paper accepted May 28th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ing various biological contaminants, including food borne hazards, and altering their occurrence, virulence and distribution and increasing the exposure of consumers (*FAO*, 2022). For example, the potential association between rising temperatures and increased levels of antimicrobial resistance (AMR) in certain zoonotic food (meat) borne pathogens has been observed, e.g., *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*. Furthermore, these pathogens are showing increased resistance, in particular, to Critically Important Antibiotics (CIA), reducing the efficacy and quality of clinical treatments (*Poirel et al*., 2018; *Van Puyvelde et al*., 2019; *WHO*, 2019). Another challenge related to the meat chain is its sustainability and environmental impact of the livestock production chain which contributes a certain share to anthropogenic Greenhouse Gas (GHG) emissions (*FAO*, 2022).

Mitigation strategies that include improvement of animal health and welfare can significantly reduce emissions. To achieve that goal, the specificity of livestock production and local production systems should be taken into consideration (*Özkan et al*., 2022). A new challenge is related to the process control of cell-based meat, which is based on culturing cells isolated from animals, followed by processing to produce food products that are comparable to the corresponding animal versions. The potential food safety hazards are associated with cell selection (faecal-borne pathogens), production (*Mycoplasma*), harvesting (biological components, such as growth factors and hormones from animal serum), food processing and formulation (additives, ingredients, nutrients) (*FAO*, 2022b), but can be tackled more efficiently with smart application of biosensors.

2. Biosensor application in the meat chain

Application of biosensors in the farm-abattoir continuum has a wide range of possibilities and can contribute to and provide significant benefits in the optimization of livestock farm management practices.

2.1. Definition and structure of biosensors

A biosensor is a device which recognizes a target biomarker (e.g. pathogen, stress hormone, acute phase protein, viruses, etc.) via an immobilized sensing element called a bioreceptor (monoclonal antibody, RNA, DNA, aptamer, glycan, lectin, enzyme, tissue, whole cell). It has rapid, sensitive and specific detection capabilities. The typical biosensor system consists of a sensing element with bioreceptor and transducer that converts the signal into a corresponding electrical signal suitable for processing and visualization (Figure 1). The choice of biosensor type depends on the targeted biomarker, the nature of the analyte, the desired sensitivity and the intended application.

There are different types of biosensors based on the biological recognition element (bioreceptor) and the transducer used. For example, *electrochemical biosensors*, *piezoelectric biosensors*, *field-effect transistor (FET) biosensors* and *magnetic biosensors*.

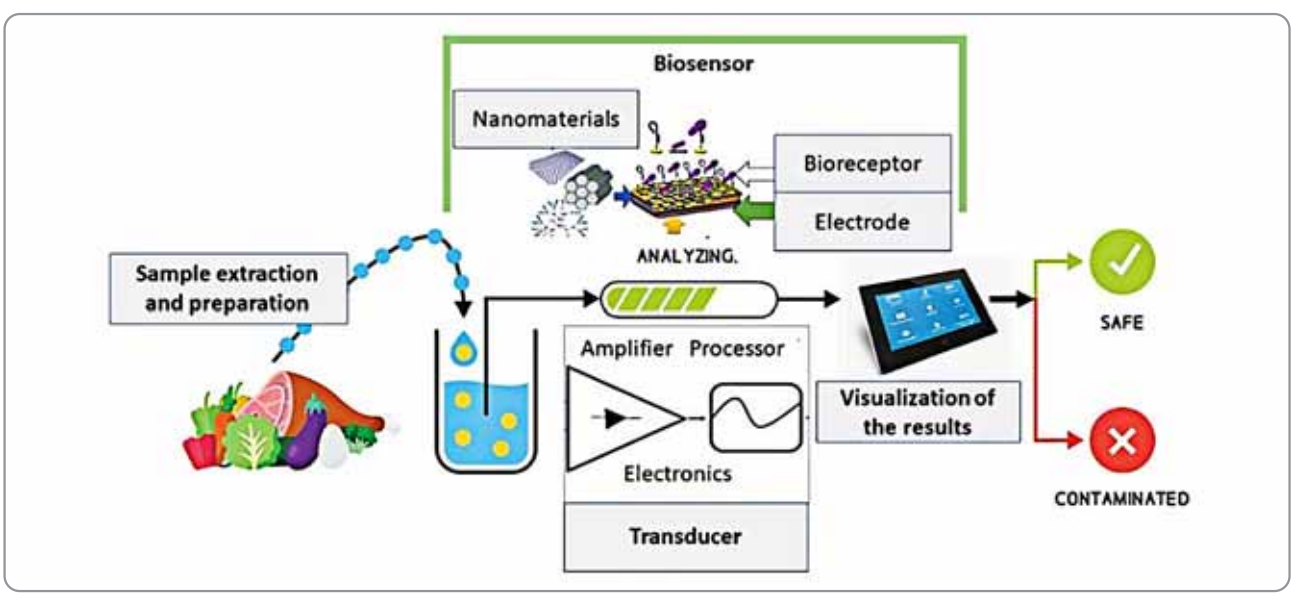


Figure 1. Biosensor with biosensing electrode (bioreceptor) and transducer that convert targeted biosignal to electrical one

2.2. Biosensors application in the farm-abattoir continuum

Biosensors, as point-of-care (PoC) devices, have the potential to detect and quantify physiological, immunological and behavioural responses of livestock and multiple animal species (*Neethirajan et al*., 2017) in the farm-abattoir continuum. They present the lab-on-a-chip concept as an alternative to the commonly used methods such as enzyme-linked immunosorbent assay (ELISA) and/or reverse transcription polymerase chain reaction (RT-PCR) that require adequate environment and space, specifically trained personnel and are time-demanding and more expensive.

2.2.1 Biosensors on the farm

Application of biosensors on-farm has a wide range of technically available opportunities related to behavioural aspects of livestock connected with their feeding dynamics, e.g. *mechanical sensors* (jaw movement) (*Rutter*, 2000) or *acceleration sensors* (feeding behaviour) (*Herinaina et al*., 2016). Furthermore, biosensors able to detect metabolic conditions are available, such as *perspiration metabolite biosensors* (e.g., physical stress via analysis of sweat for sodium and lactate levels) (*Schazmann et al*., 2010), *biosensors for salivary detection of metabolites* (cortisol) (*Yamaguchi et al*., 2013) or *tears analysis biosensors* (glucose sensor) (*La Belle et al*., 2014) or *breath analyses biosensors* (detection of Volatile Organic Compounds — VOCs, e.g. ketosis) (*Leopold et al*., 2014), Bovine Respiratory Diseases (BRD) (*Burciaga-Robles et al*., 2009), brucellosis (*Knobloch et al*., 2009), bovine tuberculosis (*Fend et al*., 2005), Johne`s diseases (*Kumanan et al*., 2009), ketoacidosis (*Mottram et al*., 1999), foot and mouth (FMD) disease (*Christensen et al*., 2011). *Other biosensors* for the detection of animal disease include detection of H7N1 antibodies for Avian Influenza virus (AIV) (*Wang et al*., 2009) or detection of specific acute phase proteins such as biosensor for detection of mastitis (based on haptoglobin detection) (*Martins et al*., 2019). *Biosensors for the detection of stress in fish* also have been developed to respond to stressors (changes in water chemistry, dissolved oxygen content, pH and metal toxicity) associated with water pollution and changes in climate, including behavioural changes (attacking behaviour and visual irritation) (*Wu et al*., 2015a).

2.2.2 Biosensors in the abattoir

The regulatory-based or routine usage of biosensors for the purposes of meat production control and monitoring is not available. However, the recent advancement in design of biosensors enabled rapid and reliable qualitative and quantitative detection of food(meat)borne pathogens, such as lateral flow aptamer-based biosensors for point-of-care detection of *Salmonella enteritidis* and *Escherichia coli* O157:H7 with sensitivity level of 10^1 CFU/ml, respectively (*Fang et al*., 2014; *Wu et al*., 2015b), *Campylobacter* in meat (poultry) samples with detection level of 1.5×10^1 CFU/g (DNA-based sensor) (*Manzano et al*., 2015), toxins of *Clostridium perfringens* (mammalian cell-based sensors) (*Yoo et al*., 2016), *Escherichia coli* (antibody-based or conductometric-based biosensors) at detection levels from 1 to 10^3 CFU/mL (*Jaffrezic-Renault et al*., 2007; *El Ichi et al*., 2014). Biosensors in the abattoir can be also used for environmental control/monitoring of the condition of abattoir wastewater via detection of Biochemical Oxygen Demand (BOD), which is a widely used parameter to describe the level of organic pollution in water and wastewaters (*Chee et al*., 1999). However, the performance of biosensors in the farm-abattoir continuum is constrained *in vitro* with the enriched bacterial suspensions encountered, and there is scarcity of data regarding the matrix (e.g. straw, faeces, blood) from the real operational environment (farm, abattoir), which requires further and deeper research.

2.3. Biosensors, meat production and climate change

Livestock is a potential climate change driver, generating up to 14.5% of total anthropogenic GHG emissions (*Cheng et al*., 2022). The conclusions drawn from similar studies should be taken with precaution, having in mind that these studies mainly considered the intensive farming livestock production systems and not extensive systems (e.g., rotational grazing system), which might even have a positive environmental impact by allowing vegetation to recover and reducing gas emissions via enhancing carbon storage and reducing the need for intensive feed production. Biosensing technology, integrated in precision livestock farming, can be an important tool in monitoring solutions for reduction of GHG emissions that originate from intensive livestock farming and, thus, facilitating the climate change mitigation, including environmen-

tal and agricultural sustainability (*Griesche and Baeumner*, 2020; *Wang et al*., 2022). This type of biosensor technology should become the key component of climate-smart agriculture and "4th revolution" in the agri-food chain (*FAO*, 2015).

2.4. Biosensors and cell-based meat

The in-line monitoring of the bio-process of meat cultivation in bioreactors can improve the efficiency and consistency of cell-based (`cultured` or `cultivated` or `clean`) meat production. Recently, cell-based food production (growing animal-based agricultural products directly from cell cultures), has been explored as a sustainable alternative to the conventional livestock and food of animal origin system, to satisfy the needs of increasing global demand for animal-source protein (*OECD-FAO*, 2022; *FAO/WHO*, 2023). The prototype biosensors are under development to enable in-situ measurements of biomass, nutrient and metabolite quantities in specific growth media (*Good Food Institute*, 2020).

3. Conclusions

The regular and routine introduction of biosensors can facilitate the transformation of the whole food (meat) value chain `from farm to fork` (via advanced Food Chain Information flow in the farm-abattoir continuum), by enabling continuous monitoring and/or early detection of animal disease and food safety hazards, so providing more sustainable and climate-friendly meat production, by reducing GHG emissions (via optimized nutrition, animal health and welfare), and by reduction of food waste.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by Technology Transfer Programme of the Innovation Fund, Republic of Serbia within the project "Development and Integration of Multiplex Microfluidic Biosensors for Meat Safety monitoring in farm-to-slaughterhouse continuum (DIBMES)" [Grant Agreement TTP 1125] & programme `IDEAS` of the Science Fund, Republic of Serbia, project "Microfluidic Lab-on-a-Chip platform for fast detection of pathogenic bacteria using novel electrochemical aptamer-based biosensors (MicroLabAptaSense)" [Grant Agreement No. 7750276].

References

Burciaga-Robles, L. O., Holland, B. P., Step, D. I., Krehbiel, C. R., McMillen, G. I., Richards, C. J., Sims, L. E., Jeffers, J. D., Namjou, K. & McCann, P. J. (2009). Evaluation of breath biomarkers and serum haptoglobin concentration for diagnosis of bovine respiratory disease in heifers newly arrived at a feedlot. *American Journal of Veterinary Research*, 70(10), 1291–1298.

Chee, G.-J., Nomura, Y., Ikebukuro, K. & Karube, I. (1999). Development of highly sensitive BOD sensor and its evaluation using preozonation. *Analaltical Chimica Acta,* 394, 65–71.

Cheng, M., McCarl, B. & Fei, C. (2022). Climate Change and Livestock Production: A Literature Review. *Atmosphere,* 13, 140, https:// doi.org/10.3390/atmos13010140

Cho, I.-H., Kim, D.H. & Park, S. (2020). Electrochemical biosensors: perspective on functional nanomaterials for on-site analysis. *Biomaterials Research,* 24(6), https:// doi.org/10.1186/s40824-019-0181-y

Christensen, L. S., Brehm, K. E., Skov, J., Harlow, K.W., Christensen, J. & Haas, B. (2011). Detection of foot-and-mouth disease virus in the breath of infected cattle using a hand-held device to collect aerosols. *Journal of Virolsology Methods*, 177(1), 44–48.

El Ichi, S., Leon, F. & Vossier, L. et al. (2014). Microconductometric immunosensor for label-free and sensitive detection of gram-negative bacteria. *Biosens Bioelectron*, 54, 378–384.

Fang, Z., Wu, W., Lu, X. & Zeng, L. (2014). Lateral flow biosensor for DNA extraction-free detection of *Salmonella* based on aptamer mediated strand displacement amplification. *Biosens Bioelectron*, 56, 192–197.

FAO/WHO, (2023). *Food safety aspects of cell-based food,* https://www.fao.org/3/cc4855en/cc4855en.pdf (accessed on 11 May 2023).

FAO, (2022). Thinking about the future of food safety. A foresight report. Rome, https://www.fao.org/3/cb8667en/cb8667en (accessed on 27 April 2023).

FAO, (2022b). *Food safety aspects of cell-based food — Background document two: Generic production process.* Rome, https://www.fao.org/3/cc2502en/cc2502en.pdf (accessed on 27 April 2023).

FAO, (2015). Climate Smart Agriculture Sourcebook, https://www.fao.org/climate-smart-agriculture-sourcebook/en/ (accessed on 10 May 2023).

Fend, R., Geddes, R., Lesellier, S., Vordermeier, H.-M., Corner, L. A. L., Gormley, E., Costello, E., Hewinson, R. G., Marlin, D. J., Woodman, A. C. & Chambers, M. A. (2005). Use of an electric nose to Diagnose *Mycobacterium*

bovis infection in badgers and cattle. *Journal of Clinical Microbiology,* 43(4), 1745–1751.

Good Food Institute, (2020). Real Sense: Integrating biosensors for cultivated meat, https://gfi.org/researchgrants/realsense-integrating-biosensors-for-cultivated-meat/ (accessed on 11 May 2023).

Griesche, C. & Baeumner, A. J. (2020). Biosensors to support sustainable agriculture and food safety. *TrAC Trends in Analytical Chemistry* 128, 115906.

Herinaina, A. I., Bindelle, J., Mercatoris, B. & Lebeau, F. (2016). A review on the use of sensors to monitor cattle jaw movements and behaviour when grazing. *Biotechnology Agronomy Sociology and Environment,* 23(S1), 273–286.

Jaffrezic-Renault, N., Martelet, C., Chevolot, Y. & Cloarec, J. P. (2007). Biosensors and bio-bar code assays based on biofunctionalized magnetic microbeads. *Sensors*, 7, 589–614.

Knobloch, H., Kohler, H., Commander, N., Reinhold, P., Turner, C., Chambers, M., Pardo, M. & Sberveglieri, G. (2009). Volatile organic compounds (VOC) analysis for disease detection: proof of principle for field studies detecting paratuberculosis and brucellosis. AIP Conference Proceedings, 195–197.

Kumanan, V., Nugen, S.R., Baeumner, A. J. & Chang, Y.-F. (2009). A biosensor assay for the detection of *Mycobacterium avium subsp. paratuberculosis* in fecal samples. *Journal of Veterinary Science*, 10(1), 35–42.

La Belle, J. T., Engelschall, E., Lan, K., Shah, P., Saez, N., Maxwell, S., Adamson, T., Abon-Eid, M., McAferty, K., Patel, D. R. & Cook, C. B. (2014). A disposable tear glucose biosensor-part 4: preliminary animal model study assessing efficacy, safety, and feasibility. *Journal of Diabetes Science and Technology*, 8(1), 109–116.

Leopold, J. H., van Hooijdonk, R. T., Sterk, P. J., Abu-Hanna, A., Schultz, M. J. & Bos, L. D. (2014). Glucose prediction by analyses of exhaled metabolites — a systematic review. *BMS Anesthesiology*, 14, 46.

Manzano, M., Cecchini, F. & Fontanot, M. et al. (2015). OLED-based DNA biochip for *Campylobacter* spp. detection in poultry meat samples. *Biosens Bioelectrony*, 66, 271–276.

Martins, S. A. M., Martins, V. C., Cardoso, F. A., Germano, J., Rodrigues, M., Duarte, C., Bexiga, R., Cardoso, S. & Freitas, P. P. (2019). Biosensors for On-Farm Diagnosis of Mastitis. *Frontiers in Bioengineering and Biotechnology*, 7, 186, 1–19.

Mottram, T., Dobbelaar, P., Schukken, Y., Hobbs, P. & Bartlett, P. (1999). An experiment to determine the feasibility of automatically detecting hyperketonaemia in dairy cows. *Livestock Production Science*, 61(1), 7–11.

Neethirajan, S., Tuteja, S. K., Huang, S.-T. & Kelton, D. (2017). Recent advancement in biosensors technology for animal and livestock health management. *Biosensors and Bioelectronics*, 98, 398–407.

OECD-FAO, (2022). Agricultural Outlook 2022–2031, Chapter 6. Meat, https://doi.org/10.1787/f1b0b29c-en (accessed on 11 may 2023)

Özkan, Ş., Teillard, F., Lindsay, B., Montgomery, H., Rota, A., Gerber P., Dhingra M. & Mottet, A. (2022). The role of animal health in national climate commitments. Rome, FAO, https://doi.org/10.4060/cc0431en. file:///C:/Users/Ivan/Downloads/cc0431en.pdf (accessed on 19 April 2023).

Poirel, L., Madec, J.Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P. & Schwarz, S. (2018). Antimicrobial Resistance in *Escherichia coli*. Microbiology Spectrum, 6(4), https://doi: 10.1128/microbiolspec.ARBA-0026-2017

Rutter, S. M. (2000). Graze: a program to analyze recordings of the jaw movements of ruminants. *Behavior Research Methods, Instruments & Computers*, 32(1), 86–92.

Schazmann, B., Morris, D., Slater, C., Beirne, S., Fay, C., Reuveny, R., Moyna, N. & Diamond, D. (2010). A wearable electrochemical sensor for the real-time measurement of sweat sodium concentration. *Analalitical Methods* 2(4), 342–348.

UNFCCC, (1992). United Nations Framework Convention on Climate Change, https://unfccc.int/resource/docs/convkp/conveng.pdf (accessed on 24 April 2023).

Van Puyvelde, S., Pickard, D. & Vandelannoote, K. et al. (2019). An African *Salmonella Typhimurium* ST313 sublineage with extensive drug-resistance and signatures of host adaptation. *Natural Communication*, 10, 4280.

Laca, E. A. & Wallis De Wries, M. (2001). Acoustic measurement of intake and grazing behaviour of cattle. *Grass Forage Science*, 55(2), 97–104.

Wang, R., Wang, Y., Lassiter, K., Li, Y., Hargis, B., Tung, S., Berghman, L. & Bottje, W. (2009). Interdigitated array microelectrode based impedance immunosensor for detection of avian influenza virus H5N1. *Talanta,* 79(2), 159–164.

Wang, X., Luo, Y., Huang, K. & Cheng, N. (2022). Biosensor for agriculture and food safety: Recent advances and future perspectives. *Advanced Agrochemistry*, 1(1), 3–6.

WHO, (2019). Critically Important Antimicrobials for human medicine : 6th Revision. Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use, https://www.who.int/publications/i/item/9789241515528 (accessed on 25 April 2023).

Wu, H., Aoki, A., Arimoto, T., Nakano, T., Ohnuki, H., Murata, M., Ren, H. & Endo, H. (2015a). Fish stress become visible: A new attempt to use biosensor for real-time monitoring fish stress. *Biosensors and Bioelectronics*, 67, 503–510.

Wu, W., Zhao, S., Mao, Y., Fang, Z., Lu, X. & Zeng, L. (2015b). A sensitive lateral flow biosensor for *Escherichia coli* O157:H7 detection based on aptamer mediated strand displacement amplification. *Analytica Chimica Acta*, 861, 62–68.

Yamaguchi, M., Matsuda, Y., Sasaki, S., Sasaki, M., Kadoma, Y., Imai, Y., Niwa, D. & Shetty, V. (2013). Immunosensor with fluid control mechanism for salivary cortisol analysis. *Biosens Bioelectron*, 41, 186–191.

Yoo, S. M. & Lee, S. Y. (2016). Optical biosensors for the detection of pathogenic microorganisms. *Trends in Biotechnology*, 34, 7–25.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523

ID: 126605833

https://doi.org/10.18485/meattech.2023.64.2.18

Original scientific paper

Changes in bacterial status and aw values during the maturation of fermented sausages

Radmila Mitrović[a], Jelena Janjić[b], Vesna Janković[a], Brankica Lakićević[a], Lazar Milojević[a], Branko Velebit[a] and Branislav Baltić[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Enterobacteriaceae
LAB
Sausage diameter

ABSTRACT

This study presents the results related to changes in a_w value and bacteriological status of fermented sausages during maturation without and with added starter culture, stuffed into a narrower and wider casing. Values of a_w of narrower and wider diameter sausages with and without added starter cultures decreased during ripening, and were close to values of 0.9. *Enterobacteriaceae* in narrower diameter sausages were not detected on day 18, i.e., the end of the ripening process, and these bacteria were not detected in wider diameter fermented sausages on day 25 or at the end of ripening (day 35). The increase in the lactic acid bacteria in narrow and wider diameter sausages without added starter culture was slower than the increase in the number of these bacteria in sausages with added starter culture.

1. Introduction

Fermented sausages are among the oldest and most valuable meat products. Today, this type of sausage is produced in all parts of the world, especially since the advent of controlled microclimatic conditions in chambers (temperature, humidity, air circulation) for drying, smoking, and maturation. It is understood that the characteristics of fermented sausages vary greatly worldwide. Even within the same geographic region, or rather locality, there are different varieties of fermented sausages with typical regional recipes and established production technologies (*Leroy et al*., 2015). They are most commonly made from ground meat and fat tissue (mostly firm pork fat) with the addition of salt and spices, and are stuffed into natural (pork, sheep, beef) or artificial (collagen and cellulose) casings (*Savić & Savić*, 2002).

The basic and most important strategy for meat preservation, including fermented sausages, has been based on salting for centuries and its importance in controlling the growth of spoilage bacteria (especially pathogens), achieving the desired texture, and enabling slicing (*Montanari et al*., 2022). The use of starter cultures in the production of fermented sausages began after 1950, and just ten years later, a mixture of different bacteria (lactic acid bacteria, micrococci) was used as a starter (*Laranjo et al*., 2019). In the mid-1990s, the meat industry started using selected bacterial cultures that act antagonistically towards pathogenic bacteria and bacteria that produce toxins. Water content is closely related to the a_w (water activity) value of meat products, and fermented sausages are considered shelf-stable if their a_w value is around 0.92 (https://www.meatsandsausages.com/sausage-types/fermented-sausage).

***Corresponding author:** Radmila Mitrović, *radmila.mitrovic@inmes.rs*

Paper received Jun 30th 2023. Paper accepted July 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

The aim of this study was to investigate changes in a_w values and selected bacterial groups during the maturation and drying of two different diameter fermented sausages, with and without the addition of a starter culture.

2. Materials and methods

For the purposes of the experiment, pork meat of the first and second category (back meat, shoulder, leg, meat with fat and connective tissue), as well as a certain proportion of pork back fat, were used. A meat grinder was used to grind these ingredients to the desired degree of fineness. The ground meat was left to cool at 0 to 5°C. After 24 hours, a spice mixture for tea sausage (containing glucose, table salt, spices, and spice extracts) and nitrite salt were added in a quantity of 2.3%, and after that the ground meat was divided into four groups. The first group (KI) consisted of narrower diameter sausages of the control group without a starter culture, the KII group consisted of wider diameter sausages of the control group without a starter culture, the KIII group consisted of narrower diameter sausages of the control group with a starter culture, and the KIV group consisted of wider diameter sausages of the control group with a starter culture. The starter culture contained lactic acid bacteria (*Lactobacillus sakei, Staphylococcus carnosus*, and *Staphylococcus xylosus*). The starter culture was added at a rate of 20 g per 200 kg of ground meat. The filling was stuffed into casings of narrower (34 mm) and wider diameter (55 mm). The sausages were labeled by groups, and placed on racks in the smokehouse (smoking at a temperature of 20–23°C, drying at 17°C and a relative air humidity of 75%). The production process lasted 18 days for sausages of narrower diameter and 35 days for sausages of wider diameter.

Microbiological analyses were conducted using the following methods: *Enterobacteriaceae* count according to ISO (2009); lactic acid bacteria count according to ISO (1998). Water activity (a_w) values were determined using the *Gimenéz and Dalgaard* (2004) method.

The obtained results were compared through statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). Mean values and measures of variation for the bacterial count and a_w value were calculated. The mean bacterial counts and a_w values in the sausages are presented graphically.

3. Results

The changes in the a_w value of sausages with a narrower diameter, with and without the addition of a starter culture, are shown in comparison with the changes in enterobacteria and lactic acid bacteria (LAB) in Figures 1–4.

The a_w value of the sausages with a narrower diameter without a starter culture (group KI) was 0.9695 ± 0.0006 on day 0 and decreased during the maturation process to 0.9110 ± 0.0010 on day 18 (Figures 1a, 2a). On day 0, the a_w value of the sausages with a narrower diameter and the addition of a starter culture (group KIII) was 0.9697 ± 0.0007, and on day 18, at the end of the maturation process, it was 0.9211 ± 0.0006 (Figure 1b, 2b).

The average number of enterobacteria in the group KI sausages was 4.64 ± 0.32 log CFU/g on day 0, which decreased to 3.01 ± 0.09 log CFU/g on day 7. In the group KIII sausages, the average number of enterobacteria was 4.64 ± 0.08 log CFU/g on day 0, and on day 7, it was 2.47 ± 0.08 log CFU/g. Enterobacteria were not detected in the sausages with a nar-

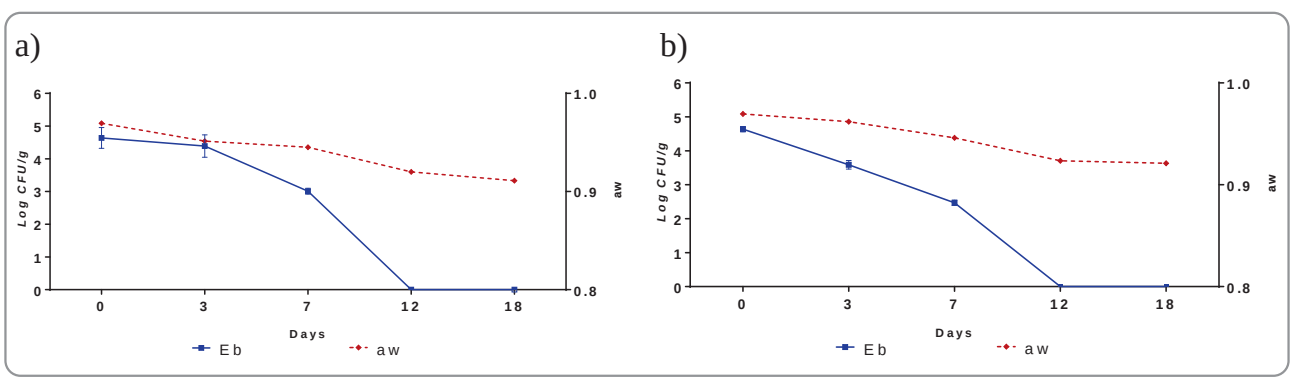


Figure 1. Numbers of *Enterobacteriaceae* (Eb) and a_w values during maturation of narrower diameter sausages without (a) group KI, and with added starter culture (b) – group KIII.

rower diameter, both with and without the addition of a starter culture, on days 12 and 18 of maturation (Figures 1a and 1b). The LAB counts in the sausages in groups KI and KIII during maturation are presented in Figures 3a and 3b. The average LAB count in group KI sausages was 5.09 ± 0.25 log CFU/g on day 0, while in group KIII, it was 5.21 ± 0.08 log CFU/g. The highest LAB count in group KI sausages was observed on day 12 of maturation (9.25 ± 0.08 log CFU/g) (Figure 2a), while in group KIII, it was on day 7 of maturation (9.56 ± 0.01 log CFU/g) (Figure 2b).

The a_w values of sausages with a wider diameter, with and without the addition of a starter culture, are shown in comparison with the bacterial status of sausages in Figures 3 to 4. The average a_w value of sausages with a wider diameter without the addition of starter cultures (group KII) was 0.9695 ± 0.0006 on day 0 and decreased during maturation to 0.9346 ± 0.0003 on day 35 (Figure 3a, 4a). Similar results were obtained for the sausages with added starter culture (group KIV). On the day 0, the a_w value of the filling was 0.96950 ± 0.0008, and it

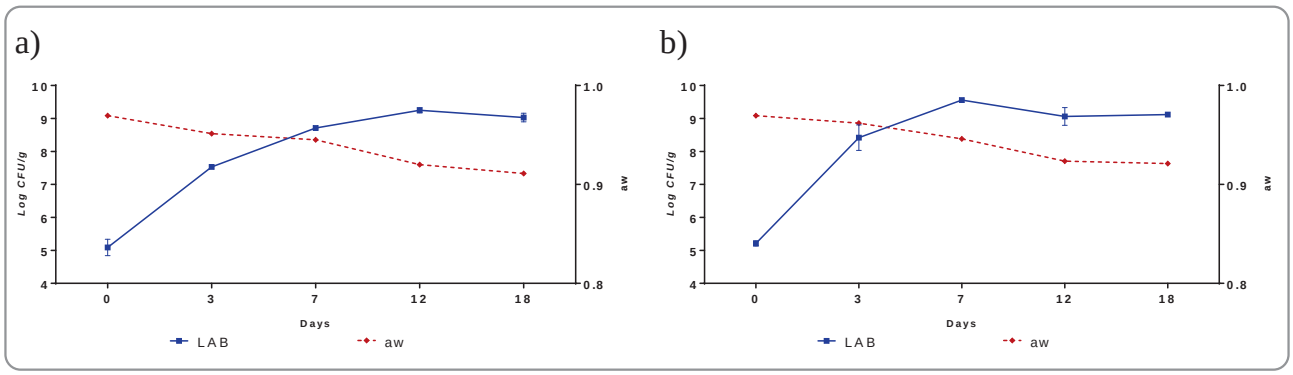


Figure 2. Numbers of lactic acid bacteria (LAB) and a_w values during maturation of narrower diameter sausages without (a) – group KI, and with added starter culture (b) – group KIII.

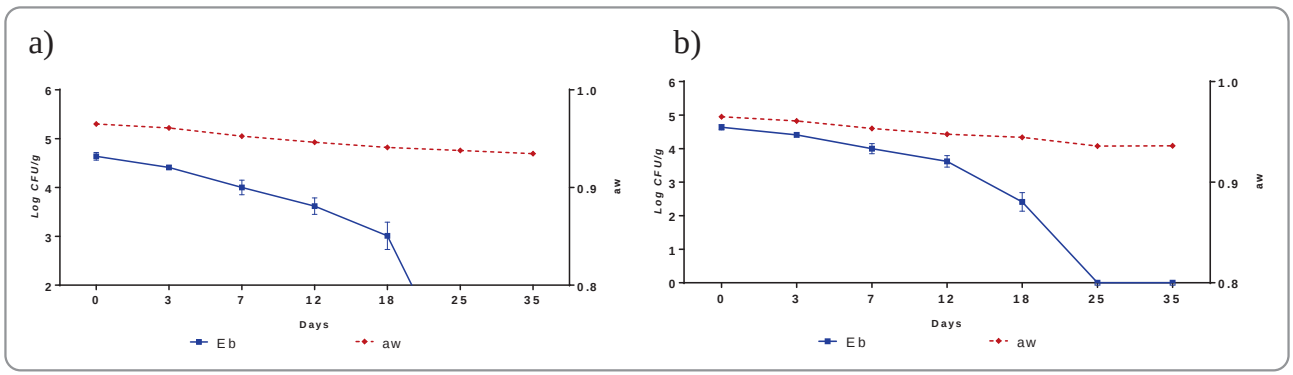


Figure 3. Numbers of *Enterobacteriaceae* (Eb) and a_w values during maturation of wider diameter sausages without (a) – group KII, and with added starter culture (b) – group KIV.

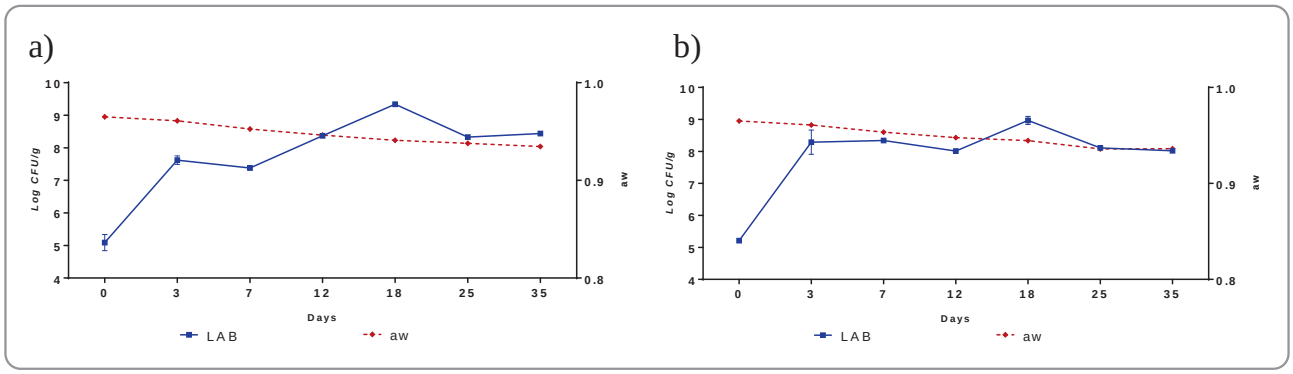


Figure 4. Numbers of lactic acid bacteria (LAB) and a_w values during maturation of wider diameter sausages without (a) – group KII, and with added starter culture (b) – group KIV

decreased during maturation to 0.9361 ± 0.0006 on day 35 (Figure 3b, 4b).

On the day of maturation, the average number of enterobacteria in the filling of group KII sausages was 4.46 ± 0.32 log CFU/g, and it did not significantly change until day 7. On day 12, it was 3.85 ± 0.12 log CFU/g, and on day 18, it was 3.01 ± 0.05 log CFU/g (Figure 3a). The average number of enterobacteria initially in the group KIV sausages was 4.64 ± 0.08 log CFU/g, and it consistently decreased during maturation, reaching 2.41 ± 0.28 log CFU/g on day 18 (Figure 3b). Enterobacteria were not detected in the filling of sausages in groups KII and KIV on days 25 and 35 of maturation (Figures 3a and 3b). The average LAB count initially in the filling of sausages in group KII was 5.09 ± 0.25 log CFU/g, and it increased until day 18 of maturation, when it reached 9.34 ± 0.22 log CFU/g. It then decreased by one logarithm, measuring 8.33 ± 0.16 log CFU/g on day 25 and 8.44 ± 0.22 log CFU/g at the end of maturation on day 35 (Figure 4a). In the filling of group KIV sausages, the average LAB count at the beginning of testing was 5.21 ± 0.11 log CFU/g, and it rapidly increased by day 3, reaching 8.29 ± 0.38 log CFU/g. It then remained stable until the day 35, measuring 8.02 ± 0.06 log CFU/g (Figure 4b).

4. Discussion

In industrial and traditional meat processing, fermented sausages have significant economic importance. The traditional production of fermented sausages with spontaneous fermentation contributes to the characteristic attributes associated with specific locations or rural environments. These attributes include physical properties (sausage diameter), physicochemical properties (pH and a_w), and the microbiota of sausages (total bacterial count, enterobacteria, enterococci, LAB, yeasts) *(Leroy et al*., 2015; *Palavecino et al.*, 2021). Traditional fermented sausages, especially those with geographical indication protection, are mainly produced without the addition of starter cultures. The preservation strategy relies on the addition of salt to reduce the water activity, control the growth of pathogens, and promote the formation of a desirable product texture.

In traditional fermented sausages, the a_w values are generally higher in sausages with a larger diameter. The a_w value depends on the water and salt content, as increasing water content in sausages increases the a_w, while increasing salt content reduces it. However, in industrial food production, the salt content is being reduced in all meat products, including fermented sausages, for health and nutritional reasons. Therefore, special importance is given to the use of starter cultures that contribute to the safety and quality of the final product. Lactic acid bacteria have the ability to lower the pH of the filling, thereby inhibiting the growth of spoilage bacteria, while also bringing the meat proteins to their isoelectric point, promoting water loss and the maturation phenomenon of sausages (*Montanari et al*., 2022).

The use of starter cultures in the production of fermented sausages dates back to the mid-20th century, initially using a mixture of LAB and micrococci as a starter culture. The primary goal was to achieve the desired color of the filling, control bacterial spoilage, and shorten the production process. Later, attention was given to starter cultures that prevent the growth of pathogens and bacteria that produce toxins. Today, the most commonly used starter cultures include LAB, *Staphylococcus*, and yeasts (*Laranjo et al*., 2019; *Cocconcelli & Fontana*, 2014; *Berni*, 2014; *Holck et al.*, 2017). According to *Montanari et al.* (2022), changes in a_w values during the maturation of fermented sausages are not dependent on the starter culture and casing selection. In the tested groups of sausages with different diameters and with the addition of different starter cultures, the a_w values were 0.97 in fresh filling, 0.96 after four days, and at the end of maturation, i.e., 30 days, ranged from 0.92 to 0.93. Numerous bacteria species are tolerant to an a_w value of 0.92. For example, *C. botulinum* does not grow below an a_w value of 0.93, *B. cereus*, *C. jejuni*, and *Salmonella* spp. do not grow below 0.95, *E. coli* does not grow below 0.93, and *L. monocytogenes* does not grow below 0.92 (source: https://www.meatsandsausages.com/sausage-types/fermented-sausage). *Baka et al.* (2011) determined that the initial LAB count (different strains of *L. sakei*) at the beginning of their study ranged from 5.7 to 6.9 log CFU/g. By the eighth day, for most strains, it increased to between 8 and 9 log CFU/g and remained relatively stable until the 28th day, the end of the maturation process. In sausages without the addition of starter cultures, the bacterial count was 4 log CFU/g at the beginning, 7.5 log CFU/g on the 16th day, and 8 log CFU/g at the end of the 28th day. In all sausage groups (experimental and control), the count of enterobacteria was 3.5 log CFU/g and decreased to below the detection limit by the 16th day. When different starter cultures and casings were used in fermented sausages (*Montanari et al.*, 2022), on day 0 of the study, the LAB

count ranged from 6.94 to 7.08 log CFU/g, on the fourth day it ranged from 8.02 to 8.84 log CFU/g, and on the 30th day, it ranged from 7.50 to 8.44 log CFU/g. In the same sausage groups, the count of enterobacteria on day zero ranged from 1.81 to 2.00 log CFU/g, on the fourth day it ranged from 1.00 to 1.77 log CFU/g, and on the 30th day, it ranged from 1.30 to 2.16 log CFU/g (*Montanari et al.*, 2022). In addition to the production of organic acids (mainly lactic acid), the pH decrease, and the a_w reduction, the preserving effect of LAB is also based on the production of antimicrobial components known as bacteriocins. Our results regarding a_w values and the bacteriological status of fermented sausages largely align with the findings of other authors.

5. Conclusion

During the ripening of fermented sausages, there is a decrease in the a_w value and number of enterobacteria, and an increase in lactic acid bacteria. The production of fermented sausages with a narrower diameter ends at 18 days, and with a wider diameter at 35 days.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143) and Contract number — Grant No. 451-03-47/2023-01/200050 from 03.02.2023

References

Baka, A. M., Papavergou, E. J., Pragalaki, T., Bloukas, J. G. & Kotzekidou, P. (2011). Effect of selected autochthonous starter cultures on processing and quality characteristics of Greek fermented sausages. *LWT-Food Science and Technology*, 44(1), 54–61.

Barbieri, F., Tabanelli, G., Montanari, C., Dall'Osso, N., Šimat, V., Smole Možina, S.... & Gardini, F. (2021). Mediterranean spontaneously fermented sausages: spotlight on microbiological and quality features to exploit their bacterial biodiversity. *Foods*, 10(11), 2691.

Berni, E. (2014). Molds. *Handbook of Fermented Meat and Poultry*, 147–153.

Cocconcelli, P. S. & Fontana, C. (2014). Bacteria. *Handbook of fermented meat and poultry*, 117–128.

Giménez, B. & Dalgaard, P. (2004). Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage micro-organisms in cold-smoked salmon. *Journal of Applied Microbiology*, 96(1), 96–109.

Holck, A., Axelsson, L., McLeod, A., Rode, T. M. & Heir, E. (2017). Health and safety considerations of fermented sausages. *Journal of Food Quality*, 2017.

ISO. (2009). 21528-2:2009. Microbiology of food and animal feedingstuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count method.

ISO, (1998). 15214:1998. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of mesophilic lactic acid bacteria — Colony-count technique at 30 degrees C.

Laranjo, M., Potes, M. E. & Elias, M. (2019). Role of starter cultures on the safety of fermented meat products. *Frontiers in Microbiology*, 10, 853.

Leroy, F., Scholliers, P. & Amilien, V. (2015). Elements of innovation and tradition in meat fermentation: Conflicts and synergies. *International Journal of Food Microbiology*, 212, 2–8.

Montanari, C., Barbieri, F., Gardini, G., Magnani, R., Gottardi, D., Gardini, F. & Tabanelli, G. (2022). Effects of Starter Cultures and Type of Casings on the Microbial Features and Volatile Profile of Fermented Sausages. *Fermentation*, 8(12), 683.

Palavecino Prpich, N. Z., Camprubí, G. E., Cayré, M. E. & Castro, M. P. (2021). Indigenous microbiota to leverage traditional dry sausage production. *International Journal of Food Science*, 2021.

Savic, Z. & Savic, I. (2002). Sausage casings. *Victus*.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 616-056.3:663/664

ID: 126610185

https://doi.org/10.18485/meattech.2023.64.2.19

Review paper

Major allergens — the big nine

Vesna Janković[a], Branko Velebit[a], Brankica Lakićević[a], Radmila Mitrović[a] and Lazar Milojević[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Food allergens
Big nine
Food safety

ABSTRACT

Legal standards specifically describe the need for allergy labelling on foods, beverages and other non-pre-packaged items because food allergies have developed as a concealed problem that seriously jeopardizes public health. The growing number of people who have food allergies has made it difficult for the foodservice business to control them appropriately. Current efforts in order to protect vulnerable customers include recognized standards for producers aiming to eliminate an allergen from their products and the inclusion of food allergy education in the training for people in food service and those in charge of enforcing food standards.

1. Introduction

A life-threatening chronic illness, food allergy significantly reduces a person's quality of life. The management of these allergies is an important aspect of public health policy. Children and adults are affected, and it has a significant negative impact on health, the medical system and growing economies (*Greenhawt*, 2016). According to *Muraro et al*. (2014), food-specific IgE antibodies, cellular processes, or both may play a role in the development of food allergy, which is defined as a health problem brought on by a specific immune-mediated reaction that occurs consistently after eating a particular food (*Wang and Sampson*, 2011). The European Academy of Allergology and Clinical Immunology (EAACI) describes food allergy as a subclass of allergic reactions where the immune system is involved. Based on the action mechanism, food allergies can be classified as IgE-mediated, non-IgE-mediated, or other types of reactions (*Wang and Sampson*, 2011). Food allergy is acknowledged as a significant public health concern that must be adequately addressed by a variety of stakeholders, including the food sector. To ensure the safety of customers who have allergies, the food industry has attempted to manage allergens, but this has proven to be extremely difficult due to the distinctive characteristics of food allergies and food allergens. The cornerstones of efficient allergen management include accurate allergen labelling, minimizing random allergen presence and safe use of relevant precautionary labelling where necessary. There are currently no effective treatments for food allergies. The best way to manage allergies is to strictly avoid allergens because they can cause serious and occasionally life-threatening allergic reactions (*Muraro et al*., 2014).

2. Big nine

Non-ingredient allergenic components that can be generated through cross-contact, such as during manufacturing or packing, are not covered by the EU food information control. In cas-

***Corresponding author:** Vesna V. Janković, *vesna.jankovic@inmes.rs*

Paper received Jun 6th 2023. Paper accepted Jun 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

es where unexpected allergens are present, food manufacturers might adopt voluntary precautionary allergen labelling (PAL) to notify and protect allergy sufferers (*Muraro et al*., 2014). Unfortunately, incorrect labelling and sporadic use of PAL that is not based on a quantitative assessment of allergy risk could cause a mismatch between labelling and allergen presence (*Crotty and Taylor*, 2010; *Remington et al.,* 2015), putting allergic consumers at risk and limiting their choices of products (*Holzhauser et al*., 2020). In Serbia, the Ordinance on declaring, labelling, and advertising of food (*Republic of Serbia,* 2017–2020) and the Ordinance on the health safety of dietary foods (*Republic of Serbia*, 2010–2018) provide legislation regarding allergens that takes into account 14 food ingredients that can cause allergic reactions. The information on the nine top allergenic foods (often known as the "big nine") will be presented in this study. More than 170 foods have been reported to cause allergic reactions, but the vast majority are caused by the "big nine": milk, eggs, nuts, fish, crustaceans, shellfish, wheat, soy and sesame (*FDA*, 2022).

2.1 Eggs and egg products

A wide range of culinary products, including bread and confectionery, gourmet (soups, sauces, dressings), and meat products, regularly contain eggs and egg components or additives. The edible portion of an egg is made up of 63% egg white, 27.5% yolk, and 9.5% egg shell, including the membrane that lines the shell. According to *Lee, Loh and Tang* (2018), 1.8 to 2% of children under the age of five have allergies to eggs, making it one of the most prevalent allergenic foods. The majority of the proteins connected to egg hypersensitivity are found in egg white (*Réhault-Godbert et al.*, 2019). Both egg white and egg yolk contain clinically significant allergenic egg proteins. Egg allergies can result in symptoms ranging from a mild rash to anaphylaxis. The anticipated prevalence is between 0.2% to 7% (*Rona et al*., 2007; *Lee*, 2017). The precise amount of a food allergen needed to cause an allergic reaction is rarely determined due to variations in patient sensitivity and the specificity of the allergen. According to *Taylor et al.* (2002), the cumulative doses of whole raw eggs that can cause allergies range from 0.13 mg to 200 mg of dry protein.

2.2 Milk and milk products

Since milk provides the needed proteins, minerals, fats, and carbohydrates for human health, it is regarded as a complete food (*Pereira et al*., 2012). Although milk has many nutritional advantages and is widely recommended (*Lucarini*, 2017; *Chalupa-Krebzdak et al*., 2018; *Silva et al.*, 2020), milk consumption in Western countries is rapidly declining. Milk contains high-quality proteins, fats, vitamins, and minerals (such as potassium, phosphorus, and calcium). Babies and young children frequently have cow's milk allergies, which typically go away by the age of six. The immune system's reaction to a particular milk protein causes it to show up as an allergic reaction (*Edwards and Younus*, 2021). Numerous cow's milk proteins are immunologically active and antigenic, and it has been discovered that many people are sensitive to a variety of cow's milk proteins. According to studies done on large populations of allergic patients, the most prevalent proteins in cow's milk, particularly lactoglobulins, caseins, and α-lactalbumin (ALA), are the main allergens; however, proteins present in low quantities, like bovine serum albumin, lactoferrin and immunoglobulins, have also proven to be important in causing milk allergies (*Silva et al*., 2020).

2.3 Cereals containing gluten and cereal products

For baked goods (bread, pastries, pizza), pasta (noodles, pasta, spaghetti), some confections (cakes, biscuits, gingerbread), and prepared foods (cream soups, sauces, etc.), wheat flour is used as a raw material (*Popov-Raljić*, 2016); *Psodorov*, 2014). Infants are most likely to develop allergic reactions to wheat and other cereals, and these reactions often subside within the first few years of life. Cereal allergy symptoms that are IgE-mediated range from mild local skin or gastrointestinal reactions to more severe, frequently fatal anaphylactic crises. Examples of wheat allergies include bakers' asthma (occupational exposure to grain flour dust) and, less frequently, IgE-mediated allergy related to exercise, also known as wheat-dependent exercise-induced anaphylaxis. People who are sensitive to wheat-related crops (barley, oats, and rye) can usually tolerate rice. In Europe and America, allergies to rice are rare, but they might be more prevalent in Asia. With an estimated 5% global incidence, gluten-related diseases have grown in epidemiological relevance. Wheat allergy, non-celiac gluten sensitivity, and celiac disease are all conditions linked to gluten (*Rubio-Tapia and Murray*, 2010).

2.4 Fish, crustaceans, molluscs and their products

Although seafood is essential for human nutrition, health and economics, it can have serious IgE antibody-mediated adverse reactions in individuals who are vulnerable. Seafood includes fish (cod, salmon and tuna), shellfish (shrimp, crab and lobster), and molluscs (squid, shellfish and snails). According to *Sharp and Lopata* (2014), eating seafood can result in severe acute hypersensitivity reactions, including fatal anaphylaxis. There are over 20,000 edible fish species, although just a few groups (*Actinopterygii*) produce the fish that are frequently eaten. People who are allergic to fish should avoid eating any fish because they are typically allergic to a wide range of species. According to *Freidl et al.* (2017), adverse reactions to seafood can be immunological, such as IgE allergy, which is mediated by the antibody for which the trigger is taken. They can also be non-immunologic, such as toxins or pathogenic components. Worldwide, 0.3% of people have a fish allergy, while 0.6% have a shellfish allergy.

2.5 Soybeans and soybean products

Due to their high concentration of physiologically active compounds that can have positive effects, legumes like soybeans grown under specific conditions are also included in the functional food list (*Popov-Raljić*, 2016). Soybean allergy is less common than each of the other seven major allergens, which has been used to argue that soybean could be eliminated from the eight major allergens without endangering the general population (*Messina and Venter*, 2020). In four adult surveys, allergies to milk/dairy products and shellfish were more prevalent than allergies to soy protein. Worldwide, 0.1% to 0.6% of the population were found to be allergic to soybeans.

2.6 Sesame

Since sesame allergy causes severe/systemic adverse immunological reactions in sesame-allergic people, it is typically a life-persisting allergy. Native to the Middle East and Africa, where it has been grown as an oilseed crop for more than 3,000 years, sesame (*Sesamum indicum*) is a seed. Traditionally, it is eaten as a paste known as tahini or as a sweet known as halva. Western nations sometimes utilize it as a topping for breads and crackers. In the USA and Canada, estimates of the population prevalence of self-reported sesame allergy range from 0.1% to 0.2%. According to studies, prevalence rates range from 0.1% in Canadian children to up to 0.8% in Australian children (*Dalal et al*. 2002; *Adatia et al*., 2017). Sesame-related anaphylaxis rates vary greatly by geographic location, with rates in the Middle East being substantially higher than those in North America. Sesame has been identified as the second most frequent meal to trigger anaphylaxis in Israeli children, accounting for 43% of cases, and the third most frequent food to trigger anaphylaxis in Saudi Arabia, although it was only shown to be the source of 2.8% of food-induced anaphylaxis cases in Canadian children. At least 8 allergenic epitopes associated with IgE-mediated reactions have been found, to date, in sesame seeds, and three sesame oil constituents have been connected to allergic contact dermatitis (*Adatia et al*., 2017). Members of the seed storage compounds, the oleosin, and profilin families have been identified as epitopes that can act as mediators of rapid hypersensitivity, whereas lignins are linked to delayed-type hypersensitivity. Sesame allergy is important to recognize due to the rarity of sesame allergy outgrowth and the high danger of unexpected reactions, despite sesame allergy's relatively low frequency in the EU and North America (*Segal et al*., 2017).

2.7 Peanuts and peanut products

Peanut allergy is a common, enduring, and sometimes fatal food allergy that is becoming more prevalent in Western countries. It is one of the most frequent IgE-mediated food reactions (*EFSA*, 2014). Only 20% of people acquire a tolerance to peanuts, it usually manifests in infancy, and it is often identified between the ages of 6 and 24 months. Additionally, it lasts longer than allergies to milk or eggs. Numerous (n = 18) peanut allergens have been identified so far: All allergens cause significant peanut allergies: Ara h 1: cupin, Ara h 2: conglutin (2S albumin), Ara h 3: cupin, Ara h 4: renamed as Ara h 3.02, Ara h 5 is profilin, Ara h 6 and Ara h 7 are conglutin, Ara h 8 is a pathogenesis-related protein, and PR-10 is a member of the bet v1 family. Ara h 9 is nonspecific lipid-transfer protein type 1, Ara h 10, 1, 14, and 15 are oleosins, Ara h 12 and 13 are defensins, Ara h 16 and 17 are nonspecific lipid transfer protein 2 and 1, and Ara h 18 is cyclophilin, peptidyl-prolyl cis-trans-isomerase (www.allergen.org/allergen nomenclature).

2.8 Nuts

Nuts can be found in many different forms, from raw seeds to baked appetizers. The average daily intake of nuts and peanuts for the overall population in the EU was 2.23 g. Total daily nut intake in Europe ranged

from 0.61 g in Sweden to 4.83 g in Spain, from northern to southern regions. Walnuts, almonds, pistachios and hazelnuts are the most popular nuts in Europe (*Lack*, 2008). Both children and adults develop dietary allergies due to this common cause, and the clinical reaction can be lethal. In the UK, 1.7% of people in the overall population had a documented allergy to nuts and almonds. Around 9% of people, including individuals who have had severe reactions in the past, outgrow this type of allergy (*Fleischer*, 2012). Like fish and peanut allergies, stone fruit allergies are lifelong, and because the majority of stone fruit allergens are homologous to one another, there is frequently cross-reactivity. According to estimates by *Siecherer et al.* (2003), 20–50% of those who are allergic to peanuts are also allergic to nuts.

3. Conclusion

Food allergy is a significant problem for public health. The severity of allergic reactions can range from gastrointestinal issues and skin rashes to anaphylaxis, shock and even death. Allergy sufferers must avoid foods that contain allergenic components to prevent allergic reactions. Customers, therefore, rely on food labels to alert them to the existence of allergenic ingredients. The controls needed to ensure that allergens that are intended to be present in a food have been identified on the label, and that unexpected allergens are absent, must be developed, put into position, and maintained by food manufacturers. The use of warning phrases like “may contain [allergen]” or “produced on equipment that also processes [allergen]” is insufficient to prevent contact with allergens. Prerequisite programs need to be used, along with HACCP plan controls that guarantee proper product labelling, to manage allergens. In order to improve the health and quality of life of Serbians with allergic diseases, as well as reduce the burden of allergic diseases on individuals, healthcare systems and the community, a national allergen strategy (NAS) must be developed in Serbia in collaboration with key stakeholder organizations.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the Contract on research financing in 2023 (451-03-47/2023-01/200050 od 03.02.2023).

References

Adatia, A., Clarke, A. E, Yanishevsky, Y. & Ben-Shoshan, M. (2017). Sesame allergy: current perspectives. *Journal of Asthma Allergy*, 10, 141–151.

Blok, B. M. (2014). European Academy of Allergy and Clinical Immunology. EAACI Food Allergy and Anaphylaxis Guidelines. Food allergy health-related quality of life measures. *Allergy*, 69(7), 845–853, doi: 10.1111/all.12405

Chalupa-Krebzdak, S., Long, C. J. & Bohrer, B. M. (2018). Nutrient density and nutritional value of milk and plant-based milk alternatives. *International Dairy Journal*, 87, 84–92.

Crotty, M. P. & Taylor, S. L. (2010). Risks associated with foods having advisory milk labeling. *Journal of Allergy and Clinical Immunology*, 125(4), 935–937.

Dalal, I. et al. (2002). Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. *Allergy,* 57(4), 362–365.

Edwards, C. W. & Younus, M. A. (2021). Cow Milk Allergy, https://www.ncbi.nlm.nih.gov/books/NBK542243. Factors affecting the determination of threshold doses for allergenic foods: how much is too much. *Journal of Allergy and Clinical Immunology*, 109, 24–30.

Fleischer et al., (2012). Allergic reactions to foods in preschool-aged children in a prospective observational food allergy study. *Pediatrics*, 130(1), 25–32.

Freidl, R., Gstoettner, A., Baranyi, U., Swoboda, I., Stolz, F., Focke-Tejkl, M., Wekerle, T., van Ree, R., Valenta, R. & Linhart, B. (2017). Blocking antibodies induced by immunization with a hypoallergenic parvalbumin mutant reduce allergic symptoms in a mouse model of fish allergy. *Journal of Allergy and Clinical Immunology*, 139(6), 1897–1905.

Greenhawt, M. (2016). Food allergy quality of life and living with food allergy. *Current Opinion in Allergy and Clinical Immunology*, 16(3), 284–90.

Holzhauser, T., Johnson, P., Hindley, J. P., O’Connor, G., Chan, C. H., Costa, J., Fæste, C. K., Hirst, B. J., Lambertini, F., Miani, M., Robert, M. C., Röder, M., Ronsmans, S., Bugyi, Z., Tömösközi, S. & Flanagan, S. D. 2020. Are current analytical methods suitable to verify VITAL® 2.0/3.0 allergen reference doses for EU allergens in foods. *Food and Chemical Toxicology*, 145, 111709, https://doi.org/10.1016/j.fct.2020.111709

https://www.fda.gov/food/food-labeling-nutrition/food-allergies

Lack, G. (2008). Food allergy, T*he New England Journal of Medicine*, 1254, 359.

Lee, S. (2017). IgE-mediated food allergies in children: prevalence, triggers, and management. *Korean Journal of Pediatrics*, 60(4), 99–105, doi: 10.3345/kjp.2017.60.4.99

Loh, W. & Tang M. L. K. (2018). The epidemiology of food allergy in the global context. *International Journal Environmental Research and Public Health*, 15(9), 2043.

Lucarini, M. (2017). Bioactive peptides in milk: From encrypted sequences to nutraceutical aspects. *Beverages*, 3(3),41.

Messina, M. & Venter, C. (2020). Recent surveys on food allergy prevalence. *Nutrition Today*, 55(1), 22–29.

Muraro, A., Dubois, A. E., DunnGalvin, A., Hourihane, J. O., de Jong, N. W., Meyer, R., Panesar, S. S., Roberts, G., Salvilla, S., Sheikh, A., Worth, A. & Flokstra-de (?). *Pediatric Allergy and Immunology*, 29(7), 762–772.

Official Gazette of the Republic of Serbia, (2010–2018). Ordinance on the health safety of dietary foods. *Official Gazette of the Republic of Serbia*, No. 45/2010, 27/2011, 50/2012, 21/2015, 75/2015, 7/2017 and 103/2018.

Official Gazette of the Republic of Serbia, (2017–2020). Ordinance on declaring, labelling, and advertising of food. *Official Gazette of the Republic of Serbia*, No. 19/2017, 16/2018, 17/2020 and 118/2020.

Popov-Raljić, J. (2016). Ishrana, Univerzitet u Novom Sadu, PMF, Departman za geografiju, turizam i hotelijerstvo, 41–50.

Psodorov, Đ. (2014). Osnovi pekarstva i poslastičarstva, Univerzitet u Novom Sadu, PMF, Departman za geografiju, turizam i hotelijerstvo, 18–24.

Réhault-Godbert, S., Guyot, N. & Nys, Y. (2019). The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. *Nutrients*, 11, 684, 17–26.

Remington, B. C., Krone T. & Koppelman, S. J. (2015). Quantitative risk reduction through peanut immunotherapy: Safety benefits of an increased threshold in Europe.

Rona, R., Keil, T., Summers, C, Gislason, D., Zuidmeer, L., Sodergren, E., Sigurdardottir, S., Lindner, T., Goldhahn, K., Dahlstrom, J., McBride, D. & Madsen, C. (2007). The prevalence of food allergy: A meta-analysis. *American Academy of Allergy Asthma & Immunology*, 120 (3), 638–646.

Rubio-Tapia, A. & Murray, J. A. (2010). Celiac disease. *Current Opinion in Gastroenterology*, 26(2), 116–122.

Rulebook on health correctness of diet foods (Official Gazette of RS, No. 45/2010, 27/2011, 50/2012, 21/2015, 75/2015, 7/2017 and 103/2018 — other rulebook).

Segal, L., Ben-Shoshan, M., Alizadehfar, R….& et al. (2017). Initial and accidental reactions are managed inadequately in children with sesame allergy. *Journal of Allergy and Clinical Immunology Practice,* 5(2), 482–485.

Sharp, M. & Lopata, A. (2014). Fish Allergy: In Review. *Clinical Reviews in Allergy & Immunology*, 46(3), 258–271.

Silva, A., Silva, M. & Ribeirob, D. (2020). Health issues and technological aspects of plant-based alternative milk. *Food Research International*, 131, 1–14.

Wang, J. & Sampson, H. (2011). Food allergy. *Journal of Clinical Investigation*, 121 (3), 827–835.

www. allergen. org-allergen nomenclature.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.03

ID: 126620169

https://doi.org/10.18485/meattech.2023.64.2.20

Review paper

Agricultural waste: a source of bioactive compounds for potential application in meat products

Milica Glišić[a], Marija Bošković Cabrol[a,b], Nikola Čobanović[a], Milan Ž. Baltić[a], Vladimir Dražković[a], Stevan Samardžić[d] and Zoran Maksimović[c]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar oslobođenja 18, 11000 Belgrade, Serbia*
[b] *University of Padova, Department of Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), Viale dell'Università 16, 35020 Legnaro, Padova, Italy*
[c] *University of Belgrade, Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Agro-industrial by-products
Natural antioxidants
Phenolic compounds
Food additives

ABSTRACT

Globalization and population growth have led to the development of a modern agricultural system that currently produces millions of tons of waste. This waste is disposed of by burning, dumping or accumulating in landfills, resulting in environmental, health, and economic issues. The agro-industrial residues are abundant with phenolic bioactive compounds, such as phenolic acids, flavonoids, tannins, and carotenoids, which, among others, exhibit antioxidant and antimicrobial capacities and have good potential as food flavorings and colorants. The most common method for isolating these compounds is solvent extraction. However, there is a trend towards eco-innovative extraction methods that offer better possibilities for implementation on an industrial scale. The oxidation of lipids and proteins is one of the main causes of quality deterioration in meat and meat products during processing and storage. Therefore, the application of natural antioxidants extracted from these new, unconventional raw materials could be a sustainable alternative to synthetic antioxidants. This review summarizes the data on natural antioxidants derived from agro-industrial by-products and their incorporation in various meat product formulations. It also addresses limiting factors related to safety and changes in sensory properties.

1. Introduction

Approximately 1,300 million tons of waste from the agricultural sector are produced worldwide annually, with a tendency to increase due to the demand for greater production as a result of economic growth and rising living standards (*Amran et al*., 2021). Failure to ensure proper disposal procedures or treatment for up to 50% of this waste represents one of the main causes of environmental pollution with a harmful effect on human and animal health and the economy (*Amran et al*., 2021). In Serbia, data regarding the quantity of agro-industrial waste from processed crops, fruits and vegetables are very scarce. According to Serbian government data, the total amount of waste in 2021 was 72,183 kt, to which agriculture contributed 0.8% and the processing industry 2.1%, while household waste makes up 82.4%, with the estimation that only 5% of the total produced waste is recycled (*Anon*, 2023).

Considering that most agricultural waste is untreated and underutilized, mainly disposed of by burning, dumping, or unplanned landfilling, the strategies and technology for conversion of agricultural wastes into valuable by-products are constantly devel-

***Corresponding author:** Milica Glišić, *glisic.mica@gmail.com*

Paper received July 3rd 2023. Paper accepted July 19th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

oping for the purpose of ensuring economically sound, sustainable, cleaner, and socially beneficial production (*Santana-Méridas et al.*, 2012). Agricultural waste can be converted directly, through different physical, chemical, and biochemical processes, or separated into components to produce fuels, energy, fiber-based products, and chemical-based high-value products (*Spatafora & Tringali*, 2012; *Sadh et al.,* 2018). The potential of crop residues for phytochemical extraction has not yet been fully explored (*Sadh et al*., 2018), but conversely, there has been a growing interest in agro-industrial residues as low-value raw materials abundant with different bioactive compounds having antioxidant and antimicrobial properties (*Amran et al*., 2021).

2. Agro-industrial waste as a source of natural antioxidants

Industrial by-products generated in the form of peels, cores, seeds, leaves, etc., account for more than 50% of the raw material that is generally discarded by the food industry. *Diamanti et al*. (2017) indicated that for every ton of pomegranate juice produced, nine tones of by-products are obtained. However, in most cases, these non-edible parts contain high nutritional properties and are excellent sources of dietary fiber, carbohydrates, proteins, flavorings, colorants, minerals, and especially phenolic compounds (*Coman et al*., 2020). For example, jabuticaba (*Myrciaria cauliflora*) residues from jelly and liquor-processing industries are an excellent source of natural pigments with antioxidant properties (anthocyanins and flavonoids) (*Baldin et al.*, 2016). The processing of grapes for wine production generates up to 30% waste, including pomace, peels, and seeds, which are considered a source of flavonoids and phenolic acids (*Carpes et al*., 2020). Other good sources of functional compounds are apple pomace and olive pomace (*Lourenço et al.*, 2019). The phenolic compound content in peels of lemons, oranges and grapefruits is 15% higher than the peeled fruits. The total phenolic content in pineapple by-products is higher than in fresh pulp (*da Silva et al.*, 2013). A higher concentration of lycopene, ascorbic acid, and phenolic compounds is also found in tomato peels compared to pulp (*George et al.*, 2004).

3. Extraction technologies

The quality of plant-originated antioxidants depends on the features of the raw materials and the technology used for their extraction. There is no standard procedure for the extraction, because these compounds have various physical and chemical properties and are constrained in different vegetal matrices (*Lourenço et al*., 2019). The most common method used is solvent extraction, which comprises different solvents, separately or in mixtures, including ethanol, acetone, methanol, hexane, petroleum ether, ethyl ether, ethyl acetate, and water (*Lai et al*., 2017). From the aforementioned solvents, only water, ethanol, ethyl acetate and acetone have GRAS (generally recognized as safe) status for use in the preparation of food ingredients (*Marriott*, 2010). This conventional method has several disadvantages, such as the use of a large amount of solvent, the use of toxic solvents (hexane and chloroform), evaporation, compound thermal degradation, and the long extraction process (*Azmir et al*., 2013). In this regard, great efforts have been made to develop eco-innovative technologies in the extraction process, so-called "green extraction methods", to replace potentially harmful organic solvents with non-toxic or food-safe ones (water, aqueous ethanol solutions, carbon dioxide, natural deep eutectic solvents), to speed up the extraction process and make it more efficient, reduce the size of the equipment, and reduce the harmful impact on the environment (*Pateiro et al*., 2021). Some of these technologies are accelerated solvent extraction, enzyme-assisted extraction, supercritical fluid extraction, high hydrostatic pressure extraction, pressurized liquid extraction, infrared-assisted extraction, pulsed electric field extraction, ultrasound-assisted extraction, and microwave-assisted extraction (*Lourenço et al.*, 2019). The current technologies were developed only at a laboratory scale, so recent research is dedicated to the possibilities of their implementation at the plant level in order to establish commercial sustainability. Promising results in scaling up extraction processes were obtained with solvent extraction, solvent-free microwave extraction, and supercritical fluid extraction (*Lourenço et al*., 2019).

4. The safety of natural antioxidants application

Many plant-derived compounds can act as antioxidants, but only a small percentage of them are safe for human consumption. The natural antioxidants must undergo a safety evaluation by the regulatory bodies including the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA) in order to be approved as food additives. This procedure implies a multi-step standard methodology: specification of the

chemical structure and physicochemical properties, risk assessments overview, proposed uses, exposure assessment, and toxicological studies (*EFSA*, 2012).

Beyond safety issues, the selection of natural additives is equally conditioned by organoleptic characteristics (especially odor and flavor), bearing in mind that they can significantly change the sensory attributes of the product, which could be unacceptable for consumers (*Mansour and Khalil*, 2000). From the application point of view, natural antioxidants must meet requirements similar to other food additives. Accordingly, they have to be compatible with the food matrix, easy to use, effective in low concentrations (0.001%–0.01%), stable during processing and shelf-life, economical, and must not negatively affect color, odor, or taste (*Hadidi et al*., 2022). However, since natural antioxidants usually exhibit lower antioxidant activities compared to synthetic ones, this implies that they would have to be used in higher concentrations, so for these compounds, the GRAS safety criterion should be fulfilled even in much higher doses (*Lourenço et al.,* 2019).

An additional aggravating factor in the application of by-products is the extensive use of various herbicides, insecticides and fungicides in agriculture, which consequently accumulate in agricultural residues. Byproduct safety hazards are also associated with mycotoxins (oil seed cake, corn by-products), heavy metals (arsenic in rice bran) and bacterial contamination of agricultural crops (*Lai et al*., 2017). Accordingly, the characterization and separation of toxins from agro-industrial raw materials are necessary so that bioactive compounds obtained from these sources are safe for use in value-added products, both for human health and for the environment (*Fritsch et al*., 2017).

5. Natural antioxidants in meat products

Lipid and protein oxidation is a common deterioration process responsible for the generation of undesirable, potentially toxic, chemical compounds, such as aldehydes, ketones, and organic acids, and for inducing protein modification through changes in amino acid composition, protein polymerization, and loss of proteolytic activity (*Hadidi et al.*, 2022). The high concentration of unsaturated fatty acids, heme pigments, metal catalysts, and oxidizing agents makes meat and meat products prone to oxidation, and consequently discoloration, off-flavor/odor development, nutrient loss, and drip loss during storage (*Amoli et al.*, 2021). Furthermore, meat and meat products are highly susceptible to bacterial spoilage and contamination by pathogenic microorganisms. Therefore, different measures, including good manufacturing and good hygienic practices, salting, heat treatments, drying, smoking, fermentation, use of additives, active packaging, and low temperatures during storage are implemented to prolong shelf life and preserve the safety and quality of meat products (*Gonçalves et al*., 2021).

The use of preservatives during the processing of meat products plays an important role in maintaining the products' overall quality. Due to their availability, high stability, good performance, and low-cost, synthetic antioxidants, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) are widely used to mitigate oxidation (*Lourenço et al*., 2019). In the European Union, the list of approved additives, the conditions of their use, and labelling are prescribed by the Regulation on Food Additives No 1333/2008 (*Regulation EC*, 2008). The use of synthetic additives in meat products in Serbia is regulated by the Rulebook on Food Additives No. 53/2018-22 (*Anon*, 2018). However, controversy has arisen in recent years regarding the use of synthetic additives in food, due to research that has shown the potential carcinogenic effects of these substances and the formation of toxic and mutagenic compounds during exposure to certain conditions, such as high temperature, which is a common procedure in the manufacture of meat products (e.g., nitrosamines generation from sodium nitrite) (*Gonçalves et al*., 2021). As a consequence, increasing consumer demand for fresh, natural, and healthier food rich in natural and biologically active compounds with additional health benefits, so-called "wellness foods" became a global trend embraced worldwide in industries, including the meat industry (*Pateiro et al.*, 2021). The use of natural antioxidants in order to reduce the consumption of synthetic additives and to obtain cleaner-label meat products could be considered as one of the promising alternatives (*Gonçalves et al.*, 2021, *Pateiro et al*., 2021).

However, the addition of natural antioxidants, rich in phenolic compounds, in the meat matrix results in unpleasant taste and aroma, notably a perceived astringency. Encapsulation technologies, such as micro- and nanoencapsulation, developed to overcome deteriorated sensory attributes of the product, offer enhanced stability against light and

Table 1. Natural antioxidants derived from agro-industrial waste incorporated in the formulation of meat products

Byproducts	Meat product Extract dose Storage	Application and Bioactive Compounds	Main Outcomes	Reference
Microencapsulated jabuticaba residue peels and seeds, water extract	- Fresh sausage - 2% and 4% - 15 days	- Natural dyes; antioxidant and antimicrobial - Phenolic compounds, mainly anthocyanins	↓TBARS (<0.1 mg MDA/kg) ↓Aerobic psychrotrophic count - Negatively influenced sensory color	*Baldin et al*., 2016
Lyophilized and microencapsulated grape pomace, ethanolic extracts	- Chicken pâté - 3 mg/g - 42 days	- Natural antioxidant - Gallic acid, trans-resveratrol, ferulic acid, coumaric acid, vanillic acid, caffeic acid	↓TBARS (≤2.5 mg MDA/kg)	*Carpes et al*., 2020
Rice bran extract	- Pork burgers - 0.5%, 1%, 2% - 21 days	- Natural antioxidant - Phenolic compounds and γ-oryzanol	↓Protein oxidation ↑b* value; ↑C* ↑Unpleasant taste	*Martillanes et al.*, 2020
Pomegranate peel water, acetone extract	- Uncured dry sausages - 1% and 2% - 28 days drying period	- Sodium nitrite substitute; natural antioxidant - Phenolic compounds, tannins, flavonoids	↓TBARS w(1.1-1.4 mg MDA/kg) ↓Carbonyls (10.5–14 nmol/mg protein) ↓Thiols (12.9–23.2 nmol Cys eq/mg protein) ↓a* value; ↑ b* value	*Cava & Ladero*, 2023
Ground buckwheat husk	- Frankfurter-type sausages - 1%, 2% and 3% - 14 days	- Natural antioxidant - Phenolic compounds (vitexin, quercetin), amino acid, mineral, fiber	↑Amino acid, Mn, Ca, K, Mg ↑Hardness ↓L* value; ↓b* value ↓Sensory acceptability	*Salejda et al*., 2022
Persimmon flour	- Liver pork pâté - 3% and 6%	- Natural antioxidant; colorant; nitrite-reducing agent - Carotenoids and phenolic acids	↓Residual nitrite levels ↓Emulsion stability ↓TBARS (<0.5 mg MDA/kg) ↓L* value; ↑ a* value ↑Sensory color intensity	*Lucas-González et al.*, 2019
Avocado varieties "Hass" and "Fuerte" peel, acetone/water extracts	- Porcine patties - 5% extract water solution -15 days	- Natural antioxidant - Catechins, procyanidins, hydroxycinnamic acids	↑% inhibitions against TBARS ↑% inhibitions against protein carbonyls	*Rodríguez--Carpena et al*., 2011
Sunflower and maize stalk residue, ethanolic extracts	- Liver pork pâté - 1% - 90 days	- Natural antioxidant and antimicrobial - Flavonoids, flavonolignans	↓TVC; ↓LAB; ↓Psychrotrophic count ↓L* value; ↑ b* value ↓Sensory acceptability	*Glišić et al.,* 2023

temperature, controlled release, and increased bioavailability of active compounds during meat processing and storage (*dos Santos Silva et al*., 2022).

Several papers have been published with the purpose of studying the incorporation of natural antioxidants extracted from different agro-industrial wastes into meat products, and some of them are presented in Table 1.

6. Conclusion

To replace synthetic antioxidants in the meat industry with active compounds from agro-industrial residues, efficient extraction methods and identification of active compounds are essential. Testing antioxidant activity *in vitro* and *in producto* while considering various processing conditions, including cooking, pressure, pH, ingredients, meat

matrix, etc., is crucial. However, sensory properties and consumer acceptance may be affected by natural antioxidants. Nutritional and toxicological studies are necessary to ensure safety, and consumer perception should be considered for adopting these new additives in meat products.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The research was supported by the Science Fund of the Republic of Serbia, No. 7752847, "Value-Added Products from Maize, Wheat and Sunflower Waste as Raw Materials for Pharmaceutical and Food Industry — PhAgroWaste" and by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Amoli, P. I., Hadidi, M., Hasiri, Z., Rouhafza, A., Jelyani, A. Z., Hadian, Z., Khaneghah, A. M. & Lorenzo, J. M. (2021). Incorporation of low molecular weight chitosan in a low-fat beef burger: Assessment of technological quality and oxidative stability. *Foods*, 10(8), Article 1959, https://doi.org/10.3390/foods10081959

Amran, M. A., Palaniveloo, K., Fauzi, R., Satar, N. M., Mohidin, T. B. M., Mohan, G., Razak, S. A., Arunasalam, M., Nagappan, T. & Sathiya Seelan, J. S. (2021). Value-added metabolites from agricultural waste and application of green extraction techniques. *Sustainability*, 13(20), 11432, https://doi.org/10.3390/su132011432

Anon, (2018). Pravilnik o prehrambenim aditivima. *Službeni glasnik RS*, broj 53/2018-22. Retrieved from https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2018/53/1/reg. Accessed June 26, 2023.

Anon, (2023). Statistički kalendar Republike Srbije, Republički zavod za statistiku, Republika Srbija, Beograd. Retrieved from https://publikacije.stat.gov.rs/G2023/Pdf/G202317016.pdf. Accessed June 26, 2023.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N. & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436, https://doi.org/10.1016/j.jfoodeng.2013.01.014

Baldin, J. C., Michelin, E. C., Polizer, Y. J., Rodrigues, I., de Godoy, S. H. S., Fregonesi, R. P., Pires, M. A., Carvalho, L. T., Fávaro-Trindade, S., Gonçalves de Lima, C., Fernandes, A. M. & Trindade, M. A. (2016). Microencapsulated jabuticaba (*Myrciaria cauliflora*) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity. *Meat Science*, 118, 15–21, https://doi.org/10.1016/j.meatsci.2016.03.016

Carpes, S. T., Pereira, D., Moura, C. D., Reis, A. S. D., Silva, L. D. D., Oldoni, T. L. C., Almeida, J. F. & Plata-Oviedo, M. V. S. (2020). Lyophilized and microencapsulated extracts of grape pomace from winemaking industry to prevent lipid oxidation in chicken pâté. *Brazilian Journal of Food Technology*, 23, Article e2019112, https://doi.org/10.1590/1981-6723.11219

Cava, R. & Ladero, L. (2023). Pomegranate peel as a source of antioxidants for the control of lipid and protein oxidation during the ripening of Iberian dry uncured sausages. *Meat Science*, 202, Article 109198, https://doi.org/10.1016/j.meatsci.2023.109198

Coman, V., Teleky, B. E., Mitrea, L., Martău, G. A., Szabo, K., Călinoiu, L. F. & Vodnar, D. C. (2020). Bioactive potential of fruit and vegetable wastes. *Advances in Food and Nutrition Research*, 91, 157–225, https://doi.org/10.1016/bs.afnr.2019.07.001

da Silva, D. I., Nogueira, G. D., Duzzioni, A. G. & Barrozo, M. A. (2013). Changes of antioxidant constituents in pineapple (*Ananas comosus*) residue during drying process. *Industrial Crops and Products*, 50, 557–562, https://doi.org/10.1016/j.indcrop.2013.08.001

Diamanti, A. C., Igoumenidis, P. E., Mourtzinos, I., Yannakopoulou, K. & Karathanos, V. T. (2017). Green extraction of polyphenols from whole pomegranate fruit using cyclodextrins. *Food Chemistry*, 214, 61–66, https://doi.org/10.1016/j.foodchem.2016.07.072

dos Santos Silva, M. E., Grisi, C. V. B., da Silva, S. P., Madruga, M. S. & da Silva, F. A. P. (2022). The technological potential of agro-industrial residue from grape pulping (*Vitis* spp.) for application in meat products: A review. *Food Bioscience*, Article 101877, https://doi.org/10.1016/j.fbio.2022.101877

EFSA, (2012). Panel on food additives and nutrient sources added to food (ANS). Guidance for submission for food additive evaluations. *EFSA Journal*, 10(7), 2760, https://doi.org/10.2903/j.efsa.2012.2760

Fritsch, C., Staebler, A., Happel, A., Cubero Márquez, M. A., Aguiló-Aguayo, I., Abadias, M., Gallur, M., Cigognini, I. M., Montanari, A., López, M. J., Suárez-Estrella, F., Brunton, N., Luengo, E., Sisti, L., Ferri, M. & Belotti, G. (2017). Processing, valorization and application of bio-waste derived compounds from potato, tomato, olive and cereals: A review. *Sustainability*, 9(8), Article 1492, https://doi.org/10.3390/su9081492

George, B., Kaur, C., Khurdiya, D. S. & Kapoor, H. C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry*, 84(1), 45–51, https://doi.org/10.1016/S0308-8146(03)00165-1

Gonçalves, L. A., Lorenzo, J. M. & Trindade, M. A. (2021). Fruit and agro-industrial waste extracts as potential antimicrobials in meat products: A brief review. *Foods*, 10(7), Article 1469, https://doi.org/10.3390/foods10071469

Hadidi, M., Orellana-Palacios, J. C., Aghababaei, F., Gonzalez-Serrano, D. J., Moreno, A. & Lorenzo, J. M. (2022). Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. *LWT*, 169, Article 114003, https://doi.org/10.1016/j.lwt.2022.114003

Lai, W. T., Khong, N. M., Lim, S. S., Hee, Y. Y., Sim, B. I., Lau, K. Y. & Lai, O. M. (2017). A review: Modified agricultural by-products for the development and fortification of food products and nutraceuticals. *Trends in Food Science & Technology*, 59, 148–160. https://doi.org/10.1016/j.tifs.2016.11.014

Lourenço, S. C., Moldão-Martins, M. & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, 24(22), Article 4132, https://doi.org/10.3390/molecules24224132

Lucas-González, R., Pellegrini, M., Viuda-Martos, M., Pérez-Álvarez, J. Á. & Fernández-López, J. (2019). Persimmon (*Diospyros kaki* Thunb.) coproducts as a new ingredient in pork liver pâté: influence on quality properties. *International Journal of Food Science & Technology*, 54(4), 1232–1239, https://doi.org/10.1111/ijfs.14047

Mansour, E. H. & Khalil, A. H. (2000). Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry*, 69(2), 135–141, https://doi.org/10.1016/S0308-8146(99)00234-4

Marriott, R. J. (2010). Greener chemistry preparation of traditional flavour extracts and molecules. *Agro Food Industry Hi-Tech*, 21(2), 46–48.

Martillanes, S., Ramírez, R., Amaro-Blanco, G., Ayuso-Yuste, M. C., Gil, M. V. & Delgado-Adámez, J. (2020). Effect of rice bran extract on the preservation of pork burger treated with high pressure processing. *Journal of Food Processing and Preservation*, 44(1), Article e14313, https://doi.org/10.1111/jfpp.14313

Pateiro, M., Gómez-Salazar, J. A., Jaime-Patlán, M., Sosa-Morales, M. E. & Lorenzo, J. M. (2021). Plant extracts obtained with green solvents as natural antioxidants in fresh meat products. *Antioxidants*, 10(2), Article 181, https://doi.org/10.3390/antiox10020181

Regulation EC, (2008). Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Retrieved from https://www.legislation.gov.uk/eur/2008/1333/data.pdf. Accessed June 22, 2023,

Rodríguez-Carpena, J. G., Morcuende, D., Andrade, M. J., Kylli, P. & Estévez, M. (2011). Avocado (*Persea americana* Mill.) phenolics, *in vitro* antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, 59(10), 5625–5635, https://doi.org/10.1021/jf1048832

Sadh, P. K., Duhan, S. & Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresources and Bioprocessing*, 5(1), Article 1, https://doi.org/10.1186/s40643-017-0187-z

Salejda, A. M., Olender, K., Zielińska-Dawidziak, M., Mazur, M., Szperlik, J., Miedzianka, J., Zawiślak, I., Kolniak-Ostek, J. & Szmaja, A. (2022). Frankfurter-type sausage enriched with buckwheat by-product as a source of bioactive compounds. *Foods*, 11(5), Article 674, https://doi.org/10.3390/foods11050674

Santana-Méridas, O., González-Coloma, A. & Sánchez-Vioque, R. (2012). Agricultural residues as a source of bioactive natural products. *Phytochemistry Reviews*, 11, 447–466, https://doi.org/10.1007/s11101-012-9266-0

Spatafora, C. & Tringali, C. (2012). Valorization of vegetable waste: identification of bioactive compounds and their chemo-enzymatic optimization. *The Open Agriculture Journal*, 6(1), 9– 16, https://doi.org/10.2174/1874331501206010009

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 639.215.2.09:595.34

ID: 126443017

https://doi.org/10.18485/meattech.2023.64.2.21

Review paper

Zoonotic potential of *Eustrongylides spp.* in freshwater fish meat

Dragana Ljubojević Pelić[a], Miloš Pelić[a], Nikolina Novakov[b], Milica Živkov Baloš[a] and Vesna Đorđević[c]*

[a] *Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia*
[b] *University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*
[c] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Nematodes
Zoonoses
Freshwater fish
Fishborne parasites

ABSTRACT

Parasites from the genus *Eustrongylides* spp. are widespread throughout the world in numerous species of freshwater fish and represent a significant hazard for humans. The development cycle of this parasite is complex. Definitive hosts are fish-eating birds while fish are intermediate host or paratenic host. Humans are accidental hosts and some clinical cases requiring surgical intervention have been reported, making eustrongylidosis a serious zoonotic disease. The aim of the paper is to present the most important characteristics of this parasite, previous findings in various fish species around the world, clinical cases in humans, prevention measures, as well as some aspects of current national and European regulations.

1. Introduction

In recent years, a constant trend of increased fish consumption has been noticeable around the world (*Tacon*, 2023). Fish is an important part of a healthy diet due to its nutritional properties (*Ljubojević et al.*, 2017; *Chen et al.*, 2022). However, the fact that fish can be a source of zoonoses must not be ignored (*Ljubojević et al.*, 2016; *Williams et al.*, 2022). Parasites, as well as their larval forms in fish meat, lead to sensory changes in fish meat, and fish that is visibly invaded by parasites is assessed as hygienically unfit for human consumption. In addition, certain parasites that may be present in freshwater fish have significant zoonotic potential, pose a serious risk and can lead to serious disease in humans (*Ljubojević et al.*, 2012). Roundworms are nematodes belonging to the genus *Eustrongylides* in the family *Dioctophymatidae* are present worldwide in many fish species, including species that are commercially important. The aim of the paper is to present the most important features of this parasite, its worldwide distribution, recorded clinical cases in humans, preventive measures, as well as some points of current national and European legislation, as well as various recommendations of relevant bodies.

2. Development cycle of *Eustrongylides* spp.

The development cycle of parasites from the genus *Eustrongylides* is very complex. Adult forms of the parasite are found in fish-eating birds, which are also the main host of this parasite. These birds that live near different water surfaces spread parasite eggs in the aquatic ecosystem with their faeces. *Oligochaeta* eat the eggs that reach the aquatic ecosys-

***Corresponding author:** Dragana Ljubojević Pelić, dragana@niv.ns.ac.rs*

Paper received May 19th 2023. Paper accepted May 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

tem, and in them, the development cycle continues by releasing the larvae. The next transitional hosts are fish that feed on the oligochaetaes, while the parasitological stage developed in them is infectious for the final host, i.e. for fish-eating birds, but also for predatory fish (*Novakov et al.*, 2013). Predatory fish are paratenic hosts and can transmit the parasite to piscivorous birds. Larvae reach sexual maturity quickly in fish-eating birds, where they lay eggs and complete their life cycle (*Orajić et al.*, 2023). Humans are accidental hosts.

3. Worldwide distribution of *Eustrongylides spp.*

Undoubtedly, anthropogenic influences through environmental pollution, climate change, construction of dams, as well as changes in people's habits when it comes to migration, tourism, and methods

Table 1. Worldwide distribution of Eustrongylides spp. in different fish species

Fish species	Country and/or region	Reference
Perch (*Perca fluviatilis*); largemouth black bass (*Micropterus salmoides*); Big-scale sand smelt (*Atherina boyeri*); eel (*Anguilla anguilla*); black bullhead (*Ictalurus melas*); carp (*Cyprinus carpio*); tench (*Tinca tinca*); pumpkinseed sunfish (*Lepomis gibbosus*)	Central Italy, the Trasimeno Lake	*Franceschini et al*., 2022
Perca fluviatilis, Lepomis gibbosus, Micropterus salmoides	Northern Italy, Lake Garda	*Menconi et al*., 2021
Big-scale sand smelt (*Atherina boyeri*)	Italy, Lake Massaciuccoli	*Guardone et al*., 2021
European perch (*Perca fluviatilis)*, largemouth black bass (*Micropterus salmoides*), sand smelt (*Atherina boyeri)*	Italy, Trasimeno Lake	*Branciari et al*., 2016
Perch (*Perca fluviatilis*, Linnaeus)	Central Italy, Trasimeno Lake (Umbria region)	*Dezfuli et al*., 2015
39 species of prey fish species and piscivorous fish species	USA, Florida	*Coyner et al*., 2002
European catfish (*Siluris glanis*)	Serbia, Danube-Tisa-Danube Canal in the territory of Novi Sad	*Novakov et al*., 2013
Pike-perch (*Sander lucioperca*)	Serbia, Vojvodina, Danube-Tisa-Danube Canal in the city area of Novi Sad	*Bjelić-Čabrilo et al*., 2013
Glossogobius giuris (Ham.)	India	*Kaur et al*., 2013
Odontobutis obscurus, *Silurus asotus*, *Culter mongolicus*, *Acanthogobius flavimanus*, *Monopterus albus*, *Channa argus*, *Channa asiatica*	China, nine localities	*Xiong et al*., 2013
Bigmouth sleeper (*Gobiomorus dormitory*)	Mexico, El Mezquital, Matamoros Tamaulipas,	*Salgado-Maldonado*, 2006
Bigmouth sleeper (*Gobiomorus dormitory*)	Northeastern Mexico, four coastal localities of Tamaulipas	*Garrido-Olvera et al*., 2022
Murray cod and Murray cod-trout cod hybrids	Australia	*Shamsi et al*., 2023
Hoplia malabaricus	Brazil, the Brazilian Amazon	*Correa et al*., 2023

of preparing and eating fish, contribute to changes in the prevalence of fish parasites, and contribute to parasites becoming global, not only health but also a significant economic problem (*Baltić et al*., 2013). Nematode larvae *Eustrongylides* spp. are distributed worldwide in numerous fish species, and some of the findings are shown in Table 1.

Parasites were mostly recorded in fish from wild catch, but some cases were also recorded in farmed fish. *Mitchell et al.* (2009) reported a case of mortality caused by *Eustrongylides ignotus* in commercially farmed sunshine bass, a hybrid cross of female-white bass, and male-striped bass in the USA. *Hernández-Ocampo et al.* (2012) described the occurrence of parasites in farmed fish in Mexico. *Kundu et al.* (2016) reported the presence of parasites in *Channa punctatus* fish (17–21 cm in length) collected from fish farms in Naihati and Kalyani, West Bengal.

4. Public health issues

The importance of parasites from the genus *Eustrongylides* from the aspect of public health is mostly in the fact that the larvae are not only present in the digestive tract but also in the meat of various types of fish (*Ljubojević et al.*, 2012; *Novakov et al.*, 2013). Findings of *Eustrongylides* larvae and clinical cases of the disease have been described in humans after consumption of raw or insufficiently thermally processed fish carrying third or fourth stage larvae (Table 2). The majority of patients required abdominal surgery.

Diseases caused by fish parasites are most often associated with Asia, but the risk has increased significantly in other parts of the world due to changes in aquaculture, tourism and increased transport and distribution of both fish and people. Additionally, methods of fish meat preparation are one of the most important factors contributing to the appearance

Table 2. Reported clinical cases of eustrongylidosis in humans

Region/Country	Number and age of patients	Symptoms	Reference
California, USA	One adult man	Under the skin granulomas in the chest contained the *Eustrongylides* nematodes	*Beaver and Theis*, 1979
Maryland, USA	Two fishermen	Progressive spastic pains of the stomach area 24 hours after the parasites got into gastrointestinal tract	*Guerin et al*., 1982
Baltimore, USA	Three adult patients	Parasites emerging from a patient's intestinal wall into the abdominal cavity were observed by laparoscopy. Two patients required abdominal surgery while one was treated medically.	*Gunby*, 1982
New York City, USA	A college student	A 10-hour history of pain in stomach	*Wittner et al*., 1989
New Jersey, USA	17-year-old youth	Intense abdominal pain in the right lower quadrant. Two large living nematodes were surgically removed from the peritoneal cavity	*Eberhard et al*., 1989
USA	A 17-year-old white male patient	Right lower quadrant pain, laparotomy for suspected acute appendicitis, a temperature of 38°C	*Narr et al*., 1996
South Sudan	Two women, 23 and 24 years of age	Fourth-stage larvae of *Eustrongylides* emerged from the lower limb of patients	*Eberhard and Ruiz-Tiben*, 2014

of diseases caused by parasites. *Eiras et al.* (2017) reported that people travelling abroad can also transfer parasites. According to the available data, no cases of disease in humans have been recorded in Serbia so far. However, the presence of this parasite in fish caught in rivers or canals in Serbia have been recorded (*Bjelić-Čabrilo et al.*, 2013; *Ćirković et al.*, 2013; *Novakov et al.*, 2013), which is very significant, but more extensive research is necessary in order to get a better picture of the epidemiology of this parasite in our country and to determine adequate measures in the form of monitoring, but also for potential sanitary measures. The big problem is that when it comes to fish-borne parasites there are very little data, many cases are unreported and those that are reported are mostly discovered by chance.

5. Diagnosis

A reliable diagnosis is achieved by visual inspection and dissection of a group of fish, as well as considering the places where parasites appear and the characteristic appearance of the parasites. Parasites of the genus *Eustrongylides* are relatively long, smooth and round in shape, and due to the presence of haemoglobin, they are a red colour (*Oraić et al.*, 2023). Beside visual inspection, candling is also applied. Both methods are highly dependent on the training and skill of the operator, as well as the type of sample being examined. In addition to these methods, other methods are also applied, such as the UV-press method (*ISO 23036-1*, 2021) the digestion method (*ISO 23036-2*, 2021) and the method that involves placing all internal organs in a container filled with water and leaving it to incubate at room temperature overnight (*Shamsi and Suthar*, 2016). These methods are very difficult to apply in routine work by food business operators and are mainly used in laboratories. The importance of fish-borne parasites is recognized, but there is still a problem that there are not enough standardized analytical methods and validated procedures for testing fish for the presence of parasites (*Chalmers et al.*, 2020).

6. Prevention and control

Prevention is possible by interrupting the developmental cycle of the parasite, i.e., by removing one of the transitional hosts or by removing the final host from the parasite's developmental cycle. Sanitation of fish ponds is a preventive solution for farmed fish, and as previously noted, very few cases of eustrongylidosis have been reported in aquacultured fish. The most important measure of prevention is the consumption of thermally well-processed fish. Recommendations are to avoid raw or thermally insufficiently treated fish in the diet. Very little data is available on the impact of different processing technologies on the survival of parasites in fish. Thermal treatment with the correct application of the ratio of time and temperature is one of the safest measures (*Ljubojević et al.*, 2015). In addition, adequate freezing is an adequate measure for parasites from the genus *Eustrongylides*, while fish preservation methods in the form of brining or pickling can reduce but not eliminate or reduce to an acceptable level the hazard for humans from *Eustrongylides* parasites (FDA, 2022). The presence of visible parasites means that the fish is not suitable for human consumption according to both European and Serbian regulations (*EC 178*, 2002; *Official Journal of RS, 41*, 2009 ; *17*, 2019), which causes significant economic consequences. *Franceschini et al.* (2022) reported in details the parasitological risk management carried out by food business operators to detect and remove visible parasites during different fish processing methods in order to prevent the placing on the market of fish contaminated with visible parasites according to *EC 2074* (2005). The larvae, due to their characteristic appearance, are relatively visible both in the organs and in the flesh of the fish, which enables them to be detected and removed by food business operators. Furthermore, in case of heavy infestation, fish could be discarded. Also, there are guides from various relevant organizations when it comes to processing fish in order to eliminate the risk of parasites (*Codex Alimentarius*, 2009; *ESSA*, 2018). The correct application of preventive procedures according to the European legislation for risk management that exists for fish from the sea is very important, i.e., that the same procedures are applied to freshwater fish, especially taking into account the changes in people's behaviour and habits when it comes to food, by which they have impact on the spread of zoonoses through the consumption of infected fish.

7. Conclusion

The best preventive measure when it comes to the risk of *Eustrongylides* spp. from fish to human health is visual inspection of fish by entities in the food business along with proper fish preparation. Continuous veterinary sanitary control of fish and fish products is necessary when placing them on the market, bearing in mind the zoonotic poten-

tial of this parasite, i.e., that it is a danger to human health. Certainly, there is a need for a multidisciplinary approach to the problem in terms of the cooperation of different sectors, i.e., the inclusion of state institutions, scientific institutions, professional organizations of the veterinary and agricultural and biological professions and production entities in order to conduct wider research and establish the real impact of parasites on production in aquaculture, and including losses due to damage caused by parasites. All this should contribute to the adoption of more detailed regulations related to this issue.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

References

Baltić, M., Bošković, M., Djordjević, V., Marković, R., Dimitrijević, M. & Pavličević, N. (2013). Fish-borne parasitic zoonoses with special reference to anthropogenic impact. 6. International Conference "Water & Fish" Faculty of Agriculture, Belgrade-Zemun, Serbia, 129–135.

Beaver, P. C. & Theis J. H. (1979). Dioctophymid larval nematode in a subcutaneous nodule from man in California. *American Journal of Tropical Medicine and Hygiene*, 28, 206–212, https://doi.org/10.4269/ajtmh.1979.28.206

Bjelić-Čabrilo, O., Novakov, N., Ćirković, M., Kostić, D., Popović, E., Aleksić, N. & Lujić, J. (2013). The first determination of *Eustrongylides excisus* Jägerskiöld, 1909: larvae (Nematoda: Dioctophymatidae) in the pike-perch *Sander lucioperca* in Vojvodina (Serbia). *Helminthologia*, 50, 291–294, https://doi.org/10.2478/s11687-013-0143-1

Branciari, R., Ranucci, D., Miraglia, D., Valiani, A., Veronesi, F., Urbani, E., Lo Vaglio, G., Pascucci, L. & Franceschini, R. (2016). Occurrence of parasites of the genus *Eustrongylides* spp. (Nematoda: Dioctophymatidae) in fish caught in Trasimeno lake, Italy. *Italian Journal of Food Safety*, 5, 206–209, https://doi.org/10.4081/ijfs.2016.6130

Chalmers, R., Robertson, L., Dorny, P., Jordan, S., Kärssin, A., Katzer, F., La Carbona, S., Lalle, M., Lassen, B., Mladineo, I., Rozycki, M., Bilska-Zajac, E., Schares, G., Mayer-Scholl, A., Trevisan, C., Tysnes, K., Vasilev, S. & Klotz, C. (2020). Parasite detection in food: Current status and future needs for validation. *Trends in Food Science & Technology*, 99, 337–350, https://doi.org/10.1016/j.tifs.2020.03.011

Chen, J., Jayachandran, M., Bai, W. & Xu, B. (2022). A critical review on the health benefits of fish consumption and its bioactive constituents. *Food Chemistry*, 369, 130874, https://doi.org/10.1016/j.foodchem.2021.130874.

Codex Alimentarius, (2009). Code of Practice for fish and fishery products. First edition World Health Organization Food And Agriculture Organization Of The United Nations Rome, Italy. Retrieved from https://www.fao.org/3/a1553e/a1553e00.pdf, Accessed May 2023.

Correa, L. L., Soares, G. B., Müller, M. I. & Adriano, E. A. (2023). First morphological and molecular characterization of the genus *Eustrongylides* nematode larvae infecting fish in the Brazilian Amazon. *Biologia*, 1–8, https://doi.org/10.1007/s11756-023-01422-6

Coyner, D. F., Spalding, M. G. & Forrester, D. J. (2002). Epizootiology of *Eustrongylides* ignotus in Florida: Distribution, density, and natural infections in intermediate hosts. *Journal of Wildlife Diseases*, 38(3), 483–499, https://doi.org/10.7589/0090-3558- 38.3.483

Ćirković, M., Novakov, N., Petrović, J., Ljubojević, D., Apić, J., Babić, J. & Teodorović, V. (2013). Finding of parasitic nematodes of fishes present in the market. *Archives of Veterinary Medicine*, 6(2), 3–13.

Dezfuli, B. S., Manera, M., Lorenzoni, M., Pironi, F., Shinn, A. P. & Giari, L. (2015). Histopathology and the inflammatory response of European perch, muscle infected with *Eustrongylides* sp. (Nematoda). *Parasites & Ve*ctors, 8, 227, https://doi.org/10.1016/j.foodres.2014.06.038

Eberhard, M. L. & Ruiz-Tiben, E. (2014). Case report: Cutaneous emergence of *Eustrongylides* in two persons from South Sudan. *The American Journal of Tropical Medicine and Hygiene*, 90(2), 315–317, https://doi.org/10.4269/ajtmh.13-0638

Eberhard, M. L., Hurwitz, H., Sun, A. M. & Coletta, D. (1989). Intestinal perforation caused by larval *Eustrongylides* (Nematoda: Dioctophymatoidea) in New Jersey. *The American Journal of Tropical Medicine and Hygiene*, 40, 648–650, https://doi.org/10.4269/ajtmh.1989.40.648

EC, 178/2002 (2002). Commission Regulation (EC) No 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Union*, 31, 1–24.

EC, 2074/2005 (2005). Commission Regulation (EC) No. 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending. *Official Journal of the European Union*, 50, 27–59.

Eiras, J. C., Pavanelli, G. C., Takemoto, R. M. & Nawa, Y. (2018). An overview of fish-borne nematodiases among returned travelers for recent 25 years–unexpected diseases sometimes far away from the origin. *The Korean Journal of Parasitology*, 56(3), 215–227, https://doi.org/10.3347/kjp.2018.56.3.215

ESSA, (2018). European guide to good practice for smoked and/or salted and/or marinated fish. Retrieved from https://food.ec.europa.eu/system/files/2018-10/biosafety_fh_guidance_essa_smoked-salted-marinated-fish.pdf. Accessed on April 2023.

FDA, (2022). Fish and fishery products hazards and controls guidance. Retrieved from: https://www.fda.gov/media/80637/download. Accessed 06 May 2022.

Franceschini, R., Guardone, L., Armani, A., Ranucci, D., Roila, R., Valiani, A., Susini, F. & Branciari, R. (2022). Five-years management of an emerging parasite risk (*Eustrongylides* sp., Nematoda) in a fishery supply chain located on Trasimeno Lake (Italy). *Food Control*, 136, 108858, https://doi.org/10.1016/j.foodcont.2022.108858

Garrido-Olvera, L., García-Prieto, L., Osorio-Sarabia, D., Sánchez-Martínez, J. G., Rábago-Castro, J. L., Hernández-Mena, D. I. & de León, G. P. P. (2022). Parasites with zoonotic potential found in commercially important fish in Tamaulipas, Northeastern Mexico. *Parasitology International*, 88, 102550, https://doi.org/10.1016/j.parint.2022.102550

Guardone, L., Ricci, E., Susini, F., Polsinelli, E., Guglielmone, G. & Armani, A. (2021). First detection of *Eustrongylides excisus* (nematoda: Dioctophymatidae) in big-scale sand smelt (*Atherina boyeri*) from the Lake Massaciuccoli (northwest Tuscany, Italy): Implications for public health and seafood quality. *Food Control*, 120, 107517, https://doi.org/10.1016/j.foodcont.2020.107517

Guerin, P. F., Marapendi, S. & Grail, L. (1982). Intestinal perforation caused by larval *Eustrongylides*. *The Morbidity and Mortality Weekly Report*, 31, 383–389.

Gunby, P. (1982). One worm in the minnow equals too many in the gut. *JAMA*, 248(2), 163.

Hernández-Ocampo, D., Pineda-López, R. F., Ponce-Palafox, J. T. & Arredondo-Figueroa, J. L. (2012). Parasitic helminth infection in tropical freshwater fishes of commercial fish farms, in Morelos State, Mexico. *International Journal of Animal and Veterinary Advances*, 4(5), 338–343.

ISO, 23036-1, (2021). Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 1: UV-press method. Retrieved from https://www.iso.org/standard/74372.html. Accessed May 2023.

ISO, 23036-2, (2021). Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method. Retrieved from https://www.iso.org/standard/74373.html. Accessed May 2023.

Kaur, P., Shrivastav, R. & Qureshi, T. A. (2013). Pathological effects of *Eustrongylides* sp. larvae (Dioctophymatidae) infection in freshwater fish, *Glossogobius giuris* (Ham.) with special reference to ovaries. *Journal of Parasitic Diseases*, 37(2), 245–250, https://doi.org/10.1007/s12639-012-0173-5

Kundu, I., Bandyopadhyay, P. K., Mandal, D. R. & Gürelli, G. (2016). Study of pathophysiological effects of the nematode parasite *Eustrongylides* sp. on freshwater fish *Channa punctatus* by hematology, serum biochemical, and histological studies. *Türkiye Parazitolojii Dergisi*, 40(1), 42–47, DOI: 10.5152/tpd.2016.4551

Ljubojevic, D., Novakov, N., Djordjevic, V., Radosavljevic, V., Pelic, M. & Cirkovic, M. (2015). Potential parasitic hazards for humans in fish meat. *Procedia Food Science*, 5, 172–175, https://doi.org/10.1016/j.profoo.2015.09.049

Ljubojevic, D., Pelic, M., Djordjevic, V., Milojevic, L. & Cirkovic, M. (2016). Bacterial hazards in fish meat: The aetiologic agents of foodborne diseases. *Meat Technology*, 57(1), 31–42.

Ljubojević, D., Bjelić-Čabrilo, O., Novakov, N., Ćirković, M., Davidov, I., Jovanović, M. & Aleksić, N. (2012). *Eustrongylides* sp. in freshwater fish species as a potential hazard for humans. In Proceedings of the International Conference Biological Food Safety and Quality. Faculty of Agriculture, Novi Sad , Serbia.

Ljubojević, D., Đorđević, V. & Ćirković, M. (2017). Evaluation of nutritive quality of common carp, *Cyprinus carpio* L. In IOP conference series: Earth and Environmental Science, 85(1), 012013, IOP Publishing, Zlatibor, Serbia.

Menconi, V., Tedesco, P., Pastorino, P., Confortini, I., Esposito, G., Tomasoni, M., Mugetti, D., Gustinelli, A., Dondo, A., Pizzul, E., Fioravanti, M. L. & Prearo, M. (2021). Could fish feeding behaviour and size explain prevalence differences of the nematode *Eustrongylides excisus* among species? The case study of Lake Garda. *Water*, 13(24), 3581, https://doi.org/10.3390/w13243581

Mitchell, A. J. (2009). Eustrongylides ignotus infecting commercial bass, *Morone chrysops* female X *Morone saxatilis* male, and other fish in the southeastern USA. *Journal of Fish Diseases*, 32, 795–799, https://doi.org/10.1111/j.1365-2761.2009.01051.x

Narr, L. L., O'Donnell, J. G., Libster, B., Alessi, P. & Abraham, D. (1996). Eustrongylidiasis: a parasitic infection acquired by eating live minnow. *Journal of Osteopathic Medicine*, 96, 400–402, https://doi.org/10.7556/jaoa.1996.96.7.400

Novakov, N., Bjelic-Cabrilo, O., Cirkovic, M., Ljubojevic, D., Lujic, J., Davidov, I., & Jovanovic, M. (2013). Eustrongylidosis of European catfish (*Siluris glanis*). *Bulgarian Journal of Agricultural Science*, 19(1), 72–76.

Official Journal of RS 41/2009, 17/2019. Food Safety Law.

Oraić, D., Giovanna Zupičić, I. & Zrnčić, S. (2023). Eustrongilidoza riba: Pregledni članak. *Veterinarska Stanica*, 54(1), 107–113, https://doi.org/10.46419/vs.54.1.5

Salgado-Maldonado, G. (2006). Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa*, 1324(1), 1–357, https://doi.org/10.11646/zootaxa.1324.1.1

Shamsi, S. & Suthar, J. (2016). A revised method of examining fish for infection with zoonotic nematode larvae. *International Journal of Food Microbiology*, 227, 13‑16, https://doi.org/10.1016/j.ijfoodmicro.2016.03.023

Shamsi, S., Francis, N., Masiga, J., Barton, D. P., Zhu, X., Pearce, L. & McLellan, M. (2023). Occurrence and characterisation of *Eustrongylides* species in Australian native birds and fish. *Food and Waterborne Parasitology*, 30, e00189, https://doi.org/10.1016/j.fawpar.2023.e00189

Tacon, A. G. (2023). Contribution of fish and seafood to global food and feed supply: An analysis of the FAO food balance sheet for 2019. *Reviews in Fisheries Science & Aquaculture*, 31(2), 274–283, https://doi.org/10.1080/23308249.2022.2124364

Williams, M., Hernandez-Jover, M. & Shamsi, S. (2022). Parasites of zoonotic interest in selected edible freshwater fish imported to Australia. *Food and Waterborne Parasitology*, 26, e00138, https://doi.org/10.1016/j.fawpar.2021.e00138

Wittner, M., Turner, J. W., Jacquette, G., Ash, L. R., Salgo, M. P. & Tanowitz, H. B. (1989). Eustrongylidiasis: a parasitic infection acquired by eating sushi. The New England *Journal of Medicine*, 320, 1124–1126, https://www.nejm.org/doi/pdf/10.1056/NEJM198904273201706

Xiong, F., Li, W. X., Wu, S. G., Zou, H. & Wang, G. T. (2013). Molecular phylogeny and host specificity of the larval *Eustrongylides* (Nematoda: Dioctophmidae) from freshwater fish in China. *The Journal of Parasitology*, 99(1), 137–144, https://doi.org/10.1645/GE-3163.1

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.564.05

ID: 126450441

https://doi.org/10.18485/meattech.2023.64.2.22

Review paper

Toxoplasma gondii — control measures for reducing risks in the pork production chain

Nikola Betić[a], Ivana Branković Lazić[a], Nedjeljko Karabasil[b], Dragan Vasilev[b], Aleksandra Uzelac[c] and Ivana Klun[c]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*
[c] *University of Belgrade, Institute for Medical Research, Centre of Excellence for Food- and Vector-borne Zoonoses, Dr. Subotića 4, 11129 Belgrade, Serbia*

ARTICLE INFO

Keywords:
T. gondii
Pigs
Pork
Prevalences
Control measures

ABSTRACT

Parasites are highly significant pathogens that are transmitted through food. Their specific life cycles, transmission routes, and usually a lengthy period between infection and the first symptoms of the disease make them a substantial risk to public health. Additionally, there are challenges in detection, diagnosis, and treatment. Toxoplasmosis is considered the most widespread parasitic infection on a global scale. It is caused by the protozoan *Toxoplasma gondii*, one of the most successful parasites of animals and humans due to its ability to parasitize within the nuclei of a wide range of hosts. Because of its importance in both veterinary and human medicine, *T. gondii* is one of the most extensively studied parasites. Existing data show seroprevalences differ across continents, countries, and even within states and among specific communities. Consuming undercooked meat presents one of the greatest risk factors for human infection with the *T. gondii* parasite, with pork being recognized as a dominant source of infection.

1. Distribution of Toxoplasma gondii infection on pig farms

While *T. gondii* infection in domestic animals is generally not a significant clinical issue, it does pose a serious economic problem due to substantial losses resulting from spontaneous abortions. It also presents a public health concern due to a significant zoonotic risk. Consuming meat derived from asymptomatically infected animals is the most notable risk factor for human infection. The presence of *T. gondii* has been documented in all domestic animals used for human consumption, including pigs (*Dubey*, 2010). Pigs are one of the most commonly raised animal species worldwide, and pork constitutes a primary source of protein for millions of people across various cultures and geographic regions. As of April 2022, there were 778.64 million pigs raised worldwide, and in the same year, the European Union produced 134.2 million pigs (*Statista*, 2023). Pork production in Serbia for 2020 amounted to around 300 thousand tonnes (*RZS*, 2021).

Studies based on the detection of specific antibodies in pig serum indicate that the *T. gondii* parasite is present in pigs worldwide, with prevalence differing between continents, from country to country, and even within regions of the same country. Furthermore, prevalence differs depending on the

***Corresponding author:** Nikola Betić, *nikola.betic@inmes.rs*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

category of pigs examined, such as between sows and fattening pigs, as well as between pigs raised on farms with biosecurity systems compared to pigs kept in free-range systems (*Dubey*, 2009; *Roqueplo et al*., 2011). Based on a meta-analysis encompassing samples from five continents, the global seroprevalence of *T. gondii* infection in pigs stands at 19%. Prevalence differs between continents and is highest in Africa and North America (25%), followed by South America (23%), Asia (21%), and is the lowest in Europe, 13% (*Foroutan et al.*, 2019). Prevalences in Europe show a wide range, from 0.9% in Austria (*Edelhofer*, 1994) to 46.8% in backyard-raised pigs in Romania (*Pastiu et al*., 2019). In Serbia, several studies have been conducted regarding the presence of *T. gondii* infection in pigs. In the first seroepidemiological study, *T. gondii*-specific IgG antibodies were detected in 15.2% of fattening pigs and in 40.9% of culled sows (*Klun et al*., 2006), while the latest study conducted showed a similar trend, with a presence in 15.1% of fattening pigs and in 43.6% of culled sows (*Betić et al*., 2022).

2. Impact of *Toxoplasma gondii* on food safety

Given that pigs are a significant reservoir of this parasite due to the presence of numerous tissue cysts in nearly all edible parts (*Dubey*, 1986), it is not surprising that a recent study conducted in the US found the economic burden on pork meat caused solely by the *T. gondii* parasite to be estimated at nearly $US 2 billion (*Scharff*, 2020).

The prevalence of *T. gondii* in animals raised for human consumption is the main indicator for assessing the public health risk of toxoplasmosis. Indirect detection methods (serological tests) are commonly used to estimate the animal infection rate, but they only determine seroprevalence. Although seroprevalence is valuable for epidemiological studies, it often does not correlate directly with the presence of tissue cysts in meat, i.e., with findings from direct detection methods. *Opsteegh et al*. (2016) investigated the correlation between direct and indirect detection in various species of meat animals, and demonstrated that the probability of detecting the parasite in seropositive individuals is highest in pigs, 58.8%. Despite the advantages of serological testing, the negative aspect is that parasites are detected in a certain number of seronegative pigs as well (up to 4.9%), suggesting that a negative serological result does not guarantee parasite-free meat. Therefore, serological testing cannot be used for individual control of pig carcasses.

3. Control measures on farm levels

The differences in seroprevalence across different geographical regions can be attributed to various climatic factors, domestic animal husbandry systems, and the level of biosecurity measures implemented in primary production. Lower seroprevalences within the European continent can be explained by the widespread adoption of intensive pig farming methods, resulting in reduced exposure to infection compared to animals in other continents (*Herrero et al*., 2016). Several studies have shown that implementing biosecurity measures on farms, such as effective rodent control programs, restricting access to cats, maintaining hygiene in production facilities, and using uncontaminated water and feed, can lead to a reduction in the prevalence of *T. gondii* in pigs. Additionally, independent factors influencing infection include farms that rear multiple animal species and smaller pig farms that focus solely on finishing (*Betić et al*., 2022). Animals raised in free-range systems are more likely to become infected with *T. gondii* (*Dubey*, 2009); this likelihood is also directly correlated with the management system and level of biosecurity on farms (*Papatsiros et al*., 2016).

However, as consumer awareness regarding animal welfare, organic and sustainable production, and environmental conservation increases, pork from pigs raised in free-range systems is gaining higher market value, and such systems are becoming more prevalent across Europe (*Zander et al*., 2013). Research has indicated that pigs raised in such systems, in comparison to intensive production, pose a greater risk for transmitting certain pathogenic microorganisms to humans, due to the lesser control inherent in these systems compared to enclosed intensive production systems (*Kuruca*, 2017).

4. Control measures in meat processing

The significance of pork as a reservoir of human infection is also highlighted by a study that ranked the risk of various pathogens in combination with food, where the *T. gondii* parasite in conjunction with pork took a high second place (*Batz et al*., 2012). Based on a study conducted in the United States, pork has a higher likelihood of being contaminated with the *T. gondii* parasite compared to beef

and poultry (*Dubey*, 2005). Since only undercooked meat poses a risk of human infection, adequate cooking temperature is a critical factor in preventing parasite infection. However, a study from 2007 found that approximately 9% of consumers cooked pork at temperatures lower than 48°C, which is insufficient to inactivate *T. gondii* cysts (*Ecolab-Ecosure*, 2007). According to recommendations from the European Food Safety Authority, meat should be treated to reach an internal temperature of at least 67°C before consumption (*EFSA*, 2007).

Various preservation methods are used to produce meat products, including smoking, salting, freezing, heating, irradiation, or high-pressure treatment, which generally contribute to lower contamination of these products with the *T. gondii* parasite compared to fresh meat (*Mie et al*., 2008*; Klun & Djurkovic-Djakovic*, 2021). However, there are instances where the presence of the parasite has been confirmed in processed pork products, such as dry-cured ham and sausages (1.5%) in the United Kingdom (*Warnekulasuriya et al*., 1998). Since some pork products are consumed raw (ham, fermented sausages, cured meat products), such products can potentially serve as a source of parasite infection.

5. Conclusion

Consuming undercooked *T. gondii*-contaminated meat, especially pork, is recognized as one of the most significant risks for human infection with this parasite. It is necessary to observe protective measures on farms in terms of biosecurity measures that effectively reduce the risk of infection, and to introduce monitoring of *T. gondii*. In order for food to be safe for consumers, it is necessary to implement actions and activities to prevent or eliminate food safety hazards in accordance with the Codex Alimentarius (*Codex Alimentarius*, 2005). Finally, it is important to emphasize that the consistent application of preventive measures at all mentioned levels, from farms and slaughterhouses to meat processing and consumers, is crucial for their effectiveness.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The work was supported by grants from the Ministry of Science, Technological Development and Innovations of the Republic of Serbia to the Institute for Medical Research (451-03-47/2023-01/200015, from 03.02.2023), to the Institute of Meat Hygiene and Technology (451-03-47/2023-01/200050), and to the Faculty of Veterinary Medicine, Belgrade (contract no. 451-03-47/2023-01/200143).

References

Batz, M. B., Hoffmann, S. & Morris, J. G. (2012). Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *Journal of Food Protection*, 75, 1278–1291.

Betić, N., Karabasil, N., Djurković-Djaković, O., Ćirković, **V., Bobić, B., Branković Lazić, I., Djordjevic, V. & Klun, I. (2022).** Seroprevalence, direct detection and risk factors for *Toxoplasma gondii* infection in pigs in Serbia, and influence of biosecurity measures. *Microorganisms*, 10(5), 1069.

Codex Alimentarius, (2005). CAC/RCP58 Code of Hygienic Practice for Meat.

Dubey, J. P. (1986). A review of toxoplasmosis in pigs. *Veterinary Parasitology,* 19, 181–223.

Dubey, J. P. (2009). Toxoplasmosis in pigs — The last 20 years. Veterinary *Parasitology*. 164, 89–103.

Dubey, J. P. (2010). Toxoplasmosis of Animals and Humans. CRC Press, Boca Raton, FL, 336.

Dubey, J. P., Hill, D. E., Jones, J. L., Hightower, A. W., Kirkland, E., Roberts, J. M., Marcet, L. P., Lehmann, T., Vianna, M. C. B., Miska, K., Sreekumar, C., Kwok, O. C. H., Shen, S. K. & Gamble, H. R. (2005). Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. *Journal of Parasitology,* 91(5), 1082–1093.

Ecolab-Ecosure, (2007). Cold temperature report. Retrieved from http:/foodrisk.org/exclusives/ecosure/ Accessed February 14, 2020.

Edelhofer, R. (1994). Prevalence of antibodies against *Toxoplasma gondii* in pigs in Austria–an evaluation of data from 1982 and 1992. *Parasitology Research,* 80, 642–644.

EFSA, (2007). Scientific opinion of the panel on biological hazards on a request from EFSA on surveillance and monitoring of *Toxoplasma* in humans, foods and animals. *EFSA Journal*, 583,1–64.

Foroutan, G., Koethe, M., Bangoura, B., Geuthner, A. C., Randau, F., Ludewig, M., Maksimov, P., Sens, M., Bärwald, A., Conraths, F. J., Villena, I., Aubert, D., Opsteegh, M. & Van der Giessen, J. (2018). *Toxoplasma gondii* infections in chickens — performance of various antibody detection techniques in serum and meat juice

relative to bioassay and DNA detection methods. *International Journal for Parasitology*, 48(9–10), 751–762.

Klun, I., Djurković-Djaković, O., Katić-Radivojević, S. & Nikolić, A. (2006). Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. *Veterinary Parasitology*, 135(2), 121–131.

Klun, I., & Djurkovic-Djakovic, O. (2021). Toxoplasma gondii in pork & pork products — too much on our plate? *Veterinarski Glasnik*, 75(1), 42–56.

Kuruca, Lj. (2017). *Toxoplasma gondii* infection in pigs: serological, parasitological and molecular findings as meat safety parameters. University of Novi Sad. Doctoral dissertation.

Mie, T., Pointon, A. M., Hamilton, D. R. & Kiermeier, A. (2008). A qualitative assessment of *Toxoplasma gondii* risk in ready-to-eat smallgoods processing. *Journal of Food Protection*, 71, 1442–1452.

Opsteegh, M., Maas, M., Schares, G. & Giessen, J. (2016). Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat (GP/EFSA/BIOHAZ/2013/01) An extensive literature review, Final report. *EFSA Supporting Publications*, 13, 294.

Papatsiros, V. G., Athanasiou, L. V., Stougiou, D., Papadopoulos, E., Maragkakis, G. G., Katsoulos, P. D., Lefkaditis, M., Kantas, D., Tzika, E. D., Tassis, P. D. & Boutsin, S. (2016). Cross-sectional serosurvey and risk factors associated with the presence of *Toxoplasma gondii* antibodies in pigs in Greece. *Vector-Borne Zoonotic Diseases*, 16(1), 48–53, https://doi.org/10.1089/vbz.2015.1845

Pastiu, A. I., Cozma-Petruț, A., Mercier, A., Balea, A., Galal, L., Mircean, V., Pusta, D. L., Bogdan, L. & Gyorke, A. (2019). Prevalence and genetic characterization of *Toxoplasma gondii* in naturally infected backyard pigs intended for familial consumption in Romania. *Parasites Vectors,* 12**,** 586, https://doi.org/10.1186/s13071-019-3842-8

Roqueplo, C., Halos, L., Cabre, O. & Davoust, B. (2011). Toxoplasma gondii in wild and domestic animals from New Caledonia. *Parasite*, 18, 345–348.

Scharff, R. (2020). Food attribution and economic cost estimates for meat and poultry related illnesses. *Journal of Food Protection*, 83(6), 959–967, https://doi.org/10.4315/JFP-19-548

Statista, (2023). Number of pigs worldwide. Retrieved from http://www.statista.com/statistics/263964/number-of-pigs-in-selected-countries 1. (accessed on 1 June 2023)

RZS [Statistical Office of the Republic of Serbia]. (2021). Statistical Yearbook, 20.10.2021. 54(226), https://publikacije.stat.gov.rs/G2021/Pdf/G20212054.pdf

Warnekulasuriya, M. R., Johnson, J. D. & Holliman. R. E. (1998). Detection of *Toxoplasma gondii* in cured meats. *International Journal of Food Microbiology*, 45, 211–215.

Zander, K., Stolz, H. & Hamm, U. (2013). Promising ethical arguments for product differentiation in the organic food sector: a mixed methods research approach. *Appetite*, 62, 133–142.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5

ID: 126459145

https://doi.org/10.18485/meattech.2023.64.2.23

Review paper

Sustainable meat production

Nedjeljko Karabasil[a], Tamara Bošković[b], Nataša Kilibarda[c], Nikola Čobanović[a], Ivan Vićić[a] and Mirjana Dimitrijević[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia*
[b] *Ministry of Ariculture, Forestry and Water Management, Veterinary Directorate, Omladinskih brigada 1, Belgrade, Serbia*
[c] *Singidunum University, Danijelova 32, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Ivestock
Pork
Beef
Poultry
Consumer

ABSTRACT

Nowadays, we are more aware than ever that intensive livestock and meat production and consumption have negative, sometimes detrimental effects on the environment and animal and human health. Habitats, biodiversity and soil quality have been greatly affected by the growth of agriculture. Good animal welfare and proper protection are essential for producing high-quality meat. For the past few decades, production from the poultry and pig sector expanded much faster than the bovine or ovine sector. Despite many barriers, global meat production is still a growing sector. Therefore, there is a need for a sustainable approach to the production of meat and meat products. The meat sector has to transform in such a manner as to be an industry that cares for the environment, animals and consumers.

1. Introduction

Despite many barriers, global meat production is still a growing sector. According to data given by the Food and Agricultural Organization of the United Nations, as the world population increases, consumption of meat or meat-related products is forecast to more than double by 2050 (*FAO*, 2017), with most products manufactured in industrial premises (*Hübel & Schaltegger*, 2022). World meat production is expected to be 364 million tonnes in 2023, and there is a slight increase from 2022 (*FAO*, 2023). For the past few decades, production from the poultry and pig sector expanded much faster than the bovine or ovine sector. Poultry meat production is predominant, and it will reach 143 million tonnes in 2023, followed by pig meat at 121,7 million tonnes, bovine meat at 76 million tonnes, ovine meat at 17 million tonnes, and international trade of meat or meat products at 42 million tonnes in 2023.

Consumer perceptions of livestock production and meat consumption are regularly discussed by the scientific and general public and are well highlighted in many studies (*Hocquette*, 2023; *Liu et al.*, 2023; *de Boer & Aiking*, 2022;): topics include impact on the environment, on human health, animal welfare, as a source of zoonotic diseases, etc. Nowadays, we are more than aware, that intensive livestock and meat production and consumption, have negative, sometimes detrimental effects on the environment, and as such, on animal and human health (*Rossi & Garner*, 2014). Therefore, there is a need for a sustainable approach to the production of meat and meat products, and the meat sector has to trans-

***Corresponding author:** Nedjeljko Karabasil, *nedja@vet.bg.ac.rs*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

form in such a manner to be an industry that cares for the environment, animals and consumers. To be more sustainable, the meat industry has to change and transform, which can include an ecological form of farming with high animal welfare and animal protection standards, how and how much is meat produced, alternative production e.g., cultivated meat etc. (*Hübel & Schaltegger*, 2022). Sustainable development will ensure food security, sufficient and nutritious food for all by sustainable agriculture with improved productivity (*de Boer & Aiking*, 2022; *Willett et al*., 2019; *UN*, 2015).

2. Sustainable meat production and consumption chain

Sustainable production needs to meet a present need without compromising the future, and therefore, consumers nowadays, especially the younger population, are more aware of the issue and very interested in supporting practice (*Hocquette*, 2023). Meat consumption is driven by various factors such as price, safety, sensory parameters, cultural diversity etc. In the developed part of the world, there is a lot of discussion about the environment, health and animal welfare, and the promotion of meat analogues. Despite that, in those counties, there is still high demand for meat. In the USA, poultry is the most consumed meat, with a decline in beef consumption, while in China, pork is the most consumed (*Wang*, 2022). Like the USA consumers, Chinese consumers recognised aspects of health, environment, and welfare issues in sustainable development as well. However, these concerns are not the prime interest in some parts of the world (e.g., Africa), because consumers are more concerned about food security and hunger.

The three pillars of sustainability are environmental health, economic prosperity, and social equity. To achieve sustainability, we need to use the raw materials we produce in the best way possible and coordinate our actions across the entire process from production to consumption. (*Galanakis*, 2019). The meat industry aims to turn raw materials into consumer products more efficiently by enhancing welfare, cutting down energy use in meat processing, making use of co- and byproducts, and improving packaging. Habitats, biodiversity, and soil quality have been greatly affected by the growth of agriculture. Land use change is a major factor that causes biodiversity loss, e.g. when grasslands are turned into farmland, or forests are cleared for grazing, most of the plants and the animals that depend on them for habitat disappear (*Cederberg*, 2014).

An important part of the food chain is animal welfare and how we protect animals in the meat sector (*Broom et al.*, 2021). In general, welfare is in direct connection with animals, and how they cope with the environment, but an aspect of protection is in the direct responsibility of humans (e.g. farmers, transporters, operators in slaughterhouses etc). Good animal welfare and proper protection are essential for producing high-quality meat (*Nenadović et al.*, 2021; *Vicic et al*., 2021; *Čobanović et al.,* 2019; *Karabasil et al.,* 2019), and we should be aware of this issue when we use animals to produce valuable proteins. Animal welfare guidelines (*WOAH*, 2019), developed by the World Organization of Animal Health are basic to follow for each sector in the meat production chain: prevent abuse and neglect, reduce suffering, and allow basic behaviour and positive emotion in relation to animal-human interactions. Principles are simple, and any conditions that deprive proper animal welfare must be eliminated (*Grandin*, 2019).

To protect the environment, and remove the constant pressure for more food and/or animal protein, maximum utilisation of raw materials must be obtained, with minimum waste. Animal waste can be reusable or non-reusable. While non-reusable waste has to go directly to incineration, all reusable waste materials can be treated (e. g. biological or thermal processes) and considered valuable resources (e. g. energy, nutrients, by-products) (*Girotto & Cossu*, 2017).

In the 21st century, food security is the goal, recognized on the global level by the United Nations, through the Sustainable Development Goals (*Djekic et al*., 2021; *UN*, 2015). Animals and the meat sector are providing highly valuable animal protein in our diet and give us better life quality, so it is upon all of us to make the best use of them by minimizing waste and taking care of the environment (*Girotto & Cossu*, 2017). To find new solutions and enough quantity of healthy and nutritionally valued food, there are suggestions to implement the breeding and consumption of alternative animal protein sources (e. g. insects, cultivated meat). This highlights the positive effects on natural resources due to the reduction of chemical products (e. g. fertilizers, pesticides, etc), and among others, reducing animal waste volumes as one of the main benefits of this practice (*Girotto & Cossu*, 2017).

3. Conclusion

For animal production and the meat industry to be sustainable, they must be proactive in changing their business practices. This includes taking into account the needs of future generations and the market, as well as the methods and technological procedures of production and the quantities produced, with minimum waste. It also involves preserving the environment and applying high standards in areas of animal welfare and protection, as well as finding and developing alternative sources of animal proteins.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143)

References

Broom, D. (2021). Farm animal welfare: a key component of the sustainability of farming systems. *Veterinarski Glasnik*, 75(2), 145–151, https://doi.org/10.2298/VETGL210514007B

Cederberg, C. (2014). Environmental impact of meat production | Primary Production/Meat and the Environment. In *Encyclopedia of Meat Sciences* (pp. 502–507), Elsevier, https://doi.org/10.1016/B978-0-12-384731-7.00170-7

Čobanović, N., Jamnikar-Ciglenečki, U., Kirbiš, A., Križman, M., Štukelj, M. & Karabasil, N. (2019). Impact of various housing conditions on the occurrence of pathological lesions in slaughtered pigs. *Veterinarski Glasnik*, 73(1), 17–29, https://doi.org/10.2298/VETGL190318010C

de Boer, J. & Aiking, H. (2022). Do EU consumers think about meat reduction when considering to eat a healthy, sustainable diet and to have a role in food system change? *Appetite*, 170, 105880, https://doi.org/10.1016/j.appet.2021.105880

Djekic I., Batlle-Bayer L., Bala A., Fullana-i-Palmer P. & Jambrak A. R. (2021). Role of the food supply chain stakeholders in achieving UN SDGs. *Sustainability*, 13, 9095, https://doi.org/10.3390/su13169095

Girotto, F. & Cossu, C. A. (2017). Animal waste and waste animal by-products generated along the livestock breeding and meat food chain. *Waste Management*, 70, 1–2, https://doi.org/10.1016/j.wasman.2017.11.028

Grandin, T. (2019). Sustainable meat production and processing. In Galanakis C. (Ed). Principles for Commercial Supply Chain Managers of Livestock and Poultry (Ch 1, Pp 1–15). Academic Press.

FAO, (2023). Food Outlook — Biannual report on global food markets. Food and Agriculture Organization of the United Nations, Rome, 2023.

FAO, (2023). The future of food and agriculture, Trends and challenges. Food and Agriculture Organization of the United Nations, Rome, 2017.

Hocquette, J.-F. (2023). Consumer perception of livestock production and meat consumption; an overview of the special issue "Perspectives on consumer attitudes to meat consumption." *Meat Science*, 200, 109163, https://doi.org/10.1016/j.meatsci.2023.109163

Hübel, C. & Schaltegger, S. (2022). Barriers to a sustainability transformation of meat production practices—An industry actor perspective. *Sustainable Production and Consumption*, 29, 128–140, https://doi.org/10.1016/j.spc.2021.10.004

Karabasil, N., Boskovic, T., Vicic, I., Cobanović, N., Dimitrijevic, M. & Teodorovic, V. 2019. Meat quality: Impact of various pre-slaughter conditions. The 60th International Meat Industry Conference MEATCON2019. IOP Conf. Series: Earth and Environmental Science 333 (2019) 012033. doi:10.1088/1755-1315/333/1/012033

Liu, J., Chriki, S., Kombolo, M., Santinello, M., Pflanzer, S. B., Hocquette, É., Ellies-Oury, M.-P. & Hocquette, J.-F. (2023). Consumer perception of the challenges facing livestock production and meat consumption. *Meat Science*, 200, 109144, https://doi.org/10.1016/j.meatsci.2023.109144

Nenadović, K., Čobanović, N., Vučinić, M., Bugarski, D., Mikuš, T., Voslarova, E., Zupan Šemrov, M., Kjosevski, M. & Karabasil, N. (2021). Welfare and meat quality of lambs. *Veterinarski Glasnik*, 75(2), 189–201, https://doi.org/10.2298/VETGL210126006N

Rossi, J. & Garner, S. A. (2014). Industrial Farm Animal Production: A Comprehensive Moral Critique. *Journal of Agricultural and Environmental Ethics*, 27(3), 479–522, https://doi.org/10.1007/s10806-014-9497-8

Vicic, I., Petrovic, M., Stajkovic, S., Cobanovic, N. & Karabasil, N. (2021). Seasonal overview of beef meat quality in a small-scale slaughterhouse. IPO Conf. Ser: Earth Environ. Sci. 854 012103.

Wang, H. H. (2022). The perspective of meat and meat-alternative consumption in China. *Meat Science*, 194, 108982, https://doi.org/10.1016/j.meatsci.2022.108982

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., De Vries, W., Majele Sibanda, L…& Murray, C. J. L. (2019). Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet*, 393(10170), 447–492, https://doi.org/10.1016/S0140-6736(18)31788-4

WOAH, 2023. World Organization for Animal Health Terrestrial Animal Health Code, Section 7, 2019. Animal Welfare, Paris France.

UN, (2015). Sustainable Development Goals. United Nations, Department of Economic and Social Affairs, Sustainable Development. Retrieved from: https://sdgs.un.org/goals, accessed by July 3, 2023.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 594.1-153:[616.98:578.82/.83

ID: 126526985

https://doi.org/10.18485/meattech.2023.64.2.24

Review paper

Viruses in shellfish — food safety risks

Mirjana Dimitrijević[a], Nevena Grković[a], Ivana Milošević[b] and Nedjeljko Karabasil[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology Bulevar Oslobođenja 18, Belgrade, Serbia*

[b] *University of Belgrade, Clinic for Infectious and Tropical Diseases, Clinical Centre of Serbia Faculty of Medicine, Bulevar Oslobođenja 16, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Shellfish
Aquaculture
Viruses
Food safety risks

ABSTRACT

Shellfish production in the EU has declined in recent decades, which is not the case with global aquaculture production of shellfish. The trend towards a healthy lifestyle and diet is becoming increasingly topical and often involves the consumption of uncooked shellfish. Unfortunately, shellfish can often be contaminated with various pathogens, especially viruses, which can endanger human health. Among the outbreaks of shellfish-borne viruses, the most notable are those caused by Norovirus (NoV) and hepatitis A virus (HAV). However, other viruses belonging to the *Herpeviridae, Picornaviridae, Adenoviridae, Astroviridae,* and *Reoviridae* can mainly cause intestinal disease in humans after consumption of contaminated shellfish. The listed viruses have been detected in shellfish worldwide and they are mostly the consequence of sewage-contaminated water. Numerous preventive and control measures are recommended to solve this problem.

1. Introduction

The search for high-quality proteins in people's diets and the provision of sufficient quantities, i.e. by another 1 million tons in 2020 compared to 2018, means that 90 million tons (51%) of the supply came from capture fisheries, whereas 88 million tonnes came from aquaculture (49 percent) (*FAO*, 2022). In Europe, Denmark produced the most mussels in 2021 (23,500 tons), but Europe is expected to increase aquaculture production. Data from the FAO indicates that worldwide production of aquaculture will decrease the supply of mussels the most by 2026, with a predicted annual growth of 0.4% (*European Mussel Trends*, 2022). Even if the production of mussels in our neighbour Montenegro is constantly rising, the capacity is still less than anticipated (*Dimitrijevic et al.*, 2022). The Serbian market is supplied with shellfish from imports, which in recent times has been about 620 tons annually of chilled or frozen shellfish. Unfortunately, shellfish can frequently contain a variety of pathogens, including viruses, that can be harmful to human health. According to *Richards* (2016), shellfish bioconcentrate pollutants, such as enteric viruses, within their edible tissues. Raw or undercooked shellfish (oysters, clams, mussels and cockles) is one of the most notable foods that may contain enteric viruses. Among the outbreaks of shellfish-borne viruses, the most notable are those caused by norovirus (NoV) and hepatitis a virus (HAV). However, shellfish can commonly become contaminated with additional viruses from the *Herpeviridae, Picornaviridae, Adenoviridae, Astroviridae* and *Reoviridae*.

***Corresponding author:** Mirjana Dimitrijević, *mirjana@vet.bg.ac.rs*

Paper received May 15th 2023. Paper accepted May 28th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Foodborne viruses in shellfish

Shellfish are most frequently contaminated with enteric viruses that can cause intestinal diseases in humans, (with diarrhoea, vomiting, nausea, abdominal cramps, fever, chills, body ache), but HAV and hepatitis E virus (HEV) may lead to further consequences. Although extreme dehydration can occasionally cause death, enteric viruses rarely cause mortal outcomes. However, this is especially true in places where rehydration medication is difficult to access (*Richards*, 2016). These viruses are all spread by the faecal-oral route and are the main viral infections to watch out for in outbreaks that are foodborne and waterborne, including those that include shellfish eating. There have been numerous reports about enteric virus contamination of shellfish products globally (*Fouillet et al.*, 2020; *Meghnath et al.*, 2019; *Miranda & Schaffner*, 2019). Lack of clean water, globalization of the supply chain, and changes in eating habits, especially an increase in the intake of food that is often eaten raw, are all factors that contribute to the growth and spread of viral foodborne disease. Effective management plans for virus monitoring in shellfish harvesting regions are usually inadequate in underdeveloped nations. Although developed nations have achieved significant progress by modernizing their sewage systems, which has decreased the viral loads in sewage, it is challenging to assess the relevance of these improvements due to the absence of regulations in place that define the acceptable limits for viruses (*Shuping, Human, Lues & Paulse*, 2023).

2.1. Norovirus — NoV

Among the outbreaks of shellfish-borne viruses, the most notable are those caused by NoV, which belonging to the family *Caliciviridae* (*De Graaf et al.*, 2016). The genogroups GI and GII, with GII.4 and GII.17, are mostly responsible for human infection, which often manifests as gastroenteritis, though some populations (young, old and immunosuppressed) can experience additional complications (*Mans, Armah, Steele & Taylor*, 2016; *Zhou et al.*, 2019). According to *Hall et al.* (2012), NoV account for an estimated 5.5 million cases of acute foodborne gastroenteritis each year in the United States, making them the most common cause of this illness. NoV outbreaks linked to shellfish have occurred in many countries (*Richards*, 2016). *Sufferdini* et al. (2020) found that in bivalve shellfish marketed in Hanoi, Vietnam, NoV was the most frequently observed virus (81.8% of samples), with NoV GI and NoV GII detected in 50.4% and 79.3% of samples respectively. In samples collected in the Gulf of Naples, Italy, NoVGII was confirmed in 39.7% of shellfish samples (*Fusco et al.*, 2019). The first comprehensive analysis of the prevalence of NoV in mussels from harvesting sites around the Adriatic Sea coast of Montenegro reveals that 43% of the samples tested positive for the virus, with a greater prevalence of genogroup GII (74.2%) (*Ilić et al.,* 2017). The Centres for Disease Control and Prevention (CDC), the US Food and Drug Administration (FDA), the Texas Department of State Health Services, and other public health partners, are investigating a multistate outbreak of norovirus illnesses linked to raw oysters from Texas and found that 298 illnesses have been reported as of December 20, 2022. At the beginning of this year, US FDA advised restaurants, retailers and consumers to avoid raw oysters from Deep Bay, British Columbia, Canada, that were gathered between January 16, 2023, and February 17, 2023, and distributed in 13 US states, as the shellfish were potentially contaminated with NoV, were not safe and should be thrown away (*FDA*, 2023).

2.2. Viruses — Hepatitis A (HAV) and Aichivirus (AiV)

HAV and AiV are enterically transmitted viruses with clinical importance that are members of the *Picornaviridae* family (*Wells & Coyne*, 2019). Depending on the virus and the susceptibility of the affected people, HAV can cause a variety of symptoms of illness. It is frequently an asymptomatic infection in healthy people with spontaneous remission but can cause liver damage and acute liver failure, which may be fatal. WHO estimated that in 2016, 7134 people died from HAV worldwide (*Randazzo & Sanchez*, 2020). The largest outbreak of HAV occurred in 1988 in and around Shanghai, China, after the consumption of harvested clams, where over 293,000 people became ill, and 47 deaths were reported (*Cooksley*, 2020). According to a prior epidemiological analysis completed by the Korea Disease Control and Prevention Agency in September 2019, salted shellfish, primarily bivalves, were identified as a source of the 2019 HAV epidemic in two South Korean locations (*Hyun, Yoon & Lee*, 2022). The ingestion of raw or undercooked shellfish has been linked to the majority of outbreaks of HAV in

shellfish (*Boxman et al.*, 2016). *Boussettine et al*. (2023) have detected HAV in 15.38% of samples from shellfish harvesting areas in Morocco. *Bazir et al.* recently discovered HAV in 46.15% of the mussel samples in Morocco *Bazir et al.,* 2022 and *Fusco et al.* (2019) found HAV in 8.9% of shellfish samples in Italy. The previous authors also found the presence of AiV in 5.6% of the samples in Gulf of Naples, Italy. AiV is currently thought to be an emerging human enteric pathogen that can cause gastroenteritis via contaminated shellfish, even though, the prevalence of AiV in cases of gastroenteritis is modest, ranging from 0.4–6.5% globally (*Macaluso et al.*, 2021).

2.3. Hepatitis E Virus (HEV)

HEV is a member of the family *Hepeviridae* within the genus *Orthopevirus*, which has five human-infecting genotypes (HEV 1, 2, 3, 4 and 7), and according to the World Health Organization (WHO), it can affect nearly 20 million people each year, resulting in roughly 3.3 million acute liver injuries, 56,600 fatalities, and significant subsequent healthcare-related economic losses (*WHO*, 2022). Also, it is considered as an emerging foodborne pathogen. Hepatitis E epidemics commonly occur in Asia and other regions of the world and only a few cases have been reported in the US, even though it can be a devastating condition (*Richards,* 2016). According to reports, the incubation period for hepatitis E is between 2 and 8 weeks, and early symptoms may include nausea, fatigue, anorexia, and low-grade fever before possibly progressing to spleen enlargement and pain in the upper right quadrant, both of which are indicative of liver involvement. *Mast & Krawczynski* (1996) indicate that clinical symptoms often go away in 4–8 weeks, except for pregnant women, who have a mortality risk of 15–25%. Virus transmission involving shellfish has occasionally been observed, but recently, *Rivadulla et al.* (2019) detected HEV in 24.4% of samples in shellfish harvesting areas from Galicia, Spain. Results of *Macaluso et al.* (2021) show that 5.56% of shellfish samples were contaminated with at least one NoV, HAV and/or HEV along the production and distribution chain in Sicily, Italy.

2.4. Astrovirus (AstV), Adenovirus (AdV) and Rotavirus (RV)

Astrovirus, Adenovirus and Rotavirus (they belong, respectively, to the family *Astroviridae, Adenoviridae* and *Reoviridae*) can also be transmitted by shellfish and often result in mild and self-limiting illness. The sudden rise in occurrences, however it is not typically linked to outbreaks. Typically, these viruses affect young infants who have not yet established immunity and symptoms include loss of appetite, occasional vomiting, abdominal pain, diarrhoea and fever (*Richards*, 2016). The incubation period is short (1–3 days) and the illness lasts up to 4 days. Even though there have been few outbreaks reported, these viruses have not been often detected in mussels or oysters (*Upfold, Luke & Knox*, 2021), but *Suffredini et al*. (2020) reported that they can be occur in high concentrations in sewage waters and provide a potential risk for contaminating shellfish. Also, this group showed an alarmingly high level of AstT, 12.4%, in samples of shellfish produced in Vietnam.

3. Conclusion

Since shellfish can be the main vector for transmission of different viruses, it is necessary to reduce viral infections through improving surveillance at all phases of shellfish production, harvesting, distribution and processing. The industry and consumers must enhance their hygiene procedures and reduce pollution. To stop the spread of foodborne viral infections, outbreaks must be better reported and epidemiologically followed up on. Also, better analytical methods for enteric virus detection in shellfish would increase shellfish safety and provide improved consumer and business protection. Recent improvements in analytical methods are projected to improve food and water monitoring capacity and lower the frequency of enteric virus infection among shellfish consumers.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Bazir, H., Hassou, N., El Mellouli, F., Zekhnini, H., Najib, S. & Ennaji, M. M. (2022). Hepatitis A and E Viruses in Mussels from Cherrat Estuary in Morocco: Detection by Real-Time Reverse Transcription PCR Analysis. *Advances in Virology,* 8066356, doi: 10.1155/2022/8066356.

Boussettine, R., Hassou, N., Maanan, M., Bessi, H. & Ennaji, M. M. (2023). Hepatitis A virus detection by RT-qPCR in shellfish samples from three Moroccan Atlantic coastal areas: Dakhla, Oualidia, and Moulay Bousselham. *Letters in Applied Microbiology,* 76(2), ovac059.

Boxman, I. L., Verhoef, L., Vennema, H., Ngui, S. L., Friesema, I. H., Whiteside, C.... & Koopmans, M. (2016). International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012. *Eurosurveillance,* 21(3), 30113.

Cooksley, W. G. E. (2000). What did we learn from the Shanghai hepatitis A epidemic? *Journal of Viral Hepatitis,* 7, 1–3.

De Graaf, M., van Beek, J. & Koopmans, M. P. (2016). Human norovirus transmission and evolution in a changing world. *Nature Reviews Microbiology,* 14(7), 421–433.

Dimitrijević, M., Zuber-Bogdanović, I., Grković, N., Aksentijević, K., Nikolić, M., Pavićević, Z. & Laušević, D. (2022). Sustainability of shellfish aquaculture in Montenegro-perspectives. *Veterinarski glasnik,* 10–10.

European Mussel Trends in 2022. Retrieved from https://www.reportlinker.com/clp/global/4428

Food and Agriculture Organization, FAO (2022). The state of world fisheries and aquaculture2022. Retrieved from https://www.fao.org/3/cc0461en/online/sofia/2022/aquaculture-production.html

Fouillet, A., Fournet, N., Forgeot, C., Jones, G., Septfons, A., Franconeri, L.... & Caserio-Schönemann, C. (2020). Large concomitant outbreaks of acute gastroenteritis emergency visits in adults and food-borne events suspected to be linked to raw shellfish, France, December 2019 to January 2020. *Eurosurveillance,* 25(7), 2000060.

Fusco, G., Anastasio, A., Kingsley, D. H., Amoroso, M. G., Pepe, T., Fratamico, P. M.... & Boccia, F. (2019). Detection of hepatitis A virus and other enteric viruses in shellfish collected in the Gulf of Naples, Italy. *International Journal of Environmental Research and Public Health,* 16(14), 2588.

Hall, A. J., Eisenbart, V. G., Etingüe, A. L., Gould, L. H., Lopman, B. A. & Parashar, U. D. (2012). Epidemiology of foodborne norovirus outbreaks, United States, 2001–2008. *Emerging Infectious Diseases,* 18(10), 1566.

Ilic, N., Velebit, B., Teodorovic, V., Djordjevic, V., Karabasil, N., Vasilev, D.... & Dimitrijevic, M. (2017). Influence of environmental conditions on norovirus presence in mussels harvested in Montenegro. *Food and Environmental Virology,* 9, 406–414.

Hyun, J. H., Yoon, J. Y. & Lee, S. H. (2022). A case-control study of acute hepatitis A in South Korea, 2019. *Osong Public Health and Research Perspectives,* 13(5), 352–359.

Macaluso, G., Guercio, A., Gucciardi, F., Di Bella, S., La Rosa, G., Suffredini, E.... & Purpari, G. (2021). Occurrence of human enteric viruses in shellfish along the production and distribution chain in Sicily, Italy. *Foods,* 10(6), 1384.

Mans, J., Armah, G. E., Steele, A. D. & Taylor, M. B. (2016). Norovirus epidemiology in Africa: a review. *PLoS One,* 11(4), e0146280.

Meghnath, K., Hasselback, P., McCormick, R., Prystajecky, N., Taylor, M., McIntyre, L.... & Galanis, E. (2019). Outbreaks of norovirus and acute gastroenteritis associated with British Columbia oysters, 2016–2017. *Food and Environmental Virology,* 11, 138–148.

Miranda, R. C. & Schaffner, D. W. (2019). Virus risk in the food supply chain. *Current Opinion in Food Science,* 30, 43–48.

Randazzo, W. & Sánchez, G. (2020). Hepatitis A infections from food. *Journal of Applied Microbiology,* 129(5), 1120–1132.

Richards, G. P. (2016). Shellfish-associated enteric virus illness: Virus localization, disease outbreaks and prevention. *Viruses in Foods,* 185–207.

Rivadulla, E. & Romalde, J. L. (2020). A comprehensive review on human Aichi virus. *Virologica Sinica,* 35(5), 501–516.

Rivadulla, E., Varela, M. F., Mesquita, J. R., Nascimento, M. S. & Romalde, J. L. (2019). Detection of hepatitis E virus in shellfish harvesting areas from Galicia (Northwestern Spain). *Viruses,* 11(7), 618.

Shuping, L. S., Human, I. S., Lues, J. F. R. & Paulse, A. N. (2023). The Prevalence of Viruses Related to the Production of Mussels and Oysters in Saldanha Bay: A Systematic Review. *Aquaculture Journal,* 3(2), 90–106.

Suffredini, E., Le, Q. H., Di Pasquale, S., Pham, T. D., Vicenza, T., Losardo, M.... & De Medici, D. (2020). Occurrence and molecular characterization of enteric viruses in bivalve shellfish marketed in Vietnam. *Food Control,* 108, 106828.

The Centres for Disease Control and Prevention CDC, (2022). Multistate Norovirus Outbreak Linked to Raw Oysters from Texas. Retrieved from https://www.cdc.gov/norovirus/outbreaks/index.html

US Food and Drug Administration FDA, (2023). Recalls, Outbreaks & Emergencies. Retrieved from https://www.fda.gov/food/alerts-advisories-safety-information/fda-advises-restaurants-retailers-and-consumers-avoid-raw-oysters-deep-bay-british-columbia-canada

Upfold, N. S., Luke, G. A. & Knox, C. (2021). Occurrence of human enteric viruses in water sources and shellfish: A focus on Africa. *Food and Environmental Virology,* 13(1), 1–31.

Wells, A. I. & Coyne, C. B. (2019). Enteroviruses: A gut-wrenching game of entry, detection, and evasion. *Viruses,* 11(5), 460.

World Health Organization WHO. Hepatitis E. Available online: https://www.who.int/en/news-room/fact-sheets/detail/hepatitis-e 19

Zhou, X., Kong, D. G., Li, J., Pang, B. B., Zhao, Y., Zhou, J. B.... & Wang, Y. H. (2019). An outbreak of gastroenteritis associated with GII. 17 norovirus-contaminated secondary water supply system in Wuhan, China, 2017. *Food and Environmental Virology,* 11, 126–137.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523.045

ID: 126529033

https://doi.org/10.18485/meattech.2023.64.2.25

Review paper

Perspectives in fat replacement in sausages

Dragan Vasilev[a], Branko Suvajdžić[a], Aleksandar Bajčić[b] and Silvana Stajković[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*
[b] *Institute of Meat Hygiene and Technology, 13 Kaćanskog, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Meat products
Sausages
Fat replacement
Low fat sausages
Functional food

ABSTRACT

Fat replacement in meat products has gained in importance during recent decades, ever since animal fat was recognized as one of the significant causes of chronic non-infectious diseases in modern human populations. Meat products with the highest fat contents include different types of sausages. As fatty tissue plays important roles in sausage quality, fat replacement is not an easy task. There are different approaches which depend on the sausage type. In fermented sausages, the fat substitute should successfully imitate the fatty tissue particles, and in emulsion-type sausages, the fat substitute should be thoroughly mixed and incorporated into the meat batter. The fat substitutes can be of protein or carbohydrate nature, and often are combined with oils rich in polyunsaturated fatty acids, aiming not only to reduce the amount of animal fat in sausages, but also to improve the fatty acid composition of the products. However, fat replacement without affecting sausage quality and shelf life was previously possible only partially and involved a relatively small percentage of replaced fat. Nowadays, some recent studies have reported 100% fatty tissue replacement without adverse effects on the products' properties, opening a new chapter in designing low fat meat products.

1. Introduction

Contemporary scientific findings, but also the growing consumer awareness of the importance of proper nutrition, led to the development of foods that, in addition to the basic nutrients, also contain ingredients capable of providing a positive impact on human health, the so-called functional foods. However, the functional food concept also strives to reduce the content of potentially harmful ingredients in food, such as animal fat (*Vasilev et al*., 2017). Animal fat is rich in saturated fatty acids and cholesterol and has a high n-6/n-3 ratio, which are all predisposing factors for the development of cardiovascular and other non-infectious chronic diseases (*Astrup et al.*, 2020). Meat products, especially some types of sausages containing more then 40% fat, are considered as some of the main sources of animal fat intake in human nutrition. However, the reduction of fat in sausages is not at all a simple task, because of the important role of fatty tissue in the appropriate sensory properties of the products, such as texture, juiciness and aroma (*Vasilev et al.*, 2013).

There are different approaches in fat reduction in sausages. The oldest and simplest, but at the same time the most unfavourable for the sensory properties of the product, is production of sausages with less fat in the recipe. More promising is the use of some fat replacers that imitate fatty tissue in the stuffing, which is especially favourable in terms of the use of substances with some functional properties, where not only low-fat, but also functionally enriched products can be obtained. That way, sau-

***Corresponding author:** Dragan Vasilev, *vasilevd@vet.bg.ac.rs*

Paper received July 31st 2023. Paper accepted August 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

sage could be simultaneously with reduced fat as well as enriched in prebiotics, fibre, n-3 fatty acids, bioactive compounds etc. (*Vasilev et al.,* 2017b; *Glišić et al.*, 2019; *Stajić and Vasilev,* 2022).

Each of the fat substitutes has its advantages and disadvantages in terms of the quantity of the fatty tissue that can be replaced without adverse effects on the product, but also considering the type of sausage in which they would be used. In fermented sausages, the fat substitute should successfully imitate the fatty tissue particles, both visually and in terms of technological features, where particles of fat substitute should be stably embedded in the stuffing among the muscle tissue particles, forming the white-red mosaic characteristic for the cross section of this type of sausage (*Suvajdžić et al*., 2018). On the contrary, in cooked emulsion-type sausages, the fat substitute should be thoroughly mixed and incorporated into the meat batter, whereby the stability of the filling is based on good emulsification and water binding. Even more, in these products, the fat substitute has to be heat resistant, as the sausages are heat treated (*Rašeta et al.*, 2018). Based on the abovementioned facts, the aim of this study was to review the achievements so far and perceive the future perspectives in fat replacement in sausages.

2. Fat replacement in fermented sausages

Fermented sausages are produced from ground meat and fatty tissue, with the addition of salt, sugar, spices and additives to prepare a mixture which is stuffed into casings and preserved by microbial fermentation, smoking and drying. The amount of added fatty tissue differs a lot, ranging from as low as 5% in some traditional products, such as Sremski kulen (*Suvajdžić et al*., 2018), up to 32% in sausages produced according to a usual recipe (*Yim et al*., 2016). As a consequence of water loss while drying, the fat content in the final product is from 10% in Sremski kulen (*Suvajdžić et al*., 2018) up to 40–50% in usual products (*Yim et al*., 2016). Because of the usually high fat content, there is a reasonable need for fat reduction in such products. Still, this is not at all an easy task bearing in mind that fatty tissue particles play a very important role in providing a balanced drying process as well as in amortising the shrinking and shrivelling of the sausages during the water loss from the meat particles. Because of that, simple fat reduction in fermented sausages usually results in wrinkled sausages, which are of hard texture and less juicy, and which especially applies to sausages stuffed into artificial collagen casings (Wirth, 1988; *Yim et al.*, 2016). However, low-fat fermented sausages of traditionally produced types, such as domestic Kulen, are specific in terms of the nature of the casings, e.g. pork appendix, being wide in diameter and with fat ends (*Wirth*, 1988). The drying and ripening process of such products is slow and requires several months (*Suvajdžić et al*., 2018), whereby the meat particles release water gradually, without adverse effects on sausage appearance.

Since the early observations of the negative impact of simple fat reduction on physico-chemical processes and sensory properties of fermented sausages (*Wirth*, 1988), numerous studies have been devoted to finding suitable substitutes capable of replacing the roles of the fat in fermented sausages. The early attempts aimed to replace a part of the animal fat with oils rich in n-3 fatty acids, aiming to reduce animal fat and to enrich the sausages with n-3 fatty acids (*Jimenez Colmenero*, 2000). However, the simple addition in fermented sausages of such oils is not possible because of their liquid state and proneness to oxidation, which led to the idea to use hydrogenated plant oils that are in solid state and are more stable (*Hilk*, 2005). The main problem with this approach was that hydrogenated fats are rich in *trans*-fatty acids that are proven to be harmful for the consumer's health (*Vasilev et al*., 2010). On the other hand, tropical plant oils, such as palm oil, seemed to be a good solution, as these oils are solid at room temperature. However, the main disadvantages were that palm oil becomes too gritty and fragile at low temperatures, and on the contrary, becomes semi liquid at relatively higher temperatures (*Dreher et al*., 2022). Moreover, as palm oil is rich in saturated fatty acids (palmitic acid, above all), fermented sausages in which the animal fat was partially replaced with palm oil contained more saturated fat than the conventional products produced with pork back fat, which is inconsistent with the basic purpose of reducing fatty tissue in sausages (*Vasilev et al*., 2010).

The idea of using plant oils was further developed through the approach of oil emulsification with plant proteins. Such prepared emulsion is choppable and could imitate the fat particles, but significantly increases the yellowness of sausages, influences aroma and affects the sausages' oxidative stability. Because of these drawbacks, such emulsions could replace a limited amount of fatty tissue in fermented sausages, since defects in sensory properties are already apparent when around 30% of the fatty tissue is replaced (*Muguerza et al.,* 2002). In order to

achieve a greater percentage of substituted fatty tissue, it is necessary to provide a better stabilization of emulsion-type systems, so different approaches for encapsulation were proposed: organogelation, which includes the use of a bi-continuous system consisting of gelators (amino acids, carbohydrates, steroid-based molecules etc.) and non-polar solvents; oil-bulking, which is a process of oil incorporation into a gel-like matrix (polysaccharides – inulin, alginate, konjac gum etc.); structured emulsions, where the oil phase is dispersed into hydrogels or organogels and; double emulsions, including oil-in-water emulsion and water-in-oil emulsion, forming a multi-layered system (*Stajić and Vasilev*, 2022).

Another promising solution to substitute a higher percentage of fatty tissue is a quite different approach, in which polysaccharides are applied. Indigestible polysaccharides are of a great significance, because they also are functional ingredients with prebiotic properties. Inulin has been widely investigated, whereby long chain inulin showed the best properties for use in fermented sausages, because after being dissolved in water it forms stable gels, which are white and tasteless. Such gels in a frozen state can be nicely chopped into small particles imitating the particles of fatty tissue (*Vasilev et al*., 2017a). Based on that, *Glišić et al.* (2019) succeeded in replacing 64 % of fatty tissue in sausages with an inulin gel suspension (inulin+water) or with an inulin gel emulsion (inulin+water+lineseed oil), without adverse effects on the sausages' sensory properties.

On the other hand, another very successful approach is the concept wherein turkey lean meat served as an emulsifier, giving a stable olive oil emulsion (*Magra et al*., 2021). Namely, turkey breast meat is a source of myofibrillar proteins that have very good emulsifying properties, but also has a pale colour, so the emulsion obtained had a colour similar to fatty tissue. This emulsion proved to be suitable for the total (100%) substitution of fatty tissue in fermented sausages, without adverse effects on the product's sensory properties (*Magra et al*., 2021).

3. Fat reduction in cooked sausages

The basis of the filling for cooked sausages consists of meat batter or meat emulsion, which are heat treated (mostly by pasteurization) after stuffing into casing. Although the fat content in these products varies widely, ranging from 8% to as much as 33% (*Honikel*, 2004), cooked sausages mostly contain over 25% fat. Fatty tissue plays a very important role in these products, influencing their aroma, colour and texture, so its reduction is also not an easy task (*Vasilev et al*., 2011).

The first attempts to produce fat-reduced cooked sausages by simply decreasing the fat content and increasing the meat content in the recipe resulted in texture failures, and even more, this approach was considered as not cost-effective, so it became clear that adequate fatty tissue substitutes should be found. Further attempts included direct incorporation of plant oils into the filling of cooked sausages followed by proper emulsification. Such an approach resulted in polyunsaturated fatty acid-enriched products, which was desirable from the nutritional point of view, but problems with lipid oxidation proneness as well as textural and aroma defects emerged (*Yilmaz et al*., 2002). In order to provide better oxidative stability of such products and to reduce the adverse effects of added oils on the sensory properties of the sausages, a variety of encapsulation systems were investigated. For emulsion-type cooked sausages, promising results were obtained with oleogels, where oils are immobilized by a gelling agent (waxes, cellulose, ceramides, phytosterols, carbohydrates etc.), as well as with oil-bulking, emulsion structuring, and double emulsion systems as described for fermented sausages (*Stajić and Vasilev*, 2022). Such oil encapsulation systems gave the best results mostly with only partial (about 50%) fatty tissue replacement in cooked sausages. Although *Wolfer et al.* (2018) reported the possibility of total (100%) fatty tissue replacement with an oleogel (soybean oil was stabilized by rice bran wax) in frankfurter-type sausages, the products were prone to fat oxidation and showed some texture problems.

The use of fibre and undigestable polysaccharides as fat substitutes was also investigated in cooked sausages, providing low fat, low energy and prebiotic-enriched products. These materials, because of their ability to retain water and maintain juiciness and their neutral flavour, are suitable for adding to emulsion-type meat products (*Kurćubić et al*., 2020). Fiber and polysaccharides could be used in small quantities of just a few percent in the sausage stuffing, but thanks to their ability to strongly bind water and give stable gels, it is possible to produce emulsion-type cooked sausage containing less fat and more water (*Vasilev et al*., 2011). For example, microcrystalline cellulose and carboxymethyl cellulose can bind water in the ratio of 1:9, forming a stable gel that could replace 50% of the fatty tissue in cooked emulsified sausages without affect-

ing their sensory properties (*Kurćubić et al*., 2020). Although the polysaccharide inulin can bind water in the ratio of only 1:3 to produce a stable gel, such inulin gel suspension can replace up to 32% of adipose tissue, as inulin can be added to the stuffing in higher amounts than can fibre (*Vasilev et al.*, 2013). However, the amount of inulin in the stuffing should not exceed 4%, because otherwise it can cause bloating and diarrhoea in consumers (*Janvary*, 2006). In order to maximize the amount of replaced fatty tissue, *Bajčić et al*. (2023) used a combination of inulin (4%) and collagen powder (1.65%), which provided a strong water binding matrix and was used for total (100%) replacement of the fatty tissue in cooked emulsified sausages without any adverse effect on the product's sensory properties.

4. Conclusion

Meat products with the highest fat contents are mostly fermented sausages and cooked sausages. As fatty tissue plays important roles in sausage quality, fat replacement is not an easy task. In fermented sausages, the fat substitute should imitate the fatty tissue particles, and in emulsion-type sausages, the fat substitute should be thoroughly mixed and incorporated into the meat batter. Fatty tissue substitutes based on oils rich in unsaturated fatty acids could be used in both type of sausages, but such oils should be stabilized by some of the various encapsulation techniques in order to hinder lipid oxidation and mitigate the oil aroma. Fibre and indigestible polysaccharides are capable of forming stable gels with water, but can be added only in limited quantities to both type of sausages. Oil-based as well as fibre/polysaccharide-based fatty tissue substitutes can usually replace about 50% of the fatty tissue in fermented and cooked sausages without adverse effects on the product's sensory properties. However, some recent studies have reported successful 100% fatty tissue replacement with substitutes, such as oil-meat emulsion in fermented sausages and inulin-collagen suspension in cooked emulsified sausages. It is noticeable that, in both cases, animal protein (myofibrillar and collagen protein, respectively, in fermented and cooked emulsified sausages) was used as stabilizers of the fat substitute. Such results have opened a new chapter in designing low fat meat products, because it is not a question any more of how much fatty tissue could be replaced, but which substitute could be another total fat substitute.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Astrup, A., Magkos, F., Bier, D. M., Brenna, J. T., de Oliveira Otto, M. C., Hill, J. O., King, J. C., Mente, A., Ordovas, J. M., Volek, J. S., Yusuf, S. & Krauss, R. M. (2020). Saturated fats and health: a reassessment and proposal for food-based recommendations: JACC State-of-the-art review. *Journal of the American College of Cardiology*, 76, 844–857, https://doi.org/10.1016/j.jacc.2020.05.077

Bajčić, A., Petronijević, R., Suvajdžić, B., Tomović, V., Stajković, S. & Vasilev, D. (2023). Use of inulin-collagen suspension for the total replacement of pork backfat in cooked-emulsified sausages. *Journal of Food and Nutrition Research*, 62(1), 35–45.

Glisic, M., Baltic, M., Glisic M., Trbovic, D., Jokanovic, M., Parunovic, N., Dimitrijevic, M., Suvajdzic, B., Boskovic, M. & Vasilev, D. (2019). Inulin-based emulsion-filled gel as a fat replacer in prebiotic- and PUFA-enriched dry fermented sausages. *International Journal of Food Science and Technology,* 54(3), 787–797, https://doi.org/10.1111/ijfs.13996

Hilk, M., (2005). Fischöl sucht Fleischwurst, omega-3-Fettsäuren machen aus Fleischwaren funktionelle Lebensmittel. *Fleischwirtschaft*, 85(3), 62–64.

Honikel, K., (2004). Die Zusammensetzung deutscher Fleischerzeugnisse. *Forschungs Report*, 2, 32–34.

Janvary, L. (2006). Fleischerzeugnisse mit Mehrwert/L, Fettreduzierte, nährwertoptimierte wursterzeugnisse mit gesundheitlichem Zusatznutzen. *Fleischwirtschaft*, 86(11), 51–54.

Jimenez Colmenero, F. (2000). Relevant factors in strategies for fat reduction in meat products. *Trends in Food Science & Technology*, 11, 56–66, https://doi.org/10.1016/S0924-2244(00)00042-X

Magra, T., Soultos, N., Dovas, C., Papavergou, E., Lazou, T., Apostolakos, I., Dimitreli, G. & Ambrosiadis, I. (2021). Dry fermented sausages with total replacement of fat by extra virgin olive oil emulsion and indigenous lactic acid bacteria. *Food Technology and Biotechnology*, 59(3), 267–281, https://doi.org/10.17113/ftb.59.03.21.7114

Muguerza, E., G. Fista, D. Ansorena, I. Astiasaran & J. G. Bloukas, (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, 61, 397–404, https://doi.org/10.1016/S0309-1740(01)00210-8

Kurćubić, V., Okanović, Đ., Vasilev, D., Ivić, M., Čolovć, D., Jokanović, M. & Džinić, N. (2020). Effect of replacing pork backfat with cellulose fiber in Pariser sausages. *Fleischwirtschaft*, 100(6), 82–88.

Rašeta, M., Mrdović, B., Đorđević, V., Polaček, V., Becskei, Z., Branković-Lazić, I. & Vasilev, D. (2018). Determination of Co-value as an indicator of nutritive value of pâté sterilised by regular and optimized regime. *Veterinarski Glasnik*, 72, 101–111, https://doi.org/10.2298/VETGL180711011R

Stajić S. & Vasilev D. (2022). Encapsulation of Meat Product Ingredients and Influence on Product Quality, In: Lević Steva, Nedović Viktor, Bugarski Branko (Eds.) Encapsulation in Food Processing and Fermentation, CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA, 256–280, https://doi.org/10.1201/9780429324918

Suvajdžić, B., Petronijević, R., Teodorović, V., Tomović, V., Dimitrijević, M., Karabasil, N., Vasilev, D. (2018). Qualität der Rohwurst Sremski Kulen — Produktion unter traditionellen und industriellen Bedingungen in Serbien. *Fleischwirtschaft*, 98(6), 93–99.

Vasilev, D., Vuković, I., Tomović, V., Jokanović, M., Vasiljević, N., Milanović-Stevanović, M. & Tubić, M. (2009). Some important physical, physico-chemical and sensory quality properties of functional fermented sausages. *Meat Technology*, 50(5–6), 342–350.

Vasilev, D., Vuković, I., Saičić, S., Vasilljević, N., Milanović--Stevanović, M. & Tubić, M., (2010). The composition and significant changes in fats of functional fermented sausages. *Meat Technology*, 51(1), 27–35.

Vasilev, D., Vuković, I. & Saičić, S. (2011). Some quality parameters of functional fermented, cooked and liver sausages. *Meat Technology*, 52(1), 141–153.

Vasilev, D., Saičić, S. & Vasiljević, N. (2013). Qualität und Nährwert von mit Inulin und Erbsenfasern als Fettgewebe-Ersatzstoffe hergestellten Rohwürsten. *Fleischwirtschaft*, 93(3), 123–127.

Vasilev D., Djordjević V., Karabasil N., Dimitrijević M., Petrović Z., Velebit B. & Teodorović V. (2017a). Inulin as a probiotic and fat replacer in meat products. *Theory and Practice of Meat Processing*, (2), 4–13, https://doi.org/10.21323/2414-438X-2017-2-2-4-13

Vasilev, D., Glišić, M., Janković, V., Dimitrijević, M., Karabasil, N., Suvajdžić B. & Teodorović, V. (2017b). Perspectives in production of functional meat products. *IOP Conference Series: Earth and Environmental Science*, 85, 012033, https://doi.org/10.1088/1755-1315/85/1/012033

Yim, D. G., Jang, K. H. & Chung, K. Y. (2016). Effect of fat level and the ripening time on quality traits of fermented sausages. *Asian-Australasian Journal of Animal Science,* 29(1), 119 –125, https://doi.org/10.5713/ajas.15.0180

Yılmaz, I., Şimşek, O. & Işıklı, M. (2002). Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Science*, 62(2), 253–258, https://doi.org/10.1016/S0309-1740(01)00255-8

Wirth, F. 1988. Technologies for making fat-reduce meat products. What possibilities are there? *Fleischwirtschaft*, 68, 1153–1156.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.513:577.112
636.4:616.89-008

ID: 126539785

https://doi.org/10.18485/meattech.2023.64.2.26

Review paper

Acute phase proteins as biomarkers of pre-slaughter stress in pigs

Silvana Stajković[a] and Dragan Vasilev[a]

[a] University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar Oslobođenja 18, Belgrade, Serbia

ARTICLE INFO

Keywords:
Pre-slaughter stress
Acute phase proteins
Haptoglobin
Pig-MAP
Serum amyloid A

ABSTRACT

Pre-slaughter handling, which include transportation, housing, social stress, heat, and dietary changes, is one of the main causes that produces stress in pigs. The appropriate biomarkers and objective laboratory criteria to evaluate pre-slaughter stress are lacking. Behavioral and physiological markers are commonly used for this reason, but these parameters may increase for reasons unrelated to stress. Acute phase proteins are considered to be markers of inflammation that have been proposed as indicators for farm animal stress monitoring. The major acute phase proteins in swine are haptoglobin, serum amyloid A, c-reactive protein, and pig major acute phase protein. Serum or plasma obtained from blood are the most used matrixes for the measurement of acute phase proteins, the collection of which involves an invasive collection method that is harmful and stressing. The use of saliva and meat juice instead of blood might overcome these disadvantages, since its collection is non-invasive and stress-free. For any assay measuring acute phase proteins, adequate analytical validation must be performed, as well as harmonization and standardization of analytical procedures. The aim of this paper is to emphasize the possibilities of use of acute phase proteins as biomarkers of pre-slaughter stress, as well as to provide survey of methodologic assays and fluids that are presently available to measure acute phase proteins.

1. Introduction

In the current pig production system, increasing attention is paid to high animal welfare standards as this is seen to be an indicator for safe, healthy, and high quality food (*Klauke et al.*, 2013). Animal welfare problems are also ethically important (*Marco-Ramell et al*., 2011). During the pre-slaughter period, pigs are exposed to many environmental stressors (*Stajkovic et al.*, 2017). An animal's response to a stressor involves a variety of adaptive physiological mechanisms designed to restore homeostasis (*Salamano et al*., 2008). Behavioral (aggression, immobilization, exploration, etc.) and physiological (plasma levels of cortisol and catecholamines, i.e., adrenaline and noradrenaline) markers are commonly used to assess level of stress, but these parameters may increase for reasons unrelated to stress (*Levine*, 1985; *Terlow*, 2005). Hence, the selection of suitable biomarker is a basic necessity in order to objectively and rapidly evaluate animal stress at any given time. Acute phase proteins (APPs) are a group of species-specific plasma proteins which respond to infection, inflammation and/or trauma and have been proposed as indicators for farm animal stress monitoring (*Diack et al*., 2011; *Petersen et al.*, 2004; *Murata,* 2007).

***Corresponding author:** Silvana Stajković, *silvana@vet.bg.ac.rs*

Paper received August 14th 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

The objective of this study was to evaluate the effect of pre-slaughter stress on changes on concentration of acute phase proteins in pigs and to evaluate methods and fluids for APP determination.

2. Pre-slaughter stress

Stress is a general term used to describe environmental factors soliciting adaptation mechanisms and the response to these challenges (*Mormède et al*., 2007). Pre-slaughter stress induced by transport, housing, and slaughter include psychological stimuli (exposure to new social group, personnel, smells, and noises, or any other change to the familiar situation) and physical stimuli (food and water restriction, extreme thermal conditions, fatigue due to movements of the lorry, etc.) that might be aversive for the animals. Stress levels of the animal depend indirectly on the situation, and directly on the animal's evaluation of the situation, but they can only be indirectly assessed, using behavioral and physiological measurements (*Terlow*, 2005).

3. Acute phase proteins

APP are primarily synthesized by hepatocytes as part of the acute phase response (APR) which is part of the early-defense or innate immune system triggered by stress, infection, trauma, neoplasia, and inflammation (*Cray et al*., 2009). The APR is a complex reaction mediated by proinflammatory cytokines, such as interleukins-1β and –6 (IL-1β and IL-6), and tumor necrosis factor-α (TNF-α). During the APR, the serum concentration of the APP changes dramatically and can be increased or decreased after a triggering event. APPs are classified as positive (major, moderate, or minor) or negative. Depending on the magnitude of increase during the APR, major proteins increase 10- to 100-fold (pig major acute phase protein (pig-MAP), haptoglobin (Hp), serum amyloid A (SAA), c-reactive protein (CRP)), moderate proteins increase 2- to 10-fold (α-1-acid glycoprotein (AGP)), and minor proteins undergo only a slight increase (fibrinogen (Fb)) (*Ceron et al*., 2005; *Saco and Bassols*, 2023). Albumin is the major negative APP in swine which, during the APR, decreases in blood concentration, as does apolipoprotein A1 (Apo-A1) (*Ceron et al*., 2005; *Saco and Bassols*, 2023). The specific effect of pre-slaughter stress on APP concentrations is difficult to evaluate, since the handling of animals for slaughter consists of a series of procedures (transport, lairage, stunning, etc.) that are unusual for them and, therefore, stressful. Also, there are concurrent subclinical infections and traumatic lesions inherent in the crowding of animals. The kinetics of the APR should also be taken into account. The serum concentration of the rapid reacting first-line APPs (such as SAA and CRP) increases within four hours (*Petersen*, 2004). Second-line APPs are Hp, CRP and Pig-MAP (*Weschenfelder et al.*, 2012; *Salamano et al*., 2008). Also, in order to adequately determine the impact of stress on concentration of APP, the range of concentration of APP in healthy and sick animals must be established, as well as differences in their concentration between sex, and age.

The concentration of Pig-MAP and Hp increases in pigs confronted with stressful situations and compromising animal welfare, such as longer transport, crowding, mixing unfamiliar pigs, or inadequate handling of feed (*Marco-Ramell et al.*, 2011; *Piñeiro et al.*, 2009; *Piñeiro et al.*, 2004; *Piñeiro et al.,* 2007a; *Piñeiro et al.,* 2007b). Extreme hot temperature elevated the concentration of Hp. Pig-MAP was the only APP whose concentration differed in pigs housed at different stocking densities. High-density pens had higher pig-MAP concentrations (*Marco-Ramell et al.*, 2011). The pig-MAP biomarker has the advantage of relatively low variability in its normal state compared to Hp and other APPs (*Diack, et al.*, 2011; *Piñeiro et al.,* 2009). CRP and SAA concentrations increase after shorter transport, probably because they are first-line APPs (*Saco and Bassols*, 2023). Higher concentrations of CRP may be also induced by stressful situations, such as alterations in feeding patterns and access to water and food (*Piñeiro et al.*, 2007a). Correlations between Hp, Pig-MAP, and CRP and stress status were found in research on mixing stress and human-animal relationships (*Valent et al.,* 2017). Stressors, such as social isolation and short road transport elevated levels of saliva SAA (*Soler et al.,* 2013).

4. Sample types and main methodologic techniques for quantification of APP

The most commonly used matrixes for the measurement of APP are serum or plasma obtained from blood, since blood can reflect the overall picture of the biochemical changes occurring in the body (*Franco-Martinez et al.*, 2020; *Saco and Bassols*, 2023). Determination of APP in blood can be used for monitoring animal health and welfare on farms. The blood collection is highly stressful and painful, both for the animal and the staff in charge of the sam-

pling (*Cerón et al.,* 2022). APP measurements in other fluids, such as meat juice and saliva, can be practical. The use of such non-invasive samples can offer various advantages compared to blood because they are in most cases pain and stress-free, they are faster and easier to obtain, and do not need specialized staff for their collection (*Franco-Martinez et al.*, 2020). However, it is important to point out the question of whether the concentrations of APPs always reliably reflect the concentrations of these molecules in blood. Meat juice can be easily obtained at slaughter, and with a standardized meat juice extraction protocol, which includes harmonization of muscle type and size, the results of APPs might be universally comparable. Concentrations of Pig-MAP and Hp in meat juice are closely correlated with those in plasma. These results open new possibilities for the assessment of animal health in pig production, with implications for food safety and meat quality (*Piñeiro et al.*, 2009). Saliva can be obtained by easy procedures, and repeated specimens can be obtained anytime and anywhere, leading to the possibility of more frequent analysis and better control of health and welfare. Saliva could substitute for blood in some cases, such as for measuring Hp and SAA, indicators of the health status of farms and some stress conditions such as transport, housing, isolation, and restraint. There is a good agreement between APPs quantified in saliva and serum (*Saco et al.,* 2023). Some salivary APPs can be influenced by sex and by circadian patterns (*Gutiérrez et al.,* 2013).

Analytical techniques for the determination of APP in swine include colorimetric (Hp), ELISA (Hp, CRP, Pig-MAP, SAA), immunoturbidimetric (Hp, CRP, Pig-MAP), radial immunodiffusion (RID) (Hp, SAA), and point-of-care type of analysis (Hp, CRP, SAA). Other technologies and biosensors have been adapted more recently (*Saco and Bassols*, 2023). All those methodologic assays have advantages and disadvantages and are still being improved to increase their specificity, sensitivity, economic availability, and user-friendliness. All of them, except the colorimetric method, are species-specific. When a large number of samples have to be assayed, automated techniques such as colorimetric and immunoturbidimetric are advisable.

For adequate interpretation of the results obtained by these methods, accurate reference intervals (RIs) of APP are necessary. Calculation of RIs has to take into account the influence of variables such as age, sex, breed, and type of housing. Also, an adequate analytical validation must be performed in order to assure analytical test specificity, reliability, accuracy, and repeatability, as well as to ensure harmonization and standardization of analytical procedures.

5. Conclusion

APP concentrations change in pigs confronted with situations causing stress and compromising animal welfare, such as transport, crowding, mixing with other pigs, or inadequate handling of feed. The use of APPs as biomarkers of pre-slaughter stress needs to be recognized and further explored. The most commonly used matrixes for the determination of APP are serum or plasma. Other sample types, saliva and meat juice, show potential uses. Reference materials for the calibration of reagents are necessary to expand the use of analytical techniques to determine APPs. The main problem in considering APPs as valid to assess pre-slaughter stress is the lack of standard basal values from healthy animals from different farm conditions, ages, and sex.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Ceron, J. J., Eckersall, P. D. & Martynez-Subiela, S. (2005). Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Veterinary Clinical Pathology*, 34, 85–99, https://doi.org/10.1111/j.1939-165x.2005.tb00019.x

Cerón, J. J., Contreras-Aguilar, M.D, Escribano, D., Martínez-Miró, S., López-Martínez, M. J., Ortín-Bustillo, A., Franco-Martínez, L., Rubio, C. P., Muñoz-Prieto, A., Tvarijonaviciute, A., López-Arjona, M., Martínez-Subiela S. & Tecles F. (2022). Basics for the potential use of saliva to evaluate stress, inflammation, immune system, and redox homeostasis in pigs. *BMC Veterinary Research*, 18(1), 81, https://doi.org/10.1186/S12917-022-03176-W

Cray, C., Zaias J. & Altman, N. H. (2009). Acute phase response in animals: a review. *Comparative Medicine*, 59(6), 517–526.

Diack, A. B., Gladney, C. D., Mellencamp, M. A., Stear, M. J., & Eckersall, P. D. (2011). Characterisation of plasma acute phase protein concentrations in a high health boar herd. *Veterinary Immunology and Immunopathology*, 139, 107–112, https://doi.org/10.1016/j.vetimm.2010.09.003

Franco-Martinez, L., Martinez-Subiela, S., Ceron, J. J., Tecles, F., Eckersa, P. D., Oravcov, K. & Tvarijonaviciute A. (2020). Biomarkers of health and welfare: A One Health perspective from the laboratory side. *Research in Veterinary Science*, 128, 299–307, https://doi.org/10.1016/j.rvsc.2019.12.012

Gutiérrez, A. M., Escribano, D., Fuentes, M. & Cerón, J. J. (2013). Circadian pattern of acute phase proteins in the saliva of growing pigs. *The Veterinary Journal*, 196(2), 167–170, https://doi.org/10.1016/j.tvjl.2012.10.003

Klauke, T. N., Piñeiro, M., Schulze-Geisthövel, S., Plattes, S., Selhorst, T. & Petersen, B. (2013). Coherence of animal health, welfare and carcass quality in pork production chains. *Meat Science*, 95(3), 704–711, https://doi.org/10.1016/j.meatsci.2013.03.022

Levine, S. (1985). A definition of stress. In: Moberg, G.P. (Ed.), Animal Stress. Waverly Press, Baltimore, MA, USA, pp. 51–69.

Marco-Ramell, A., Pato, R., Peña, R., Saco, Y., Manteca, X., Ruiz de la Torre, J. L & Bassols, A. (2011). Identification of serum stress biomarkers in pigs housed at different stocking densities. *The Veterinary Journal*, 190(2), 66–71, https://doi.org/10.1016/j.tvjl.2011.01.003

Mormède, P., Andanson, S., Aupérin, B., Beerda,B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C., G., Richard, S. & Veissier, I. (2007). Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiology & Behavior*, 92, 317–339, https://doi.org/10.1016/j.physbeh.2006.12.003

Murata, H. (2007). Stress and acute phase protein response: an inconspicuous but essential linkage. *The Veterinary Journal*, 173, 473–474, https://doi.org/10.1016/j.tvjl.2006.05.008

Petersen, H. H., Nielsen, J. P. & Heegaard, P. M. (2004). Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, 35(2), 163–187, https://doi.org/10.1051/vetres:2004002

Piñeiro, C., Morales, J., Piñeiro, M., Lampreave, F. & Mateos, G. G. (2004). Effects of induced mixing and pen size on performance and serum concentration of acute phase proteins in growing pigs. *Journal of Animal Science*, 82, 505 (abstr).

Piñeiro, C., Piñeiro, M., Morales, J., Andres, M., Lorenzo, E., del Pozo, M., Alava, M. A. & Lampreave, F. (2009). Pig-MAP and haptoglobin concentration reference values in swine from commercial farms. *The Veterinary Journal*, 179, 78–84, https://doi.org/10.1016/j.tvjl.2007.08.010.

Piñeiro, C., Piñeiro, M., Morales, J., Carpintero, R., Campbell, F. M. & Eckersall, P. D., Toussaint, M. J., Alava, M. A., & Lampreave, F. (2007a). Pig Acute-Phase Protein levels after stress induced by changes in the pattern of food administration. Animal, 1(1), 133–9, https://doi.org/10.1017/S1751731107283909

Piñeiro, M., Gymnich, S., Knura, S., Piñeiro, C. & Petersen, B. (2009). Meat juice: an alternative matrix for assessing animal health by measuring acute phase proteins. Correlations of pig-MAP and haptoglobin concentrations in pig meat juice and plasma. *Research in Veterinary Science*, 87, 273–276, https://doi.org/10.1016/j.rvsc.2009.03.013

Piñeiro, M., Piñeiro, C., Carpintero, R., Morales, J., Campbell, F. M., Eckersall, P. D., Toussaint, M. J. & Lampreave, F. (2007b). Characterisation of the pig acute phase protein response to road transport. *The Veterinary Journal*, 173(3), 669–674, https://doi.org/10.1016/j.tvjl.2006.02.006

Saco, Y. & Bassols, A. (2023). Acute phase proteins in cattle and swine: A review. *Veterinary Clinical Pathology*, 52(1), 50–63, https://doi.org/10.1111/vcp.13220

Salamano, G., Mellia, E., Candiani, D., Ingravalle, F., Bruno, R, Ru G. & Doglione, L. (2008). Changes in haptoglobin, C-reactive protein and pig-MAP during a housing period following long distance transport in swine. *The Veterinary Journal*, 177(1), 110–115, https://doi.org/10.1016/j.tvjl.2007.03.015

Soler, L., Gutiérrez, A., Escribano, D., Fuentes, M. & Cerón, J. J. (2013). Response of salivary haptoglobin and serum amyloid A to social isolation and short road transport stress in pigs. *Research in Veterinary Science*, 95, 298–302, https://doi.org/10.1016/j.rvsc.2013.03.007

Stajkovic, S., Teodorovic, V., Baltic, M. & Karabasil, N (2017). Pre-slaughter stress and pork quality. *IOP Conference Series Earth and Environmental Science*, 85 012034 https://doi.org/10.1088/1755-1315/85/1/012034

Terlow, C. (2005). Stress reactions at slaughter and meat quality in pigs: genetic background and prior experience: A brief review of recent findings. *Livestock Production Science*, 94(1–2), 125–135, https://doi.org/10.1016/j.livprodsci.2004.11.032

Valent, D., Arroyo, L., Peña, R., Yu, K., Carreras, R., Mainau, E., Velarde, A. & Bassols, A. (2017). Effects on pig immunophysiology, PBMC proteome and brain neurotransmitters caused by group mixing stress and human-animal relationship. *PLoS One*, 12(5), e0176928, https://doi.org/10.1371/journal.pone.0176928

Weschenfelder A.V., Torrey, S., Devillers, N., Crowe, T., Bassols, A., Saco, Y., Piñeiro, M., Saucier, L. & Faucitano, L. (2012). Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance. *Journal of Animal Science*, 90(9), 3220–3231, https://doi.org/10.2527/jas.2012-4676

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.1/.3

ID: 126545673

https://doi.org/10.18485/meattech.2023.64.2.27

Review paper

Non-thermal technologies for milk and dairy processing

Tijana Ledina[a], Jasna Đorđević[a], Marija Kovandžić[a] and Snežana Bulajić[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar oslobođenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Non-thermal technologies
Dairy processing
Functional foods

ABSTRACT

Modern consumers demand minimally processed, high-quality, sustainably produced, and ethically sourced food. The food industry strives to meet these demands without compromising food safety. Non-thermal technologies offer a solution by using different physical hurdles to ensure microbiological safety and extended shelf life. In the dairy industry, high-pressure processing, ultrasound, ultraviolet processing, cold plasma and pulsed electric fields show promise as effective non-thermal technologies. These methods achieve microbial inactivation by altering cell membrane structures and damaging genetic material, although the specific mechanisms may vary. Moreover, non-thermal technologies have the potential to enhance product quality and facilitate the development of functional dairy products, with high-intensity ultrasound and supercritical carbon dioxide as particularly noteworthy. Despite the expanding research and development in the field of non-thermal technologies in dairy industries, several challenges persist, including equipment costs, enzyme inactivation efficiency, the absence of validation procedures, regulatory hurdles and consumer acceptance.

1. Introduction

Modern consumers are becoming more informed and aware of the food they purchase — they search for the food that is minimally processed, has high quality, and a "fresh-like" appeal (*Neokleous et al*., 2022; *Zhang et al*., 2018). Additionally, sustainability and ethics have emerged as crucial elements that consumers take into account when choosing food for purchase (*de Toledo Guimarães*, 2018). The food industry strives to meet consumers' demands, but at the same time, food safety must not be compromised, and long shelf-life needs to be achieved (*Mir et al.*, 2016).

Conventional heat treatments are routinely applied in the dairy industry in order to achieve microbiological safety, inactivate enzymes and prolong shelf life, but they also result in loss of nutritional and sensory properties of the product (*Delorme et al.*, 2020). Moreover, the issue of sustainability in milk processing is gaining more attention, due to the high carbon footprint associated with the milk supply chain (*Grandsir et al.*, 2023). Namely, milk is recognized as a second most polluting drink in the world after coffee (*Poore and Nemecek*, 2018).

While the search for new preservation and processing technologies has been ongoing for more than 100 years, global trends in food production have accelerated research in the field of novel food processing technologies. At present, the research focus on novel food-processing is divided into two distinct fields. The first field is focused on novel thermal technologies that utilize heat generated through unconventional ways, such as microwaves and rad-

***Corresponding author:** Tijana Ledina, *tijana.ledina@vet.bg.ac.rs*

Paper received July 13th 2023. Paper accepted July 28th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

iofrequency. The second field involves non-thermal technologies that rely on physical hurdles to achieve desired goals (*Barbosa-Cánovas & Bermúdez-Aguirre*, 2010)

Non-thermal technologies are defined as the technologies which are applied at the ambient or sublethal temperatures (*Cullen et al.*, 2012), without direct exposure of the product to heat (*Chacha et al.*, 2021). Non-thermal technologies rely on the use of electrical, electromagnetic, light or mechanical forces instead of thermal energy (*Rodriguez-Gonzales et al.*, 2015). Since thermisation is the mildest heat treatment applied in the dairy industry (57–68°C for 10–20s), in this context, processing milk at temperatures below 57°C can be considered as non-thermal treatment (*Scudino et al.*, 2020). However, it should be taken into consideration that EU legislation defines milk as raw only if it has not been heated at the temperatures above 40°C, or exposed to any other treatments with an equivalent effect (*EFSA*, 2015).

The most promising and most investigated non-thermal technologies in the dairy industry are high-pressure processing (HPP), ultrasound, ultraviolet processing, cold plasma and pulsed electric fields (PEF).

The aim of this review is to give brief overview of these technologies, their potential for the future application, but also current limitations. Membrane filtration, as one of the most important non-thermal technologies, is not covered by this review, since it is already widely used in the dairy industry.

2. Application of non-thermal technologies in the dairy industry

Initially, non-thermal technologies were meant to be employed to ensure food safety. However, other potential uses also emerged over time. An overview of the non-thermal technologies which have the potential for application in the dairy industry, mechanisms through which they achieve microbial inactivation and other potential uses are shown in Table 1.

Microbial inactivation by non-thermal technologies is accomplished through two mechanisms: alteration of the cell membrane structure and destruction of the genetic material (*Zhang et al*., 2018). However, the specific ways in which these effects are achieved may vary among different non-thermal technologies (Table 1). Microbial inactivation depends on many factors, such as cell shape and size, initial number and phase of growth, but also properties of the food matrix and process variables (*Abrahamsen & Narvhus*, 2022; *Soltanzadeh et al*., 2020). Due to the limited effectiveness of individual non-thermal technologies in microbial inactivation, the focus has shifted towards exploring the potential of combining different non-thermal technologies or integrating non-thermal technologies with heat treatments. This approach aims to achieve the desired level of microbial inactivation by synergistic effects (*Evrendilek*, 2014). Attention has been paid to studying the impact of combining ultrasound with heat, known as "thermosonication"; the effects of combining ultrasound, heat, and pressure, referred to as "manothermosonication", as well as the combination of ultrasound with ultraviolet irradiation, known as "photosonication" (*Abrahamsen & Narvhus*, 2022). The combination of pulsed electric field (PEF) and mild heat treatments has demonstrated promising results, surpassing the microbicidal effects achieved by either PEF or heat pasteurization alone (*Sharma et al*., 2014; *Alirezalu et al*., 2020). This combined approach offers microbial safety, but at the same time, superior quality in terms of nutritional profile and sensory attributes (*Soltanzadeh et al.,* 2020).

Additional advantages of non-thermal technologies were recognized, such as the potential to improve product quality and to help the development of novel food products (*Barbosa-Cánovas & Bermúdez-Aguirre*, 2010). Several non-thermal technologies are being extensively researched for their potential for the production of functional dairy products. High-intensity ultrasound and supercritical carbon dioxide have emerged as the most promising ones (*de Toledo Guimaraes et al*., 2018). Ultrasound treatment has proven to be effective in achieving a fermented milk product with increased levels of biologically active compounds (*Potoroko et al*., 2018). It has also demonstrated its ability to improve lactose absorption by bifidobacteria and promote the production of organic acids (*Nguyen et al.*, 2012), and enhance the viability of the probiotic strain *Lactobacillus acidophilus*-La5 (*Barukčić et al.*, 2015). Additionally, it has been utilized to improve the antioxidant and antihypertensive activities, as well as the bioavailability of phenolic compounds and bioactive peptides in chocolate milk beverages (*Monteiro et al*., 2018). Lastly, ultrasound treatment has been employed in the manufacturing of γ-aminobutyric acid (GABA)-enriched products (*Shokri et al.*, 2021).

Table 1. Non-thermal technologies for milk processing and their potential use in the dairy industry

Process	Definition	Mechanism of microbial inactivation	Other potential uses, use/ beneficial effect
High pressure processing	Transfer of high hydrostatic pressure (in the range 100–1000 MPa) to the food matrix, using a medium (water or oil-alcohol mixtures) (*D'Incecco et al*., 2021)	Under the influence of high pressure, the cell wall and cell membrane of microorganisms undergo irreversible destruction, resulting in a notable change in their permeability. Secondary, tertiary and quaternary structures of large molecules ae changed and these molecules lose their function (*Zhang et al.*, 2018).	Cheese ripening. Enhancing sensory properties of yogurt (texture and creaminess). Prevention of post-acidification due to inactivation of starter cultures, yeasts and moulds. Manufacture of reduced-fat and stabilizer-free ice cream (*Voigt et al*., 2015). Improvement of functional properties (*de Toledo Guimarães et al.*, 2018).
Ultrasound	Longitudinal sound waves, occurring at frequencies beyond the human hearing threshold (approximately 20kHz). Ultrasound creates a sequence of compressions and expansions within a medium (food matrix). This process triggers the formation of vacuum bubbles, which subsequently grow and collapse (*D'Incecco et al.*, 2021).	Micro bubbles in milk generated during the treatment undergo expansion and contraction, in the process known as cavitation. These bubbles gradually increase in size until they collapse, resulting in the generation of localized high temperature and pressure. In such conditions, the bacterial cell membranes are ruptured and destroyed. Chemical compounds that break down the cell walls are also formed (*Abrahamsen & Narvhus*, 2022).	Separation and extraction of milk fat. Homogenization Improvement of lactose crystallization in ice-cream. Improvement of emulsifying properties of dairy emulsion. Reduction of viscosity and control of age thickening in concentrated milk (*Abrahamsen & Narvhus*, 2022).
Ultraviolet radiation	Non-ionizing form of invisible light in the portion of electromagnetic spectrum between visible light and X-rays, with the wavelength between 100 and 400 nm (*Delorme et al*., 2020).	Formation of photoproducts, which interfere with DNA transcription and replication processes (*Delorme et al*., 2020).	
Cold plasma	Plasma, an ionized gas considered the fourth state of matter, comprises electrons and positive and negative ions, which can transfer energy through collisions with gas molecules (gas is in a quasi-neutral state due to the existence of equal number of positive and negative charges carried by different species) (*Neoκleous et al.*, 2022). Consequently, this process generates highly reactive species like reactive hydroxyl radicals, hydrogen peroxide, ozone, nitrogen oxide and UV radiation. (*Niemira*, 2012).	Damage of the cell surface by reactive species. Volatilization of compounds and intracellular desorption of UV photons. Destruction of genetic material (*Coutinho et al.*, 2018).	Enhancement of proteins' physical and chemical properties (*Jadhav et al*., 2021).
Pulsed electric field (PEF)	Passage of an alternating current using electrodes. Food matrix is placed between the electrodes, and is treated with short high-voltage electric pulses, generated by high-voltage pulse generator (*D'Incecco et al.*, 2021).	Electric field generated by PEF inactivates microorganisms through the process of electroporation (*Alirezalu et al.*, 2020).	

3. Non-thermal technologies in dairy industry — considerations for future use

Despite the promising and expanding research and development in the field of non-thermal technologies in dairy industries, numerous challenges still remain to be addressed.

One of the current limitations of non-thermal technologies for industrial use is the requirement for significant initial investments, primarily due to the high cost of equipment. As a result, these technologies are more suitable for premium-priced products rather than mass-market applications (*Voigt et al.*, 2015; *D'Incecco et al.*, 2021). Furthermore, the existing equipment is designed for in-batch processing, thereby limiting the amount of product that can be processed at one time (*Voigt et al*., 2015; *Arshad et al*., 2020). For industrial implementation, it is necessary to develop equipment that can operate continuously in-line with the production process (*D'Incecco et al.,* 2021).

In order for the process to be considered a valid alternative to heat-treatments, it is crucial that the native or bacterial enzymes in milk can be effectively inactivated. Various non-thermal technologies, including ultrasound, HPP, and PEF, have demonstrated effectiveness in enzyme inactivation. However, when used individually, these methods require high-intensity treatment parameters, which can have detrimental effects on the nutritional and sensory properties of milk. A combination of different non-thermal technologies or the integration of non-thermal technologies with mild heat treatments can be employed, in order to achieve desired results (*Ahmad et al*., 2019).

Effects of non-thermal technologies on sensory properties of the products have not been studied extensively to date (*Neoκleous et al*., 2022). Some studies reported possible negative impacts of non-thermal technologies on flavour and aroma of the products, mainly due to the oxidation processes (*Priyadarshini et al.*, 2019; *Choudhary & Bandla*, 2012; *Delorme et al*., 2020; *Ribeiro et al.*, 2021; *Marchesini et al.*, 2015).

The efficacy of high temperature-short time pasteurization is typically evaluated by the activity of alkaline phosphatase, with remaining activity indicating an inadequate process. However, for non-thermal technologies, standardized process parameters and validation procedures have not yet been established. Consequently, the absence of validation procedures, but also harmonized labelling, represent regulatory hurdles for the non-thermal technologies (*Barbosa-Cánovas & Bermúdez-Aguirre*, 2010; *D'Incecco et al*., 2020).

Consumer acceptance of novel products remains a challenge (*Tuorilla & Hartmann*, 2020), as there is a perception that these products are overpriced and pose a higher risk compared to conventionally processed alternatives *(Coutinho et al.*, 2021). It is crucial, therefore, to educate consumers about the potential advantages of non-thermal technologies to achieve better acceptance (*Coutinho et al.*, 2021).

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Abrahamsen, R. K. & Narvhus, J. A. (2022). Can ultrasound treatment replace conventional high temperature short time pasteurization of milk? A critical review. *International Dairy Journal*, 131, 105375, https://doi.org/10.1016/j.idairyj.2022.105375

Ahmad, T., Butt, M. Z., Aadil, R. M., Inam-ur-Raheem, M., Abdullah, Bekhit, A. E.-D., Guimarães, J. T., Balthazar, C. F., Rocha, R. S., Esmerino, E. A., Freitas, M. Q., Silva, M. C., Sameen, A. & Cruz, A. G. (2019). Impact of nonthermal processing on different milk enzymes. *International Journal of Dairy Technology*, 72(4), 481–495, https://doi.org/10.1111/1471-0307.12622

Evrendilek, G. (2014). Non-thermal processing of milk and milk products for microbial safety. *Dairy Microbiology and Biochemistry: Recent Developments*, 322, 322–355, http://dx.doi.org/10.1201/b17297-14

Alirezalu, K., Munekata, P. E. S., Parniakov, O., Barba, F. J., Witt, J., Toepfl, S., Wiktor, A. & Lorenzo, J. M. (2020). Pulsed electric field and mild heating for milk processing: a review on recent advances. *Journal of the Science of Food and Agriculture*, 100(1), 16–24, https://doi.org/10.1002/jsfa.9942

Arshad, R. N., Abdul-Malek, Z., Munir, A., Buntat, Z., Ahmad, M. H., Jusoh, Y. M. M., Bekhit, A. E.-D., Roobab, U., Manzoor, M. F. & Aadil, R. M. (2020). Electrical

systems for pulsed electric field applications in the food industry: An engineering perspective. *Trends in Food Science & Technology*, 104, 1–13, https://doi.org/10.1016/j.tifs.2020.07.008

Barbosa-Cánovas, G. V., Bermúdez-Aguirre, D., Franco, B. G., Candoğan, K. & Shin, G. Y. (2022). Novel food processing technologies and regulatory hurdles. In Ensuring global food safety (pp. 221–228). Academic Press, https://doi.org/10.1016/B978-0-12-816011-4.00006-9

Barukčić, I., Lisak Jakopović, K., Herceg, Z., Karlović, S. & Božanić, R. (2015). Influence of high intensity ultrasound on microbial reduction, physico-chemical characteristics and fermentation of sweet whey. *Innovative Food Science & Emerging Technologies*, 27, 94–101, https://doi.org/10.1016/j.ifset.2014.10.013

Chacha, J. S., Zhang, L., Ofoedu, C. E., Suleiman, R. A., Dotto, J. M., Roobab, U., Agunbiade, A. O., Duguma, H. T., Mkojera, B. T., Hossaini, S. M., Rasaq, W. A., Shorstkii, I., Okpala, C. O. R., Korzeniowska, M. & Guiné, R. P. F. (2021). Revisiting Non-Thermal Food Processing and Preservation Methods—Action Mechanisms, Pros and Cons: A Technological Update (2016–2021). *Foods*, 10(6), 1430, https://doi.org/10.3390/foods10061430

Choudhary, R. & Bandla, S. (2012). Ultraviolet pasteurization for food industry. *International Journal of Food Science and Nutrition Engineering*, 2(1), 12–15, 10.5923/j.food.20120201.03

Coutinho, N. M., Silveira, M. R., Guimarães, J. T., Fernandes, L. M., Pimentel, T. C., Silva, M. C., Borges, F. O., Fernandes, F. A., Rodrigues, S., Freitas, M. Q., Esmerino, E. A. & Cruz, A. G. (2021). Are consumers willing to pay for a product processed by emerging technologies? The case of chocolate milk drink processed by cold plasma. *LWT*, 138, 110772, https://doi.org/10.1016/j.lwt.2020.110772

Coutinho, N. M., Silveira, M. R., Rocha, R. S., Moraes, J., Ferreira, M. V. S., Pimentel, T. C., Freitas M. Q., Silva M. C., Raices, R. S. L., Senaka Ranadheera, C., Borges, F. O., Mahias, S. P., Fernandes, F. A. N., Rodrigues, S. & Cruz, A. G. (2018). Cold plasma processing of milk and dairy products. *Trends in Food Science & Technology*, 74, 56–68, https://doi.org/10.1016/j.tifs.2018.02.008

Cullen, P. J., Tiwari, B. K. & Valdramidis, V. P. (2012). Status and Trends of Novel Thermal and Non-Thermal Technologies for Fluid Foods. In *Novel Thermal and Non-Thermal Technologies for Fluid Foods*, (pp. 1–6). Academic Press, https://doi.org/10.1016/B978-0-12-381470-8.00001-3

de Toledo Guimarães, J., Silva, E. K., de Freitas, M. Q., de Almeida Meireles, M. A. & da Cruz, A. G. (2018). Non-thermal emerging technologies and their effects on the functional properties of dairy products. *Current Opinion in Food Science*, 22, 62–66, https://doi.org/10.1016/j.cofs.2018.01.015

Delorme, M. M., Guimarães, J. T., Coutinho, N. M., Balthazar, C. F., Rocha, R. S., Silva, R., Margalho, L. P., Pimentel, T. C., Silva, M. C., Freitas, M. Q., Granato, D., Sant'Ana, A. S., Duart, M. C. K. H. & Cruz, A. G. (2020). Ultraviolet radiation: An interesting technology to preserve quality and safety of milk and dairy foods. *Trends in Food Science & Technology*, 102, 146–154, https://doi.org/10.1016/j.tifs.2020.06.001

D'Incecco, P., Limbo, S., Hogenboom, J. A. & Pellegrino, L. (2021). Novel technologies for extending the shelf life of drinking milk: Concepts, research trends and current applications. *LWT*, 148, 111746, https://doi.org/10.1016/j.lwt.2021.111746

EFSA (EFSA Panel on Biological Hazards), (2015). Scientific opinion on the public health risks related to the consumption of drinking raw milk. *EFSA Journal*, 13, Article 3940, https://doi.org/10.2903/j.efsa.2015.3940

Grandsir, C., Falagán, N. & Alamar, M. C. (2023). Application of novel technologies to reach net-zero greenhouse gas emissions in the fresh pasteurised milk supply chain: A review. *International Journal of Dairy Technology*, 76(1), 38–50, https://doi.org/10.1111/1471-0307.12926

Jadhav, H. B., Annapure, U. S. & Deshmukh, R. R. (2021). Non-thermal Technologies for Food Processing. *Frontiers in Nutrition*, 8, 657090, https://doi.org/10.3389/fnut.2021.657090

Marchesini, G., Fasolato, L., Novelli, E., Balzan, S., Contiero, B., Montemurro, F., Andrighetto, I. & Segato, S. (2015). Ultrasonic inactivation of microorganisms: A compromise between lethal capacity and sensory quality of milk. *Innovative Food Science & Emerging Technologies*, 29, 215–221, https://doi.org/10.1016/j.ifset.2015.03.015

Mir, S. A., Shah, M. A. & Mir, M. M. (2016). Understanding the role of plasma technology in food industry. *Food and Bioprocess Technology*, 9, 734–750, https://doi.org/10.1007/s11947-016-1699-9

Monteiro, S. H. M. C., Silva, E. K., Alvarenga, V. O., Moraes, J., Freitas, M. Q., Silva, M. C., Raices, R. S. L, Sant'Ana, A. S., Meireles, M. A. A. & Cruz, A. G. (2018). Effects of ultrasound energy density on the non-thermal pasteurization of chocolate milk beverage. *Ultrasonics Sonochemistry*, 42, 1–10, https://doi.org/10.1016/j.ultsonch.2017.11.015

Neokleous, I., Tarapata, J. & Papademas, P. (2022). Non-thermal processing technologies for dairy products: Their effect on safety and quality characteristics. *Frontiers in Sustainable Food Systems*, 6, 856199, https://doi.org/10.3389/fsufs.2022.856199

Nguyen, T. M. P., Lee, Y. K. & Zhou, W. (2012). Effect of high intensity ultrasound on carbohydrate metabolism of bifidobacteria in milk fermentation. *Food Chemistry*, 130(4), 866–874, https://doi.org/10.1016/j.foodchem.2011.07.108

Niemira, B. A. (2012). Cold plasma reduction of *Salmonella* and *Escherichia coli* O157: H7 on almonds using ambient pressure gases. *Journal of Food Science*, 77(3), M171–M175, https://doi.org/10.1111/j.1750-3841.2011.02594.x

Poore, J. & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*, 360(6392), 987–992, https://doi.org/10.1126/science.aaq0216

Potoroko, I., Kalinina, I., Botvinnikova, V., Krasulya, O., Fatkullin, R., Bagale, U. & Sonawane, S. H. (2018). Ultrasound effects based on simulation of milk processing properties. *Ultrasonics Sonochemistry*, 48, 463–472, https://doi.org/10.1016/j.ultsonch.2018.06.019

Priyadarshini, A., Rajauria, G., O'Donnell, C. P. & Tiwari, B. K. (2019). Emerging food processing technologies and factors impacting their industrial adoption. *Critical Reviews in Food Science and Nutrition*, 59(19), 3082–3101, https://doi.org/10.1080/10408398.2018.1483890

Ribeiro, K. C. S., Coutinho, N. M., Silveira, M. R., Rocha, R. S., Arruda, H. S., Pastore, G. M., Neto, R. P. C., Tavares, M. I. B., Pimentel, T. C., Silva, P. H. F., Freitas, M. Q., Esmerino, E. A., Silva, M. C., Duarte, M. C. K. H. & Cruz, A. G. (2021). Impact of cold plasma on the techno-functional and sensory properties of whey dairy beverage added with xylooligosaccharide. *Food Research International*, 142, 110232, https://doi.org/10.1016/j.foodres.2021.110232

Rodriguez-Gonzalez, O., Buckow, R., Koutchma, T. & Balasubramaniam, V. M. (2015). Energy Requirements for Alternative Food Processing Technologies—Principles, Assumptions, and Evaluation of Efficiency. *Comprehensive Reviews in Food Science and Food Safety*, 14(5), 536–554, https://doi.org/10.1111/1541-4337.12142

Scudino, H., Silva, E. K., Gomes, A., Guimarães, J. T., Cunha, R. L., Sant'Ana, A. S., Meireles, M. A. A. & Cruz, A. G. (2020). Ultrasound stabilization of raw milk: Microbial and enzymatic inactivation, physicochemical properties and kinetic stability. *Ultrasonics Sonochemistry*, 67, 105185, https://doi.org/10.1016/j.ultsonch.2020.105185

Sharma, P., Oey, I. & Everett, D. W. (2014). Effect of pulsed electric field processing on the functional properties of bovine milk. *Trends in Food Science & Technology*, 35(2), 87–101, https://doi.org/10.1016/j.tifs.2013.11.004

Shokri, S., Terefe, N. S., Shekarforoush, S. S. & Hosseinzadeh, S. (2021). Ultrasound-assisted fermentation for enhancing metabolic and probiotic activities of *Lactobacillus brevis*. *Chemical Engineering and Processing — Process Intensification*, 166, 108470, https://doi.org/10.1016/j.cep.2021.108470

Soltanzadeh, M., Peighambardoust, S. H., Gullon, P., Hesari, J., Gullón, B., Alirezalu, K. & Lorenzo, J. (2020). Quality aspects and safety of pulsed electric field (PEF) processing on dairy products: a comprehensive review. *Food Reviews International*, 38, 96–117, https://doi.org/10.1080/87559129.2020.1849273

Tuorila, H. & Hartmann, C. (2020). Consumer responses to novel and unfamiliar foods. *Current Opinion in Food Science*, 33, 1–8, https://doi.org/10.1016/j.cofs.2019.09.004

Voigt, D. D., Kelly, A. L. & Huppertz, T. (2015). High-Pressure Processing of Milk and Dairy Products. In Datta, N. & Tomasula, P. M. (Eds.), Emerging Dairy Processing Technologies: Opportunities for the Dairy Industry (pp. 71–92). John Wiley & Sons, Ltd, https://doi.org/10.1002/9781118560471.ch3

Zhang, Z.-H., Wang, L.-H., Zeng, X.-A., Han, Z. & Brennan, C. S. (2018). Non-thermal technologies and its current and future application in the food industry: a review. *International Journal of Food Science & Technology*, 54(1), 1–13, https://doi.org/10.1111/ijfs.13903

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.04:669.018.674

ID: 126553353

https://doi.org/10.18485/meattech.2023.64.2.28

Review paper

Levels and accumulation of selected heavy metals in the One Health approach

Nevena Grković[a], Nikola Čobanović[a], Branko Suvajdzić[a], Dragoljub Jovanović[a] and Mirjana Dimitrijević[a]*

[a] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia

ARTICLE INFO

Keywords:
Meat
Heavy metals
Cadmium
Lead
Risk assessments

ABSTRACT

Meat and meat products are main sources of human nutrients, including protein, minerals, vitamins, and fats**.** One of the main potential risks of meat consumption, to public health, is the accumulation of heavy metals. Their concentrations in the environment are increasing with the rapid development of human civilization as well as the exploitation of geological resources. Because they are so prevalent in the environment, heavy metals can infiltrate the food chain. Food contamination consequently has the potential to negatively impact consumer health. Heavy metals, including lead (Pb), cadmium (Cd), and mercury (Hg), that are frequently present in food have toxicological reference values, and their primary dietary sources are known. Their levels in all kinds of food, including meat, are assessed by comparing them with the maximum permissible limits set by the European Union. European Commission Regulation EC 2023/915 sets maximum levels (MLs) of heavy metals allowed in traded meats from domesticated bovine animals, sheep, pigs, and poultry, but also from less frequently eaten meats from wild animals, including cephalopods and bivalve mollusks.

1. Introduction

Meat is a rich source of nutrients important for human diet and provides the well-known proteins, minerals, vitamins and trace element contents. In recent years, much attention has been focused on the risk of heavy metal contamination in meat, because of the toxic nature of these metals even at relatively small levels (*Lukáčová et al*., 2013). Metals are a large group of elements that are classified as essential trace elements, macroelements (sodium, calcium, and magnesium) and toxic or heavy metals. While some elements (iron, copper, manganese, cobalt, and zinc) are essential for human health and play a specific role in body metabolism, others (lead, cadmium, chrome, mercury, and arsenic) are recognized as dangerous, and their presence can cause biochemical and neurological problems in humans. Heavy metals can be involved in different signaling pathways in carcinogenesis, interfere with ATP synthesis, and change protein synthesis (*Rudy et al*., 2007).

Metals are found in all living organisms and in the environment, where they are formed by burning fossil fuels, and are in pesticides and herbicides, waste disposal sites, and municipal sewage. They have a long life in the environment and can move up the food chain. Heavy metal contamination of meat and livestock products does not occur during processing; rather, the main contributing factors include giving contaminated feed to livestock and poultry

***Corresponding author:** Nevena Grković, *nevena.ilic@vet.bg.ac.rs*

Paper received Jun 19th 2023. Paper accepted Jun 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

or keeping them in close proximity to contaminated areas (*Singh et al.*, 2011). The target tissues, like muscle, the kidneys, and the liver, are the primary sites for the precipitation of heavy metals in the animal body (*Emami et al.*, 2023). There is a greater probability of toxic metal traces being found in higher levels in food from animals that are from areas with mining and anthropogenic activities.

The diet, particularly the consumption of meat and other animal products, is usually the main source of human exposure to heavy metals. Although acute ingestion of these elements through food can infrequently result in poisoning, the accumulation of them in the body has a negative impact on human health (*Ahmad et al.,* 2018). The WHO has concluded, after extensive evaluation studies on food additives and their toxicity, that even low amounts of particular metals, such as lead and cadmium, can result in disease in humans (*WHO*, 2001). Lead, cadmium, arsenic, mercury, and chromium are the heavy metals in meat and animal products that should primarily be controlled. Therefore, the EU Scientific Committee on Food has established a maximum limit for these specific pollutants in products, which is periodically monitored by the European Food Safety Authority (EFSA) (*Reg EC 2023/915*), with adjustments and additions for cadmium (*Reg EC 2021/1323*) and lead (*Reg EC 2021/1317*) in recent years. In accordance with these regulations, the Republic of Serbia published a currently valid regulation on maximum levels for certain contaminants in food *(Official Gazette of the RS, No. 127 of November 18*, 2022).

Lead (Pb) is a dangerous heavy metal that occurs naturally in the environment, in rocks, soil, vegetation, and the hydrosphere. Bipolar lead forms interfere with the normal function of enzymes and inhibit their action. If the bonding capacity for blood protein is exceeded, lead passes into the bone marrow, liver, and kidneys (*Levin et al*., 2020). Table 1 shows the maximum permitted values of lead in the meat of different animal species according to *Regulation EC 2021/1317*. Cadmium (Cd) is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Foodstuffs like cereals, vegetables, and meat and meat products are the main

Table 1. Maximum lead (Pb) levels (mg/kg wet weight) in meat of different animal species (Reg EC 2021/1317; Official Gazette of the RS, No. 127/2022)

Foodstuffs	Maximum level of Pb (mg/kg wet weight)
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.10
Offal of bovine animals and sheep	0.20
Offal of pig	0.15
Offal of poultry	0.10
Muscle meat of fish	0.30
Bivalve mollusks	1.50

Table 2. Maximum cadmium (Cd) levels (mg/kg wet weight) in meat of different animal species (Reg EC 2021/1323; Official Gazette of the RS, No. 127/2022)

Foodstuffs	Maximum level of Cd (mg/kg wet weight)
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.050
Horsemeat (excluding offal)	0.20
Liver of bovine animals, sheep, pig poultry and horse	0.50
Kidney of bovine animals, sheep, pig poultry and horse	1.0
Muscle meat of fish	0.050
Bivalve mollusks	1.0

source of cadmium exposure. The specified maximum cadmium values for meat products according to *Regulation EC 2021/1323* are shown in Table 2. Arsenic (As) is found in high concentrations in nature as a result of anthropogenic pollution (use of herbicides, fungicides, pesticides, glass and smelter manufacturing). There is no need to restrict or avoid eating meat and animal products in order to lower exposure to arsenic because they contain little to no arsenic (*Nachman et al.*, 2017). Mercury (Hg) is global toxic heavy metal, even in trace amounts, and its presence in food must be monitored at all times. Mercury can be present in contaminated air, soil, and water, and it can also be converted by microorganisms into more accessible organic forms that are then ingested at higher levels of the food chain (*Rodríguez-Estival et al.*, 2020).

2. The presence of heavy metals in meat of different animal species

Different animal species have various bio-accumulation capacities for heavy metals. The level of heavy metals in animals and meat thereof also depends on environmental conditions and industrialization development in the environment where the animals are raised (*Badis et al.,* 2014). For example, mutton accumulates lower levels of these metals than do other meat animals, which may be caused by the specific feeding of sheep with grass and less mineral supplement (*Han et al.*, 2022). Accumulation of cadmium and lead in kidney, liver, and spleen of horse was found to be relatively higher than in tissues of other animal species (*Mor et al.*, 2005). Generally, liver was found to have the highest significant level of metals, and the meat and blood has the lowest levels. Due to frequent the high concentrations of metals in the sea, we can find high levels of arsenic, cadmium, lead, and mercury in marine fish above the regulatory limits. Seafood is the main dietary source of arsenic in humans, because these foods contain several times the amount of this metal than other foods (*Bosch et al.*, 2016). Besides this, mercury levels found in some seafood products are of great concern, exceeding the limits set for fishery products by the European Regulation (1.0 mg/kg) (*Regulation EC 2022/617*). Because lead ammunition is used in hunting, it is common knowledge that game meat occasionally contains high quantities of lead, especially around the bullet entry and exit points on the carcasses. While non-lead alternatives to lead shotgun and rifle ammunition have been developed, there is no European rule requiring their usage for game shooting (*Mateo & Kanstrup*, 2019). Some other heavy metals can also appear in somewhat higher levels in wildlife than in meat from domesticated animals.

3. General health effects of heavy metals

The high exposure to heavy metals is a serious problem in the food chain because of their toxicity and bioaccumulation. The competence of the body to exude toxic metals is generally slower than the intake, thus leading to their accumulation in organs and to long-term health impacts. Carcinogenic effects, central and peripheral nervous system damage, and blood circulatory effects are some of health effects that can be expected (*Durkalec et.*, 2015).

For example, excessive intake of lead in the human body can lead to developmental disabilities in children, nervous system dysfunction of the fetus and infants, kidney and blood problems, reproductive dysfunction, and cardiovascular disease. Cadmium manifests in several pathological processes, especially being toxic to the kidneys, and particularly to proximal tubular cells, where it accumulates over time and can cause kidney dysfunction. Besides this, chronic exposure of cadmium can cause liver harm and bone and blood damage. One consequence of mercury poisoning is damage to the nervous system of newborn children (*Han et al*., 2022). According to the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel), the tolerable weekly intake (TWI) of cadmium is 2.5 µg/kg b.w. (body weight of the person) per week, which is equivalent to 2.5/7 = ~0.36 µg/kg b.w. per day (*EFSA*, 2011). For lead, the benchmark dose level (BMDL10) of 0.63 µg/kg b.w. per day is considered as health-based guidance value (HBGV), since EFSA concluded that the current provisional tolerable weekly intake (PTWI) of 25 µg/kg b.w. is no longer appropriate, as there is no evidence for a threshold for critical lead-induced effects (*EFSA*, 2010).

Metallic mercury poisoning often results from mercury vapor, which enters the alveoli through the respiratory tract and is transported throughout the body through the blood circulation, causing movement disorders, headache, dizziness, etc. The persistent occurrence and accumulation of heavy metals from numerous sources resulted in them being ranked by the Agency of Toxic Substances and Disease Registry (ATSDR) as the most hazardous and toxic substances in the environment (*ATSDR*, 2011).

4. Conclusion

In the human diet, meat has an important function and is a rich source of nutrients. In addition to its useful components, meat contains some hazardous components such as heavy metals, whose levels must be periodically monitored to ensure the safety of public health. Preventive and regulatory measures for controlling contamination sources are the best way to reduce contamination in meat and meat products. Implementation of good agricultural practices, farm management systems, water testing, feed protection from contamination, and the selection of uncontaminated areas to keep animals can help identify and control the risks of heavy metals occurring in meats. Developed and some developing countries have responded to the potential menace of the over-intake of these metals by regulating the amounts of toxic metals permissible in different types of meat and meat products. The promotion of the One Health concept in meat production from farm to fork is one strategy that can be used to prevent the contamination of meat by heavy metals.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-68/2020- 14/200143).

References

Agency for Toxic Substances and Diseases Registry (ATSDR), 2011. Available from http://www.atsdr.cdc. gov/substances/toxsearch.asp

Ahmad, R. S., Imran, A. & Hussain, M. B. (2018). Nutritional composition of meat. *Meat Science and Nutrition*, 61(10.5772), 61–75.

Badis, B., Rachid, Z. & Esma, B. (2014). Levels of selected heavy metals in fresh meat from cattle, sheep, chicken and camel produced in Algeria. *Annual Research & Review in Biology*, 1260–1267.

Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E. & Hoffman, L. C. (2016). Heavy metals in marine fish meat and consumer health: a review. *Journal of the Science of Food and Agriculture*, 96(1), 32–48.

Commission Regulation (EU) 2021/1317 of 9 August 2021. Commission Regulation (EU) 2021/1317 of 9 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of Lead in certain foodstuffs (Text with EEA relevance).

Commission Regulation (EU) 2021/1323 of 10 August 2021. Commission Regulation (EU) 2021/1323 of 10 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of Cadmium in certain foodstuffs (Text with EEA relevance).

Commission Regulation (EU) 2022/617 of 12 April 2022. Commission Regulation (EU) 2022/617 of 12 April 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of Mercury in fish and salt.

Commission Regulation (EU) 2023/915 of 25 April 2023. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.

Durkalec, M., Szkoda, J., Kolacz, R., Opalinski, S., Nawrocka, A. & Zmudzki, J. (2015). Bioaccumulation of lead, cadmium and mercury in roe deer and wild boars from areas with different levels of toxic metal pollution. *International Journal of Environmental Research,* 9(1), 205–212.

EFSA, 2010. Scientific opinion on lead in food. Panel on contaminants in the food chain (CONTAM). *EFSA Journal*, 8 (4), 1570. Parma, Italy.

EFSA, 2011. Panel on contaminants in the food chain (CONTAM); scientific opinion on tolerable weekly intake for cadmium. *EFSA Journal*, 9(2), 19.

Emami, M. H., Saberi, F., Mohammadzadeh, S., Fahim, A., Abdolvand, M., Dehkordi, S. A. E. ... & Maghool, F. (2023). A review of heavy metals accumulation in red meat and meat products in the Middle East. *Journal of Food Protection*, 100048.

Han, J. L., Pan, X. D. & Chen, Q. (2022). Distribution and safety assessment of heavy metals in fresh meat from Zhejiang, China. *Scientific Reports*, 12(1), 3241.

Levin, R., Vieira, C. L. Z., Mordarski, D. C. & Rosenbaum, M. H. (2020). Lead seasonality in humans, animals, and the natural environment. *Environmental Research,* 180, 108797.

Lukáčová, A., Binkowski, Ł. & Golian, J. (2021). Comparison of mercury concentration in meat products of different origin. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 31–33.

Mateo, R. & Kanstrup, N. (2019). Regulations on lead ammunition adopted in Europe and evidence of compliance. *Ambio,* 48(9), 989–998.

Mor, F., Sonal, S. & Cerit, H. (2005). Lead and cadmium levels in tissues of horses in Bursa, Turkey. *Fresenius Environmental Bulletin*, 14(9), 773–776.

Nachman, K. E., Love, D. C., Baron, P. A., Nigra, A. E., Murko, M., Raber, G. ... & Navas-Acien, A. (2017). Nitarsone, inorganic arsenic, and other arsenic species in Turkey meat: exposure and risk assessment based on a 2014 US market basket sample. *Environmental Health Perspectives,* 125(3), 363–369.

Official Gazette of the RS, No. 127/2022, (2022). Regulation on the maximum levels for certain contaminants in food.

Rodríguez-Estival, J., Ortiz-Santaliestra, M. E. & Mateo, R. (2020). Assessment of ecotoxicological risks to river otters from ingestion of invasive red swamp crayfish in metal contaminated areas: Use of feces to estimate dietary exposure. *Environmental Research,* 181, 108907.

Rudy, M., Znamirowska, A. & Zin, M. (2007). Level of accumulation of selected heavy metals in horse tissue as a function of age. *Medicine Weter,* 63(11), 1303–1306.

Singh, R., Gautam, N., Mishra, A. & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*, 43(3), 246.

World Health Organisation (WHO), (2001). Cadmium. In: Safety Evaluation of Certain Food Additives and contaminants. Fifty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: WHO Food Additives Series 46. pp 247–305

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.1.033

ID: 126557705

https://doi.org/10.18485/meattech.2023.64.2.29

Review paper

Horse carcass and meat quality — current knowledge and research gaps

Nikola Čobanović[a], Nevena Grković[a], Branko Suvajdžić[a], Ivan Vićić[a] and Nedjeljko Karabasil[a]*

[a] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia

ARTICLE INFO

Keywords:
Carcass conformation
Carcass fat cover
Meat colour
Water-holding capacity

ABSTRACT

Horsemeat can be considered a good alternative for conventional meats due to its potential dietetic and health benefits linked with its specific nutritional composition. The aim of this review was to provide information on the carcass and meat quality of horses, as well as methods for their examination based on currently available scientific literature in order to expand knowledge in this field and determine the direction of future research. The most important horse carcass quality indicators are the carcass conformation and carcass fat cover, while the most important horsemeat quality traits are pH, colour, water-holding capacity and texture. However, more research is needed to establish a classification system for horse carcasses as well as threshold values for colour and water-holding capacity traits that might be used for horsemeat classification into quality classes.

1. Introduction

Horsemeat can be considered a good alternative for conventional meats (chicken, pork, sheep or beef) due to its potential dietetic and health benefits linked with high content of protein, iron, minerals (P, Fe, Zn and Cu) and vitamins (A, E, C and B group) (*Lorenzo et al.*, 2014, 2019). This meat type has a low fat content, low cholesterol content and favourable fatty acid profile, with a high content of unsaturated fatty acids relative to saturated acids and provides a large amount of essential amino acids (*Lee et al.*, 2007).

However, horsemeat can only be considered as an alternative for conventional meat types if the production chain is under strict control to guarantee traceability and high quality and safety of raw meat and meat products (*Lorenzo et al.*, 2014, 2019). It is also important to define horse carcass and meat characteristics, appropriate assessment methods and reference ranges for good or poor carcass/meat quality to ensure that product quality meets consumers expectations. Therefore, the aim of this review was to provide information on the carcass and meat quality of horses, as well as methods for their examination based on currently available scientific literature in order to expand knowledge in this field and determine the direction of future research.

2. Carcass characteristics

The horse carcasses exhibit a large degree of variability, which arises from differences in horse origins, slaughter ages, breeds, types and production systems (*Lorenzo et al.*, 2019). However, the unique attributes of the horse carcass are a dark colour that can change to brown or black with a bluish tinge

***Corresponding author:** Nikola Čobanović, cobanovic.nikola@vet.bg.ac.rs

Paper received Jun 29th 2023. Paper accepted July 12th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

upon exposure to air, the absence of significant fatty deposits, and the presence of yellow-coloured fat (*Lorenzo et al.*, 2014, 2019). Certain features, such as neck length, rib number (18 pairs), non-lobulated kidney and bony structures, can help differentiate horse carcasses (*Lorenzo et al.*, 2019). The most important factors that affect horse carcass quality are age, slaughter weight, breed, gender, production system, finishing feeding and anatomical location of measurements (*Franco et al.*, 2011; *Lorenzo et al*, 2013a; *Domínguez et al*, 2015). Horse carcass traits are evaluated by different parameters such as: slaughter weight, carcass weight, dressing percentage, carcass conformation, carcass fat cover and carcass linear measurements (*Lorenzo et al*., 2014; *Znamirowska*, 2005).

Slaughter weight and dressing percentage are measures of the animal's development and are important for determination of the fattening performance (daily weight gain) (*National Research Council*, 1988). Indirectly, considering that heavier carcasses usually have more musculature, weight can also indicate horse carcass meatiness. In horses, slaughter weight can be determined on a balance scale or with horse weight tape on the farm, at the lairage or, most often, 1 hour before slaughter (*De Palo et al*, 2013). The dressing percentage of horses is mainly the ratio of dressed hot carcass weight to the slaughter weight, expressed as a percentage (hot dressing percentage) (*Lorenzo et al.*, 2014). Extremely high slaughter weight and dressing percentage can be a disadvantage if they are a consequence of the large amount of fat, bones and organs (*National Research Council*, 1988).

A horse carcass is defined as the carcass from which the skin, head, lower legs (separated at the tarsal and carpal joints), tail and all internal organs of the thoracic, abdominal and pelvic cavities (except for the kidneys and renal fat tissue) have been removed during processing (*Polidori et al*, 2015). Carcass weight is usually determined on a balance scale 45 minutes (hot carcass weight) or 24 hours *postmortem* when the internal carcass temperature drops below 7°C (cold carcass weight). Cold carcass weight can also be calculated by reducing hot carcass weight by 2% (*European Commission*, 2008).

Carcass conformation is the most important indicator when classifying carcasses because it directly indicates the amount of meat (*Pečiulaitienė et al*, 2015). It describes the development of carcass profiles, in particular the essential parts, such as legs (round and rump), back and shoulders (chuck), and indicates the sum of muscle and fat in relation to the bones (*Ekiz et al*, 2021). Carcass conformation is visually evaluated at the end of the slaughterline, i.e., immediately after determination of hot carcass weight (45 minutes *postmortem*). The currently available ONIBEV (*Office National Interproffessionnel du Betail et des Viandes*) classification system for horse carcasses, established in 1979 in France, takes into consideration the horse age, carcass conformation, carcass fat cover and the slaughter weight. However, some authors (*Fàbregas and Such*, 2001; *Lorenzo et al.*, 2019; *Cittadini et al,* 2021) consider that the ONIBEV system (1979) for classifying horse carcasses is limited, and therefore, the EUROP standard for beef carcass classification is used as an alternative in commercial conditions and in research studies (*Cittadini et al*., 2021). Since horsemeat has a regular group of consumers, addressing the issue of objective classification of horses and their carcasses will increasingly gain importance (*Znamirowska*, 2005). The absence of an official classification system for horse carcasses intended for meat consumption in the European Union necessitates the development of such a system to ensure an adequate supply of desired quality meat to meet consumer demands (*Lorenzo et al.*, 2014). The implementation of a standardised classification system would enable comparative analyses of horsemeat across different countries, between time periods and between studies, and the grouping of meat batches for export based on uniform parameters or criteria, neither of which is possible at the moment (*Znamirowska*, 2005). This would also provide positive incentives for primary producers to produce horses of higher quality standards and facilitate crossbreeding efforts aimed at obtaining premium-quality raw meat, for which they could potentially receive higher prices (*Znamirowska*, 2005).

Carcass fat cover is one of the most important parameters in the horse carcass classification, and is usually determined at the same time as the carcass conformation (45 minutes *postmortem*) (*Lorenzo et al.*, 2019). The carcass fat cover describes the amount of fat on the outside of the carcass and in the thoracic cavity (*Ekiz et al.*, 2021). From the carcass quality aspect, favourable fat cover is described when a carcass has uniformly and evenly distributed, continuous, but not too thick layer of fat tissue (*European Commission*, 2008). Sufficient carcass fat cover insulates the carcass and decreases cooling loss (*Albertí et al*, 2022), slowing down surface meat spoilage (Gill, 1983), increases antioxidative

stability and improves meat aroma (juiciness, taste and odour) (*Alberti et al*., 2017). The subcutaneous fat on a horse carcass is not abundant, and the kidney fat depots and flank region are relatively lean, even in well-fed animals (*Lorenzo et al*., 2014, 2019). The fat cover of horse carcasses can be determined using the same methodology as for beef carcasses (*Cittadini et al.,* 2021). Five classes are defined, using the visual carcass evaluation on the dorsal side and on thoracic cavity, represented by the incremental scale ranges from 1 (denotes the least fat) to 5 (denotes the most fat) (*European Commission*, 2008).

Carcass linear measurements of horses are can be determined using the same indicators as for beef carcass quality assessment (*De Boer et al*, 1974). These are general indicators, because it is difficult to objectively define the relationship between the carcass morphometric measurements and muscle development in slaughter animals (*Lonergan et al*, 2019). However, linear measurements directly indicate the muscle development in the most valuable anatomical parts (chest, back, shoulders and legs) on the horse carcasses (*Gurgel et al.*, 2021). Horse carcass linear measurements are measured 24 hours *postmortem* in centimetres using a tape and/or calliper in the following order: carcass length, carcass compactness index, chest depth, leg length, leg width, leg circumference and leg compactness index (*Znamirowska*, 2005).

3. Meat quality

Horsemeat has a very dark red colour, yellow fat and high carbohydrate content (intramuscular glycogen) with a typical sweetish smell and taste, which can be considered a significant disadvantage from the consumer's perspective (*Stanisławczyk et al*, 2021a; *Driessen et al,* 2022). The most important factors that affect horsemeat quality are age, slaughter weight, breed, gender, production system and anatomical location of the measurements (*Franco et al.*, 2011; *Lorenzo et al*, 2013b, *Lorenzo et al.*, 2019). Horsemeat quality are evaluated by different traits such as: pH, temperature, colour, water-holding capacity and texture (*Franco et al.*, 2011; *Seong et al.*, 2016; *Lorenzo et al.*, 2014, 2019).

Physicochemical indicators (pH and temperature) are important horsemeat quality traits for monitoring the processes taking place during the *postmortem* conversion of muscle into meat. After slaughter, the horsemeat pH rapidly drops below 6, and the onset of *rigor mortis* starts to take place at 48 hours *postmortem* (*Lorenzo et al*., 2019). Under normal conditions (without stress), horsemeat reaches pH values between 5.4 and 5.9 within 24–48 hours *postmortem* (*Seong et al.*, 2016; *Walker*, 2017; *Stanisławczyk et al*, 2019). Considering that meat pH also depends on the temperature, the optimal horsemeat temperature ranges from 37.2°C to 38.5°C 45 minutes *postmortem* (*Green et al*, 2005) and from 0 to 7ºC 24 hours after slaughter (*Walker*, 2017). Compared to other meat types, horsemeat has high glycogen and ATP content, and consequently, it is highly resistant to spoilage and is a high durability raw material (*Lorenzo et al.*, 2019; *Stanisławczyk et al.*, 2021a). This can be attributed to the specific *postmortem* changes that occur within the glycogen-rich horse muscles, which involve prolonged anaerobic glycolysis and lactic acid production as the end product (*Stanisławczyk et al.*, 2021a). This results in a sustained acidification within the muscles and, thus, low ultimate horsemeat pH (*Stanisławczyk et al.*, 2021a). The measures of horsemeat pH can be taken at different times, from 45 minutes to 6 days after slaughter (*Sarriés and Beriain*, 2005; *Franco et al*, 2013; *Stanisławczyk et al*, 2020; *Cittadini et al*., 2021), but in most studies, this physicochemical indicator was determined 24 hours *postmortem* in *Musculus longissimus dorsi*, using a portable pH meter (*Franco et al*., 2011; *Domínguez et al*., 2015; *López-Pedrouso et al.*, 2023).

Horsemeat is characterised by a relatively good water-holding capacity (*Stanisławczyk et al*., 2021a), that ranges from 67.3% to 73.9% (*Strashynskyi and Fursik*, 2020). The good water-holding capacity of horsemeat contributes to low fluid loss during heat treatment, resulting in high yield and good quality final meat products, which indicates that this meat type is a good raw material for producing different meat products (*Stanisławczyk et al*., 2021a). The following methods are most often used to determine the water-holding capacity of horse meat: (i) (forced) drip loss (*Domínguez et al*., 2015; *Franco et al*., 2011; *Lorenzo et al*., 2013a); (ii) thawing loss (*De Palo et al*., 2013); (iii) cooking loss (*Franco et al*., 2011; Lorenzo et al., 2013a; Domínguez et al., 2015; *Seong et al.*, 2016; *Stanisławczyk et al.*, 2020; *López-Pedrouso et al*., 2023); and (iv) centrifugation (*De Palo et al*., 2013). In most investigations, horsemeat water-holding capacity was determined 24 hours *postmortem* in *Musculus longissimus dorsi* (*Franco et al*., 2011, 2013; *Domínguez et al*., 2015; *Stanisławczyk et al*., 2020). Unlike pork and poultry meat (*Kralik et al*, 2018; Čobanović et al., 2020),

there are no reference values in the available scientific literature for horsemeat that could be used for classification into meat quality classes. Future research should establish cut-off values for horsemeat water-holding capacity to assess whether the meat is of good or low quality.

One quality characteristic that distinguishes horsemeat, even from beef, is its relatively dark-red colour with a subtle brownish hue, which rapidly darkens and turns into a black-brown shade upon exposure to air (*Stanisławczyk et al*., 2021a). This horsemeat property is attributed to its elevated myoglobin content (7.4 mg/g) compared to beef (3.8 mg/g) and pork (ranges from 0.79 to 1.44 mg/g) (*Lorenzo et al.*, 2014, 2019*; Stanisławczyk et al.*, 2021a). The elevated myoglobin concentration in horsemeat facilitates its colour transformation, rendering it more visible to the eye compared to pork or veal, and, thus, fresh horsemeat colour stability is relatively low, shortening the shelf-life (*Lorenzo et al*., 2014, 2019*; Stanisławczyk et al.*, 2021a). It has been reported that horsemeat darkens as the animal ages, while the fat tissue turns yellowish or even orange in colour (*Stanisławczyk et al*, 2021b). However, the myoglobin content in horse muscle tissue increases during the first years two of life, and then decreases during the ten following years (*Lorenzo et al*., 2019). Horsemeat colour can be measured 24 hours *postmortem* in the *Musculus longissimus dorsi* after 30 minutes of blooming time at 4°C using portable colorimeter based on the colorimetric scale CIE L*, a*, b* (*Franco et al*., 2011, 2013; *Lorenzo et al*., 2013a; *Domínguez et al.*, 2015; *Stanisławczyk et al*., 2020). Compared to pork and poultry meat (*Kralik et al.*, 2018*; Čobanović et al.*, 2020), there are no reference values in the available scientific literature for horsemeat colour that could be used for classification into quality classes. Consequently, future research should establish cut-off values for horsemeat colour to assess whether the meat is of good or low quality.

Horsemeat texture is undesirable, as it exhibits extreme stringiness and hardness, particularly in older animals, even after undergoing heat treatment (*Stanisławczyk et al.*, 2021a). This can be attributed to a higher proportion of connective tissue, specifically collagen (3.5% of the total protein content in horsemeat), compared to pork (less than 0.5%) and beef (ranges from 0.49% to 1.0%) (*Stanisławczyk et al.*, 2021a, 2021b). Conversely, horsemeat from younger animals generally displays superior tenderness, surpassing in this aspect other meat types, especially, beef (*Lorenzo et al.*, 2014, 2019; *Stanisławczyk et al*., 2021a). As animals age, connective tissue mechanical stability increases as a consequence of collagen crosslinking (*Stanisławczyk et al*., 2021a, 2021b). Consequently, collagen within the intermuscular connective tissue becomes stiffer, harder and more resistant to heat denaturation, leading to progressive meat toughening and requiring greater force for cutting (*Stanisławczyk et al*., 2021a). Horsemeat texture can be determined 24 hours *postmortem* on cooked samples obtained from *Musculus longissimus dorsi* by using the Warner-Bratzler shear force (WBSF) test (*De Palo et al.*, 2013; *Franco et al.*, 2011, 2013; *Lorenzo et al*., 2013b; *Stanisławczyk et al*., 2020; *Stanisławczyk et al*., 2021b). Based on texture values obtained by using the WBSF test, horsemeat can be classified in the same manner as beef (*Stanisławczyk et al*., 2021a): very tender (WBSF < 3.2 kg), tender (3.2 < WBSF < 3.9 kg), intermediate (3.9 < WBSF < 4.6 kg) and tough (WBSF > 4.6 kg).

4. Conclusion

Based on the analysis of existing scientific literature, it can be concluded that the most important horse carcass quality indicators are the carcass conformation and carcass fat cover, while the most important horsemeat quality traits are pH, colour, water-holding capacity and texture. However, more research is needed to establish a classification system for horse carcasses as well as threshold values for colour and water-holding capacity traits that might be used to classify horsemeat into quality classes.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Albertí, P., Campo, M. M., Beriain, M. J., Ripoll, G. & Sañudo, C. (2017). Effect of including whole linseed and vitamin E in the diet of young bulls slaughtered at two fat covers on the sensory quality of beef packaged in two different packaging systems. *Journal of the Science of Food and Agriculture*, 97(3), 753–760, https://doi.org/10.1002/jsfa.7792

Cittadini, A., Sarriés, M. V., Domínguez, R., Indurain, G. & Lorenzo, J. M. (2021). Effect of breed and finishing diet on growth parameters and carcass quality characteristics of Navarre autochthonous foals. *Animals*, 11(2), 488, https://doi.org/10.3390/ani11020488

Čobanović, N., Stanković, S. D., Dimitrijević, M., Suvajdžić, B., Grković, N., Vasilev, D. & Karabasil, N. (2020). Identifying physiological stress biomarkers for prediction of pork quality variation. *Animals*, 10(4), 614, https://doi.org/10.3390/ani10040614

De Boer, H. D. B. L., Dumont, B. L., Pomeroy, R. W. & Weniger, J. H. (1974). Manual on EAAP reference methods for the assessment of carcass characteristics in cattle. *Livestock Production Science*, 1(2), 151–164, https://doi.org/10.1016/0301-6226(74)90055-4

De Palo, P., Maggiolino, A., Centoducati, P. & Tateo, A. (2013). Slaughtering age effect on carcass traits and meat quality of Italian heavy draught horse foals. *Asian-Australasian Journal of Animal Sciences*, 26(11), 1637, https://doi.org/10.5713/ajas.2013.13174

Domínguez, R., Crecente, S., Borrajo, P., Agregán, R. & Lorenzo, J. M. (2015). Effect of slaughter age on foal carcass traits and meat quality. *Animal*, 9(10), 1713–1720, https://doi.org/10.1017/s1751731115000671

Driessen, B., Marlin, D. & Buyse, J. (2022). Horses. In L. Faucitano (Ed.), Preslaughter Handling and Slaughter of Meat Animals (pp. 194-221). Wageningen Academic Publishers, https://doi.org/10.3920/978-90-8686-924-4

Ekiz, B., Kecici, P. D., Ograk, Y. Z., Yalcintan, H. & Yilmaz, A. (2021). Evaluation of the functionality of EUROP carcass classification system in thin-tailed and fat-tailed lambs. *Meat Science*, 181, 108603, https://doi.org/10.1016/j.meatsci.2021.108603

European Commission (2008). EC Regulation No. 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcases and the reporting of prices thereof. ECOJ No. L3373–30.

Fàbregas, X. & Such, X. (2001). Clasificación de canales equines: I. Distribución de clases. *ITEA*, 2, 706–708.

Franco, D., Rodríguez, E., Purriños, L., Crecente, S., Bermúdez, R. & Lorenzo, J. M. (2011). Meat quality of "Galician Mountain" foals breed. Effect of sex, slaughter age and livestock production system. *Meat Science*, 88(2), 292–298, https://doi.org/10.1016/j.meatsci.2011.01.004

Franco, D., Crecente, S., Vázquez, J. A., Gómez, M. & Lorenzo, J. M. (2013). Effect of cross breeding and amount of finishing diet on growth parameters, carcass and meat composition of foals slaughtered at 15 months of age. *Meat Science*, 93(3), 547–556, https://doi.org/10.1016/j.meatsci.2012.11.018

Franco, D. & Lorenzo, J. M. (2014). Effect of muscle and intensity of finishing diet on meat quality of foals slaughtered at 15 months. *Meat Science*, 96(1), 327–334, https://doi.org/10.1016/j.meatsci.2013.07.018

Gill, C. O. (1983). Meat spoilage and evaluation of the potential storage life of fresh meat. *Journal of Food Protection*, 46(5), 444 – 452, doi.org/10.4315/0362-028x-46.5.444

Green, A. R., Gates, R. S. & Lawrence, L. M. (2005). Measurement of horse core body temperature. *Journal of Thermal Biology*, 30(5), 370–377, https://doi.org/10.1016/j.jtherbio.2005.03.003

Gurgel, A. L. C., Difante, G. D. S., Emerenciano Neto, J. V., Fernandes de Araújo, C. G., Costa, M. G., Ítavo, L. C. V.... & Fernandes, P. B. (2021). Prediction of carcass traits of Santa Inês lambs finished in tropical pastures through biometric measurements. *Animals*, 11(8), 2329, https://doi.org/10.3390/ani11082329

Kralik, G., Kralik, Z., Grčević, M. & Hanžek, D. (2018). Quality of chicken meat. In B. Yucel & T. Taskin (Eds.), Animal husbandry and nutrition (pp. 63–94), IntechOpen, https://doi.org/10.5772/intechopen.69938

Lee, C. E., Seong, P. N., Oh, W. Y., Ko, M. S., Kim, K. I. & Jeong, J. H. (2007). Nutritional characteristics of horsemeat in comparison with those of beef and pork. *Nutrition Research and Practice*, 1(1), 70 –73, https://doi.org/10.4162/nrp.2007.1.1.70

Lonergan, S. M., Topel, D. G. & Marple, D. N. (2019). Methods to measure body composition of domestic animals. In S. M. Lonergan, D. G. Topel, and D. N. Marple (Eds.), The Science of Animal Growth and Meat Technology (pp. 125–146), Elsevier, https://doi.org/10.1016/b978-0-12-815277-5.00008-1

López-Pedrouso, M., Lorenzo, J. M., Cittadini, A., Sarries, M. V., Gagaoua, M. & Franco, D. (2023). A proteomic approach to identify biomarkers of foal meat quality: A focus on tenderness, color and intramuscular fat traits. *Food Chemistry*, 405, 134805, https://doi.org/10.1016/j.foodchem.2022.134805

Lorenzo, J. M., Sarriés, M. V. & Franco, D. (2013a). Sex effect on meat quality and carcass traits of foals slaughtered at 15 months of age. *Animal*, 7(7), 1199–1207, https://doi.org/10.1017/s1751731113000189

Lorenzo, J. M., Pateiro, M. & Franco, D. (2013b). Influence of muscle type on physicochemical and sensory properties of foal meat. *Meat Science*, 94(1), 77–83, https://doi.org/10.1016/j.meatsci.2013.01.001

Lorenzo, J. M., Sarriés, M. V., Tateo, A., Polidori, P., Franco, D. & Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review. *Meat Science*, 96(4), 1478–1488, https://doi.org/10.1016/j.meatsci.2013.12.006

Lorenzo, J. M., Maggiolino, A., Sarriés, M. V., Polidori, P., Franco, D., Lanza, M. & De Palo, P. (2019). Horsemeat: Increasing Quality and Nutritional Value. In Lorenzo, J. M., Munekata, P. E., Barba, F. J. & Toldrá, F. (Eds.), More than Beef, Pork and Chicken–The Production, Processing,

and Quality Traits of Other Sources of Meat for Human Diet (pp. 31–69), Springer, https://doi.org/10.1007/978-3-030-05484-7_3

National Research Council, (1988). Live animal and carcass composition measurement. In Designing Foods: Animal Product Options in the Marketplace. National Academies Press (US).

ONIBEV, (1979). Catalogue de Classement des Équidés (en carcasse). Office National Interproffessionnel du Betail et des Viandes, Paris Cedex.

Pečiulaitienė, N., Jukna, V., Meškinytė-Kaušilienė, E., Kerzienė, S. & Moleikaitienė, S. (2015). Effects of weight and age on carcass yield and conformation of cattle. *Biotechnology in Animal Husbandry*, 31(1), 73–84, https://doi.org/10.2298/bah1501073p

Polidori, P., Pucciarelli, S., Ariani, A., Polzonetti, V. & Vincenzetti, S. (2015). A comparison of the carcass and meat quality of Martina Franca donkey foals aged 8 or 12 months. *Meat Science*, 106, 6–10, https://doi.org/10.1016/j.meatsci.2015.03.018

Sarriés, M. V. & Beriain, M. J. (2005). Carcass characteristics and meat quality of male and female foals. *Meat Science*, 70(1), 141–152, doi.org/10.1016/j.meatsci.2004.12.006

Seong, P. N., Park, K. M., Kang, G. H., Cho, S. H., Park, B. Y., Chae, H. S. & Van Ba, H. (2016). The differences in chemical composition, physical quality traits and nutritional values of horse meat as affected by various retail cut types. *Asian-Australasian Journal of Animal Sciences*, 29(1), 89–99, doi.org/10.5713/ajas.15.0049

Stanisławczyk, R., Rudy, M., Gil, M. & Duma-Kocan, P. (2019). Influence of cold and frozen storage on the chemical content, hydration properties and texture parameters of horse meat. *Medycyna Weterynaryjna*, 75(4), 242–246, https://doi.org/10.21521/mw.6207

Stanisławczyk, R., Rudy, M. & Gil, M. (2020). Quality characteristics of horse meat as influence by the age of horse. *International Journal of Food Properties*, 23(1*)*, 864–877, https://doi.org/10.1080/10942912.2020.1764579

Stanisławczyk, R., Rudy, M. & Rudy, S. (2021a). The quality of horsemeat and selected methods of improving the properties of this raw material. *Processes*, 9(9), 1672, https://doi.org/10.3390/pr9091672

Stanisławczyk, R., Rudy, M., Gil, M., Duma-Kocan, P. & Żurek, J. (2021b). Influence of horse age, marinating substances, and frozen storage on horse meat quality. *Animals*, 11(9), 2666, https://doi.org/10.3390/ani11092666

Strashynskyi, I. & Fursik, O. (2020, December). Using of horsemeat as an additional source of raw materials for expanding the range of meat products. Poster session presentation at the meeting of the Specialized and multidisciplinary scientific researches, Amsterdam, The Netherlands, pp. 38–40, https://doi.org/10.36074/11.12.2020.v2.10

Walker, B. D. (2017). Impact of carcass chill time on the microbiology of horse meat, University of Alberta, Canada.

Znamirowska, A. (2005). Prediction of horse carcass composition using linear measurements. *Meat Science*, 69(3*)*, 567–570, https://doi.org/10.1016/j.meatsci.2004.10.008

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.1/.3(497.11)

ID: 126578185

https://doi.org/10.18485/meattech.2023.64.2.30

Review paper

Production and trade of milk and dairy products in Serbia

Jasna Đorđević[a], Tijana Ledina[a], Marija Kovandžić[a] and Snežana Bulajić[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Keywords:
Milk
Dairy products
Milk production

ABSTRACT

Milk plays a significant role in human nutrition, and more than 6 billion people worldwide consume milk and dairy products. In 2019, total milk production in Serbia reached 1,597 million litres, cow's milk accounting for almost 95%. Out of the total amount of cow's milk produced in 2021, 58.2% was purchased. The share of milk delivered to dairies increased by over 50% in the previous decade. The results in the dairy sector are directly influenced by dairy cattle farming in Serbia, which has been experiencing a decrease, but there has been a consistent increase in milk yield over the past few years. Production of dairy products in Serbia recorded a decrease during the last ten years, except for dry products (cream powder, whole and partially-skimmed milk powder), which recorded an increase. Trade in dairy products is very volatile, as dairy trade flows can be affected by the overall economic situation in a country, fluctuations in supply and demand, changing exchange rates and political measures. The largest dairy product trade in Serbia is conducted in milk and cream, followed by cheese, and then milk and cream powder.

1. Introduction

More than 6 billion people worldwide consume milk and dairy products (*Visioli and Strata*, 2014; *Grandsir et al*., 2023). Milk plays a significant role in human nutrition, contributing approximately 8 to 9% of the dietary energy supply, 19% of the dietary protein supply and 12 to 14% of dietary fat supply in Europe (*FAO*, 2013).

In 2021, global milk production amounted to approximately 928 million tonnes, reflecting a 1.3% growth compared to 2020. This increase in production was observed across various geographical regions, except for Europe and Oceania, which experienced a decline in milk production. Milk production in Europe in 2021 underwent a slight decrease of 0.4% compared to 2020. This decline can be attributed primarily to decreases in production within Ukraine (*OECD-FAO*, 2021; *FAOSTAT*, 2022). Out of the total global milk production, cow's milk contributes to 81% of the milk, followed by buffalo's (13%), goat's, sheep's and camel's milk combined (4%; *FAO*, 2019). In Europe, raw milk production in 2021 amounted to 161 million tonnes, of which 96.4% was cow's milk, 1.9% ewe's milk, 1.5% goat's milk and 0.2% buffalo's milk (*Eurostat*, 2022). Developing countries have witnessed a remarkable increase of their share in global production of milk. Additionally, there has been an increase in per capita milk and dairy products consumption (*Hemme*, 2003; *Knips*, 2005). Most of the milk quantity (80 to 90%) in developing countries is processed by small-scale processors who produce a variety of milk products. The type of processing can vary depending on country and region, and relates to local tastes, dietary habits, culinary traditions and market demand (*FAO*, 2023).

***Corresponding author:** Jasna Đorđević, *jasna.djordjevic@vet.bg.ac.rs*

Paper received Jun 5th 2023. Paper accepted July 13nd 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Milk and dairy product production in Serbia

Household consumption of milk and dairy products in Serbia is shown in Table 1. The annual per capita milk consumption in Serbia decreased by 33% from 2012 to 2019 (55.1 litres in 2012; 36.4 litres in 2019). The consumption of white cheese is higher compared to other dairy products in Serbia, with an average of around 10 kg per person annually from 2012 to 2019, followed by consumption of approximately 1 kg of kajmak per person per year and consumption of butter ranging from 0.1 to 0.3 kg per person annually.

The total milk production in 2019 in Serbia on agricultural holdings amounted to 1,597 million litres, with cow's milk production reaching 1,509 million litres, accounting for almost 95% of milk produced (Table 2; *Statistical Office of the Republic of Serbia*, 2022). The overall milk quantity includes all the milk obtained through milking, as well as colostrum which is used as animal feed.

Production of cow's milk contributes 6.3% of the total value of agricultural production, making it one of the most money-making products of Serbian agriculture. Out of the total amount of cow's milk produced (estimated at around 1,473 million litres), approximately 858 million litres or 58.2% were purchased in 2021. The share of milk delivered to dairies increased by over 50 percent in the previous decade (in 2012 — 49.4%; in 2011 — 47.5%; *Statistical Office of the Republic of Serbia*, 2022). However, a significant amount of milk (approximately 35%) still remains to be sold on farm, which is substantially more in comparison to the European Union, where almost 94% of

Table 1. Household consumption of milk and dairy products in Serbia*

Type of product	Measurement unit	2017	2018	2019	2021
Milk (raw, pasteurized and sterilized)	L	90.8	102.6	97.5	91.3
Fermented dairy products	L	78.8	83.6	83.5	88.8
White cheeses	kg	22.7	25.9	25.0	21.2
Other dairy products	kg	22.1	20.4	22.3	28.1

*Due to the COVID-19 pandemic, the Statistical Office of the Republic of Serbia suspended the "Household Consumption Survey" field research in mid-March 2020

Data are from the Statistical Office of the Republic of Serbia (2022)

Table 2. Production and use of cow's, sheep's and goat's milk from 2013 to 2021 (million litres)

Year	Cow's milk	The amount of cow's milk used for human consumption and processing	Ewe's milk	The amount of sheep's milk used for human consumption and processing	Goat's milk	The amount of goat's milk used for human consumption and processing
2013	1,451	1,416	18	18	34	32
2014	1,492	1,457	20	19	38	37
2015	1,501	1,470	19	19	44	43
2016	1,504	1,467	17	16	37	36
2017	1,506	1,467	14	13	33	32
2018	1,493	1,457	18	17	34	33
2019	1,509	1,467	11	11	31	30
2020	1,495	1,457	9	9	34	33
2021	1,473	1,441	10	10	34	34
2022	1,425	1,394	9	8	34	33

Statistical Office of the Republic of Serbia, 2022.

Table 3. Products from cow's milk obtained in dairies for the market (in thousand tons)

Year	Milk[1]	SC	FP	CM	SMP	Butter	Cheese	CP/WMP/PSMP
2013	20.92	4.02	17.85	0.09	0.10	0.08	0.52	2.35
2014	19.77	2.50	16.73	0.04	0.2	0.17	0.70	2.81
2015	19.89	2.47	17.55	0	0.10	0.21	0.40	3.38
2016	19.64	2.48	18.22	0	0.40	0.16	0.33	3.68
2017	18.73	2.34	17.12	0	0.10	0.16	0.33	4.14
2018	19.08	2.29	16.46	0	cd	0.11	0.35	4.39
2019	17.84	2.41	16.60	0	cd	cd	0.44	4.67
2020	19.56	2.70	17.46	0	cd	cd	0.45	4.43
2021	17.41	2.74	18.39	0	0	cd	0.44	4.42
2022	17.59	2.70	17.62	0	cd	cd	0.41	4.25

[1]Pasteurized and sterilized milk; SC- Sour cream; FP – Fermented products; CM – Concentrated milk (condensed and evaporated); CP – Cream powder; WMP – Whole milk powder; PSMP – Partially skimmed milk powder; SMP – Skimmed milk powder; cd – confidential data
Statistical Office of the Republic of Serbia

milk is delivered to dairies (*Eurostat*, 2022; *Statistical Office of the Republic of Serbia*, 2022).

The results in the dairy sector are directly influenced by dairy cattle farming in Serbia, which has been experiencing a decrease and noticeable decline in the number of milking cows. In brief, the number of milking cows in 2014 amounted to 437,000 (a short-term stabilization compared to the previous period), but in 2017, the decline in the number of milking cows had continued, reaching 429,000. By 2022, the number of milking cows had decreased further to 374,000. On the other hand, dairy cattle farming showed a slight, but consistent increase in average milk yield per cow over the past few years, which can be the result of improved breeding and selection of dairy cows, education and support of producers with supportive agricultural policies including subsidies, loans, and other, as well as improvement of nutrition and husbandry conditions (*Statistical Office of the Republic of Serbia*, 2022).

The milk delivered to dairies is processed into different dairy products. In September 2022, dairy production in Serbia recorded a decrease of approximately 6.4% compared to September 2021. Production of cream powder and whole and partially-skimmed milk powders increased, while production of sour cream and fermented products was relatively constant over the last ten years (Table 3). The inventory levels have increased by about 2.5%. However, the overall sales for the period from January to September 2022 were lower by 0.5% compared to the same period in 2021.

3. Trade of milk and dairy products

The dairy sector is highly localised, as milk is a perishable product, and dairy products are mostly consumed in the country or region of production. Only a small fraction of global production is traded internationally (*Knips*, 2005). One of the characteristics that makes the dairy sector unique is the differentiation between into non-tradable (fluid "drinking" milk) and tradable ("manufacturing" milk). Trade differs depending on the product and its suitability for trade. Hence, trade is of different importance depending on the product (and its suitability for trade), with milk powders globally having the highest share, as 30% of skimmed milk powder, 10 to 15% of butter and retail packed condensed milks, 3% of yogurt and other fresh dairy products, and less than 0.5% of packed cream and packed liquid milks is traded internationally (*IDF*, 2002). Whole and skimmed milk powders account for about half of the total dairy trade, and are almost exclusively imported by developing and transition countries (*Knips*, 2005). The limited participation of developing countries, in which Serbia belongs, can be partly explained by the fact that most countries in the region strive for self-sufficiency in food, despite the fact that milk production is very important in the region. Trade in dairy products is very volatile, as dairy trade flows can be affected by the overall economic situation in a country, fluctuations in supply and demand, changing exchange rates and political

Table 4. The trade of milk and dairy products in Serbia (quantity in tonnes and value in thousands of €)

	Product type		
	Milk and cream	**Milk and cream powder**	**Cheese**
Export			
2018			
Quantity (t)	28.847	1.475	14.903
Value (000 €)	1	1.640	45.490
2019			
Quantity (t)	76.347	1.118	15.789
Value (000 €)	24.349	1.578	47.587
2020			
Quantity (t)	45.789	532	15.104
Value (000 €)	15.549	768	45.043
2021			
Quantity (t)	36.980	92	15.685
Value (000 €)	15.639	279	47.126
Import			
2018			w
Quantity (t)	33.064	5.727	6.034
Value (000 €)	18.191	10.984	23.061
2019			
Quantity (t)	37.061	3.226	7.769
Value (000 €)	20.623	7.061	29.957
2020			
Quantity (t)	41.658	4.618	9.393
Value (000 €)	23.428	19.443	35.632
2021			
Quantity (t)	35.282	3.857	11.523
Value (000 €)	22.269	9.363	45.520

*Statistical Office of the Republic of Serbia

measures. According to the data from the Statistical Office of the Republic of Serbia, trade varies from year to year. The largest trade is conducted in milk and cream, followed by cheese, and then milk and cream powder, and it varies from year to year (Table 4; *Statistical Office of the Republic of Serbia*, 2022).

4. Conclusion

The manuscript provides insights into the milk and dairy industry in Serbia, contributing to better understanding of production trends and trade dynamics.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Visioli, F. & Strata, A. (2014). Milk, dairy products, and their functional effects in humans: A narrative review of recent evidence. *Advances in Nutrition*, 5(2), 131–143, doi: 10.3945/an.113.005025

FAO, (2013). Chapter 3: Milk and dairy products composition. In E. Muehlhoff, A. Bennett & D. McMahon (Eds.), Milk and Dairy Products in Human Nutrition (pp. 41–102).

FAO, (2022). Dairy Market Review: Overview of global dairy market and policy developments in 2021, Rome.

OECD-FAO, (2021). Dairy and dairy products. OECD-FAO agricultural outlook 2022–2031. Retrieved from https://www.fao.org/dairy-production-products/resources/publications/en/

FAO, (2017). Gateway to dairy production and products. Milk and Milk Products.

FAO, (2019). Dairy and dairy products. In OECD-FAO Agricultural Outlook 2019–2028 © OECD/FAO 2019.

Eurostat. Milk and milk product statistics. Retrieved from https://ec.europa.eu/eurostat/statistics.explained/index.php?title=Milk_and_milkproduct_statistics

FAO, (2023). Gateway to dairy production and products. Retrieved from https://www.fao.org/dairy-production-products/production/production-systems/en/

Statistical Office of the Republic of Serbia, (2022). Statistical yearbook.

Knips, V. (2005). Developing Countries and the Global Dairy Sector Part I Global Overview. PPLPI Working Paper No. 30. FAO — Animal Production and Health, Rome.

Hemme, T. (2003). Revolution in dairy farming. Paper presented at the IDF World Dairy Summit 2003 in Brugge, International Farm Comparison Network (IFCN), Global Farm GbR, Braunschweig/Germany.

FAOSTAT, Online database http://faostat.external.fao.org/

IDF, (2002). World Dairy Situation 2002. Bulletin of the International Dairy Federation, 378/2002, Brussels.

Grandsir, C., Falagán, N. & Alamar, M.C. (2023). Application of novel technologies to reach net-zero greenhouse gas emissions in the fresh pasteurised milk supply chain: A review. *International Journal of Dairy Technology*, 76, 38–50, https://doi.org/10.1111/1471-0307.12926

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.55:639.112

ID: 126534921

https://doi.org/10.18485/meattech.2023.64.2.31

Review paper

The nutritional profile and technological properties of rabbit meat

Branko Suvajdžić[a], Nikola Čobanović[a], Nevena Grković[a], Ivan Vićić[a] and Dragan Vasilev[a]

[a] *University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bulevar oslobođenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Rabbit meat
Rabbit meat production
Chemical composition
Technological properties

ABSTRACT

The production and consumption of rabbit meat are declining worldwide, even though rabbit meat offers a nutritional profile that satisfies modern consumer aspirations. Consumers are not sufficiently familiar with the dietetic properties of rabbit meat and have prejudices about its consumption. From a nutritional and technological aspect, rabbit meat is suitable for the production of different meat products as well as products with added value. Therefore, this paper highlights the importance of rabbit meat, its nutritional and technological characteristics, and promotes the development of rabbit meat products that, due to their nutritional value, should conquer the market and break consumers' prejudices.

1. Introduction

Rabbit meat has excellent nutritional and dietetic characteristics. Its high levels of proteins and essential amino acids, moderately high energy value, low fat and cholesterol content, low sodium but high phosphorus levels, and significant content of B vitamins (especially vitamin B12), make it healthy (*Dalle Zotte & Szendrő*, 2011; *Szendrő et al.,* 2020). Therefore, rabbit meat is used in the production of baby foodstuffs and is highly recommended for adolescents, pregnant women, the elderly, and convalescents (*Cury et al.*, 2011; *Składanowska-Baryza & Stanisz*, 2019).

However, the rabbit meat industry is currently going through a difficult period, primarily because of a progressive decrease in consumption and structural weaknesses, as well as criticism concerning welfare conditions and other moral concerns (*Cullere & Dalle Zotte*, 2018). People reluctantly purchase rabbit meat due to its price and a typical gamey flavour, which is unacceptable for many consumers (*Hoffman et al.*, 2004). Many consumers, especially young people, refuse to buy a whole rabbit carcass, which is repulsive to them. Additionally, in many countries, consumption of rabbit meat is not common since rabbits are considered as pets (*González-Redondo & Contreras-Chacón*, 2012). In order to promote the consumption of rabbit meat and ensure the sustainability of the rabbit meat industry, new approaches are urgently needed. The development of innovative, healthier rabbit meat products can be a successful strategy to attract new consumers, particularly those who are health conscious (*Cullere & Dalle Zotte*, 2018). Consequently, this paper highlights the importance of rabbit meat, its nutritional and technological characteristics, and promotes the development of rabbit meat products

***Corresponding author:** Branko Suvajdžić, *brankos@vet.bg.ac.rs*

Paper received July 3rd 2023. Paper accepted July 19th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

that, due to their nutritional value, should conquer the market and break consumers' prejudices.

2. Rabbit meat production and trade

Today, rabbits can theoretically be characterized as the ultimate meat-producing animals, considering their short life cycle and gestation period as well as their high feed conversion capacity (*Lebas et al.*, 1997; *Honrado et al.*, 2022). Additionally, the nutritional value of rabbit meat can be greatly improved by dietary modifications and/or the inclusion of substances that promote health, since rabbits are monogastric animals (*Dalle Zotte & Cullere*, 2019). Although rabbits are bred all over the world, there are significant differences between continents or countries in terms of rabbit meat production (*FAOSTAT*, 2020).

In the period 2010–2020, the largest rabbit meat production was on the Asian continent, followed by Europe, Africa, and the Americas (Table 1). Asia accounted for 70.49% of global rabbit meat production in 2020. During the mentioned period, the world's top producer was China, followed by the North Korea, the European Union (EU), and Egypt. The largest producers in the EU were Spain, Italy, and France. According to data retrieved from *FAOSTAT*, in the period 2010–2015, global rabbit meat production increased by 9.32%, while after 2015 there was a decrease in production (−31.17%). However, trends varied between countries. Estimated values retrieved from *FAOSTAT* show that rabbit meat production in China has progressively decreased from 783457 tons in 2015 to 488000 tons in 2020 (−37.71%). A similar trend was estimated in North Korea and the EU, where rabbit meat production decreased by 7.20% and 45.60%, respectively. In recent years, countries in the Americas have maintained rabbit meat production at the same levels as previously. Going against the global regression in rabbit meat production during the mentioned period, an increase in rabbit meat production of 6.46% occurred in Africa's top producer, Egypt. Egypt accounted for 71.19% of Africa's rabbit meat output in 2020, followed by Algeria and Sierra Leone.

Table 1. Rabbit meat production in the world (FAOSTAT, 2020)

Continent/Country	Production Quantity (Tonnes)		
	2010	2015	2020
Asia	828,586.09	940,767.28	634,024.78
China	690,000.00	783,457.38	488,000.00
North Korea	133,900.00	153,878.11	142,793.92
Europe	260,672.91	256,647.84	153,150.08
European Union	230,582.91	223,237.64	121,433.79
Russian Federation	14,429.00	17,374.00	18,364.00
Africa	78,638.00	92,794.85	97,122.49
Egypt	52,282.00	64,946.00	69,144.76
Algeria	7,500.00	8,223.70	8,428.45
Sierra Leone	7,600.00	7,920.06	8,103.75
Americas	17,549.50	17,016.53	15,429.12
Mexico	4,350.00	4,399.96	4,481.78
Peru	3,360.00	3,359.13	3,402.13
Colombia	3,185.00	3,182.25	3,212.37
World	1,185,446.51	1,307,226.51	899,726.47

In 2020, Europe held 91.89% and 82.40% of the global rabbit meat imports and exports, respectively. The main importing countries were Germany (4,398.58 tonnes), Belgium (3,902.97 tonnes), and Portugal (1,884.2 tonnes), while Spain (6,771.64 tonnes), Hungary (4,783.22 tonnes), France (4,002.39 tonnes), and Belgium (3,319.25 tonnes) were the largest exporting countries in the Europe. As a world's top producer of the rabbit meat in 2020, China was just the third biggest exporting country in the world (4266.30 tonnes), while there were no official import data for this country (*FAOSTAT*, 2020).

3. Nutritional profile of rabbit meat

Rabbit meat is characterized by high levels of proteins, essential amino acids, and polyunsaturated fat acids, low fat and cholesterol content, low sodium but high phosphorus levels, and a significant content of vitamin B12 (*Dalle Zotte & Szendrő*, 2011). Its moderately high energy value (603–899 kJ/100 g, depending on the carcass part) depends mostly on high protein content, which provides 80% of the energy value (*Dalle Zotte & Szendrő*, 2011). Protein content in rabbit meat ranges from 18.6 g/100 g in forelegs to 22.4 g/100 g in the loin. Looking at the whole carcass, rabbit meat has approximately 20.3 g/100 g of proteins (*Dalle Zotte & Szendrő*, 2011). As a source of valuable proteins, rabbit meat provides all essential amino acids, especially lysine (2.12 g/100 g), threonine (2.01 g/100 g), leucine, (1.73 g/100 g), valine (1.19 g/100 g), isoleucine (1.15 g/100 g), and phenylalanine (1.04 g/100 g) (*Hernàndez & Dalle Zotte*, 2010). Furthermore, rabbit meat is a lean meat with a fat content ranging from 1.8 g/100 g to 8.8 g/100 g, depending on the part of carcass (*Dalle Zotte & Cullere*, 2019). Rabbit intramuscular fat contains mostly unsaturated fatty acids (60.5%), while saturated fatty acids are less represented (38.9%) (*Dalle Zotte & Szendrő*, 2011). Among the unsaturated fatty acids, polyunsaturated fatty acids are predominant, primarily linoleic (18:2 n-6) and α-linolenic fatty acid (18:3 n-3), both essential fatty acids. Linoleic acid is accounts for >20% of all fatty acids in rabbit meat (*Hernàndez & Dalle Zotte*, 2010). In addition, rabbit meat contains significantly more linolenic acid (3%) than do beef (0.70%), pork (0.95%), and lamb (1.37%) (*Enser et al.*, 1996). Long-chain n-6 and n-3 polyunsaturated fatty acids are synthesized from the aforementioned essential fatty acids. Rabbit meat also contains long-chain n-3 polyunsaturated fatty acids, such as docosahexaenoic acid (0.31 mg/100 g) and eicosapentaenoic acid (0.15 mg/100 g) (*Dalle Zotte & Szendrő*, 2011). Mainly, n-3 polyunsaturated fatty acids have an antiatherogenic effect and, consequently, reduce the risk of cardiovascular diseases. Additionally, balancing the ratio of n-6/n-3 polyunsaturated fatty acids is essential for the normal course of different physiological processes (*Park et al*., 2022). The optimal n-6/n-3 ratio should be about 1–2:1, since n-6 and n-3 polyunsaturated fatty acids are metabolized by competitive, common enzymatic reactions (*Tortosa-Caparrós et al.*, 2017; *Park et al*., 2022). Compared to meats of other animal species, rabbit meat offers a fairly low n-6/n-3 ratio, which amounts to 7:1 for the loin (*Dalle Zotte & Szendro*, 2011). Through the rabbit's diet, it is possible to increase the level of n-3 polyunsaturated fatty acids in the rabbit meat and, consequently, decrease the n-6/n-3 ratio, which would be closer to the optimal. Moreover, rabbit meat has a lower cholesterol content compared to other meat types, which amounts about 47 mg/100 g and depends on the rabbit's diet (*Hernàndez & Dalle Zotte*, 2010). Taking into account the potential consequences for human health of cholesterol intake, this fact is significant, and all feeding strategies for rabbits must aim to achieve the lowest cholesterol content in the meat (*Dalle Zotte & Szendro*, 2011). As a white meat type, rabbit meat contains lower levels of iron (1.1–1.3 mg/100 g) and zinc (0.55 mg/100 g) than do the red meats, beef, lamb, and pork (*Parigi Bini et al*., 1992; *Lombardi-Boccia et al*., 2005). Furthermore, rabbit meat has very low sodium content (37–47 mg/100 g), which makes it suitable in diets for hypertension (*Dalle Zotte & Szendrő*, 2011; *Dalle Zotte & Cullere*, 2019). On the other hand, rabbit meat is rich in other minerals, such as potassium (428–431 mg/100 g), phosphorus (222–234 mg/100 g), and selenium (9.3–15 μg/100 g), which are included in the regulation of different physiological functions (*Dalle Zotte & Szendrő*, 2011; *Dalle Zotte & Cullere*, 2019). In terms of vitamins, rabbit meat is a good source of B vitamins. Accordingly, 100 g of rabbit meat provides 8% of daily vitamin B2, 12% of daily vitamin B5, 21% of daily vitamin B6, and 77% of daily vitamin B3 needs, as well as the fulfilment of daily vitamin B12 needs (*Combes,* 2004). Compared to meats of other animal species, rabbit meat is richer in vitamin B12 (8.7 mg/100 g), which is essential for the formation of red blood cells, DNA synthesis, and the proper development of nervous system functions

(*Dalle Zotte & Szendro*, 2011). It is known that vitamin B12 prevents pernicious anaemia and disorders of the nervous system (*Stabler & Allen*, 2004).

4. Technological properties of rabbit meat

As known, the pH value is crucial parameter during the selection of meat for processing and also is an indicator of meat quality and shelf life (*Carrillo-Lopez et al.*, 2021). The pH of rabbit meat is mainly measured in *Musculus biceps femoris* and *Musculus longissimus dorsi*, 45 minutes and 24 hours after slaughter (Kumar et al., 2023). *Musculus biceps femoris* has expressed oxidative metabolism, lower glycolytic potential and, consequently, higher pH compared to *Musculus longissimus dorsi* (Kumar et al., 2023). During the conversion of muscle to meat under normal conditions (without stress), the muscle pH gradually decreases due to the accumulation of lactic acid, and 45 minutes after the slaughter of rabbits, it ranges between 6.1 and 6.9 (*Carrillo-Lopez et al.*, 2021). In comparison to meat of other animal species, rabbit meat has an inferior shelf life since its pH after 24 hours is between 5.6 and 5.85 (*Kozioł et al.*, 2015). The meat pH directly affects water holding capacity, which is essential parameter for meat processing, since poor water holding can result in technological and economic losses (*de Oliveira Paula et al.*, 2020). A low ultimate pH leads to decreased water holding capacity and increased drip losses as well as cooking losses (*Sampels & Skoglund*, 2021). Consequently, rabbit meat with such characteristics is not suitable for use in heat treated products, but may be considered for production of dried meat products and fermented sausages. Rabbit meat with a high (rather than low) water holding capacity has intact, more soluble proteins, and consequently greater protein binding and fat emulsifying capacities, so it is suitable for emulsified sausages production (*Ramos & Gomide*, 2017).

Rabbit meat is especially tender because of its lower elastin content and the high solubility of its collagen, compared to other meat types. Collagen content in rabbit meat ranges from 5.71 to 7.97 mg/g, depending on the carcass part (*Bueno et al*., 2023). Collagen is the main protein of the intramuscular connective tissue, and its quantity and solubility contribute to the development of meat toughness and/or tenderness. High collagen content leads to increased meat toughness (*Pascual & Pla*, 2008). Collagen solubility in muscles is determined by the presence of trivalent thermo-stable collagen crosslinks, which increase during animal growth. Additionally, the low amount of collagen in the meat batter favours the fat globule stabilization and water retention abilities during the production of emulsified sausages (*Bueno et al*., 2023).

Rabbits have a low quantity of adipose tissue, which is necessary for the production of rabbit meat sausages. In order to replace fatty tissue, numerous hydrocolloid systems, such as alginate, carrageenan, xanthan gum, cellulose derivatives, starches, and pectins, have been examined for their ability to form gels and replace fat. However, fat replacement with functional ingredients such as vegetable fibre, is more beneficial due to their technological and dietetic characteristics (*Petracci & Cavani*, 2013). Vegetable fibre can be utilized for replacement alone or in combination with plant oils, which are rich in n-3 polyunsaturated fatty acids, the significance of which has already been highlighted (*Petracci & Cavani*, 2013).

Meat colour is an essential indicator of the processing suitability of meat. As known, meat colour derives from the main pigment of muscle tissue, myoglobin, and also from its chemical state (oxymyoglobin, reduced myoglobin, or metmyoglobin). Compared to other animal species, rabbit meat contains a low amount of myoglobin (0.02% of wet muscle weight) (*Carrillo-Lopez et al.*, 2021) and, consequently, it has relatively light (L*), with a low redness (a*) and yellowness (b*) contribution. According to *Ignacio et al*. (2019), the lightness of meat products increases significantly with the addition of rabbit meat, while the redness significantly decreases. Moreover, the low myoglobin content in rabbit meat limits the development of nitrosyl-myoglobin, which is created in the reaction between myoglobin and nitrites. As already mentioned, rabbit meat is rich in polyunsaturated fatty acids, which are unstable and susceptible to oxidation, the products of which lead to the metmyoglobin formation and, consequently, a decrease in redness (*Ignacio et al*., 2019). However, lipid oxidation is less expressed in rabbit meat than in red meats, since it is poorer in iron, which acts as a pro-oxidant (*Cullere & Dalle Zotte*, 2018). Additionally, the oxidative stability of rabbit meat can be improved by adding 200 mg of α-tocopheryl acetate/kg of feed to the rabbit diet (*Dalle Zotte & Szendrő*, 2011).

5. Conclusion

Taking into account the nutritional profile and technological properties of rabbit meat, it can be concluded that rabbit meat is a good raw material for meat processing. Its nutritional profile makes it suitable for incorporation into added value products that satisfy modern consumer aspirations for healthy food. The development and promotion of new rabbit meat products could be a successful strategy to attract consumers who are not sufficiently familiar with the dietetic properties of rabbit meat and have prejudices about its consumption.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Bueno, L. O., Bittencourt, M. T., Machado, L. C., Ramos, A. D. L. S. & Ramos, E. M. (2023). Technological performance of different rabbit meat cuts in processing cured cooked ready-to-eat meat products. *Ciência Animal Brasileira*, 24, https://doi.org/10.1590/1809-6891v24e-73917E

Carrillo-Lopez, L. M., Robledo, D., Martínez, V., Huerta-Jimenez, M., Titulaer, M., Alarcon-Rojo, A. D. ... & Garcia-Flores, L. R. (2021). Post-mortem ultrasound and freezing of rabbit meat: Effects on the physicochemical quality and weight loss. *Ultrasonics Sonochemistry,* 79, 105766, https://doi.org/10.1016/j.ultsonch.2021.105766

Combes, S. (2004). Valeur nutritionnelle de la viande de lapin. *INRAE Productions Animales*, 17(5), 373–383. (in French) https://doi.org/10.20870/productions-animales.2004.17.5.3610

Cullere, M. & Dalle Zotte, A. (2018). Rabbit meat production and consumption: State of knowledge and future perspectives. *Meat Science,* 143, 137–146, https://doi.org/10.1016/j.meatsci.2018.04.029

Cury, K., Martínez, A., Aguas, Y. & Olivero, R. (2011). Characterization of rabbit meat and sausage production. *Revista Colombiana de Ciencia Animal*, 3(2), 269–282.

Dalle Zotte, A. & Szendrő, Z. (2011). The role of rabbit meat as functional food. *Meat Science,* 88(3), 319–331, https://doi.org/10.1016/j.meatsci.2011.02.017

Dalle Zotte, A. & Cullere, M. (2019). Carcass traits and meat quality of rabbit, hare, guinea pig and capybara. In *More than Beef, Pork and Chicken–The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet*, 167–210.

de Oliveira Paula, M. M., Bittencourt, M. T., de Oliveira, T. L. C., Bueno, L. O., Rodrigues, L. M., Soares, E. R. ... & Ramos, E. M. (2020). Rabbit as sustainable meat source: carcass traits and technological quality of meat and of mechanically deboned meat. *Research, Society and Development*, 9(11), e5029119906-e5029119906, https://doi.org/10.33448/rsd-v9i11.9906

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J. & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science,* 42(4), 443–456, https://doi.org/10.1016/0309-1740(95)00037-2

FAOSTAT, (2020), https://www.fao.org/faostat/en/#data

González-Redondo, P. & Contreras-Chacón, G. M. (2012). Perceptions among university students in Seville (Spain) of the rabbit as livestock and as a companion animal. *World Rabbit Science*, 20(3), 155–162, https://doi.org/10.4995/wrs.2012.1147

Hernández, P. & Dalle Zotte, A. (2010). Influence of diet on rabbit meat quality. In *Nutrition of the Rabbit* (pp. 163–178). Wallingford UK: CABI, https://doi.org/10.1079/9781845936693.0163

Hoffman, L. C., Nkhabutlane, P., Schutte, D. W. & Vosloo, C. (2004). Factors affecting the purchasing of rabbit meat: A study of ethnic groups in the Western Cape. *Journal of Consumer Sciences*, 32.

Honrado, A., Aínsa, A., Marquina, P. L., Beltrán, J. A. & Calanche, J. B. (2022). Low-fat fresh sausage from rabbit meat: An alternative to traditional rabbit consumption. *Meat Science*, 194, 108973, https://doi.org/10.1016/j.meatsci.2022.108973

Ignacio, E. O., Santos, J. M. D., Santos, S. E. D. J., Souza, C. V. B. & Barretto, A. C. D. S. (2019). Effect of the addition of rabbit meat on the technological and sensory properties of fermented sausage. *Food Science and Technology*, 40, 197–204, https://doi.org/10.1590/fst.02019

Kozioł, K., Maj, D. & Bieniek, J. (2015). Changes in the color and pH of rabbit meat in the aging process. *Medycyna Weterynaryjna*, 71(2), 104 –108.

Lebas, F., Coudert, P., Rouvier, R. & De Rochambeau, H. (1997). *The Rabbit: husbandry, health, and production* (Vol. 21). Rome: Food and Agriculture Organization of the United Nations.

Lombardi-Boccia, G., Lanzi, S. & Aguzzi, A. (2005). Aspects of meat quality: Trace elements and B vitamins in raw and cooked meats. *Journal of Food Composition and Analysis,* 18(1), 39–46, https://doi.org/10.1016/j.jfca.2003.10.007

Parigi Bini, R., Xiccato, G., Cinetto, M. & Dalle Zotte, A. (1992). Effetto dell'età, del peso di macellazione e del sesso sulla qualità della carcassa e della carne cunicola. *Zootecnica e Nutrizione Animale,* 18, 173–190. (in Italian)

Park, S., Lee, J. J., Lee, J., Lee, J. K., Byun, J., Kim, I. & Ha, J. H. (2022). Lowering n-6/n-3 ratio as an important dietary intervention to prevent LPS-inducible dyslipidemia and hepatic abnormalities in ob/ob mice. *International Journal of Molecular Sciences*, 23(12), 6384, https://doi.org/10.3390/ijms23126384

Pascual, M. & Pla, M. (2008). Changes in collagen, texture and sensory properties of meat when selecting rabbits for growth rate. *Meat Science*, 78(4), 375–380, https://doi.org/10.1016/j.meatsci.2007.07.009

Petracci, M. & Cavani, C. (2013). Rabbit meat processing: historical perspective to future directions. *World Rabbit Science*, 21(4), 217–226, doi.org/10.4995/wrs.2013.1329

Ramos, E. M. & Gomide, L. A. M. (2017). Meat quality assessment: fundamentals and methodologies (2nd ed.). Viçosa: Editora UFV. (in Portuguese)

Sampels, S. & Skoglund, J. (2021). Quality of carcasses and meat from male and female rabbits. *Теория и практика переработки мяса*, 6(3), 255–258.

Składanowska-Baryza, J. & Stanisz, M. (2019). Pre-slaughter handling implications on rabbit carcass and meat quality–A review. *Annals of Animal Science,* 19(4), 875–885, https://doi.org/10.2478/aoas-2019-0041

Stabler, S. P. & Allen, R. H. (2004). Vitamin B12 deficiency as a worldwide problem. *Annual Review of Nutrition*, 24, 299–326.

Szendrő, K., Szabó-Szentgróti, E. & Szigeti, O. (2020). Consumers' attitude to consumption of rabbit meat in eight countries depending on the production method and its purchase form. *Foods*, 9(5), 654, https://doi.org/10.3390/foods9050654

Tortosa-Caparrós, E., Navas-Carrillo, D., Marín, F. & Orenes-Piñero, E. (2017). Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Critical Reviews in Food Science and Nutrition*, 57(16), 3421–3429, https://doi.org/10.1080/10408398.2015.1126549

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

https://doi.org/10.18485/meattech.2023.64.2.32

Review paper

Meat matters: tackling food loss and waste in the meat sector

Nataša Kilibarda[a], Neđeljko Karabasil[b] and Jelena Stojanović[c]*

[a] *Singidunum University, Danijelova 32, 11000 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia*
[c] *Accreditation Body of Serbia, Vlajkovićeva 3, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Food loss
Food waste
Meat sector

ABSTRACT

Food wastage occurs throughout the entire food chain, starting from agricultural production and continuing through post-harvest handling, storage, production, distribution, consumption and disposal. Food wastage not only affects food security but also has detrimental consequences for the global economy and the environment. Food loss and waste occur throughout the meat supply chain. According to literature data, 23% of meat production is lost and wasted across all stages of the food chain, with the highest share attributed to the consumption phase. Improved farming practices, animal health management, efficient transport systems, proper storage and handling at processing and retail levels, and consumer education on responsible consumption can all contribute to reducing food loss and waste in the meat sector.

1. Introduction

Globalisation in the 21st century has the potential to bring numerous advantages, especially for developing countries. However, due to the concentration of powerful companies in developed countries and the unequal distribution of profits, the rich have greater access to the benefits of global economic integration, while the poor are left behind, despite experiencing some economic growth. The widening gap between the rich and the poor is a clear indication of the prevalence of hunger and food waste in the world (*Goldsmith*, 2014; *Kilibarda*, 2020). According to a report released by the United Nations Environment Programme (UNEP), it is estimated that approximately 931 million tons of edible food are wasted every year on a global scale (*UNEP*, 2021), and there are currently 820 million people suffering from hunger globally (*FAO*, 2013a; *Kilibarda*, 2020). This issue presents a dual challenge. On one hand, there is a significant problem of overproduction and subsequent waste of food worldwide. On the other hand, the problem of hunger continues to persist and even grow in certain regions like Africa, Latin America and Western Asia (*Karwowska et al.*, 2021; *UNEP*, 2021).

Food wastage occurs throughout the entire food chain, starting from agricultural production and continuing through post-harvest handling, storage, production, distribution, consumption and disposal (*FAO*, 2013b). Although food loss and waste happen worldwide, there is a significant disparity between developing countries and those considered developed. Developing countries primarily experience food loss during the early stages of the food chain, mainly due to limited infrastructure and technological capabili-

***Corresponding author:** Nataša Kilibarda, *nkilibarda@singidunum.ac.rs*

Paper received July 21st 2023. Paper accepted August 3rd 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ties (*Kilibarda*, 2020; *Parfitt et al.*, 2010; *Wang et al.,* 2017). In contrast, more developed countries particularly contribute to food waste during the consumption phase (*FAO*, 2013a). Food wastage not only affects food security, but also has detrimental consequences for the global economy and the environment. It leads to increased emission of harmful gases, water wastage, loss of arable land and destruction of biodiversity (*Betz et al.*, 2015; *FAO,* 2013a; *FAO*, 2014; *Wang et al*. 2017). As food is wasted further along the food chain, additional resources and inputs are expended (*Kilibarda*, 2020; *Wang et al*. 2017). The ecological significance of food losses depends on various factors such as the type of food, the stage in the food chain where it occurs, and how it is managed after being wasted or lost (*Bilska et al.*, 2020a). Certain food products, like beef and dairy, are associated with higher consumption of natural resources and have a potentially greater negative environmental impact (*Moult et al.*, 2018). The relationship between food waste and climate change, particularly in terms of greenhouse gas (GHG) emissions, has been a topic of concern (*Bilska et al.*, 2020a). However, studies conducted by *Bryngelsson et al.* (2016) suggest that reducing food waste alone may only result in a modest 1%–3% reduction in emissions, which does not significantly contribute to achieving climate goals. However, taking action to tackle food waste is essential for creating a sustainable tomorrow (*Karwowska et al.*, 2021). Furthermore, promoting shifts towards more sustainable dietary patterns that include reduced meat consumption and increased consumption of plant-based alternatives can contribute to lowering GHG emissions associated with the livestock sector (*FAO*, 2017). By implementing strategies to reduce food waste, improve production efficiency, and adopt sustainable practices, the meat sector can play a vital role in mitigating its environmental impact and working towards a more sustainable and environmentally conscious food system (*Ganeson*, 2023; *Karwowska et al.*, 2021).

2. From farm to fork: addressing food loss and waste in the meat supply chain

Meat products play a significant role in the modern human diet, with an increasing global demand that has led to a steady rise in meat production worldwide. Global meat production has reached approximately 252.6 million metric tons (MT). This production includes 99.1 million MT of chicken, 95.8 million MT of pork and 57.7 million MT of beef (*Wang et al*., 2022). Per capita meat consumption has also shown an upward trend globally, and in 2014, the average person consumed approximately 43 kg of meat annually (*Ritchie & Roser*, 2017). The rise in meat consumption is anticipated to escalate in developing regions, primarily driven by high population levels and growth rates. It is projected that the volume of meat consumption in developing countries will increase approximately fivefold compared to developed countries (*OECD/FAO*, 2020). However, the production of meat and meat products has a significant environmental impact, necessitating the responsible management of the entire production chain, including production, processing, transport and consumption stages (*FAO*, 2017). According to *Gerber et al.* (2013), food animal production contributes to approximately 14.5% of the total human-induced GHG emissions annually, equivalent to 7.1 gigatons of CO_2. Beef production accounts for the largest share of GHG emissions at 35.3%, followed by dairy cattle at 30.1%, swine at 9.5%, and poultry at 8.7%. Methane emissions from ruminants and methane and N_2O emissions from manure storage contribute to approximately 39.1% and 9.5%, respectively, of the total GHG emissions attributed to food animal production.

Meat loss and waste occur throughout the meat supply chain. According to *Lipinski* (2020), 23% of meat production is lost and wasted across all stages of the food chain, with the highest share attributed to consumption (64%), followed by manufacturing (20%), distribution (12%) and primary production and post-harvest (3.5%) (*Caldiera et al.*, 2019). This pattern aligns with the well-known trend in developed regions like Europe, where most waste occurs towards the end of the food chain, particularly at the retail and consumer levels (*Kilibarda*, 2020). While meat accounts for only about 4% of global food loss and waste, data presented by *Flanagan et al.* (2019) and *Ranganathan et al.* (2016) highlight its higher economic value and environmental impact compared to other food groups (cereals, fruits and vegetables). *Buzby & Hyman* (2012) estimated the total value of meat product waste in the United States at approximately 83 million $US. According to *Bux & Amicarelli* (2022), approximately 0.45–0.50 million tons of fresh meat are estimated to be wasted throughout the entire Italian agri-food chain. This waste equates to a value of over €242–268 million. Additionally, there are additional losses in terms of energy and water, amounting to €435–481 million. These findings underscore the importance of

addressing food waste in the meat sector, both from an economic and environmental perspective (*Karwowska et al*., 2021; *Mosna et al.*, 2021).

The reasons for food waste in the meat sector vary depending on the stage of the food supply chain (*Karwowska et al.*, 2021). In their research, *Magalhães et al.* (2020) highlights the "*lack of transportation infrastructures*", "*inadequate handling*", "*poor operational performance*", "*variety of products available in supermarkets*" and "*unhealthy animals and outbreaks of disease*" as the most influential causes of food loss and waste in the Brazilian beef supply chain. Losses at the stage of primary production, as a part of meat sector, can be attributed to farming/rearing conditions and transport to the slaughterhouse. Factors such as poor farming practices, inadequate animal welfare conditions and unfavourable transport conditions can lead to mortality and losses, while some animals are rejected during slaughter due to health or quality concerns (*Gustavsson,* 2011). Disease outbreaks among livestock can result in the loss of animals. To ensure food safety and prevent the spread of diseases, sick animals can be removed from the supply chain, leading to food loss and waste (*Jaja et. al.*, 2018; *Lipinski*, 2020). In the meat processing and manufacturing stage, losses can occur due to trimming, packaging defects and quality control issues. *Dora et al.* (2019) identified several fundamental causes of food losses during the processing stage, including incorrect transportation, product alterations, human error and product defects. Meat and meat products have a short shelf life and require cold storage. If the temperature is not controlled, they can spoil quickly. Additionally, if meat products are not sold before their expiry date, they are wasted, especially at the retail stage. Meat is highly conducive to the growth of microorganisms. The most common risk in relation to meat is the presence of pathogenic microorganisms. When hazards are detected in meat and meat products, that results in losses for food producers and in food waste. During transportation and distribution, factors such as inadequate refrigeration, improper handling and delays can lead to spoilage and loss of meat products (*Lipinski*, 2020). Improper packaging, packaging design, material, and atmosphere influence the product's susceptibility to mechanical damage and microbial contamination, thereby affecting its quality (*Bogataj et al*., 2020; *Zhang et al.*, 2015). The consumption stage plays a significant role in food losses and waste within the food supply chain. According to the literature, the consumption stage, which includes households and food services, is responsible for the largest share of food waste generation in Europe (*Caldeira at al.*, 2019; *Kilibarda et al.*, 2020). Consumers can discard meat products due to improper storage conditions, inadequate knowledge about food preparation, over-purchasing, or personal preferences, packaging size (purchasing larger packages, leading to leftovers that may go uneaten and eventually be wasted) and confusion related to date labels (misunderstandings regarding date labels, such as "use-by" and "best before" dates) (*Bilska et al*., 2020b; *Lipinski*, 2020; *Neff et al*., 2019). Retailers can also generate waste due to overstocking, sensory imperfections or product expiration (*Lipinski*, 2020). Specifically, when it comes to meat, food waste generated in households and food services accounts for 64% of the total food waste in the meat supply chain (*Caldeira at al*., 2019). However, households tend to generate a much larger amount of food waste compared to food service establishments for various food groups, including cereals, potatoes, eggs, dairy and meat.

3. Strategies and innovative approaches to minimise and valorise food loss and waste in the meat sector

In addition to meat, a slaughterhouse produces various other organs and tissues, such as fatty tissues, horns, hoofs, feet, skull, entrails and internal organs, which are mechanically separated from the meat (*Ockerman et al*., 2017). Approximately 45–60% of an animal's total weight is considered suitable for human consumption, while the remaining portion is considered waste (*Chowdhury et al*., 2022). The waste obtained from this sector is known for its complex nature, mainly due to factors such as its pathogenic nature, high water content, propensity for rapid auto-oxidation, and elevated levels of enzymatic activity (*Jayathilakan et al.*, 2012). The waste generated from a slaughterhouse can be broadly categorised into solid waste and liquid waste. For the treatment of solid waste and possibly its valorisation, *Sabumon* (2023) suggests following strategies within waste management: anaerobic digestion, composting, rendering, alkaline hydrolysis and enzyme application. For wastewater management from slaughterhouses, *Sabumon* (2023) states as possible solutions, land application, physicochemical treatment, biological treatment, advanced oxidation processes and combined processes, while for blood waste management, the author suggests proper col-

lection and separation and then its utilisation and treatment. Bones, as waste from meat sector, contain valuable elements such as phosphorus and calcium that can be used as the basis for other products. By utilising thermal methods, it is possible to obtain significant amount of hydroxyapatite ash from this waste, which can serve as a substitute for phosphorites and has the potential to be used to produce food-grade phosphoric acid and mono- and dicalcium feed phosphates (*Kowalski et al*., 2021). The recycling of residual animal fat from industrial meat processing for biodiesel production offers an attractive renewable energy source (*Skoronski et al*., 2016). Using food waste as animal feed presents a solution that addresses waste management and food security challenges. By incorporating these food wastes into animal diets, we can maximise their value, minimise waste and contribute to a more sustainable and efficient food system (*Rajeh et al*., 2020). This is evident in various aspects, including a reduction in GHG emissions by 56.40%, a decrease in water consumption by 22.62%, a significant reduction in land use by 87.50%, and a decrease in fossil resource scarcity by 21.78% (*Mosna et al*. 2021). According to *Araújo dos Santos et al*. (2023), handling poultry litter for biogas production and reusing poultry waste to produce meat meals in feed production resulted in the avoidance of methane and ammonia emissions, leading to a reduction of over 55% in environmental indicators such as climate change, terrestrial acidification, and freshwater eutrophication.

Smart packaging materials (active and intelligent packaging) are a rapidly emerging technology that offers various features for monitoring the condition of packaged products. These systems enhance the interaction between consumers and food products, offering a unique consumer experience while providing clear data, traceability and trackability, and decreased risk of food wastage (*Ganeson et al.*, 2023).

Consumer education, awareness campaigns and strategies for better meal management and food storage can help reduce food waste in households. Efforts to reduce food waste at the consumer stage should focus on promoting proper storage practices, providing consumer education on food preparation and portion control, and improving understanding of date labelling. By empowering consumers with knowledge and tools to minimise waste, significant progress can be made in reducing food loss and waste in the meat sector (*Martin-Rios et al*., 2018; *Reynolds et al*., 2019). Furthermore, while food service establishments generate a smaller proportion of food waste than do households, they still have a role to play in waste reduction. Improving portion control, menu planning, inventory management and donation programs for excess food can help minimise waste in the food service sector (*Kilibarda*, 2020; *Kilibarda et al*., 2020).

4. Conclusion

Addressing the unequal distribution of the benefits of globalisation, as well as combating hunger and reducing food waste, is crucial. Efforts should be made to promote fair economic exchange and ensure that the advantages of globalisation are accessible to all. Furthermore, initiatives should focus on improving infrastructure and technology in developing countries to reduce food loss, while in developed countries, consumer behaviour and attitudes towards food should be addressed to minimise food waste and its negative impact on the environment. Addressing causes of food loss and waste in the meat sector requires interventions at each stage of the supply chain. Improved farming practices, animal health management, efficient transport systems, proper storage and handling at processing and retail levels, and consumer education on responsible consumption can all contribute to reducing food loss and waste in the meat sector. By addressing these causes, the meat sector can contribute to minimising food waste and maximising the utilisation of valuable resources.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Araújo Dos Santos, R., Silva da Costa, J., Maranduba, H. L., Almeida Neto J. A., & Rodrigues, L. B. (2023). Reducing the environmental impacts of Brazilian chicken meat production using different waste recovery strategies. *Journal of Environmental Management*, 341, 118021, https://doi.org/10.1016/j.jenvman.2023.118021

Betz, A., Buchli, J., Göbel, C. & Müller, C. (2015). Food waste in the Swiss food service industry — Magnitude and potential for reduction. *Waste Management* (New York, N.Y.), 35, 218–226, https://doi.org/10.1016/j.wasman.2014.09.015

Bilska, B., Tomaszewska, M. & Kołożyn-Krajewska, D. (2020b). Managing the risk of food waste in foodservice establishments. *Sustainability*, 12(5), 2050, http://dx.doi.org/10.3390/su12052050

Bilska, B., Tomaszewska, M., Kołożyn-Krajewska, D., Szczepański, K., Łaba, R. & Łaba, S. (2020a). Environmental aspects of food wastage in trade — a case study. *Environmental Protection and Natural Resources*, 31(2) 24–34, https://doi.org/10.2478/oszn-2020-0009

Bogataj, D., Hudoklin, D., Bogataj, M., Dimovski, V. & Colnar, S. (2020). Risk mitigation in a meat supply chain with options of redirection. *Sustainability*, 12, 8690, https://doi.org/10.3390/su12208690

Bryngelsson, D., Wirsenius, S., Hedenus, F. & Sonesson, U. (2016). How can the EU climate targets be met? A combined analysis of technological and demand-side changes in food and agriculture. *Food Policy*, 59, 152–164, https://doi.org/10.1016/j.foodpol.2015.12.012

Buzby, J. C. & Hyman, J. (2012). Total and per capita value of food loss in the United States. *Food Policy*, 37(5), 561–570, https://ideas.repec.org/a/eee/jfpoli/v37y2012i5p561-570.html>

Caldeira, C., De Laurentiis, V., Corrado, S., van Holsteijn, F. & Sala, S. (2019). Quantification of food waste per product group along the food supply chain in the European Union: a mass flow analysis. *Resources, Conservation, and Recycling*, 149, 479–488, https://doi.org/10.1016/j.resconrec.2019.06.011

Chowdhury, M.W., Nabi, M.N., Arefin, M.A., Rashid, F., Islam, M.T., Gudimetla, P. & Muyeen, S. M. (2022). Recycling slaughterhouse wastes into potential energy and hydrogen sources: an approach for the future sustainable energy. *Bioresource Technology Reports*, 19, 101–133, https://doi.org/10.1016/J.BITEB.2022.101133

Dora, M., Wesana, J., Gellynck, X., Seth, N., Dey, B. L. & Steur, H. D. (2019). Importance of sustainable operations in food loss: evidence from the Belgian food processing industry. *Annals of Operations Research*, 290, 47–72.

FAO, (2013a). Food Wastage Footprint: Impacts on Natural Resources: Summary Report. FAO, Rome.

FAO, (2013b). Toolkit: Reducing the Food Wastage Footprint. FAO, Rome.

FAO, (2014). Food Wastage Footprint Full-cost Accounting: Final Report. Food Wastage Footprint, Rome.

FAO, 2017. *The Future of Food and Agriculture — Trends and Challenges.* Rome.

Flanagan, K., Robertson, K., Hanson, C. & Timmermans, A. J. M. (2019). *Reducing food loss and waste: Setting a Global Action Agenda*. World Resources Institute (WRI), https://wriorg.s3.amazonaws.com/s3fs-public/reducing-food-loss-waste-global-action-agenda_0.pdf

Ganeson, K., Mouriya, G. K., Bhubalan, K., Razifah, M. R., Jasmine, R., Sowmiya, S., Amirul, A. A, Vigneswar, S. & Ramakrishna, S. (2023). Smart packaging − A pragmatic solution to approach sustainable food waste management, *Food Packaging and Shelf Life*, 36, 101–044, https://doi.org/10.1016/j.fpsl.2023.101044

Goldsmith, E. (2014). Development as colonialism. In: Goldsmith, E. & Mander, J. (Eds.), The Case Against the Global Economy: and For a turn Towards Localization (pp. 42–71). 2nd ed. Routledge, Abingdon.

Gustavsson, J., Cederberg, C., Sonesson, U. & Emanuelsson, A. (2011). The Methodology of the FAO Study: Global Food Losses and Food. Waste-Extent, Causes and Prevention; FAO: Rome, Italy.

Jaja, I. F., Mushonga, B., Green, E. & Muchenje, V. (2018). Factors responsible for the post-slaughter loss of carcass and offal's in abattoirs in South Africa. *Acta Tropica*, 178, 303–310, https://doi.org/10.1016/j.actatropica.2017.12.007

Jayathilakan, K., Sultana, K., Radhakrishna, K. & Bawa, A. S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, 49(3), 278–293, https://doi.org/10.1007/s13197-011-0290-7

Karwowska, M., Łaba, S. & Szczepański, K. (2021). Food loss and waste in meat sector—Why the consumption stage generates the most losses? *Sustainability*, 13(11), 6227, https://doi.org/10.3390/su13116227

Kilibarda, N. (2020). Food Safety and Waste in Hospitality. In: Leal Filho, W., Azul, A. M., Brandli, L., Özuyar, P. G. & Wall, T. (Eds.), Zero Hunger. Encyclopedia of the UN Sustainable Development Goals (pp 338–347). Springer, Cham, https://doi.org/10.1007/978-3-319-95675-6_107

Kilibarda, N., Djokovic, F. & Suzic, R. (2020). Food waste management — reducing and managing food waste in hospitality. *Meat Technology* 60(2), 134–142, https://doi.org/10.18485/meattech.2019.60.2.8

Kowalski, Z., Kulczycka, J., Makara, A. & Harazin, P. (2021). Quantification of material recovery from meat waste incineration — An approach to an updated food waste hierarchy. *Journal of Hazardous Materials*, 416, 126021, https://doi.org/10.1016/j.jhazmat.2021.126021

Lipinski, B. (2020). Why Does Animal-Based Food Loss and Waste Matter?. *Animal frontiers: the review magazine of animal agriculture*, 10(4), 48–52, https://doi.org/10.1093/af/vfaa039

Magalhães, V. S. M., Ferreira, L. M. D. F., César, A. D. S., Bonfim, R. M. & Silva, C. (2021). Food loss and waste in the Brazilian beef supply chain: an empirical analysis". *The International Journal of Logistics Management*, 32(1), 214–236, https://doi.org/10.1108/IJLM-01-2020-0038

Martin-Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C. (2018). Food waste management innovations in the foodservice industry. *Waste Management* (New York, N.Y.), 79, 196–206, https://doi.org/10.1016/j.wasman.2018.07.033

Mosna, D., Bottani, E., Vignali, G. & Montanari, R. (2021). Environmental benefits of pet food obtained as a result of the valorisation of meat fraction derived from packaged food waste. *Waste Management*, 125, 132–144.

Moult, J., Allan, S., Hewitt, C. N. & Berners-Lee, M. (2018). Greenhouse gas emissions of food waste disposal options for UK retailers. *Food Policy*, 77, 50–58.

Neff, R. A., Spiker, M., Rice, C., Schklair, A., Greenberg, S. & Leib, E. B. (2019). Misunderstood food date labels and reported food discards: A survey of U.S. consumer attitudes and behaviors. *Waste Management*, 86, 123–132, https://doi.org/10.1016/j.wasman.2019.01.023

Ockerman, H. W., Basu, L. & Toldr´a, F. (2017). Edible by-products. In: Lawrie's Meat Science, eighth ed., 679–696, https://doi.org/10.1016/B978-0-08-100694-8.00022-4

OECD/FAO, (2020). *OECD-FAO Agricultural Outlook 2020-2029*, OECD Publishing, Paris/FAO, Rome, https://doi.org/10.1787/1112c23b-en

Parfitt, J., Barthel, M. & Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. Philosophical transactions of the Royal Society of London. *Series B, Biological Sciences*, 365(1554), 3065–3081, https://doi.org/10.1098/rstb.2010.0126

Rajeh, C., Saoud, I., Kharroubi, S., Nalbandian, S. & Abiad, M. (2020). Food loss and food waste recovery as animal feed: a systematic review. *Journal of Material Cycles and Waste Management*, 23, 1–17, https://doi.org/10.1007/s10163-020-01102-6

Ranganathan, J., Vennard, D., Waite, R., Searchinger, T., Dumas, P. & Lipinski, B. (2016). *Shifting diets: Toward a sustainable food future*. Global Food Policy Report. International Food Policy Research Institute (IFPRI). Washington, D.C (Chapter 8) http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/130216

Reynolds, C., Goucher, L., Quested, T., Bromley, S., Gillick, S., Wells, V. K., Evans, D., Koh, L., Carlsson Kanyama, A., Katzeff, C., Svenfelt, Å. & Jackson, P. (2019). Review: Consumption-stage food waste reduction interventions — What works and how to design better interventions. *Food Policy*, 83, 7–27, https://doi.org/10.1016/j.foodpol.2019.01.009

Ritchie, H. & Roser, M. (2017). "Meat and Dairy Production". Published Online at OurWorldInData.org. 2017. Available online: https://ourworldindata.org/meat-production (accessed on 17 July 2023).

Skoronski, E., Cardoso de Oliveira, D., Fernandes, M., Felippe da Silva, G., de Lourdes Borba Magalhães, M. & João, J. J. (2016). Valorization of agro-industrial by-products: analysis of biodiesel production from porcine fat waste. *Journal of Cleaner Production*, 112(4), 2553–2559, https://doi.org/10.1016/j.jclepro.2015.10.026

Skoronski, E., Oliveira, D., Fernandes, M., Silva, G., Magalhães, M. & João, J. (2016). Valorization of agro-industrial by-products: analysis of biodiesel production from porcine fat waste. *Journal of Cleaner Production*, 112, 2553–2559, https://doi.org/10.1016/J.JCLEPRO.2015.10.026

UNEP Food Waste Index Report, 2021. Available online: https://www.unep.org/resources/report/unep-food-waste-index-report-2021 (accessed on 17 July 2023).

Wang, L., Liu, G., Liu, X., Liu, Y., Gao, J., Zhou, B., Gao, S. & Cheng, S. (2017). The weight of unfinished plate: A survey based characterization of restaurant food waste in Chinese cities. *Waste Management*, 66, 3–12.

Wang, S., Jena, U. & Das, K. C., (2022). Long term performance of pilot methanogenic digester filled with seashell wastes treating slaughterhouse wastes: biogas production and environmental impact. *Biochemical Engineering Journal,* 187, 108651 https://doi.org/10.1016/j.bej.2022.108651

Zhang, H., Hortal, M., Dobon, A., Bermudez, J. M. & Lara-Lledo, M. (2015). The Effect of Active Packaging on Minimizing Food Losses: Life Cycle Assessment (LCA) of Essential Oil Component-enabled Packaging for Fresh Beef. *Packaging Technology and Science*, 28(9), 761–774, Portico, https://doi.org/10.1002/pts.2135

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.056
579.67

ID: 126603785

https://doi.org/10.18485/meattech.2023.64.2.33

Original scientific paper

Composition and diversity of microbial communities of industrial environment objects

Yuliya Yushina[a], Elena Zaiko[a], Anzhelika Makhova[a], Oxana Kuznetsova[a], Anastasia Semenova[a], Dagmara Bataeva[a], Maria Grudistova[a] and Nasarbay Nasyrov[a]*

[a] *V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Talalikhina st., 26, 109316, Moscow, Russia*

ARTICLE INFO

Keywords:
Microflora
Pathogen microorganism
Resistance

ABSTRACT

One of the main sources of bacteria that cause spoilage is microorganisms harbored on surfaces and objects in the production environment of food enterprises. This paper presents the results of the taxonomic composition of the microbiota of objects in the production environment of pork processing enterprises, with the identification of key groups of microorganisms. The microbiota in the facilities of the production environment of the meat processing enterprise was mainly represented by 12 phyla. Among these, Proteobacteria, Bacteroidota, Actinobacteria, and Firmicutes were predominant. The results of 16S rRNA amplicon sequencing were analyzed for the presence of pathogenic bacteria in the studied samples. Potentially pathogenic *Shigella* bacteria were found in two samples. In addition to pathogenic bacteria, bacteria detected in the samples cause spoilage of meat and meat products; these were the genera *Brochothrix* and *Pseudomonas*. Pathogenic microorganisms were studied by the molecular method with accumulation. Representatives of pathogenic microorganisms were present on the objects in the production environment. *Listeria monocytogenes* was found in three samples, *Salmonella* spp. in two samples, and *Campylobacter* spp. in one of the samples studied.

1. Introduction

The food industry is developing with serious complications concerning reduced susceptibility to foodborne pathogenic bacteria during lapses (*EFSA and ECDC,* 2016). One of the main sources of food spoilage are microorganisms from objects in the production environment in food enterprises, which was identified in previously (*Stellato et al.,* 2016).

Although this is a global economic and health problem, until recently, little was known about microbial diversity in slaughterhouses and meat processing plants, and it was difficult to track and control ways to reduce the risk of meat spoilage. Moreover, certain taxa or strains with good biofilm-forming capacity are able to survive in sanitary conditions and/or increase adaptive responses to stress. The meat industry continues to rely on standard microbiological methods for hygienic production control. Such methods can be useful in determining the general condition of an object, but they do not allow us to describe the complex microbial communities formed within a particular production complex (*Zwirzitz et al.,* 2020).

In the last decade, attempts have been made to characterize the microbiome in the food industry using molecular genetic methods (*Bokulich et*

***Corresponding author:** Yushina Yulia, *yu.yushina@fncps.ru*

Paper received July 13th 2023. Paper accepted July 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

al., 2015*; Yang et al,* 2016). Techniques such as next generation sequencing (NGS) allow the detection of microorganisms in food without prior culture and without isolation of species-specific fragments from total DNA and amplification of genes encoding rRNA (*Ercolini*, 2013*; Lozupone and Knight,* 2005). In addition, it allows the creation of object-specific bacterial flux maps that show unique transmission patterns for individual taxa. This approach helps to increase knowledge about the transmission of microorganisms, thereby improving hygiene standards in the food industry to improve food safety.

The aim of the work was to study the taxonomic composition of microbiota on the production environment surfaces and objects in a pork processing enterprise, with the identification of key groups of microorganisms.

2. Materials and methods

2.1 Research subjects

The objects of the study were 36 samples taken in September 2020 from production surfaces and equipment at a meat processing enterprise (21 samples). The list of analyzed samples is presented in Table 1.

2.2 DNA isolation, amplification, and sequencing of 16S rRNA gene fragments

Isolation of total DNA was performed using a modified Birnboim-Doly alkaline isolation procedure and Wizard technology from Promega. (США) (*Bulygina et al.,* 2002). DNA concentration was measured on a spectrophotometer Smart-

Table 1. Environmental samples taken in the meat processing plant

Number	Zone	Point
A1		sorting table 1
A2		conveyor belt 1
A3		equipment case
A4		cleaning equipment (hose)
A5		sorting table 2
A6		conveyor belt for carcasses 2
A7		work aprons
A8		cleaning equipment (mop)
A9	Preparation for cutting and deboning	equipment maintenance tool
A10		by-product conveyor belt
A11		conveyor belt 3
A12		work gloves
A13		waste bin (external part)
A14		carcass (external part)
A15		carcass (internal part)
A16		knives for cutting
A17		plastic container
A18	Chilling	wall
A19		carcass cutting saw
A20	Cutting and deboning	table
A21		hooks for pork heads

Spec 3000 (BioRad, USA). The DNA concentration in the preparations ranged from 10 to 40 ng/µl, whereby A260/A280 = 1.8−1.9. Determination of the nucleotide sequence of the total amplification of the 16S rRNA gene fragments (V3-V4 region) was carried out by high-throughput sequencing on the platform MiSeq (Illumina, США). The variable V3-V4 region of the 16S rRNA gene was amplified using universal primers 341F (5'-CCTAYG GGDBGCWSCAG) and 806R (5'-GGA CTA CNVGGG THTCTAAT) (Frey et al., 2016). The resulting library was sequenced on MiSeq (Illumina, San Diego, CA, USA) using Miseq Reagent Kit V3 in the format of 2×300 nucleotide pair-end reads.

2.3. Bioinformatics analysis

Paired readings were combined using the FLASH v.1.2.11 program (*Magoč and Salzberg*, 2011). After merging, low-quality reads, singletons, and chimeras were excluded. The remaining readings were clustered into operational taxonomic units (OTUs) with at least 97% identity. To determine the proportion of OTUs in each of the samples, original reads (including low-quality and singletons) were superimposed on representative OTU sequences with a minimum identity of 97% over the entire length of the reading. To perform all these procedures, the USEARCH v.11 software package (*Edgar*, 2010) was used. Taxonomic identification of microorganisms by 16S rRNA gene sequences was performed using the VSEARCH v.2.14.1 algorithm in the Silva v.138 database (*Rognes* et al., 2016).

2.4 Detection of pathogenic bacteria

The detection of *Listeria monocytogenes, Salmonella* spp., and *Campylobacter* spp. was conducted by the commercial LAMP-based kit (3M Molecular Detection Assay Listeria monocytogenes; 3M — for Listeria monocytogenes detection; 3M Molecular Detection Assay Salmonella — for Salmonella spp. detection; 3M Molecular Detection Assay Listeria monocytogenes — for Listeria monocytogenes detection), used according to the manufacturer's manual. All samples identified as positive by molecular analysis were confirmed by standard laboratory tests in accordance with Russian Standard Methods. Confirmation of *L. monocytogenes* was performed according ISO 11290. Confirmation of *Campylobacter* spp. was performed according to GOST 10272 part 1. Confirmation of *Salmonella* prevalence was performed according the Russian Standard method for the detection of *Salmonella* spp. GOST 31659.

3. Results

After processing the sequencing data, the sequences for all accessions were pooled into a cluster of 4020 operational taxonomic units with a minimum identity of 0.97.

The taxonomic classification of the obtained OTUs was carried out according to the Silva 16S rRNA sequence database (*Quast et al., 2013*). The results of the taxonomic analysis of the composition of microbial communities according to the 16S rRNA gene sequence are shown in Figure 1.

The microbiota on the production environment surfaces and objects in the meat processing enter-

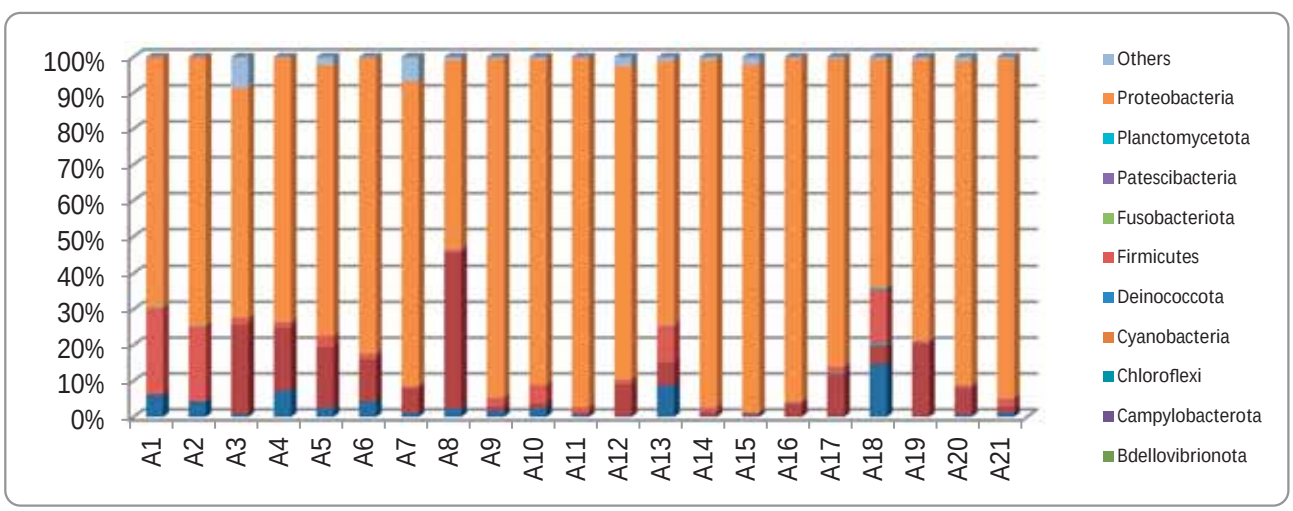


Figure 1. Taxonomic composition of microbial communities of samples taken from the meat processing plant A1-A21

Table 2. The results of sequencing of 16S rRNA amplicons for the presence of pathogenic bacteria

Bacteria	Number OTU identity	OTU
Salmonella	Missing	-
Listeria monocytogenes	Missing	-
Shigella	A1-0.02 %, A11- 0.02%	Otu4
Brucella	Missing	-
Campylobacter jejuni	Missing	-
Clostridium perfringens	Missing	-
Staphylococcus aureus	Missing	-

prise was mainly represented by 12 phyla. Among these, Proteobacteria, Bacteroidota, Actinobacteria and Firmicutes were predominant. Proteobacteria dominated in samples A9, A11, A14, A15, and A16, in which they accounted for more than 90% of the data obtained. Sample A8 contained representatives of the phylum Bacteroidota to the greatest extent (43.29%). Slightly fewer Bacteroidota were present in samples A3, A4, A5, A6, and A19, where the percentage ranged from 11.62% to 24.79% depending on the sample. Firmicutes were found in all studied samples; however, their maximum percentage was noted in samples A1 and A1, at 23.20% and 20.39%, respectively. Actinobacteria were presented in various amounts in the samples, and the maximum percentage of this phylum, 14.64%, was in A18. Minor groups included representatives of Bdellovibrionota, Campylobacterota, Chloroflexi, and Fusobacteria.

The results of sequencing of 16S rRNA amplicons were analyzed for the presence of pathogenic bacteria in the studied samples. At the same time, potentially pathogenic *Shigella* were found in samples A1 and A11 (Table 2). However, the number of *Shigella* 16S rRNA reads in the samples did not exceed 0.02% of the total number of reads.

In addition to pathogenic bacteria, bacteria of the genera *Brochothrix* and *Pseudomonas*, which cause spoilage of meat and meat products, were detected in the samples. Bacteria of the genus *Brochothrix* were found in samples A3, A4, A5, A7-A10, A12, A13, A16-F18. The number of readings of these bacteria ranged from 0.01% to 6.14% of the total number of readings. Bacteria of the genus *Pseudomonas* were found in all samples; the number of readings of these bacteria ranged from 0.19% to 78.47% of the total number of readings. The largest proportions of bacteria of the genus *Pseudomonas* were observed in samples A21 (78.47%), A20 (69.95%), A14 (67.81%), A13 (66.85%), A9 (64.72%) and A11 (61.68%).

Pathogenic microorganisms were investigated by the molecular method with accumulation. Pathogenic bacteria were present on the production environment surfaces. *L. monocytogenes* were found in three samples A2, A11, and A17 (Table 3).

Table 3. Pathogenic bacteria in environmental samples

Bacteria	Sample number	Point
Listeria monocytogenes	A2	conveyor belt 1
	A11	conveyor belt 3
	A17	plastic container
Salmonella spp.	A2	conveyor belt 1
	A5	sorting table 2
Campylobacter spp.	A19	carcass cutting saw

Samples A2 and A5 were contaminated with *Salmonella*. One of the natural samples of A19 contained *Campylobacter* spp.

4. Discussion

During the analysis of the microbiome, the main phyla of the meat processing enterprise were Proteobacteria and Firmicutes. It is known that these microorganisms are representatives of the intestinal microflora of pigs and are often found on the surface of biotic and abiotic objects of meat processing enterprises (*Zhang et al.,* 2020). The distribution of these phyla on the surface of carcasses is interesting: there is evidence that Proteobacteria are more often found on the surface of the upper part of the carcass, and Firmicutes on the surface of the lower part. This is probably due to the characteristics of primary processing and surface contamination in one way or another (*Steven et al.,* 2022*; Braley et al.,* 2022). Bacteria of the genus *Pseudomonas* were found in all samples, and the number of readings of these bacteria ranged from 0.1% to 43.6% of the total number of readings (*Yu et al*., 2020). This is confirmed in scientific works of recent years, where *Pseudomonas* is a significant part of the microbial community of abiotic objects in meat processing enterprises (*Cobo-Díaz et al.,* 2021). In a study on shelf life, *Chen et al. (*2020) did not find *Pseudomonas* on chilled poultry carcasses, but found them on the walls of the air cooler, and by the end of 12 days of storage, they dominated the microbiota of packaged carcasses (*Chen et al,* 2020). This once again proves the influence of the bacterial status of production environment objects on the microbiota of products during processing and storage. During our study, the genus *Shigella* was found, the reservoir for which is also the gastrointestinal tract. However, the number of *Shigella* 16S rRNA reads in the samples did not exceed 0.02%. This suggests that indicator microorganisms determined by classical microbiological methods (for example, fecal contamination — *E. coli*) may represent only a fraction of the total number of organisms potentially present on the carcass or on the surface of the equipment, and do not reflect the real level of hygiene of enterprises (*Blevins et al.,* 2018). Another serious problem is the presence of pathogenic microorganisms (*L. monocytogenes* and *Salmonella*). Work surfaces can be a source for the spread of antibiotic-resistant strains of *Salmonella (Bertolatti et al.,* 2003).

The environmental samples also contained *Brochothrix*, which can grow in an environment with a low oxygen content and a high concentration of carbon dioxide. This means that if *Brochothrix* bacteria get into a meat product packed in a vacuum or a modified gas environment, they can cause its spoilage.

The study of the microbiome of food enterprises brings not only practical benefits to a particular meat processing complex, but also helps to form global databases of taxonomic profiles of microbial communities depending on the geographical location.

5. Conclusion

Sequencing of food microbiomes reveals key characteristics of food safety and quality. Pathogenic bacteria and spoilage microorganisms have been identified, which suggests that the objects and surfaces in the production environment of food enterprises can play a key role in the transfer of microorganisms to food products. The data obtained demonstrate diverse and highly variable communities of microorganisms living on various facilities in the enterprise, which is informative in the context of food safety and spoilage.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science and Higher Education of the Russian Federation (project № 075-15-2020-775).

Acknowledgements: The authors wish to thank Mardanov Andrey Vladimirovich (to the Head of the Laboratory of Microbial Genomics and Metagenomics, Federal Research Center Fundamentals of Biotechnology of RAS) for conducting sequencing.

References

Bertolatti, D., O'Brien, F. & Grubb, W. (2003). Characterization of drug-resistant *Staphylococcus aureus* isolated from poultry processing plants in Western Australia. *International Journal of Environmental Health Research*, 13, 1, 43–54, https://doi.org/10.1080/0960312021000063322

Blevins, R. E., Kim, S. A., Park, S. H., Rivera, R. & Ricke, S. C. (2018). Historical, current, and future prospects for food safety in poultry product processing systems. Food and feed safety systems and analysis. *Academic Press*, 323–345, https://doi.org/10.1016/B978-0-12-811835-1.00018-X

Bokulich, N. A., Bergsveinson, J., Ziola, B. & Mills, D. A. (2015). Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *Elife*, 4, e04634, https://doi.org/10.7554/eLife.04634

Bulygina, E. S., Kuznetsov, B. B., Marusina, A. I., Kravchenko, I. K., Bykova, S. A., Kolganova, T. V. & Galchenko, V. F. (2002). Study of nucleotide sequences of nifH genes in representatives of methanotrophic bacteria. *Microbiology*, 71(4), 500–508.

Chen, S. H., Fegan, N., Kocharunchitt, Ch., Bowman, J. P. & Duffy, L. L. (2020). Impact of poultry processing operating parameters on bacterial transmission and persistence on chicken carcasses and their shelf life. *Applied and Environmental Microbiology*, 86(12), e00594–20, https://doi.org/10.1128/AEM.00594-20

Cobo-Díaz, J. F., Alvarez-Molina, A., Alexa, E. A., Walsh, C. J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter P. & Alvarez-Ordóñez A. (2021). Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. *Microbiome*, 9, 204, https://doi.org/10.1186/s40168-021-01131-9

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26, 2460–2461, https://doi.org/10.1093/bioinformatics/btq461

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal*, 14(12), 4634, 231.

Ercolini, D. (2013). High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Applied Environmental Microbiology*, 79, 3148–3155, https://doi.org/10.1128/AEM.00256-13

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F. & Hartmann, M. (2016). Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiology Ecology*, 92, 3, https://doi.org/10.1093/femsec/fiw018

Lozupone, C. & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, 71(12), 8228–8235, https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Magoč, T. & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963, https://doi.org/10.1093/bioinformatics/btr507

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue), D590–D596, https://doi.org/10.1093/nar/gks1219

Ricke, S. C., Dittoe, D. K., Brown, J. A. & Thompson, D. R. (2022). Practical opportunities for microbiome analyses and bioinformatics in poultry processing. *Poultry Science*, 101(5), 101787, https://doi.org/10.1016/j.psj.2022.101787

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584, https://doi.org/10.7717/peerj.2584

Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F. & Ercolini, D. (2016). Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. *Applied and Environmental Microbiology*, 82(13), 4045–4054, https://doi.org/10.1128/AEM.00793-16

Yang, H., Huang, X., Fang, S., Xin, W., Huang, L. & Chen, C. (2016). Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Scientific Reports*, 6, 27427, https://doi.org/10.1038/srep27427

Yu, Z., Joossens, M. & Houf, K. (2020). Analyses of the bacterial contamination on Belgian broiler carcasses at retail level. *Frontiers in Microbiology*, 11, 539540, https://doi.org/10.3389/fmicb.2020.539540

Zhang, X., Peng, Z., Li, P., Mao, Y., Shen, R., Tao, R., Diao, X., Liu, L., Zhao, Y. & Luo, X. (2020). Complex internal microstructure of feather follicles on chicken skin promotes the bacterial cross-contamination of carcasses during the slaughtering process. *Frontiers in Microbiology*, 11, 571913, https://doi.org/10.3389/fmicb.2020.571913

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F. F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M. & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *NPJ Biofilms and Microbiomes*, 6(1), 26, https://doi.org/10.1038/s41522-020-0136-z

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5:636.2

ID: 126616329

https://doi.org/10.18485/meattech.2023.64.2.34

Review paper

Nutritional approaches to enhance fatty acid composition of beef: a review

Mirjana Lukić[a], Sara Simunović[a] and Stefan Simunović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Beef
Fatty acids
Forage feeding
Oilseed/oil supplementation

ABSTRACT

Intramuscular fat content and fatty acid composition are important factors contributing to the nutritional value of meat. Beef meat is characterized by a high content of saturated fatty acids (SFA) which can contribute to elevated serum cholesterol levels and coronary heart disease in humans. Consumer concerns regarding the association between beef consumption and chronic cardiovascular diseases have motivated increased interest in developing strategies for reducing the lipid content and improving the fatty acid (FA) composition of beef. Although beef fat quality is determined by many factors, such as breed, gender, age, live weight, fatness degree, but the bovine diet has a key role in enhancing the nutritional quality of beef lipids. Nutritional strategies to improve the FA composition of beef focussed mainly on reducing the SFA and the n-6:n-3 poly-unsaturated fatty acid (PUFA) ratio, simultaneously increasing the PUFA of beef fat.

1. Introduction

Beef is a high-quality source of protein and essential micronutrients, but consumers recognize it as red meat with a high content of SFA (*McAfee et al*., 2010). The processes of microbial lipolysis and biohydrogenation in the rumen, which result in the conversion dietary PUFA to more saturated end products, are major reasons why beef fats are highly saturated. A high intake of SFA and a high ratio n-6:n-3 PUFA are typical of Western and, increasingly, global diets and is associated with an increased risk of cardiovascular disease, obesity, type 2 diabetes and several form of cancer, especially in people with genetic predispositions (*Trbović et al.*, 2020). The feeding system has important effects on beef quality since the nutrient composition and energy intake of the diet affect commercial quality attributes of the carcass and the nutritional composition of the meat, particularly the amount of intramuscular fat and FA composition (*Liu et al.*, 2022). In comparison to new breeding strategies, manipulation of beef quality through feeding is cost-effective and more practical, and a variety of nutritional interventions have been successfully implemented in feedlots (*Weeb*, 2006). Growing demand for foods with potentially beneficial effects on consumer health has motivated increased interest in developing strategies for improving the nutritional quality of beef and producing meat more desirable for the consumers.

This paper reviews nutritional strategies for improving the lipid composition of beef by increasing its content of PUFA.

***Corresponding author:** Mirjana Lukić, *mirjana.lukic@inmes.rs*

Paper received Jun 13th 2023. Paper accepted Jun 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Forages and fatty acid composition of beef

Grass, either fresh or conserved, is usually the cheapest and most important source of cattle feed in temperate climates. Typical ruminant diet mainly consists of carbohydrates, (up to 70% of dry matter). Lipids from grasses and legumes, mainly present in the form glycolipids, are important components of the ruminant diet even though they constitute only small fraction of dry matter (up to 8 %). Forages such as grass and clover contain a high proportion α -linolenic acid (ALA, 18:3n-3) (~70%) and linoleic acid (LA, 18:2n-6) (~20%). In contrast, cereal (maize, barley, sorghum) grain lipids are richer in LA (~60%) compared to ALA (~4%) (*Davis et al*., 2022). Ruminants are generally supplied with unsaturated fatty acids (UFA) from the forage portion of their diet. The findings of available studies conducted in Europe and South America are generally consistent and showed differences in meat FA composition from pasture- and grain-fed animals, and higher PUFA content, especially ALA in pasture-fed groups (*Alfaia et al.*, 2009, *De la Fuente et al.*, 2009, *Leheska et al.*, 2008, *Garcia et al.*, 2008). Pasture-based feeding of beef cattle results in increased ALA content in muscle lipids, but also in increased content long chain n-3 PUFA, eicosapentaenoic acid (EPA 20:5n-3), docosapentaenoic (DPA 22:5n-3), and docosahexaenoic acid (DHA 22:6n-3), due to the elongation and desaturation of ALA in body tissue. *Alfaia et al.* (2009) reported that pasture and feedlot beef contain 21.3 mg and 4.7 mg EPA/100g and 20 mg and 1.1 mg DHA/100g, respectively. Consuming pasture-fed beef could be considered as an adequate method for increasing dietary intake of n-3 PUFA, including EPA, DPA and DHA, in humans (*Butler et al.*, 2021). Higher protection of FA in fresh grass from the ruminal biohydrogenation, relative to that of grain, could be explained by the presence of secondary plant metabolites (PSM) in pasture (*Alfaia et al.*, 2009). Some PSM have the potential to modulate the microbial population in the rumen and modify ruminal fermentation, leading to increased PUFA availability for incorporation into

Table 1. Effect of forage type, oilseed and oil supplementation on the PUFA content of beef muscle (mg/100g muscle)

Diet	C18:2n-6 Linoleic	C18:3n-3 Linolenic	C20:5n-3 EPA	C22:6n-3 DHA	Total PUFA	Reference
Pasture	12.55	5.53	2.13	0.20	28.99	*Alfaia et al.* (2009)
2-Month concentrate after pasture	11.38	1.96	1.28	0.14	19.84	
4-Month concentrate after pasture	10.07	0.84	0.77	0.12	16.31	
Mixed pasture	2.59	1.17	0.54	0.09	6.11	*Duckett et al.* (2013)
Alfalfa	2.85	1.32	0.60	0.10	6.71	
Pearl millet	2.27	1.06	0.49	0.07	5.47	
Oilseed						*Mapiye et al.* (2013)
Hay + flaxseed	3.72	1.09	0.21	0.02	6.59	
Hay + sunflower-seed	4.47	0.49	0.10	0.02	6.70	
Red clover silage + flaxseed	3.73	1.38	0.26	0.03	6.99	
Red clover silage + sunflower-seed	5.17	0.39	0.10	0.02	7.63	
Oil supplementation						*González et al.* (2014)
Concentrate + linseed oil	4.70	1.59	0.44	0.08	9.02	
Concentrate + sunflower oil	5.03	0.55	0.22	0.04	7.75	
Concentrate + soybean oil	4.94	0.75	0.29	0.04	8.12	

animal muscle tissue. It has been reported that the enzyme polyphenol oxidase (PPO), which is present in red clover, may reduce the activity of plant lipases and as a result, reduce ruminal biohydrogenation of ALA. Some other plant species such as chicory, perennial ryegrass and meadow fescue, also contain a range PSM such as tannins, saponins and proanthocyanins, which can enhance protection of PUFA in the rumen and provide higher concentrations of beneficial PUFA that can be incorporated into the meat (*Kearns et al*., 2023). Generally, the impact of fresh grass on n-3 and n-6 PUFA levels in beef depends on plant species, phenological stage, plant maturity and senescence, temperature, light exposure, seasonality and time spent on pasture (*Pethik et al.*, 2021). With regard to the type of forage, *Duckett et al.* 2013 (Table 1**)** reported a difference in the proportion of ALA in muscles from steers that grazed alfalfa, pearl millet or a mixed pasture, with the highest concentration ALA found in steers grazing alfalfa. Moreover, the FA profile of beef from animals fed with botanically diverse pasture shows a higher content of n-3 PUFA than that from animals fed with pure swards (*Kalač*, 2011). The FA composition of intramuscular fat in grass-finished beef significantly improves with increasing duration of the grazing period prior to slaughter. Steers grazing perennial ryegrass for 158 days display higher n-3 PUFA (as a proportion of total FA) and lower n-6:n-3 PUFA ratios, compared to steers grazing perennial ryegrass pasture for 44 days and 99 days (*Noci et al.*, 2005a). Feeding concentrates in the finishing period to animals reared on pasture causes depletion of ALA and higher accretion of LA and a longer finishing period on concentrate, significantly attenuating all beneficial characteristics achieved by grass feeding (*Ponnampalam et al.*, 2006). Roughage conservation methods such as haying or silage production reduce the initial concentrations of antioxidants and PUFA. Consequently, proportions of ALA and long chain n-3 PUFA in meat lipids are higher from the animals fattened on fresh grass (pasture) compared to those fattened on grass silage (*French*, 2000). Feeding with wholecrop wheat silage or maize silage resulted in a low proportion of n-3 PUFA in muscles of finishing cattle, compared with those fed grass silage (*Noci et al.*, 2005b, *Dymnicka et al.*, 2004). Replacing, maize silage with a legume-cereal mixture and lucerne silage increased the concentration ALA in intramuscular fat of bulls by 2.2 times (0.63 and 1.39g/100g FA, respectively) (*Bartoň et al.*, 2010). The high dietary proportion of grass silage improved the beef FA profile, and the proportion valuable long-chain n-3 PUFA, EPA and DHA in intramuscular fat increases linearly with increased grass silage proportion in the beef cattle diet (*Keller et al.*, 2021).

3. Dietary lipid sources and fatty acid composition of beef

Dietary supplementation with oilseed improves the FA composition of beef despite ruminal biohydrogenation, because a proportion of dietary PUFA bypasses the rumen intact and is absorbed and deposited in body fat. The proportion of PUFA in meat increases linearly with PUFA levels in the diet up to 80 g/kg of dry matter intake, above which rumen function is compromised, meaning that the capacity to manipulate the fatty acid composition by use of ruminally-available fatty acids is limited. Many studies (*Marino et al*., 2019, *Fiorentini et al*., 2018, *González et al.*, 2014, *Juárez et al.*, 2011) show that supplementation of bovine diet with oilseeds, such as linseed, soybean, sunflower-seed or their oils, can improve the FA composition of beef. The FA profile of these oilseeds is various, with LA comprising 16–66 g/100 g and ALA 7–54 g/100 g of total FA. Linseed contains the highest proportion of ALA (around 54% of the total FA), and as a consequence, a low n-6:n-3 PUFA ratio. Accordingly, supplementation with linseed or linseed oil (rich in ALA) can increase the concentration of ALA in muscle tissue with desirable decrease in the n-6:n-3 PUFA ratio. Sunflower-seed (rich in LA) can increase the concentration of LA in tissue but with an associated undesirable increase in the n-6:n-3 PUFA ratio (*Scollan et al.*, 2014). In the study by *Mapiye et al.* (2013), steers fed high forage diet, with either grass hay or red clover silage (70% forage: 30% concentrate), in combination with flaxseed had higher proportions of total n-3 PUFA, ALA, EPA and DHA, than steers fed high forage diet in combination with sunflower-seed, and the authors noted the influence of dietary PUFA supplements is strongly influenced by the composition of the basal diet, especially by the amount and type of forages. *Mach et al.* (2006) offered whole canola seed (ALA content 10.6 g/100 g of total FA) or whole linseed (ALA content 54.2 g/100 g of total FA), at three lipid levels (50, 80 and 110 g/kg dry matter) to 54 Holstein bulls and noted that the concentration of n-3 PUFA in the *longissimus dorsi* muscle increased linearly with lipid level. Nevertheless, the n-6:n-3 PUFA ratio was significantly lower in whole linseed sup-

plemented beef (15.6, 8.8 and 6.3), compared to whole canola seed supplemented beef (26.2, 21.0 and 26.4) at increasing levels of supplementation, respectively. Dietary flaxseed elicits substantial changes in the intramuscular beef FA composition (*Kim et al*., 2009). Proportions of PUFA in the intramuscular fat of steers fed 10% and 15% flaxseed were increased by 42.4 % and 57.1% respectively, compared with the control group (*Kim et al.*, 2009). The physical form in which linseed is provided to animals has little to no impact on the FA composition of beef. In a study by *Raes et al*. (2004), the effects feeding of extruded or crushed linseed on intramuscular and subcutaneous FA composition was investigated, and the authors reported that both physical forms of linseed in the finishing diet of young bulls (for approximately 120 days) increased ALA supply equally. Due to costs related to oilseed supplementation, it is useful to evaluate the optimal duration of oilseed inclusion in the bovine diet. The effect of the duration of dietary supplementation with linseed on the level incorporation of n-3 PUFA into beef muscle was reported by *Marino et al.* (2019). Results of this study suggest that supplying 10% linseed for 75 days before slaughter is sufficient for the incorporation of a significant quantity of n-3 PUFA into meat from steers. *González et al.* (2014) (Table 1) compared the effect of supplementation with linseed, sunflower and soybean oils, added at 4.5 % to a commercial concentrate, on the FA composition of young beef cattle. Feeding linseed oil diet led to a doubling Σn-3 PUFA, ALA and DHA compared to feeding sunflower and soybean oil diets. Expressed quantitatively per 170 g of beef serving, this amounts to n-3 PUFA increasing from 52 mg to 110 mg and long-chain PUFA increasing from 21 mg to 39 mg, i.e., to a level that provides 16% of the recommended intake of long-chain n-3 PUFA (250mg EPA+DHA per day) suggested by the European Food Safety Authority (*EFSA*, 2010).

4. Conclusion

Despite biohydrogenation of lipids in the rumen by the ruminal microbiota, diet has a key role in enhancing the FA composition of beef. The most effective method for increasing n-3 PUFA and reducing the n-6:n-3 PUFA ratio in beef fat is inclusion of dietary ingredients that are rich in n-3 PUFA. Feeding fresh or ensiled forages, whole oilseeds or plant oil results in beneficial changes in FA composition and higher nutritional quality of the beef.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, and based on the Contract on the implementation and financing of scientific research work in 2023 (No 451-03-47/2023-01/200050 dated 03.02.2023).

References

Alfaia, C. P. M, Alves, S. P., Martins, S. I. V., Costa, A. S. H., Fontes, C. M. G. A., Lemos, J. P. C., Bessa, R. J. B. & Prates, J. A. M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry*, 114, 939–946.

Bartoň, L., Bureš, D. & Kudrna, V. (2010). Meat quality and fatty acid profile of the musculus longissimus lumborum in Czech Fleckvieh, Charolais and Charolais x Czech Fleckvieh bulls fed different types of silages. *Czech Journal Animal Science*, 55, 479–487.

Butler, G., Mohamed, A. A., Oladokun, S., Wang J. & Davis H. (2021). Forage-fed cattle point the way forward for beef. *Future Foods* 3, 100012, https://doi.org/10.1016/j.fufo.2021.100012

Davis, H., Magistrali, A., Butler, G. & Stergiadis, S. (2022). Nutritional benefits from fatty acids in organic and grass-fed beef. *Foods*, 11, 646.

De la Fuente, J., Díaz, M. T, Álvarez, I., Oliver, M. A., Font i Furnols, M., Sañudo, C., Campo M. M, Montossi, F., Nute G. R. & Cañeque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science*, 82, 331–337.

Duckett S. K., Neel, J. P. S, Lewis, R. M., Fontenot, J. P. & Clapham W. M. (2013). Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. *Journal of Animal Science*, 91, 1454–1467, doi:10.2527/jas2012-5914.

Dymnicka, M., Klupczyński, J., Lozicki, A., Miciński, J. & Strzetelski, J. (2004). Polyunsaturated fatty acids in *M. longissimus thoracis* of fattening bulls fed silage of grass or maize. *Journal Animal Feed Science*, 13, 101–104.

European Food Safety Authority (EFSA), (2010). Panel on dietetic products, nutrition, and allergies (NDA); scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, 8(3), 1461 (Available online: www.efsa. europa.eu).

Fiorentini, G., Santanaa, M. O., Messanaa, J. D. Valentea, A. L. S., Härtera, C. J., Rabeloa, C. H. S, French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., Moloney, A. P., Barberoa R. P., Lannab, D. P. D, Reisa, R. A. & Berchiellia, T. T. (2018). Effect of lipid sources on fatty acid profiles of meat from pasture- and feedlot-finished Nellore bulls. *Livestock Science,* 211, 52–60.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J. & Moloney, A. P. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science*, 78, 2849–2855.

Garcia, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, A. A. & Casa, J. J (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science*, 79, 500–508.

González, L., Moreno, T., Bispo, E., Dugan, M. E. R. & Franco, D. (2014). Effect of supplementing different oils: Linseed, sunflower and soybean, on animal performance, carcass characteristics, meat quality and fatty acid profile of veal from "Rubia Gallega" calves. *Meat Science*, 96, 829–836.

Juárez, M., Dugan, M. E. R., Aalhus, J. L, Aldai, N., Basarab, J. A, Baron, V. S. & McAllister T. A. (2011). Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. *Meat Science*, 88, 434–440.

Kalač, P. (2011). The effects of feeding fresh forage and silage on some nutritional attributes of beef: an overview. *Journal of Agrobiology*, 28(1), 1–13.

Kearns, M., Ponnampalam, E. N., Jacquier, J. C., Grasso, S., Boland, T. M., Sheridan, H. & Monahan, F. J. (2023). Can botanically-diverse pastures positively impact the nutritional and antioxidant composition of ruminant meat? — Invited review. *Meat Science*, 197, 109055.

Keller, M., Reidy, B., Scheurer, A., Eggerschwiler, L., Morel, I. & Giller, K., (2021). Soybean Meal Can Be Replaced by Fava Beans, Pumpkin Seed Cake, Spirulina or Be Completely Omitted in a Forage-Based Diet for Fattening Bulls to Achieve Comparable Performance, Carcass and Meat Quality. *Animals*, 11, 1588, https://doi.org/10.3390/ani11061588

Kim, C., Kim, J., Oh, Y., Park, E., Ahn, G., Lee, G., Lee, J. & Park, K. (2009). Effects of Flaxseed diets on performance, carcass characteristics and fatty acid composition of Hanwoo steers. Asian-Aust. *Journal of Animal Science*, 22(8), 1151–1159.

Leheska, J. M., Thompson, L. D., Howe, J. C., Hentges, E., Boyce, J., Brooks, J. C., Shriver, B., Hoover, L. & Miller M. F. (2008). Effects of conventional and grass-feeding systems on the nutrient composition of beef. *Journal of Animal Science*, 86(12), 3575–3585.

Liu, J., Ellies-Oury, M. P., Stoyanchev, T. & Hocquette, J. F. (2022). Consumer Perception of Beef Quality and How to Control, Improve and Predict It? Focus on Eating Quality. *Foods*, 11, 1732.

Mach, N., Devant, M., Dıaz, I., Font-Furnols, M., Oliver, M. A., Garcıa, J. A. & Bach, A. (2006). Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. *Journal of Animal Science,* 84, 3039–3048.

Mapiye, C., Turner, T. D., Rolland, D. C., Basarab, J. A., Baron, V. S., McAllister, T. A., Block, H. C., Uttaro, B., Aalhus J. L. & Dugan, M. E. R. (2013). Adipose tissue and muscle fatty acid profiles of steers fed red clover silage with and without flaxseed. *Livestock Science,* 151, 11–20.

Marino, R., della Malva, A., Caroprese, M., de Palo, P., Santillo, A., Sevi, A. & Albenzio, M. (2019). Effects of whole linseed supplementation and treatment duration on fatty acid profile and endogenous bioactive compounds of beef muscle. *Animal*, 13(2), 444–452.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B.W., Wallace, J. M. W., Bonham, M. P. & Fearon, A. M. (2010). Red meat consumption: an overview of the risks and benefits. *Meat Science*, 84, 1–13.

Noci, F., Monahan, F. J., French, P. & Moloney, A. P. (2005a). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture fed beef heifers: Influence of the duration of grazing. *Journal of Animal Science,* 83, 1167–1178.

Noci, F., O'Kiely, P., Monahan, F. J., Stanton, C. & Moloney, A. P. (2005b). Conjugated linoleic acid concentration in m. longissimus dorsi from heifers offered sunflower-based concentrates and conserved forages. *Meat Science,* 69, 509–518.

Ponnampalam, E. N., Mann N. J. & Sinclair, A. J., (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and *trans* fatty acids in Australian beef cuts: potential impact on human health. *Asia Pacific Journal Clinical Nutrition*, 15(1), 21–29.

Raes, K., Fievez, V., Chow, T., Ansorena, D., Demeyer, D. & De Smet, S. (2004). Effect of diet and dietary fatty acids on the transformation and incorporation of C18 fatty acids in double-muscled Belgian blue young bulls. *Journal of Agricultural and Food Chemistry*, 52, 6035–6041.

Scollan, N. D, Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, J., Hocquette J-F. & Moloney A. P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, 97, 384–394.

Trbović, D., Lukić, M., Petronijević, R., Lakićević, B., Rašeta, M., Branković Lazić I. & Parunović, N. (2020). Evaluation of n-3 polyunsaturated fatty acid content in various foods: health impact assessment. *Meat Technology*, 61(2), 174 –178.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 614.44:599.32

ID: 126624265

https://doi.org/10.18485/meattech.2023.64.2.35

Review paper

Anticoagulant rodenticides in game meat: a risk to human health

Vladimir Drašković[a], Milica Glišić[a], Ružica Cvetković[a], Radislava Teodorović[a], Ljiljana Janković[a] and Milutin Đorđević[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Rodent pest control
Anticoagulant rodenticides
Non-target animals
Wild game

ABSTRACT

Although rodents are the largest taxonomic groups of all mammals, only about 5% of them are considered pests. Rodent pest control is used to control commensal rodents such as Norway rats (*Rattus norvegicus*), roof rats (*Rattus rattus*), and house mice (*Mus musculus*). Methods used for rodent pest control are: trapping, poisons, habitat management, fertility control, barriers, repellents (acoustic and olfactory), behavioural mechanisms, predators or parasites, control of ectoparasites or pathogens, damage prevention and forecasting, etc. One of the most widespread methods in the world is the application of poisons. The most common are anticoagulant rodenticides, which are divided into first-generation anticoagulant rodenticides and second-generation anticoagulant rodenticides. Considering that anticoagulant rodenticides are indiscriminate and can affect all vertebrates, there is a high risk of unintentional poisoning of non-target wildlife or domesticated animals. Therefore, there is growing concern about the detection of second-generation anticoagulant residues in a large number of animal species. Their accumulation in the environment can cause anticoagulants to transfer along the food chain, causing potentially serious health consequences for wildlife and humans.

1. Introduction

Rodents are one of the largest taxonomic groups of all mammals. Of the 5419 mammalian species, approximately 42%, or 2,277 species, are rodents. They are found on all continents except Antarctica (*Wilson and Reeder*, 2005; *Yu et al.*, 2020). Rodents are highly adaptable animals, with a wide distribution and diverse impacts on the economy, environment, agriculture, food security and safety, biodiversity, public health, etc. (*Capizzi et al.*, 2014; *Jacoblinnert et al.*, 2022). Despite their great species diversity and widespread distribution around the world, only about 5% of rodents are considered pests (*Witmer*, 2018). Rodent pest control is used to control commensal rodents' population, such as Norway rats (*Rattus norvegicus*), roof rats (*Rattus rattus*), and house mice (*Mus musculus*), which represent not only economically important pests, but also a serious public health problem (*Quinn et al.*, 2019). It is estimated that 5% of food produced in the world is eaten or damaged by rodents (*Jurišić et al.*, 2022). In addition, rodents can transmit more than 40 zoonotic pathogens to humans in a variety of ways, both directly and indirectly (*Buckle and Smith*, 2015). The house mouse and roof rat are listed among the 100 World's Worst Invasive Alien Species by the IUCN/ISSG (Invasive Species Specialist Group).

***Corresponding author:** Vladimir Drašković, *vdraskovic@vet.bg.ac.rs*

Paper received August 1st 2023. Paper accepted August 12th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Rodent pest control

Methods used for rodent pest control are: trapping, poisons, habitat management, fertility control, barriers, repellents (acoustic and olfactory), behavioural mechanisms, predators or parasites, control of ectoparasites or pathogens, damage prevention and forecasting, etc. (*Capizzi et al.*, 2014). One of the most widely applied methods for pest control is the use of poisons. Anticoagulant rodenticides are the most commonly used of all poisons, and as of 2017, accounted for more than 95% of rodenticides approved as biocides in the European Union (*Capizzi et al*., 2014; *ECHA*, 2017; *Kotthoff et al.*, 2019).

Many different biocidal products are registered as rodenticides worldwide and classified into three main classes: acute, subacute, and chronic rodenticides. The acute and subacute poisons include compounds such as arsenic, strychnine, zinc phosphide, sodium monofluoroacetate, alphachloralose, thallium sulphate, calciferols, bromethalin and others (*Buckle and Smith*, 2015). Of these, zinc phosphide is still allowed for use in many countries, including in Serbia.

Anticoagulant rodenticides are the most effective and most commonly used biocidal products (*Regnery et al*., 2019). According to *Capizzi et al.* (2014), anticoagulants were used in 61% of cases of pest control. Anticoagulant rodenticides are divided into first-generation anticoagulant rodenticides (FGAR) (i. e. pindone, diphacinone, chlorophacinone, warfarin, and coumatetralyl) and second-generation anticoagulant rodenticides (SGAR) (i. e. difethialone, brodifacoum, bromadiolone, flocoumafen, and difenacoum). FGARs show a lethal effect on consecutive multiple oral intakes, while SGARs are more toxic, and single feeding is often sufficient for a lethal dose (*Fisher et al*., 2019).

Anticoagulant rodenticides inhibit the production of vitamin K by blocking the activity of vitamin K epoxide reductase and subsequently clotting factors (II, VII, IX, and X) involved in the blood coagulation process. Therefore, poisoned animals die from internal haemorrhage within 3 to 7 days (*Buckle and Smith*, 2015; *Damin-Pernik et al.*, 2016).

3. Anticoagulant rodenticides and impact on non-target animals and wild game

Considering that anticoagulant rodenticides are indiscriminate and can affect all vertebrates, there is a high risk of unintentional poisoning of non-target wildlife or domesticated animals (*Regnery et al.,* 2019).

Exposure of wildlife (non-target animals) to anticoagulant rodenticides occurs via three pathways: 1) direct ingestion of rodenticide bait (primary exposure), which is common in herbivores and omnivores because most baits are cereal-based, 2) direct consumption of unabsorbed rodenticides from the digestive tract of prey (secondary exposure), and 3) indirect exposure typically occurs when an animal consumes poisoned prey that carries residual concentrations of anticoagulant (tertiary exposure) (*Morzillo and Mertig*, 2011, *Regnery et al.,* 2019).

All SGARs, due to their high risk to human health and the environment, have been identified as being either persistent, bioaccumulative, and toxic or very persistent and very toxic (*Kotthoff et al.*, 2019), hence have a higher risk of poisoning non-target animals in comparison to the FGARs (*Fisher et al.*, 2019).

Widespread use of anticoagulant rodenticides can lead to the accumulation of anticoagulants in the environment, and consequently, poisoning and accumulation of anticoagulant rodenticide residues in non-target terrestrial and aquatic species. Furthermore, the accumulation of anticoagulants in the environment can lead to their transfer along the food chain, with potentially serious health consequences for wildlife and humans (*Regnery et al*., 2019). Therefore, there is growing concern about the detection of second-generation anticoagulant residues in different tissues of a large number of animal species, e.g., barn owls (*Geduhn et al*., 2016), foxes (*Geduhn et al.*, 2015), hedgehogs (*Dowding et al*., 2010) and snails (*Alomar et al.*, 2018), including game, e.g., black bear, wild pigs (*McMillin et al*., 2018) and white-tailed deer (*Stone et al*., 1999). Considering the abovementioned issues, these residues pose a potential danger to human health.

SGARs have liver half-lives of >80 to 350 days and are typically used only for rodent pest control (*Erickson and Urban*, 2004, *McMillin et al*., 2018). SGARs contain two asymmetric carbons in their chemical structure and each is a mixture of four stereoisomers assembled into two pairs of diastereoisomers (*cis*-diastereoisomers or *trans*-diastereoisomers), each pair containing two (1R,3R) (1S,3S)-isomers and (1R,3S) (1S,3R)-isomers in different proportions, with different pharmacokinetic properties and biological activities. There is always one diastereoisomeric form with a shorter half-life than the other one, so the risk of secondary poisoning in predators can differ between isomers (*Lefebvre et al*., 2017; *Alabau et al.*, 2020).

Wild animals can become exposed to rodenticides in urban, suburban and agricultural areas where the use of rodenticides against commen-

sal rodents is continuous, or in farmland areas where the use of rodenticides is periodically intensive (*López-Perea et al*., 2018). However, intensive use of rodenticides in the environment can lead to long-term chronic accumulation of SGAR in predators or can cause fatal poisonings in many different species of non-target animals with secondary poisoning in predators (*Olea et al*., 2009).

Game animals are also at risk of exposure to SGARs, especially omnivorous species, such as wild boar (*Sus scrofa*), by the direct ingestion of rodenticide baits or by the consumption of carcasses of animals poisoned by rodenticides (*Alabau et al*., 2020).

Alabau et al. (2020) indicated a high prevalence of rodenticides liver and muscle of wild boars in urban areas (60.8%), suburban areas (40%) and rural areas (7.7%). These results showed a positive relationship between the presence of SGAR residues in wildlife and human population density, most likely because of the intensive use of rodenticides for rodent pest control in urban areas, consequently leading to long-term chronic accumulation of rodenticides in wildlife. This indicates a potential risk to game meat consumers. Namely, although rodenticide doses are low, wild game in urban areas could have much higher concentrations of rodenticides in meat and other organs, which would increase the risk to human health (*Alabau et al*., 2020).

4. Anticoagulant rodenticides in wild game edible tissues and risk to human health

The use of anticoagulants. primarily warfarin and diphacinone, as antithrombotic therapy in humans is well known. However, problems regarding involuntary exposure can occur when anticoagulants enter the food chain, primarily through foods of animal origin. One example is the presence of anticoagulant residues in game meat, which is a potential hazard to human health, primarily in regions where game meat is often consumed *(López-Perea et al*., 2018).

Game meat has exceptional nutritional value, low fat content, and good digestibility. The skin of game animals is lighter and thinner than that of domestic animals, resulting in a higher meat yield in the total carcass weight. For example, the amount of meat in roe deer, European deer, and mouflon ranges from 55 to 70%. The percentage of fat in meat differs between the different types of game: from 3.85% in deer thigh meat to 0.98% in pheasant breast meat (*Zakula*, 1976).

Since game meat is not widely available to the public, it does not occupy a significant place in the diet, except for hunters. According to data from 2021, there are over 87,500 hunters in Serbia (*Anon*, 2022). The average game meat consumption per capita in Serbia is 0.14 kg, whereas per hunter, it is 17.22 kg. This consumption is much lower compared to other European countries, where Austria has the highest average game meat consumption per capita (1.21 kg), while Hungary has the highest consumption per hunter (146.86 kg).

Table 1 shows the ten-year average of planned and executed hunting of the most important big game in Serbia (*Anon*, 2022). Based on this data, it has been estimated that the production of game meat per 100 ha in Serbia is similar to in Croatia, averaging around 15.65 kg. However, this is more than seven times less than the production of game meat in Austria, over five times less than in the Czech Republic, Hungary, and Germany, and more than half of that in Slovakia (*Anon*, 2021).

Table 1. The number of animals of the four big-game species in Serbia (from 2011–2021) according the planned and executed game shooting (Anon, 2022)

Year	European deer	European fallow deer	Roe deer	Wild boar	European deer	European fallow deer	Roe deer	Wild boar
	Planned game shooting				Executed game shooting			
2011	823	169	12824	8046	653	114	8039	4962
2013	1122	351	14017	10365	870	182	8529	6475
2015	1366	309	15683	11023	1035	99	9279	7775
2017	1243	168	16962	13939	856	85	10544	11179
2019	1145	293	17689	15942	813	229	10484	12919
2021	1429	246	19503	20560	984	188	11454	15228

In the study by *Eason et al.* (1999, 2001), the accumulation of brodifacoum in various tissues of different wild game is reported. Namely, the brodifacoum concentration in wild boar was in the range from 0.007 to 1.7 mg/kg in the liver, and from 0.01 to 0.07 mg/kg in muscle; in red deer (*Cervus elaphus*) the concentration was up to 0.02 mg/kg in muscle and 0.03 mg/kg in liver; and in goat (*Capra hircus*) the brodifacoum concentration was up to 0.01 mg/kg in liver. *McMillin et al.* (2018) demonstrated that in the black bear (*Ursus americanus*) liver, the concentration of residual anticoagulants was 8.7 mg/kg. The Authors indicated that at such a high concentration of rodenticide, a 60 kg human would need to consume 2.68 kg of liver to reach mammalian LD_{50} values (0.39 mg/kg body weight for rats) of brodifacoum.

Research conducted by *Pitt et al.* (2011) found that cooking has an impact on diphacinone residues concentration in meat, thereby increasing its potential hazard to human health. In that study, the concentration of diphacinone increased in all tissues after cooking, which could indicate that water loss during heating tended to concentrate diphacinone in tissues.

Eisemann and Swift (2006) indicated the hazards of maximum concentrations of diphacinone residues in pig muscle (0.25 mg/kg), pig liver (3.07 mg/kg) and game liver (0.56 mg/kg). They reported that a 55 kg person would have to eat 28.49 kg of pork meat, 2.33 kg of pork liver or 12.77 kg of game liver to reach a dose of diphacinone equivalent to that affecting blood clotting in rats, while pregnant women of the same weight (55 kg) would have to ingest 5.50 kg, 0.45 kg or 2.46 kg, respectively, for the amount of diphacinone equivalent to the dose that has been shown to cause foetal reabsorption in rats.

Although it seems unlikely that all this could happen in one day, the risk is reflected in the facts that some SGARs are highly accumulative and that repeated exposure increases the risk of adverse effects of anticoagulants, and people who use antithrombotic therapy should be especially careful (*López-Perea et al.*, 2018).

5. Conclusion

Anticoagulant rodenticides have been used for more than half a century and today are the most commonly used biocides for rodent pest control. Widespread use of these poisons can lead to poisoning and accumulation of anticoagulant rodenticide residues in the environment and wild animals. Anticoagulant rodenticides can occur in the environment due to several pathways: during the production of the active substance, the formulation of the biocidal product, the application of baits, and the disposal of baits. Entry of rodenticides into the food chain is a big risk, which can potentially lead to serious problems for human health. Although anticoagulant residues were found only in low levels in wild game tissues, these chemicals can potentially affect human health. Given the seriousness of the problem likely in the future, more research is needed to properly deal with anticoagulant residues in game meat.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Alabau, E., Mentaberre, G., Camarero, P. R., Castillo-Contreras, R., Sánchez-Barbudo, I. S., Conejero, C., Fernández-Bocharán, M. S., López-Olvera, J. R. & Mateo, R. (2020). Accumulation of diastereomers of anticoagulant rodenticides in wild boar from suburban areas: Implications for human consumers. *Science of the Total Environment*, 738, 139828, https://doi.org/10.1016/j.scitotenv.2020.139828

Alomar, H., Chabert, A., Coeurdassier, M., Vey, D. & Berny, P. (2018). Accumulation of anticoagulant rodenticides (chlorophacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*. *Science of the Total Environment*, 610, 576–582, https://doi.org/10.1016/j.scitotenv.2017.08.117

Anon, (2021), https://www.lorist.co.rs/znacaj-mesa-divljaci-u-ishrani-ljudi/ (Assessed on July 31, 2023).

Anon, (2022). Statistical Yearbook of the Republic of Serbia, Statistical Office of the Republic of Serbia, Belgrade, https://data.stat.gov.rs/Home/Result/13040601?languageCode=sr-Latn&displayMode=table&guid=f64a952d-6d44-4cd1-9787-775c1543c09a

Buckle, A. & Smith, R. (2015). Rodent pests and their control (2nd ed.). CAB International.

Capizzi, D., Bertolino, S. & Mortelliti, A. (2014). Rating the rat: global patterns and research priorities in impacts and management of rodent pests. *Mammal Review*, 44(2), 148–162, https://doi.org/10.1111/mam.12019

Damin-Pernik, M., Espana, B., Lefebvre, S., Fourel, I., Caruel, H., Benoit, E. & Lattard, V. (2016). Management of rodent populations by anticoagulant rodenticides: toward third-generation anticoagulant rodenticides. *Drug Metabolism and Disposition*, 45(2), 160–165, https://doi.org/10.1124/dmd.116.073791

Dowding, C. V., Shore, R. F., Worgan, A., Baker, P. J. & Harris, S. (2010). Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). *Environmental Pollution*, 158(1), 161–166, https://doi.org/10.1016/j.envpol.2009.07.017

Eason, C. T., Milne, L., Potts, M., Morriss, G., Wright, G. R. G. & Sutherland, O. R. W. (1999). Secondary and tertiary poisoning risks associated with brodifacoum. *New Zealand Journal of Ecology*, 23(2), 219–224, http://www.jstor.org/stable/24054775

Eason, C. T., Wright, G. R. G., Milne, L. M. & Morriss, G. A. (2001). Laboratory and field studies of brodifacoum residues in relation to risk of exposure to wildlife and people. *Science for Conservation*, 177, 11–23.

ECHA, (2017). Biocidal products R4BP3. European_Chemicals_ Agency, Helsinki, Finland. Retrieved from https://echa.europa.eu/information-on-chemicals/biocidal-products

Eisemann, J. D. & Swift, C. E. (2006). Ecological and human health hazards from broadcast application of 0.005% diphacinone rodenticide baits in native Hawaiian ecosystems. In Proceedings of the 22nd Vertebrate Pest Conference https://doi.org/10.5070/V422110064

Fisher, P., Campbell, K. J., Howald, G. R. & Warburton, B. (2019). Anticoagulant rodenticides, islands, and animal welfare accountancy. *Animals*, 9(11), 919, https://doi.org/10.3390/ani9110919

Geduhn, A., Esther, A., Schenke, D., Gabriel, D. & Jacob, J. (2016). Prey composition modulates exposure risk to anticoagulant rodenticides in a sentinel predator, the barn owl. *Science of the Total Environment*, 544, 150–157, https://doi.org/10.1016/j.scitotenv.2015.11.117

Geduhn, A., Jacob, J., Schenke, D., Keller, B., Kleinschmidt, S. & Esther, A. (2015). Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes). PLoS One*, 10(9), Article e0139191, https://doi.org/10.1371/journal.pone.0139191

Jacoblinnert, K., Jacob, J., Zhang, Z. & Hinds, L. A. (2022). The status of fertility control for rodents—recent achievements and future directions. *Integrative Zoology*, 17(6), 964–980, https://doi.org/10.1111/1749-4877.12588

Jurišić, A., Ćupina, A. I., Kavran, M., Potkonjak, A., Ivanović, I., Bjelić-Čabrilo, O., Meseldžija, M., Dudić, M., Poljaković-Pajnik, L. & Vasić, V. (2022). Surveillance strategies of rodents in agroecosystems, forestry and urban environments. *Sustainability*, 14(15), 9233, https://doi.org/10.3390/su14159233

Kotthoff, M., Rüdel, H., Jürling, H., Severin, K., Hennecke, S., Friesen, A. & Koschorreck, J. (2019). First evidence of anticoagulant rodenticides in fish and suspended particulate matter: spatial and temporal distribution in German freshwater aquatic systems. *Environmental Science and Pollution Research*, 26, 7315–7325, https://doi.org/10.1007/s11356-018-1385-8

Lefebvre, S., Fourel, I., Queffélec, S., Vodovar, D., Mégarbane, B., Benoit, E., Siguret, V. & Lattard, V. (2017). Poisoning by anticoagulant rodenticides in humans and animals: causes and consequences. In Malangu, N. (Ed.), Poisoning-From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis, 21. (Chapter 2) http://dx.doi.org/10.5772/intechopen.69955

López-Perea, J. J. & Mateo, R. (2018). Secondary exposure to anticoagulant rodenticides and effects on predators. In Van den Brink, N. W., Elliott, J. E., Shore, R. F. & Rattner, B. A (Eds.), Anticoagulant rodenticides and wildlife (pp. 159–193), https://doi.org/10.1007/978-3-319-64377-9_7

McMillin, S. C., Poppenga, R. H., Chandler, S. C. & Clifford, D. L. (2018). Anticoagulant Rodenticide Residues in Game Animals in California. Proceedings of the 28th Vertebrate Pest Conference.

Morzillo, A. T. & Mertig, A. G. (2011). Urban resident attitudes toward rodents, rodent control products, and environmental effects. *Urban Ecosystems*, 14, 243–260, https://doi.org/10.1007/s11252-010-0152-5

Olea, P. P., Sánchez-Barbudo, I. S., Viñuela, J., Barja, I., Mateo-Tomás, P., Pineiro, A. N. A., Mateo, R. & Purroy, F. J. (2009). Lack of scientific evidence and precautionary principle in massive release of rodenticides threatens biodiversity: old lessons need new reflections. *Environmental Conservation*, 36(1), 1–4, https://doi.org/10.1017/S0376892909005323

Pitt, W. C., Higashi, M. & Primus, T. M. (2011). The effect of cooking on diphacinone residues related to human consumption of feral pig tissues. *Food and Chemical Toxicology*, 49(9), 2030–2034, https://doi.org/10.1016/j.fct.2011.05.014

Quinn, N., Kenmuir, S. & Krueger, L. (2019). A California without rodenticides: challenges for commensal rodent management in the future. *Human–Wildlife Interactions*, 13(2), 8, https://doi.org/10.26077/gegq-dg52

Regnery, J., Friesen, A., Geduhn, A., Göckener, B., Kotthoff, M., Parrhysius, P., Petersohn, E., Reifferscheid, G., Schmolz, E., Schulz, R.S., Schwarzbauer, J. & Brinke, M. (2019). Rating the risks of anticoagulant rodenticides in the aquatic environment: a review. *Environmental Chemistry Letters*, 17, 215–240, https://doi.org/10.1007/s10311-018-0788-6

Stone, W. B., Okoniewski, J. C. & Stedelin, J. R. (1999). Poisoning of wildlife with anticoagulant rodenticides in New York. *Journal of Wildlife Diseases*, 35(2), 187–193, https://doi.org/10.7589/0090-3558-35.2.187

Wilson D. & Reeder D. (2005). Mammal Species of the World a Taxonomic and Geographic Reference (3rd ed.) The Johns Hopkins University Press; Baltimore, MA: Johns Hopkins University Press, Baltimore, Maryland, USA, 745–753.

Witmer, G. W. (2018). Perspectives on existing and potential new alternatives to anticoagulant rodenticides and the implications for integrated pest management. In Van den Brink, N. W., Elliott, J. E., Shore, R. F. & Rattner, B. A (Eds.), Anticoagulant rodenticides and wildlife (pp. 357–378), https://doi.org/10.1007/978-3-319-64377-9_13

Yu, Z. M., Chen, J. T., Qin, J., Guo, J. J., Li, K., Xu, Q. Y., Wang, W., Lu, M., Qin, X.C. & Zhang, Y. Z. (2020). Identification and characterization of Jingmen tick virus in rodents from Xinjiang, China. *Infection, Genetics and Evolution*, 84, 104411, https://doi.org/10.1016/j.meegid.2020.104411

Zakula, R. (1976). Game as a protein source. *Hrana i Ishrana*, 17, 543–548.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 616.036-002
614:612.392.8

ID: 126627849

https://doi.org/10.18485/meattech.2023.64.2.36

Review paper

Reviewing the current situation and opinions of the hepatitis E virus among natural reservoirs and through the food chain

Lazar Milojević[a], Branko Velebit[a] and Nikola Betić[a]*

[a] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040, Belgrade, Republic of Serbia

ARTICLE INFO

Keywords:
Hepatitis E virus
Foodborne viruses
Pigs

ABSTRACT

Viral foodborne diseases have grown to be an important part of all foodborne illnesses that have been observed in recent years, and they are considered an increasing threat to the public's health. An increase in hepatitis E virus (HEV) cases that are not related to travel has been identified in surveillance studies carried out in developed countries in the EU. Hepatitis E genotype 3 virus was primarily associated with eating undercooked or uncooked pig meat or other wild animals, according to the research. The main reservoirs in nature are domestic pigs and wild boars. The main route of infection involves the fact that pigs infected with HEV enter the slaughterhouse as healthy animals (most often they do not have visual symptoms), and so their tissues and meat are used for human consumption. Currently, adequate heat treatment is the most effective way to prevent HEV infection from contaminating meat, liver and meat products. This virus is an ideal pathogen for full implementation of the One Health approach, where it is necessary to communicate, collaborate and coordinate in all sectors, including human health, animal health, environment, and other areas of expertise. Success in implementing the One Health approach would lead to controlling all links in this complicated chain and achieving the best health outcomes.

1. Introduction

Over the past few decades, the food business market has shifted from being locally oriented to being globally based as a result of growing urbanization and altered eating patterns. A rise in the population of middle-class people has been accompanied by an increase in the demand for meat in some parts of the world, including China, Southeast Asia, India, and Africa. As livestock production and international trade increase to meet demand, the risk of exposure to various foodborne hazards rises as well because they can spread along the global food supply chain that creates possibilities for local foodborne occurrences to travel globally (*FAO*, 2022). The global food supply may be vulnerable if there is a flaw in the process, as contamination can happen with pathogens from throughout the world, including those that have recently emerged (*Lord et al.*, 2022). Regardless of the fact that contamination prevention and control strategies are generally successful in reducing foodborne diseases, diverse and complex food systems, especially those located in less developed nations, pose a big challenge.

According to *De Aceituno et al.* (2013), noroviruses, a type of foodborne virus, are the prima-

***Corresponding author:** Lazar Milojević, *lazar.milojevic@inmes.rs*

Paper received July 21st 2023. Paper accepted August 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ry source of foodborne illness in developed countries. However, across the European Union (EU) and Asia, the prevalence of recently identified foodborne viruses like the hepatitis E virus (HEV) has increased (*Ruggeri et al*., 2013). On a worldwide basis, it is estimated that 939 million people, or 1 in 8, have had an infection caused by HEV (*Li et al*., 2020). In contrast to norovirus and hepatitis A virus, this virus is zoonotic and is considered as a growing issue in veterinary public health (*Meng et al*., 1997).

The single-stranded, positive-sense RNA genome of the small, non-enveloped icosahedral HEV is about 7.2 kb in size. Short untranslated areas and three main open reading frames (ORF1, ORF2, and ORF3) make up the RNA. HEV belongs to the Orthohepevirus genus and is a member of the Hepeviridae family (Smith et al, 2014). Furthermore, this genus is classified into four species, from Orthohepevirus A to Orthohepevirus D. The most explored, Orthohepevirus A, is further divided into 7 genotypes, and only genotypes 1–4 are capable of infecting people (*Doceul et al*., 2016). Genotypes 3 and 4 have been discovered in different animal species, and they are zoonotic. On the other hand, genotypes 1 and 2 most frequently infect people via water. Among people, HEV infection can result in a wide spectrum of symptoms (*Lhomme et al*., 2020), from asymptomatic to self-limiting icteric hepatitis and even permanent liver damage. Immunocompromised patients and those with underlying liver disease are more likely to develop serious or chronic illness after infection. Mortality is rare, happening in only about 2% of cases (*Park et al*., 2016).

Genotypes 3 and 4 can infect people via a number of different pathways. However, intake of HEV-contaminated animal tissues and food products is the most significant method of transmission (*Park et al.*, 2016). HEV infection can also develop through transplantation of organs or a blood transfusion. Genotypes 3 and 4 are capable of infecting a variety of animals, but nevertheless, the primary animal reservoirs for these genotypes are the domestic pig (*Sus scrofa domesticus*) and wild boar (*Sus scrofa*) (*Ricci et al*., 2017; *Caruso et al.*, 2016).

Faecal-oral transmission is the main way of transmitting HEV infection in pigs, and they are usually infected without showing any clinical symptoms, although some pigs develop mild to moderate acute self-limiting hepatitis that may progress to permanent liver damage (*Meng*, 2010). The virus in pigs is primarily present in the liver, where it replicates. However, it has been found that the virus can be present in other pig tissues. Pork liver and pork liver products are the most evident cause of foodborne HEV, in accordance with several research studies. Pigs infected with HEV enter the slaughterhouse as healthy animals (they do not have visual symptoms), and so their tissues and meat, used for human consumption, can pose a high risk of foodborne HEV transmission.

2. Presence of HEV in domestic pigs

2.1. HEV seroprevalence

According to *Meng et al.* (1997), there were farms with detected HEV antibodies (Ab) in all tested adult pigs. In Europe, HEV seroprevalence has been at various levels, but all studies reported a measurable presence of HEV Ab in pigs. In France, it was discovered that out of 186 randomly selected farms, 65% of them had seropositive animals (*Rose et al.*, 2011). The results were even more dramatic in Norway and Spain. In Norway, 90% of farms had pigs with HEV Ab (Ig-G) (Lange et al., 2016), while in Spain, 204 out of 208 farms had seropositive pigs (*Casas et al.*, 2009). In Denmark and the Netherlands, seroprevalence at farm level was about 55% (*Breum et al.*, 2010; *Rutjes et al.*, 2007). In their research, *Steyer et al.* (2011) reported HEV seroprevalence of farms was 33.3% in Slovenia. According to the results of *Asimoula et al.* (2009), all tested farms in the territory of northern Greece had pigs positive for anti-HEV Ab.

On the other hand, on an individual level, HEV seroprevalence depends on the age of the examined pigs as well as the territory. Consequently, *Meng et al.* (1997), during their investigation, observed that the infection begins after the second month of life in piglets and that the seroprevalence is significantly higher in pigs older than 4 months. *Salines et al.* (2017), in their comprehensive review, presented data showing that pig seroprevalence (45 analysed studies) ranges from 8 to 93%. A large retrospective study in Spain for the period from 1985 to 1997 showed that among 2,871 examined pigs, the presence of HEV Ab was recorded in 48.45% (Casas et al., 2009). In Croatia, according to *Jermisic et al.* (2017), the pig seroprevalence was 32.94%. *Tsachev et al.* (2019) published the results of a study of HEV seroprevalence in pigs in Bulgaria, according to which the overall prevalence was 60.3% and that it varied between regions and categories of examined pigs. They concluded that the highest seroprevalence

was in the sow category (80%). In Serbia, in Vojvodina, the HEV seroprevalence was 34.6%, according to *Lupulovic et al.* (2010). Furthermore, it fluctuated between different municipalities and ranged from 16.7% to 75%.

2.2. HEV prevalence

The potential presence of viral RNA can be examined in different types of animal samples (serum, faeces, liver, meat, and intestinal organs) using the reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR method. If seroprevalence is observed at the farm level, according to the data of *Salines et al.* (2017) and based on the comprehensive results of 25 analysed studies, it was determined that the prevalence of the virus in the animals ranges from 10% to 100%. *Fernández-Barredo et al.* (2007), in their research in Spain from 2002 to 2004, came to the conclusion that in 131 samples of faeces and serum, viral RNA was present in 76% of farms. Similar results were published in Sweden by *Widen et al.* (2011), who examined the presence of HEV in faeces samples. Furthermore, 40% of the samples from the 30 farms in Hungary that *Reuter et al.* (2009) examined were HEV positive. The results reported by *Forgách et al.* (2010) (39% of animals were the viral-RNA positive) provided additional evidence of the reliability of previous findings. In Serbia, *Petrovic et al.* (2008) published preliminary findings showing that HEV infection was found in 4 of the 5 farms investigated. They analysed samples were pig organ tissue and faeces from different locations.

When we observe HEV prevalence on an individual level, we can conclude that it fluctuates greatly. Consequently, *Salinas et al.* (2017), based on the results of a comprehensive analysis (which included 69 analysed studies), concluded that HEV prevalence in animals can range from 1% to 89%. Furthermore, the sample origin does not have an impact on the HEV prevalence; it oscillates greatly among all examined sample categories (blood, faeces and serum). Therefore, HEV prevalence in faeces samples from European nations fluctuated between 2.5 and 87.5% (*Widén et al*., 2011; *Kantala et al.*, 2015; *Monini et al.*, 2015). Furthermore, HEV prevalence in sera samples ranged from 0.9% to 45% (*Grierson et al*., 2015; *Ivanova et al*., 2015). Among liver samples analysed in Europe, the HEV prevalence ranged from 0% to 75%, depending on the country (*Jori et al.*, 2016; *Lainšček et al.*, 2017; *Feurer et al.*, 2018).

On the other hand, when samples were classified according to age category, the most commonly examined categories were younger than 3 months and older than 6 months. In the category older than 6 months, HEV prevalence was significantly lower than in the younger animals. So, in Serbia, there was no detectable presence of HEV in liver samples from pigs older than 6 months (*Milojević et al*., 2019), while in Slovenia, HEV prevalence in this age group was 0.25% according to *Lainšček et al.* (2017). *Kantala et al.* (2017) also did not establish the presence of HEV in this age category. According to available data, the prevalence in pigs older than 6 months in France was 2.8%, according to *Feurer et al.* (2018). A similar percentage of positive results was obtained in Germany (4.0%) as published by *Wenzel et al.* (2011). Differently from that, in the age category younger than 3 months, HEV prevalence is significantly higher. According to *Milojević et al.* (2019), HEV prevalence was 34 % in this age group in Serbia. Similar results were published by *Forgách et al.* (2010) in Hungary, where 36% of tested samples from the < 3 month category were HEV-positive. In Italy, HEV prevalence in this age group was 30% (*Ruggeri et al*., 2013). The high percentage of HEV-positive pigs in this age category noted in various studies confirms the hypothesis that piglets are the most vulnerable after 2 months; at this time, the period of passive immunity expires.

3. Presence of HEV in wild boars and deer

Aside from domestic pigs, wild boars and deer are considered to be natural reservoirs of this virus. Therefore, plenty of studies have examined the presence of HEV in various tissues of these wild animals. In Hungary, the presence of HEV was detected in 11% of the wild boar, 22% of roe deer and 10% of deer (*Forgách et al*., 2010) *Jemeršić et al.* (2017), in their comprehensive study, revealed that the total seroprevalence among wild boars in the territory of Croatia was 31.1%, while among different regions it ranged from 7.7% to 50.6%. HEV RNA was detected in 11.33% of wild boars younger than one year old. During the 2013–2015 hunting seasons, 50 samples of wild boar origin were collected in Romania. They were tested for the presence of HEV RNA and the results showed that nine samples (18%) were positive (*Porea et al.*, 2017). In contrast, in Slovenia, the seroprevalence in 288 examined sera was 30.2%, while RNA originating from HEV was detected in only one sample (*Žele et al.*,

2016). As a result of all these studies, it is concluded that wild boars and deer can contribute to a significant transmission pathway for this virus, particularly in areas where game meat consumption is high.

4. Presence of HEV in food

Various studies have confirmed that food is the main route of HEV infection for people in developed countries. Thus, one of the first recorded and proven cases among people suffering from acute hepatitis was in Japan (*Yazaki et al.*, 2003). The infections were caused by consuming the raw liver of domestic animals, as well as the meat of wild pigs. After that, cases of human HEV disease were recorded and confirmed after consuming thermally insufficiently treated food originating from domestic, wild pigs and deer. In France, *Renou et al.* (2014) confirmed that the hepatitis in sick people was caused by HEV and that the source of the infection was the traditional Corsican figatelli sausages. Furthermore, more human cases of acute hepatitis caused by HEV have been reported in France, and they have been linked to the consumption of the same sausage, but the source has not been completely confirmed (*Pavio et al.*, 2014). According to *Said et al.* (2013), sausage and ham purchased from supermarkets were the sources in the development of HEV infections in Great Britain. Similar to this, HEV was identified in one patient in Hungary and was associated with the previous eating of sausage (*Reuter et al.*, 2009). On the other hand, eating wild boar meat has been linked to autochthonous human HEV infections in Germany (*Wichmann et al.*, 2008). Researchers in Spain confirmed cases of hepatitis in people infected by consuming cooked pork (piglet meat) or wild boar meat (*Riveiro-Barciela et al.*, 2015; *Rivero-Juarez et al.*, 2017). Further analyses showed a very high percentage similarity of the analysed RNA sequences originating from HEV isolated from food and from sick patients (from 99.7% to 100%).

Examinations have focused on the possible presence at retail of HEV in consumer products. The presence of HEV in these products, especially ready-to-eat products, might represent a high risk for consumers (vulnerable categories are the most threatened). In Serbia, *Milojevic et al.* (2021) discovered a HEV prevalence of 5% in pig liver samples from retail establishments. Similar occurrences were reported in Germany and Netherlands where respective HEV prevalences were 4% and 6.5% (*Bouwknegtet al.*, 2007; *Wenzel et al.*, 2011). On the other hand, the HEV prevalence in ready-to-eat sausage was 14.6% in the Netherlands, according to *Boxman et al.* (2020). According to *Pallerla et al.* (2020), in Germany, 10% of the tested samples (liver products and pork meat products) were positive for HEV presence, while in Switzerland the prevalence of HEV was 18.9% in the sausages containing liver tissue (*Moor et al.*, 2018). *Di Bartolo et al.* (2015) collected samples of sausages containing liver tissue and examined the potential presence of HEV from grocery stores in Italy. The prevalence of HEV in fresh sausages containing liver tissue was 22.2%, while in dried sausages it was 4.3%. All the studies confirmed that food could present a very important source of HEV. Further research should establish the exact infectious dose of HEV, with the ultimate goal of obtaining definitive answers for risk analysis of HEV infection through contaminated food.

5. Conclusion

HEV and its transmission via food has been established as a growing hazard to people's health. However, since the infection shares general symptoms with other diseases (fatigue, nausea, vomiting, diarrhoea and stomach cramps), symptoms alone do not allow for fast and proper causative agent identification in most cases. The ingestion of raw or uncooked liver or meat products containing liver has been identified as the main route in the transmission HEV. Adequate heat treatment of food is the most effective way to prevent HEV infection from contaminating meat, liver and meat products. Successful implementation of the One Health approach would lead to controlling all links in this complicated chain and achieving the best health outcomes.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The Ministry of Science, Technological Development, and Innovation, Republic of Serbia (Grant No. 451-03-47/2023-01/200050, from 03.02.2023) financially supported this paper.

References

Asimoula, S., Elena, T., Alexopoulos, C., Kyriakis, S. C. & Froesner, G. (2009). First report of serological evidence of hepatitis E virus infection in swine in northern Greece. *Acta Veterinaria*, 59(2–3), 205–211, https://doi.org/10.2298/avb0903205s

Bouwknegt, M., Engel, B., Herremans, M. M. P. T., Widdowson, M. A., Worm, H. C., Koopmans, M. P. G., Frankena, K., De RodaHusman, A. M., De Jong, M. C. M. & Van Der Poel, W. H. M. (2007). Bayesian estimation of hepatitis E virus seroprevalence for populations with different exposure levels to swine in The Netherlands. *Epidemiology and Infection*, 136(4), 567–576, https://doi.org/10.1017/s0950268807008941.

Boxman, I. L. A., Jansen, C. C. C., Zwartkruis-Nahuis, A. J. T., Hägele, G., Sosef, N. P. & Dirks, R. A. M. (2020). Detection and quantification of hepatitis E virus RNA in ready to eat raw pork sausages in the Netherlands. *International Journal of Food Microbiology*, 333, 108791, https://doi.org/10.1016/j.ijfoodmicro.2020.108791.

Breum, S. Ø., Hjulsager, C. K., de Deus, N., Segalés, J. & Larsen, L. E. (2010). Hepatitis E virus is highly prevalent in the Danish pig population. *Veterinary Microbiology*, 146(1–2), 144–149, https://doi.org/10.1016/j.vetmic.2010.05.002

Caruso, C., Peletto, S., Rosamilia, A., Modesto, P., Chiavacci, L., Sona, B., Balsamelli, F., Ghisetti, V., Acutis, P. L., Pezzoni, G., Brocchi, E., Vitale, N. & Masoero, L. (2016). Hepatitis E Virus: A Cross-Sectional Serological and Virological Study in Pigs and Humans at Zoonotic Risk within a High-Density Pig Farming Area. *Transboundary and Emerging Diseases*, 64(5), 1443–1453, Portico, https://doi.org/10.1111/tbed.12533.

Casas, M., Pujols, J., Rosell, R., de Deus, N., Peralta, B., Pina, S., Casal, J. & Martín, M. (2009). Retrospective serological study on hepatitis E infection in pigs from 1985 to 1997 in Spain. *Veterinary Microbiology*, 135(3–4), 248–252, https://doi.org/10.1016/j.vetmic.2008.09.075

De Aceituno, A. M. F., Rocks, J. J., Jaykus, L. A. & Leon, J. S. (2013). Foodborne viruses. In: Guide to Foodborne Pathogens, 2nd Edn, eds R. G. Labbé and S. García. (Oxford: John Wiley and Sons, Ltd.), 352–376.

Doceul, V., Bagdassarian, E., Demange, A. & Pavio, N. (2016). Zoonotic Hepatitis E Virus: Classification, Animal Reservoirs and Transmission Routes. *Viruses*, 8(10), 270, https://doi.org/10.3390/v8100270.

FAO, **(2022).** Thinking about the future of food safety. A foresight report. Rome, https://www.fao.org/3/cb8667en/cb8667en (accessed on 27 April 2023).

Fernández-Barredo, S., Galiana, C., García, A., Gomez-Muñoz, M. T., Vega, S., Rodríguez-Iglesias, M. A. & Pérez-Gracia, M. T. (2007). Prevalence and genetic characterization of hepatitis E virus in paired samples of feces and serum from naturally infected pigs. *Canadian Journal of Veterinary Research,* 71, 236–240.

Feurer, C., Le Roux, A., Rossel, R., Barnaud, E., Dumarest, M., Garry, P. & Pavio, N. (2018). High load of hepatitis E viral RNA in pork livers but absence in pork muscle at French slaughterhouses. *International Journal of Food Microbiology*, 264, 25–30, https://doi.org/10.1016/j.ijfoodmicro.2017.10.013.

Forgách, P., Nowotny, N., Erdélyi, K., Boncz, A., Zentai, J., Szűcs, G., Reuter, G. & Bakonyi, T. (2010). Detection of hepatitis E virus in samples of animal origin collected in Hungary. *Veterinary Microbiology*, 143(2–4), 106–116, https://doi:10.1016/j.vetmic.2009.11.004

Grierson, S., Heaney, J., Cheney, T., Morgan, D., Wyllie, S., Powell, L., Smith, D., Ijaz, S., Steinbach, F., Choudhury, B. & Tedder, R. S. (2015). Prevalence of Hepatitis E Virus Infection in Pigs at the Time of Slaughter, United Kingdom, 2013. *Emerging Infectious Diseases* 21(8), 1396–1401, https://doi.org/10.3201/eid2108.141995

Ivanova, A., Tefanova, V., Reshetnjak, I., Kuznetsova, T., Geller, J., Lundkvist, Å., Janson, M., Neare, K., Velström, K., Jokelainen, P., Lassen, B., Hütt, P., Saar, T., Viltrop, A. & Golovljova, I. (2015). Hepatitis E Virus in Domestic Pigs, Wild Boars, Pig Farm Workers, and Hunters in Estonia. *Food and Environmental Virology*, 7(4), 403–412, https://doi.org/10.1007/s12560-015-9210-8.

Jemeršić, L., Prpić, J., Brnić, D., Keros, T., Pandak, N. & Đaković Rode, O. (2019). Genetic diversity of hepatitis E virus (HEV) strains derived from humans, swine and wild boars in Croatia from 2010 to 2017. *BMC Infectious Diseases*, 19(1), https://doi.org/10.1186/s12879-019-3906-6

Jori, F., Laval, M., Maestrini, O., Casabianca, F., Charrier, F. & Pavio, N. (2016). Assessment of Domestic Pigs, Wild Boars and Feral Hybrid Pigs as Reservoirs of Hepatitis E Virus in Corsica, France. *Viruses*, 8(8), 236, https://doi.org/10.3390/v8080236

Kantala, T., Heinonen, M., Oristo, S., von Bonsdorff, C.-H. & Maunula, L. (2015). Hepatitis E Virus in Young Pigs in Finland and Characterization of the Isolated Partial Genomic Sequences of Genotype 3 HEV. *Foodborne Pathogens and Disease*, 12(3), 253–260, https://doi.org/10.1089/fpd.2014.1841

Lange, H., Øverbø, J., Borgen, K., Dudman, S., Hoddevik, G., Urdahl, A. M., Vold, L. & Sjurseth, S. K. (2016). Hepatitis E in Norway: seroprevalence in humans and swine. Epidemiology and *Infection*, 145(1), 181–186, https://doi.org/10.1017/s0950268816002144

Lhomme, S., Marion, O., Abravanel, F., Izopet, J. & Kamar, N. (2020). Clinical Manifestations, Pathogenesis and Treatment of Hepatitis E Virus Infections. *Journal Clinical Medicine*, 9(2), 331, https://doi.org/10.3390/jcm9020331.

Li, P., Liu, J., Li, Y., Su, J., Ma, Z., Bramer, W. M., Cao, W., Man, R. A., Peppelenbosch, M. P. & Pan, Q. (2020). The global epidemiology of hepatitis E virus infection: A systematic review and meta-analysis. *Liver International*, 40(7), 1516–1528, Portico, https://doi.org/10.1111/liv.14468

Lord, N., Huisman, W. & Paoli, L. (2022). Food crimes, food harms and the food system — SI introduction. *Crime, Law and Social Change*, 78(5), 455–462, https://doi.org/10.1007/s10611-022-10061-y

Lupulovic, D., Lazic, S., Prodanov-Radulovic, J., Jiménez de Oya, N., Escribano-Romero, E., Saiz, J.-C. & Petrovic, T. (2010). First Serological Study of Hepatitis E Virus Infection in Backyard Pigs from Serbia. *Food and Environmental Virology*, 2(2), 110–113, https://doi.org/10.1007/s12560-010-9033-6

Meng, X. J. (2010). Hepatitis E virus: Animal reservoirs and zoonotic risk. *Veterinary Microbiology*, 140(3–4), 256–265, https://doi.org/10.1016/j.vetmic.2009.03.017

Meng, X.-J., Purcell, R. H., Halbur, P. G., Lehman, J. R., Webb, D. M., Tsareva, T. S., Haynes, J. S., Thacker, B. J. & Emerson, S. U. (1997). A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings of the National Academy of Sciences of the United States of America*, 94(18), 9860–9865, https://doi.org/10.1073/pnas.94.18.9860

Milojevic, L., Velebit, B., Dimitrijevic, M., Djordjevic, V., Jankovic, V., Grkovic, N. & Nikolic, A. (2021). Detection and quantification of hepatitis E virus genome in pig liver samples originating from Serbian retail establishments. *IOP Conference Series: Earth and Environmental Science*, 854(1), 012059, https://doi.org/10.1088/1755-1315/854/1/012059

Milojević, L., Velebit, B., Teodorović, V., Kirbiš, A., Petrović, T., Karabasil, N. & Dimitrijević, M. (2019). Screening and Molecular Characterization of Hepatitis E Virus in Slaughter Pigs in Serbia. *Food and Environmental Virology*, 11(4), 410–419, https://doi.org/10.1007/s12560-019-09393-1

Monini, M., Di Bartolo, I., Ianiro, G., Angeloni, G., Magistrali, C. F., Ostanello, F. & Ruggeri, F. M. (2015). Detection and molecular characterization of zoonotic viruses in swine fecal samples in Italian pig herds. *Archives of Virology*, 160(10), 2547–2556, https://doi.org/10.1007/s00705-015-2538-4.

Moor, D., Liniger, M., Baumgartner, A. & Felleisen, R. (2018). Screening of Ready-to-Eat Meat Products for Hepatitis E Virus in Switzerland. *Food and Environmental Virology*, 10(3), 263–271, https://doi.org/10.1007/s12560-018-9340-x.

Pallerla, S. R., Schembecker, S., Meyer, C. G., Linh, L. T. K., Johne, R., Wedemeyer, H., Bock, C. -Thomas, Kremsner, P. G. &Velavan, T. P. (2020). Hepatitis E virus genome detection in commercial pork livers and pork meat products in Germany. *Journal of Viral Hepatitis*, 28(1), 196–20, Portico, https://doi.org/10.1111/jvh.13396.

Park, W. J., Park, B. J., Ahn, H. S., Lee, J. B., Park, S. Y., Song, C. S., Lee, S. W., Yoo, H. S. & Choi, I. S. (2016). Hepatitis E virus as an emerging zoonotic pathogen. *Journal of Veterinary Science*, 17, 1–11, http://dx.doi.org/10.4142/jvs.2016.17.1.1

Pavio, N., Merbah, T. & Thébault, A. (2014). Frequent Hepatitis E Virus Contamination in Food Containing Raw Pork Liver, France. *Emerging Infection Disease*, 20(11), 1925–1927, https://doi.org/10.3201/eid2011.140891

Petrovic, T., Prodanov, J. & Lazic, S. (2008). First preliminary results on the presence of hepatitis E virus in swine population in Serbia. *Symposium on Current Developments in Food and Environmental Virology*, Pisa, Italy, (pp. 52–53).

Porea, D., Anita, A., Demange, A., Raileanu, C., Oslobanu Ludu, L., Anita, D., Savuta, G. & Pavio, N. (2017). Molecular detection of hepatitis E virus in wild boar population in eastern Romania. *Transboundary and Emerging Diseases*, 65(2), 527–533, Portico, https://doi.org/10.1111/tbed.12736

Lainšček, P., Toplak, I. & Kirbiš, A. (2017). A comprehensive study of hepatitis E virus infection in pigs entering a slaughterhouse in Slovenia. *Veterinary Microbiology*, 212, 52–58, https://doi.org/10.1016/j.vetmic.2017.11.002

Renou, C., Roque-Afonso, A.-M. & Pavio, N. (2014). Foodborne Transmission of Hepatitis E Virus from Raw Pork Liver Sausage, France. *Emerging Infection Disease*, 20(11), 1945–1947, https://doi.org/10.3201/eid2011.140791

Reuter, G., Fodor, D., Forgách, P., Kátai, A. & Szűcs, G. (2009). Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *Journal of Medicine Virology*, 44(4), 277–281, https://doi.org/10.1016/j.jcv.2009.01.008

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernandez Escamez, P. S., Herman, L., Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., … Girones, R. (2017). Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. *EFSA Journal*, 15(7), https://doi.org/10.2903/j.efsa.2017.4886

Riveiro-Barciela, M., Mínguez, B., Gironés, R., Rodriguez-Frías, F., Quer, J. & Buti, M. (2015). Phylogenetic Demonstration of Hepatitis E Infection Transmitted by Pork Meat Ingestion. *Journal of Clinical Gastroenterology*, 49(2), 165–168, https://doi.org/10.1097/mcg.0000000000000113

Rivero-Juarez, A., Frias, M., Martinez-Peinado, A., Risalde, M. A., Rodriguez-Cano, D., Camacho, A., García-Bocanegra, I., Cuenca-Lopez, F., Gomez-Villamandos, J. C. & Rivero, A. (2017). Familial Hepatitis E Outbreak Linked to Wild Boar Meat Consumption. *Zoonoses and Public Health*, 64(7), 561–565, Portico, https://doi.org/10.1111/zph.12343

Rose, N., Lunazzi, A., Dorenlor, V., Merbah, T., Eono, F., Eloit, M., Madec, F. & Pavio, N. (2011). High prevalence of hepatitis E virus in French domestic pigs. *Comparative Immunology, Microbiology and Infectious Diseases*, 34(5), 419–427, https://doi:10.1016/j.cimid.2011.07.003

Ruggeri, F. M., Di Bartolo, I., Ostanello, F. & Trevisani, M. (2013). Epidemiology of the Human HEV Infection. In: Hepatitis E Virus: An Emerging Zoonotic and Foodborne Pathogen, eds F. M. Ruggeri, I. Di Bartolo, F. Ostanello, and M. Trevisani. (New York, NY: Springer New York), 11–21.

Rutjes, S. A., Lodder, W. J., Bouwknegt, M. & de RodaHusman, A. M. (2007). Increased hepatitis E virus prevalence on Dutch pig farms from 33 to 55% by using appropriate internal quality controls for RT-PCR. *Journal of Virological Methods*, 143(1), 112–116, https://doi.org/10.1016/j.jviromet.2007.01.030

Said, B., Ijaz, S., Chand, M. A., Kafatos, G., Tedder, R. & Morgan, D. (2013). Hepatitis E virus in England and Wales: indigenous infection is associated with the consumption of processed pork products. *Epidemiology Infection*, 142(7), 1467–1475, https://doi.org/10.1017/s0950268813002318

Salines, M., Andraud, M. & Rose, N. (2017). From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review. *Veterinary Research,* 48, 31, https://doi.org/10.1186/s13567-017-0436-3

Smith, D. B., Simmonds, P., Jameel, S., Emerson, S. U., Harrison, T. J., Meng, X.-J., Okamoto, H., Van der Poel, W. H. M. & Purdy, M. A. (2014). Consensus proposals for classification of the family Hepeviridae. *Journal of General Virology — Microbiology Society*, 95(10), 2223–2232, https://doi.org/10.1099/vir.0.068429-0.

Steyer, A., Naglič, T., Močilnik, T., Poljšak-Prijatelj, M. & Poljak, M. (2011). Hepatitis E virus in domestic pigs and surface waters in Slovenia: Prevalence and molecular characterization of a novel genotype 3 lineage. *Infection, Genetics and Evolution*, 11(7), 1732–1737, https://doi.org/10.1016/j.meegid.2011.07.007.

Tsachev, I., Pepovich, R., Marutsov, P., Baymakova, M., Pishmisheva, M., Pekova, L. & Gospodinova, K. (2019). Seroprevalence of hepatitis E virus infection in pigs from Southern Bulgaria: a preliminary report. *International Journal of Infection Diseases*, 79, 63, https://doi.org/10.1016/j.ijid.2018.11.163.

Wenzel, J. J., Preiß, J., Schemmerer, M., Huber, B., Plentz, A. & Jilg, W. (2011). Detection of hepatitis E virus (HEV) from porcine livers in Southeastern Germany and high sequence homology to human HEV isolates. *Journal of Clinical Virology*, 52(1), 50–54, https://doi.org/10.1016/j.jcv.2011.06.006

Wichmann, O., Schimanski, S., Koch, J., Kohler, M., Rothe, C., Plentz, A., Jilg, W. & Stark, K. (2008). Phylogenetic and Case-Control Study on Hepatitis E Virus Infection in Germany. *Journal of Infection Diseases*, 198(12), 1732–1741, https://doi.org/10.1086/593211

Widén, F., Sundqvist, L., Matyi-Toth, A., Metreveli, G., Belák, S., Hallgren, G. & Norder, H. (2011). Molecular epidemiology of hepatitis E virus in humans, pigs and wild boars in Sweden. *Epidemiology Infection*, 139(3), 361–371, https://doi.org/10.1017/s0950268810001342

Yazaki, Y., Mizuo, H., Takahashi, M., Nishizawa, T., Sasaki, N., Gotanda, Y. & Okamoto, H. (2003). Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *Journal of General Virology*, 84(9), 2351–2357, https://doi.org/10.1099/vir.0.19242-0

Žele, D., Barry, A. F., Hakze-van der Honing, R. W., Vengušt, G. & van der Poel, W. H. M. (2016). Prevalence of Anti-Hepatitis E Virus Antibodies and First Detection of Hepatitis E Virus in Wild Boar in Slovenia. *Vector Borne Zoonotic Diseases*, 16(1), 71–74, https://doi.org/10.1089/vbz.2015.1819

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.52.057

ID: 126629385

https://doi.org/10.18485/meattech.2023.64.2.37

Review paper

Meat products and functional food

Slaviša Stajić[a], Nikola Stanišić[b] and Vladimir Kurćubić[c]*

[a] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia*
[b] *Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia*
[c] *University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia*

ARTICLE INFO

Keywords:
Meat products
Functional food
Sodium reduction
Additive replacement
n-3 fatty acids
Dietary fibres

ABSTRACT

Functional food is a term for food products fortified with ingredients that possess beneficial physiological effects. Meat products are characterized by relatively high salt, fat and saturated fatty acid contents. Moreover, phosphates and nitrites are also marked as potential harmful components. Designing meat products as functional food has been associated with replacement (and/or reduction) of these components with other, especially natural, ingredients that possess beneficial effects. The development of such products poses quite a challenge since it requires the creation of a product with improved functional properties and the same sensory quality as conventional ones. Results of numerous studies into improving the nutritional properties of meat products indicate that the meat industry has responded to the changes of lifestyle and perception of food.

1. Introduction

Meat is generally a significant source of several nutrients: proteins of high biological value, and micronutrients such as iron, zinc, phosphorus, selenium and vitamin B12 (*Pereira & Vicente*, 2013; *Williams*, 2007). Fat is a great source of energy. Moreover, meat is almost an exclusive source of several bioactive compounds with antioxidative, anti-inflammatory, anti-carcinogenic and anti-atherosclerotic properties, such as conjugated linoleic acids, carnosine, anserine and taurine (*Pereira & Vicente*, 2013; *Young et al.*, 2013). Since meat as essential food needed to be preserved, ancient people developed meat products by combining different animal tissues with salt and spices, and applying early preservation techniques — drying, heating and smoking (*Kurćubić et al.*, 2022). For centuries, these products have been an excellent source of protein, energy and other nutrients. However, in the last 50–60 years, fresh meat has become readily available and meat products lost their primary function and are more valuable because of their sensory properties (*Stajić & Vasilev*, 2022).

In the last third of the 20th century, it was observed that some food ingredients can have a negative effect on health (salt, saturated fatty acids (SFA)) if they are consumed in sufficient quantities. Conversely, other ingredients (antioxidants, n-3 fatty acids, minerals, vitamins) can be important in preventing or treating certain diseases (*Doyon & Labrecque*, 2008). This led to a different perception of food — the purpose of food is no longer only to satisfy hunger and provide energy and basic nutrients, but it could also be a tool that prevents the occurrence of diseases caused by changes in lifestyle and diet, and improves physical and mental health (*Siró et al.*, 2008). Therefore, the concept of "new food" appeared which later become "functional food".

***Corresponding author:** Stajić Slaviša, *stajic@agrif.bg.ac.rs*

Paper received Jun 9th 2023. Paper accepted Jun 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Functional food

In 1984, Japanese scientists first used the term "functional food" for food products fortified with ingredients that possess beneficial physiological effects (*Bigliardi & Galati*, 2013). Japan is also the first country where this field was regulated (in 1991), when the Ministry of Health introduced the regulation called FOSHU — Food for Specified Health Uses (*Iwatani & Yamamoto*, 2019). Since then, numerous definitions of functional food have been proposed — *Bigliardi and Galati* (2013) selected 39 definitions after extensive literature research. In general, functional food should be food that is part of regular nutrition and contains ingredients with a selective effect on one or more functions of the body, and the positive effects of which can be seen as physiologically functional (*Jiménez-Colmenero et al.* 2010; *Zhang et al.*, 2010).

3. Meat products as functional food

In general, meat products contain all the nutrients/compounds from meat that have a positive effect on health, although the content of some minerals, taurine, carnosine and thermolabile vitamins, can be reduced during the process of production, storage and preparation for consumption. Some compounds with a positive effect on health (e.g. bioactive peptides) and others with an adverse effect on health (e.g. biogenic amines and nitrosamines) can occur during the production process. Also, some ingredients such as salt (sodium), phosphates and nitrites, as well as SFA that fatty tissue is rich with, are correlated with a negative influence on health. A large number of research studies has investigated reduction and/or replacement of these potential harmful components.

3.1. Reduction of salt (sodium)

Salt is the most common non-meat ingredient used in meat processing. It is essential for technological and sensory properties, shelf-life and safety of meat products. Its influence depends on salt content, form of meat (whole muscle/ground), processing procedures (heating, drying, mixing/grinding) and can be summarized as: extraction and activation of myofibrillar proteins, meat emulsion formulation and stabilization, improvement of the water-holding capacity (WHC) and defining flavour (*Kurćubić et al.*, 2022). High sodium intake has been correlated with high blood pressure, while salt is the major source of sodium in meat products, in which it accounts for approximately 79% of the sodium (*Desmond*, 2006).

The salt content in s formulation depends on the type of meat product: usually 1–2% in burger type products, 1.8–2.5% in emulsion-type sausages, 2–3% in cooked ham, 2.5–3% in dry-fermented sausages (>3.5% after drying), >3% in dry-cured meats (>4.5% after drying). The literature data indicate that salt reduction to about 1.7% in emulsion-type sausages (without phosphates added) and to 2.3% in fermented sausages can be reached without major adverse effects on products (*Corral et al.*, 2013; *Stajić et al.*, 2022). The major impact of salt on WHC came from the Cl^- anion (*Petit et al.*, 2019); therefore, reduction of the salt content in meat products has limitations. For example, salt levels above 1.3% (with added phosphates) are needed to obtain a stable meat emulsion (*Vasquez Mejia et al.*, 2019). Another possible course of action is the reduction of the sodium content by replacing salt with other chloride salts (KCl, $CaCl_2$, $MgCl_2$) which also has limitations, because the salty taste of salt mainly comes from the Na^+ cation (*Petit et al.*, 2019), and the use of other chloride salts can alter taste — e. g. use of KCl and $MgCl_2$ leads to bitterness (*Corral et al.*, 2013). Therefore, the partial replacement of NaCl with other chloride salts and combining it with compounds/ingredients that enhance salty taste and/or mask bitter taste could be good solution (*Inguglia et al.,* 2017). Different commercial mixtures consisting of sodium salt mixtures and lysine, arginine, K-lactate, glycine, yeast and mushroom extracts have been developed with the aim of reducing the sodium level and overcoming/masking technological and sensory defects (*Kurćubić et al.*, 2022).

3.2. Replacement of phosphates

Phosphates are very important for meat products because they increase the swelling of meat fibres (WHC), promote solubilization of myofibrillar proteins, bind metal ions, and reduce viscosity of meat batters (*Sebranek*, 2009). Therefore, phosphates are of great significance for technological properties of emulsified-type sausages and whole-muscle brine-injected meat products. The reduction of the phosphate content was not in focus (as much as salt and fat reduction) because the content/addition of phosphates to meat products is limited by regulations — 8 g/kg product of total phosphorus (as

P_2O_5) in Serbia, and 5 g/kg product of added phosphates in the EU. However, due to a change in P/Ca ratio in dietary intake and the increase of consumer demand for the "clean label" products, several research studies have been conducted to examine phosphate replacement, especially in emulsion-type sausages. The phosphate reduction was based on: i) replacement with natural mineral-based ingredients e.g. calcium powders originating from shells, eggs and algae (*Bae et al*., 2017; *Stajić et al.*, 2020); ii) replacement with natural ingredients that can bind water and emulsify fat, e.g. dietary fibre and heteropolysaccharides (*Câmara et al.*, 2020; *Powell et al.*, 2019; *Stajić et al.*, 2022); iii) reduction by application of alternative processing techniques e.g. ultrasound (*Pinton et al.*, 2019).

Natural calcium powders increase pH values and, therefore, contribute to proper processing yield, while on the other hand, they alter colour and texture properties (*Bae et al.*, 2017; *Stajić et al.*, 2020). Dietary fibre contains soluble and insoluble fibre and so has gel-forming ability, water-binding capacity and oil-binding capacity (*Tungland & Meyer*, 2002). However, due to differences in the amount of soluble and insoluble fibre, different impacts occur that are amount- and product-dependent. Lower emulsion stability was obtained when phosphates were replaced with 0.3–0.6% of wheat, maize, pea, potato and 0.5–1% of citrus fibre (*Powell et al.*, 2019; *Stajić et al.*, 2022). However, this was not significant in all treatments, and similar processing yields compared to controls were obtained in treatments with 0.6% of wheat, maize and pea fibres and 0.75% of citrus fibre. Application of ultrasound for 18 minutes can be used in processing emulsion-type sausages with 50% of reduced phosphates. These strategies have certain limitations; however, they offer great potential to multi-ingredient phosphate replacement strategy.

3.3. Nitrate replacement/reduction

Nitrites are ingredients that have multiple effects in meat systems: antimicrobial effects (particularly on neurotoxin-producing *Clostridium botulinum*), pink colour and aroma formation and lipid oxidation delay (*Alirezalu et al.*, 2019). However, nitrites also participate in the creation of carcinogenic N-nitrosamines, whose content is in correlation with the content of nitrites (*Alirezalu et al.*, 2019). Nitrites are usually added in amounts of 100–150 mg/kg depending on regulations. The main part of this amount is needed for *C. botulinum* control, while about 25 mg is needed for colour formation (*Sindelar & Milkowski*, 2012). Therefore, the strategy for nitrite reduction (and thus N-nitrosamine reduction) includes the introduction of ingredients that exhibit antimicrobial and antioxidant activity. In the research of *Kurćubić et al.* (2014), nitrite was replaced with ethanol extract of *Kitaibelia vitifolia* in effective concentration of 12.5 g/kg of the initial batch of dry-fermented sausages. The results indicate the great antimicrobial and antioxidative potential of *K. vitifolia* ethanol extract during production and cold storage. Also, colour, taste and overall acceptability were not affected. In frankfurter-type sausages, *Alirezalu et al.* (2019) obtained promising results in terms of antimicrobial and antioxidant effects and sensory properties of frankfurter-type sausages during cold storage (45 days at 4°C) when replacing nitrite with 1% of chitosan and 0.2% of ε-polylysine (both in combination with a 500 ppm mixture of green tea, stinging nettle and olive leaves extracts).

3.4. Fat reduction and/or improvement of fatty acids profile

Fatty tissue is essential for the quality of meat products. This is especially significant in meat products where fatty tissue is ground together with meat, mixed with non-meat ingredients (salt, additives, spices), and subjected to different procedures (drying, fermentation, grinding, emulsification) to produce fermented sausages, emulsion-type sausages or burgers/patties (*Kurćubić et al.,* 2022). The fat content of these products can be up to 50% in dry-fermented sausages and up to 30% in emulsion-type sausages and burgers/patties (*Kurćubić et al.,* 2022). Moreover, animal fat is rich in SFA and has a low content of n-3 polyunsaturated fatty acids (PUFA). Recommendations of the World Health Organization (WHO) from two decades ago emphasized that the amount of fat in total daily energy intake should be in the 15–30% range, SFA < 10% and n-3 PUFA 1–2%. Strategies include reduction of the amount of fatty tissue and/or partial to total replacement with non-lipid replacers or oils rich in PUFA (*Kurćubić et al.,* 2022). The reduction of the amount of fatty tissue has limited effects because this reduces the acceptability of fermented sausages (*Liaros et al.,* 2009) and burgers (*Heck et al.,* 2019). A partial replacement of fatty tissue with non-lipid fat replacers (inulin, cereal, and fruit fibre) can be a strategy to improve the quality of low-fat meat products

(*Bajcic et al.*, 2023; *Kurćubić et al.,* 2020). However, this only reduces the intake of energy which originates from fat — the FA profile can be changed only by introducing oils rich in PUFA (especially n-3 PUFA) into the formulation of meat products (*Kurćubić et al.,* 2022). On the other hand, oils rich in PUFA (e.g. grapeseed, flaxseed, fish, algae and their combinations) are more susceptible to lipid oxidation, and therefore, these oils need to be stabilized (cannot be added in liquid form) before application (*Stajić & Vasilev*, 2022). Oils were immobilized and stabilized in emulsions, double emulsions, gel-like matrixes (hydrogel, oleogel, oil-bulking, structured emulsions) and encapsulated by different encapsulation techniques — spray-drying, electrostatic extrusion, etc. (*Stajić & Vasilev*, 2022). The immobilization technique together with oil type, amount of fat replacement, and the principle of fat replacement (with same amount of oil (later stabilized) or with the same amount of substitute which consists of immobilized oil) influence the technological and sensory properties of modified meat products (*Stajić et al.*, 2018; *Stajić et al.*, 2020). Nutritional properties depend on the amount of fat replacement and oil fatty acid profile.

3.5. Meat products as sources of dietary fibre

As mentioned above, food could also be a tool that prevents the occurrence of diseases. Meat products are not a source of dietary fibre, however, so cold hybrid meat products, made by combining two or more types of meat with plant proteins and other non-meat ingredients, have found their place on the market (*Galanakis et al.*, 2021). Hybrid meat products have a potential advantage over plant-based meats, because they provide the familiar meaty taste and texture while containing plant-based ingredients that can contribute to a healthier and sustainable diet (*Grasso & Javorska*, 2020). They can offer a broader range of flavours and textures while reportedly having significantly lower greenhouse gas emissions when compared to all-meat products (*Baune et al.,* 2021).

The percentage of plant ingredients (legumes, grains, fruits and vegetables) in these products can be from 10% to 50%. By definition, they are not added as extensions but as part of the product constituents (*Grasso & Javorska*, 2020). Ingredients like soy, wheat, starch and fibre have been used in the meat industry for their functional properties (emulsification, water binding/holding and gelling) and to save costs (*Singh et al.*, 2008; *Asgar et al.*, 2010). The conceptual difference between hybrid and traditional meat products is that plant-based ingredients are used not only for economic and technological purposes, but also for improving health claims, lowering the environmental impact and decreasing meat consumption (*Grasso,* 2020; *Talens et al.*, 2022). A market research study showed that a third of consumers are flexitarians, which means that although they eat meat, they regularly avoid it on certain days (*Grasso and Jaworska*, 2020). Therefore, hybrid meat products could create new business opportunities for the meat industry, as they fulfil the growing flexitarian consumer needs.

4. Conclusion

Results of numerous studies into improving the nutritional properties of meat products indicate that the meat industry has responded to the changes of lifestyle and perceptions of the food. Meat products with reduced sodium and fat contents and improved fatty acid profiles have been developed. Moreover, natural replacers for synthetic and potentially harmful ingredients have been introduced. The development of such products poses quite a challenge since it requires the creation of a product with improved functional properties and the same sensory quality as conventional ones. New research is being carried out with the aim of optimizing the developed products in order to provide the necessary sensory quality in addition to further improving the nutritional properties.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, project ref. number: 451-03-47/2023-01/200116.

References

Alirezalu, K., Hesari, J., Nemati, Z., Munekata, P. E. S., Barba, F. J. & Lorenzo, J. M. (2019). Combined effect of natural antioxidants and antimicrobial compounds during refrigerated storage of nitrite-free frankfurter-type sausage. *Food Research International,* 120, 839–850, https://doi.org/10.1016/j.foodres.2018.11.048

Asgar, M. A., Fazilah, A., Huda, N., Bhat, R. & Karim, A. A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. *Comprehensive Reviews in Food Science & Food Safety,* 9, 513–529, http://dx.doi.org/10.1111/j.1541-4337.2010.00124.x

Bae, S. M., Cho, M. G. & Jeong, J. Y. (2017). Effects of various calcium powders as replacers for synthetic phosphate on the quality properties of ground pork meat products. *Korean Journal for Food Science of Animal Resources,* 37, 456–463, http://dx.doi.org/10.5851/kosfa.2017.37.3.456

Bajcic, A., Petronijevic, R., Suvajdzic, B., Tomovic, V., Stajković, S. & Vasilev, D. (2023). Use of inulin-collagen suspension for the total replacement of pork backfat in cooked-emulsified sausages. *Journal of Food and Nutrition Research,* 62, 35–45.

Barros, J. C., Munekata, P. E. S., de Carvalho, F. A. L., Domínguez, R., Trindade, M. A., Pateiro, M. & Lorenzo, J. M. (2021). Healthy beef burgers: Effect of animal fat replacement by algal and wheat germ oil emulsions. *Meat Science,* 173, 108396, https://doi.org/10.1016/j.meatsci.2020.108396

Baune, M.-C., Jeske, A.-L., Profeta, A., Smetana, S., Broucke, K., Van Royen, G., Gibis, M., Weiss, J. & Terjung, N. (2021). Effect of plant protein extrudates on hybrid meatballs — changes in nutritional composition and sustainability. *Future Foods,* 4, 100081, https://doi.org/10.1016/j.fufo.2021.100081

Bigliardi, B. & Galati, F. (2013). Innovation trends in the food industry: The case of functional foods. *Trends in Food Science & Technology,* 31, 118–129, http://dx.doi.org/10.1016/j.tifs.2013.03.006

Câmara, A. K. F. I., Vidal, V. A. S., Santos, M., Bernardinelli, O. D., Sabadini, E. & Pollonio, M. A. R. (2020). Reducing phosphate in emulsified meat products by adding chia (*Salvia hispanica* L.) mucilage in powder or gel format: A clean label technological strategy. *Meat Science,* 163, 108085, https://doi.org/10.1016/j.meatsci.2020.108085

Corral, S., Salvador, A. & Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science,* 93, 776–785, http://dx.doi.org/10.1016/j.meatsci.2012.11.040

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science,* 74, 188–196, http://dx.doi.org/10.1016/j.meatsci.2006.04.014

Doyon, M. & Labrecque, J. (2008). Functional foods : A conceptual definition. *British Food Journal,* 110, 1133–1149, http://dx.doi.org/10.1108/00070700810918036

Galanakis, C. M., Rizou, M., Aldawoud, T. M. S., Ucak, I. & Rowan, N. J. (2021). Innovations and technology disruptions in the food sector within the COVID-19 pandemic and post-lockdown era. *Trends in Food Science & Technology,* 110, 193–200, https://doi.org/10.1016/j.tifs.2021.02.002

Grasso, S. (2020). Hybrid meat. *Food Science and Technology,* 34, 48–51, https://doi.org/10.1002/fsat.3403_12.x

Grasso, S. & Jaworska, S. (2020). Part meat and part plant: Are hybrid meat products fad or future? *Foods,* 9, 1888, https://doi.org/10.3390%2Ffoods9121888

Heck, R. T., Fagundes, M. B., Cichoski, A. J., de Menezes, C. R., Barin, J. S., Lorenzo, J. M. . . . & Campagnol, P. C. B. (2019). Volatile compounds and sensory profile of burgers with 50% fat replacement by microparticles of chia oil enriched with rosemary. *Meat Science,* 148, 164–170, https://doi.org/10.1016/j.meatsci.2018.10.017

Heck, R. T., Vendruscolo, R. G., de Araújo Etchepare, M., Cichoski, A. J., de Menezes, C. R., Barin, J. S. . . .& Campagnol, P. C. B. (2017). Is it possible to produce a low-fat burger with a healthy n-6/n-3 PUFA ratio without affecting the technological and sensory properties? *Meat Science,* 130, 16–25, https://doi.org/10.1016/j.meatsci.2017.03.010

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P. & Burgess, C. M. (2017). Salt reduction strategies in processed meat products — A review. *Trends in Food Science & Technology,* 59, 70–78, https://doi.org/10.1016/j.tifs.2016.10.016

Iwatani, S. & Yamamoto, N. (2019). Functional food products in Japan: A review. *Food Science and Human Wellness,* 8, 96–101, https://doi.org/10.1016/j.fshw.2019.03.011

Jiménez-Colmenero, F., Carballo, J. & Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. *Meat Science,* 59, 5–13, http://dx.doi.org/10.1016/S0309-1740(01)00053-5

Josquin, N. M., Linssen, J. P. H. & Houben, J. H. (2012). Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil. *Meat Science,* 90, 81–86, http://dx.doi.org/10.1016/j.meatsci.2011.06.001

Kurćubić, V., Okanović, D., Vasilev, D., Ivić, M., Čolović, D., Jokanović, M. & Džinić, N. (2020). Effects of replacing pork back fat with cellulose fiber in Pariser sausages. *Fleischwirtschaft,* 100, 82–88.

Kurćubić, V., Stajić, S., Miletić, N. & Stanišić, N. (2022). Healthier meat products are fashionable — consumers love fashion. *Applied Sciences,* 12, 10129, https://doi.org/10.3390/app121910129

Kurćubić, V. S., Mašković, P. Z., Vujić, J. M., Vranić, D. V., Vesković-Moračanin, S. M., Okanović, Đ. G. & Lilić, S. V. (2014). Antioxidant and antimicrobial activity of *Kitaibelia vitifolia* extract as alternative to the added nitrite in fermented dry sausage. *Meat Science,* 97, 459–467, http://dx.doi.org/10.1016/j.meatsci.2014.03.012

Lawrie, R. A. & Ledward, D. (2006). *Lawrie's Meat Science, Seventh Edition*: CRC Press.

Liaros, N. G., Katsanidis, E. & Bloukas, J. G. (2009). Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. *Meat Science,* 83, 589–598, http://dx.doi.org/10.1016/j.meatsci.2009.07.006

Olmedilla-Alonso, B., Jiménez-Colmenero, F. & Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science,* 95, 919–930, http://dx.doi.org/10.1016/j.meatsci.2013.03.030

Pereira, P. M. & Vicente, A. F. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat Science,* 93, 586–592, http://dx.doi.org/10.1016/j.meatsci.2012.09.018

Petit, G., Jury, V., de Lamballerie, M., Duranton, F., Pottier, L. & Martin, J.-L. (2019). Salt intake from processed meat products: Benefits, risks and evolving practices. *Comprehensive Reviews in Food Science and Food Safety,* 18, 1453–1473, https://doi.org/10.1111/1541-4337.12478

Pintado, T. & Cofrades, S. (2020). Quality characteristics of healthy dry fermented sausages formulated with a mixture of olive and chia oil structured in oleogel or emulsion gel as animal fat replacer. *Foods,* 9, 830.

Pinton, M. B., Correa, L. P., Facchi, M. M. X., Heck, R. T., Leães, Y. S. V., Cichoski, A. J. . . . & Campagnol, P. C. B. (2019). Ultrasound: A new approach to reduce phosphate content of meat emulsions. *Meat Science,* 152, 88–95, https://doi.org/10.1016/j.meatsci.2019.02.010

Powell, M. J., Sebranek, J. G., Prusa, K. J. & Tarté, R. (2019). Evaluation of citrus fiber as a natural replacer of sodium phosphate in alternatively-cured all-pork Bologna sausage. *Meat Science,* 157, 107883, https://doi.org/10.1016/j.meatsci.2019.107883

Sebranek, J. G. (2009). Basic Curing Ingredients. In R. Tarté (Ed.), *Ingredients in Meat Products: Properties, Functionality and Applications* (pp. 1–23). New York, NY: Springer, New York.

Sindelar, J. J. & Milkowski, A. L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide,* 26, 259–266, http://dx.doi.org/10.1016/j.niox.2012.03.011

Singh, P., Kumar, R., Sabapathy, S. & Bawa, A. (2008). Functional and edible uses of soy protein products. *Comprehensive Reviews in Food Science and Food Safety,* 7, 14–28, https://doi.org/10.1111/j.1541-4337.2007.00025.x

Siró, I., Kápolna, E., Kápolna, B. & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance — A review. *Appetite,* 51, 456–467, https://doi.org/10.1016/j.appet.2008.05.060

Stajić, S., Kalušević, A., Tomasevic, I., Rabrenović, B., Božić, A., Radović, P. . . .& Živković, D. (2020). Technological properties of model system beef emulsions with encapsulated pumpkin seed oil and shell powder. *Polish Journal of Food and Nutrition Sciences,* 70(2), 159–168, https://doi.org/10.31883/pjfns/118008

Stajić, S., Lilić, S., Danijela, V., Vladimir Tomović & Živković, D. (2018). *Sastav lipida fermentisanh kobasica sa dodatkom lanenog ulja*. Paper presented at the XXII Savetovanje o biotehnologiji, Čačak, Srbija.

Stajić, S., Stanišić, N., Tomasevic, I., Djekic, I., Ivanović, N. & Živković, D. (2018). Use of linseed oil in improving the quality of chicken frankfurters. *Journal of Food Processing and Preservation,* 42, e13529, http://doi.org/10.1111/jfpp.13529

Stajić, S., Tomasevic, I., Stanišić, N., Tomović, V., Lilić, S., Vranić, D. . . .& Živković, D. (2020). Quality of dry-fermented sausages with backfat replacement — Fermented sausages with high content of flaxseed oil and pre-treated with soy protein isolate and alginate. *Fleischwirtschaft,* 100(7), 74–81.

Stajić, S., Tomasevic, I., Tomovic, V. & Stanišić, N. (2022). Dietary fibre as phosphate replacement in all-beef model system emulsions with reduced content of sodium chloride. *Journal of Food and Nutrition Research,* 61, 277–285.

Stajić, S. & Vasilev, D. (2022). Encapsulation of meat products ingredients and influence on product quality. In S. Lević, V. Nedović & B. Bugarski (Eds.), *Encapsulation in Food Processing and Fermentation*. Boca Raton: CRC Press.

Talens, C., Ibargüen, M., Murgui, X., García-Muñoz, S. & Peral, I. (2022). Texture-modified meat for senior consumers varying meat type and mincing speed: Effect of gender, age and nutritional information on sensory perception and preferences. *Future Foods,* 6, 100180, https://doi.org/10.1016/j.fufo.2022.100180

Tungland, B. C. & Meyer, D. (2002). Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety,* 1, 90–109, https://doi.org/10.1111/j.1541-4337.2002.tb00009.x

Vasquez Mejia, S. M., Shaheen, A., Zhou, Z., McNeill, D. & Bohrer, B. M. (2019). The effect of specialty salts on cooking loss, texture properties, and instrumental color of beef emulsion modeling systems. *Meat Science,* 156, 85–92, https://doi.org/10.1016/j.meatsci.2019.05.015

Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics,* 64, S113-S119, https://doi.org/10.1111/j.1747-0080.2007.00197.x

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I. . . .& Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science,* 78(4), 343–358, http://dx.doi.org/10.1016/j.meatsci.2007.07.019

Young, J. F., Therkildsen, M., Ekstrand, B., Che, B. N., Larsen, M. K., Oksbjerg, N. & Stagsted, J. (2013). Novel aspects of health promoting compounds in meat. *Meat Science,* 95, 904–911, http://dx.doi.org/10.1016/j.meatsci.2013.04.036

Zhang, W., Xiao, S., Samaraweera, H., Lee, E. J. & Ahn, D. U. (2010). Improving functional value of meat products. *Meat Science,* 86, 15–31, http://dx.doi.org/10.1016/j.meatsci.2010.04.018

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.524.056

ID: 126632201

https://doi.org/10.18485/meattech.2023.64.2.38

Original scientific paper

Sensory quality, oxidative stability, textural and fatty acid profile of nitrite-reduced kulen fermented sausage during ripening

Stefan Simunović[a], Vesna Ž. Đorđević[a], Sara Simunović[a], Daniel Franco[b], Slaviša Stajić[c] and Igor Tomašević[c,d]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000, Belgrade, Serbia*
[b] *Department of Chemical Engineering, Campus Vida, Universidade de Santiago de Compostela,15782 Santiago de Compostela, Spain*
[c] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Serbia*
[d] *DIL German Institute of Food Technology, Quakenbrueck, 49610 Germany*

ARTICLE INFO

Keywords:
Nitrites
Kulen
Texture
Oxidative stability
Paprika
Nutritional value

ABSTRACT

The aim of the study was to investigate the effect of nitrite reduction on oxidative stability, chemical composition, textural and sensory properties of traditional kulen sausage. In total, three batches of kulen were made. The control batch (C) contained 110.0 mg/kg of sodium nitrite ($NaNO_2$), the second (R1) batch contained 55 mg/kg of $NaNO_2$, while the third (R2) was produced without nitrites. Nitrite removal from the sausage formulation significantly affected oxidative stability, while the reduction of nitrite from 110 mg/kg to 55 mg/kg did not affect the oxidative stability of the product. When it comes to texture, complete removal of nitrite from kulen resulted in significantly lower values of hardness, gumminess and chewiness. Sensory scores for colour were similar between all analysed batches. However, scores for aroma, taste, consistency and overall acceptability were significantly lower in R2 batch sausages. At the same time, scores of all investigated sensory parameters were similar for sausages formulated with 110 mg/kg and 55 mg/kg of $NaNO_2$.

1. Introduction

Fermented sausages are products with long tradition of manufacturing in the Europe. Traditionally, they are produced using a combination of hurdles which all together contribute to the safety of the product. These include salting, drying, fermentation, sometimes smoking and addition of different additives and spices. One of the most commonly used additives in industrial processed meat production is nitrite salts. Nitrite salts are used in the form of sodium nitrite ($NaNO_2$) or potassium nitrite (KNO_2). Their use is very important in terms of development of bright red colour of meat and antimicrobial activity against *Clostridium botulinum (Djordjevic et al.,* 2019). In addition, nitrites are powerful antioxidants and may improve taste of the product (*Simunovic et al.*, 2022). Hence, nitrites and nitrates are recognized by the meat industry as irreplaceable additives, especially due to their effect on the development of the characteristic bright red colour of cured meat.

Consumers are more interested in products produced without additives because they are more aware of their negative impact on human health (*Simunovic et al.*, 2022). Therefore, food companies, in addition to initiatives aimed at reducing greenhouse gas emissions, are changing their marketing

***Corresponding author:** Stefan Simunović, *stefan.simunovic@inmes.rs*

Paper received August 14th 2023. Paper accepted August 17th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

approach and launching new products with modified nutritional composition (*Rajic, et al.,* 2021). When it comes to nitrites, their use is considered dangerous because they participate in the formation of N-nitrosamines, which are found to induce cancer (*Flores & Toldrá*, 2021). Besides their carcinogenic effect, these compounds were also found to cause insulin resistance diseases and Alzheimer's disease (*Tong et al.*, 2009). Therefore, in the last few decades, there has been a great number of studies examining the possibility of reducing or replacing nitrite in processed meat (*Christieans et al.,* 2018; *Hospital et al.,* 2016; *Ozaki et al.*, 2021; *Simunovic et al.*, 2022). The main potential hazard regarding nitrite reduction in processed meat is growth of *C. botulinum* and formation of botulinum neurotoxins, some of which can cause botulism in humans. However, *Hospital et al.* (2016) found that reduction and removal of nitrites and nitrates from formulation of fermented sausages did not compromise safety regarding *C. botulinum* in tested conditions.

During the production of fermented sausages, nitrates can be reduced to nitrites, which is why many studies have proposed foods naturally rich in nitrates as nitrite alternatives (*Gassara et al.,* 2016; *Pennis et al.,* 2020; *Pini et al.,* 2020; *Sucu & Turp*, 2018; *Tang et al.*, 2021). Nitrates are found in high concentrations in green, leafy vegetables like spinach and also in vegetables like celery, radish, beetroot and others. Paprika (*Capsicum* spp.) is also reported to contain high amounts of nitrates (*Colavita et al.,* 2014). According to (*Vuković et al.,* 2012), paprika can contain up to around 500 mg/kg of nitrates. On the other hand, paprika is an irreplaceable ingredient in every type of kulen and its content varies in the formulation from 1 to 3% (*Tomasevic et al.,* 2022).

The aim of this study was to investigate the effect of nitrite reduction on oxidative stability, chemical composition, textural and sensory properties of traditional kulen sausage.

2. Materials and methods

2.1. Production of kulen

Three batches of traditional kulen were made using 80% of pork ham and 20% of pork firm fatty tissue. The first (C) and the second (R1) batch were produced with addition of 110 mg/kg of nitrites and 55 mg/kg of nitrites, respectively. The third (R2) batch was produced without addition of nitrites. After stuffing in casings, sausages were traditionally smoked and transferred to ripening chamber for a total of 40 days. Sampling was performed on the manufacturing day and then on each 8 days of ripening. Shelf life of sausages was assessed after 50 and 100 days of storage at 2°C.

2.2. Physicochemical analysis

Protein, fat, ash and thiobarbituric acid-reactive substances (TBARS) contents were measured as described in our previous study (*Simunovic et al.*, 2021). TBARS were expressed as mg of malondialdehyde (MDA) per kg of sample. Peroxide number and acid value were determined in compliance with ISO 3960:2017 and ISO 660:2020, respectively.

2.3. Texture analysis

Texture profile analysis (TPA) was performed according to (*Simunović, Đorđević, Rašeta, et al.*, 2022), with some modifications. Prior to compression of each sample, exact dimensions of sausage cuboids were obtained using digital calliper and were entered into the processing software.

2.4. Sensory analysis

An experienced twelve-member panel evaluated following attributes of sausages at the end of the ripening: colour, aroma, taste, consistency and overall acceptability. The panel was trained during three months according to ISO 8586:2012 (*ISO*, 2012). Participants were served tap water and apples in order to clean the palate between the samples. In addition, a total of 43 consumers who regularly consume kulen took part in a triangle test. Three samples marked with a random three-digit numbers were served to each participant, one of which was different. They were asked to choose only one sample which they thought was different. In order to reduce the number of trials needed, sequential analysis was performed according to ISO 16820:2004 (*ISO*, 2004).

2.5. Statistical analysis

Statistical analysis was performed using SPSS software (IBM, Armonk, NY, USA). This statistical software was used to calculate mean values and significant differences. The results of the survey were analysed using MS Excel (Microsoft, Redmond, WA, USA).

3. Results

3.1. Chemical content and oxidative stability

At the end of ripening and storage, peroxide values were found to be the significantly ($P < 0.01$) different among all batches, being highest in sausages of the control batch, followed by R1 and R2 (Figure 1). When it comes to secondary products of lipid oxidation, significantly lower levels of TBARS were found in sausages formulated with sodium nitrite (C and R1) than those found in nitrite-free sausages (Figure 2). This is because nitric oxide binds and inhibit activity of ferrous and ferric ions, which act as catalysts of hydroperoxide decomposition to hydroxyl, peroxy and alkoxy radicals (*Domínguez et al.*, 2019; *Wanjala et al.,* 2021). The results of our study are in accordance with those reported by *Berardo et al.* (2016) and *Karwowska et al.,* (2019), who studied the effect of nitrite reduction on lipid oxidation of fermented and cooked sausages, respectively. The authors found significantly higher TBARS levels in nitrite-free sausages than in that produced with addition of 150 mg/kg of sodium nitrite, indicating the strong antioxidative effect of nitrites. However, results of our study did not align with those reported by *Karwowska et al.* (2019), who found significant differences between TBARS values of sausages formulated with 50 and 100 mg/kg of sodium nitrite. In present study, no significant ($P < 0.01$) differences were observed between sausages treated with 110 mg/kg of sodium nitrite (C) and those containing 55 mg/kg of sodium nitrite (R1) in terms of TBARS levels. It is important to point out the importance of reduction of nitrates from paprika to nitrites during ripening which, coupled with antioxidative activity of carotenoids, provides an additional antioxidant effect in kulen (*Sebranek & Bacus*, 2007). The study of *Revilla and Vivar-Quintana* (2004) revealed that by increasing levels of paprika in sausage formulation, TBARS values are reduced. To support this, TBARS values found in this study for kulen are lower than those reported by a number of authors for different dry sausages produced without red paprika powder (*Berardo et al.*, 2016; *Ozaki, Santos, et al.*, 2021; *Tang et al.*, 2021).

3.2. Texture profile analysis

Hardness, gumminess and chewiness increased significantly throughout the ripening in all analysed batches (Table 1). Regardless of concentration, nitrite addition influenced significantly ($P < 0.01$) higher values of hardness and chewiness. This outcome is in accordance with the study of *Tang et al.* (2021), who reported significantly higher values of hardness and chewiness of sausages made with 150 mg/kg of sodium nitrite compared to those found in nitrite-free sausages. In the present study, gumminess was found to be significantly lower in nitrite-free sausages to that found in the control batch but similar to that found in sausages produced using 55 mg/kg of nitrites. On the other hand, nitrite reduction showed no significant differences in cohesiveness and springiness between the batches. Effects of nitrite on texture parameters could be explained by the formation of carbonyls, which may lead to cross-linking of muscle proteins and affect their net charge, which consequently results in their changed spatial arrangement (*Bao & Ertbjerg*, 2019).

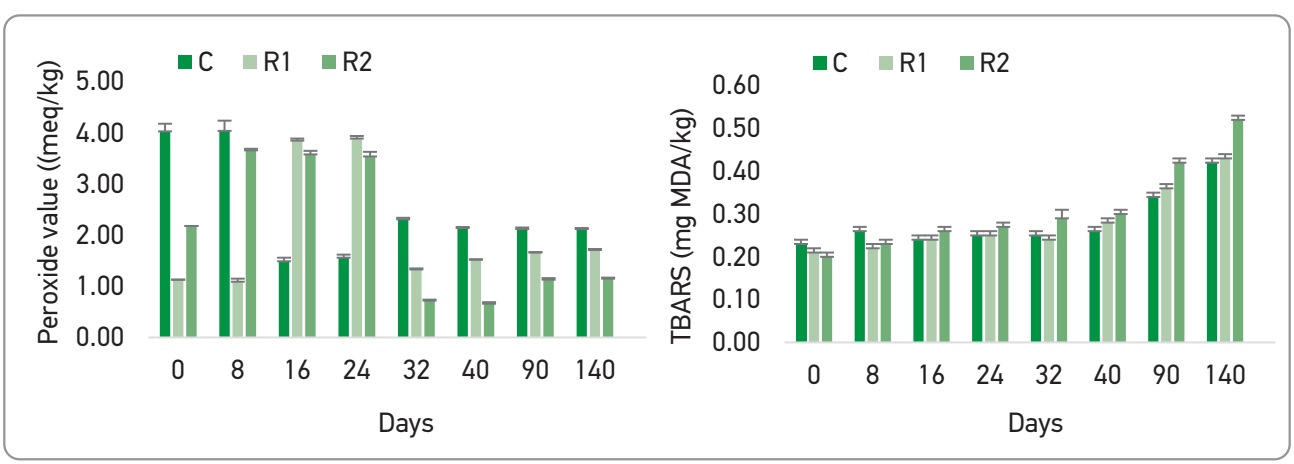


Figure 1. Evolution of peroxide values (meq/kg) and TBARS (mg MDA/kg) during production and storage of *kulen* produced with different levels of sodium nitrite (mean ± standard deviation).

Table 1. Chemical and textural changes during the first 16 days of production of nitrite reduced kulen

	Processing time (days)											
	0			SEM	8			SEM	16			SEM
	C	R1	R2		C	R1	R2		C	R1	R2	
Chemical parameters												
Fat content (%)	21.481[a]	23.712[a]	22.723[a]	0.32	24.261[b]	25.732[b]	25.242[b]	0.22	29.071[c]	26.652[c]	28.151[c]	0.36
Protein content (%)	16.201[a]	15.701[a]	15.821[a]	0.10	17.561[b]	17.561[b]	18.372[b]	0.14	21.011[c]	21.011[c]	21.282[c]	0.05
Ash content (%)	3.091[a]	3.041[a]	2.981[a]	0.02	3.351[b]	3.281[a]	3.331[b]	0.01	3.961[c]	4.221[b]	4.032[c]	0.04
Texture												
Hardness (N)	7.131[a]	7.351[a]	6.661[a]	0.21	20.641[b]	20.1212[b]	17.142[b]	0.51	37.291[c]	36.541[c]	31.322[c]	0.72
Springiness	0.771[a]	0.692[a]	0.7212[a]	0.01	0.901[b]	0.8412[b]	0.792[ab]	0.01	0.911[b]	0.861[b]	0.831[b]	0.02
Cohesiveness	0.5312[a]	0.521[ac]	0.562[a]	0.00	0.471[b]	0.481[b]	0.451[b]	0.01	0.501[abc]	0.501[ab]	0.471[b]	0.01
Gumminess (N)	4.441[a]	3.362[a]	3.611[a]	0.13	9.411[b]	11.431[b]	9.331[b]	0.33	16.531[b]	18.492[c]	15.421[c]	0.34
Chewiness (N)	3.351[a]	2.422[a]	2.432[a]	0.13	8.8612[b]	10.201[b]	7.642[b]	0.29	16.741[c]	15.541[c]	11.522[bc]	0.49

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Mean values in the same row (corresponding to the same day of ripening) not followed by a common number differ significantly (P<0.01) Mean values in the same row (corresponding to the same batch) not followed by a common letter differ significantly (P<0.01).

Table 2. Chemical and textural changes during the last 16 days of production of nitrite reduced kulen

	Processing time (days)											
	24			SEM	32			SEM	40			SEM
	C	R1	R2		C	R1	R2		C	R1	R2	
Chemical parameters												
Fat content (%)	29.38[1c]	32.12[2d]	28.82[1c]	0.51	32.12[1d]	33.81[2e]	32.81[3d]	0.25	31.28[1e]	34.10[1e]	32.47[1d]	0.41
Protein content (%)	22.15[1d]	22.57[1d]	22.27[1d]	0.11	23.93[12e]	23.72[1e]	25.19[2e]	0.25	23.91[1e]	24.21[12e]	24.82[1e]	0.15
Ash content (%)	4.05[1c]	4.29[2b]	4.04[1c]	0.04	4.52[1d]	4.38[2b]	4.43[12d]	0.02	4.90[1d]	4.95[1b]	4.96[1e]	0.07
Texture												
Hardness (N)	41.66[1c]	41.85[1d]	39.15[1d]	0.67	48.06[1d]	47.71[1e]	43.70[2e]	0.61	53.57[1d]	52.71[1e]	49.09[2f]	0.42
Springiness	0.79[1a]	0.83[1b]	0.74[1ab]	0.02	0.71[1a]	0.70[1a]	0.69[1a]	0.01	0.73[1a]	0.78[1ab]	0.70[1a]	0.01
Cohesiveness	0.52[1abc]	0.48[2b]	0.48[2b]	0.01	0.59[1ad]	0.56[1acd]	0.54[1a]	0.01	0.61[1d]	0.60[1d]	0.56[1a]	0.01
Gumminess (N)	18.18[1c]	21.01[2c]	17.85[1c]	0.60	26.43[1d]	26.96[1d]	23.22[2d]	0.50	28.94[1d]	27.36[12d]	24.15[2d]	0.59
Chewiness (N)	16.79[1c]	16.87[1c]	14.50[1cd]	0.47	17.73[1c]	17.30[1c]	15.98[1d]	0.43	19.22[1c]	19.40[1c]	16.49[2d]	0.52

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Mean values in the same row (corresponding to the same day of ripening) not followed by a common number differ significantly (P<0.01) Mean values in the same row (corresponding to the same batch) not followed by a common letter differ significantly (P<0.01).

3.3. Sensory evaluation

Sensory evaluation conducted by an experienced sensory panel showed significant ($P < 0.01$) differences between nitrite free sausages and sausages formulated with nitrite (C and R1) in terms of scores obtained for aroma, taste, consistency and overall acceptability (Table 3). However, scores of these sensory parameters were found to be similar for sausages of C and R1 batch. In terms of colour, no significant differences were observed between the three batches.

Table 3. Sensory evaluation of kulen formulated with different levels of sodium nitrite (mean±standard deviation)

Attributes	C	R1	R2
Colour	4.33±0.65[a]	4.42±0.79[a]	4.33±0.65[a]
Aroma	4.42±0.79[a]	4.25±0.96[a]	3.33±0.65[b]
Taste	4.42±0.51[a]	4.08±0.99[a]	3.42±0.67[b]
Consistency	4.17±0.58[a]	4.17±0.72[a]	3.50±0.90[b]
Overall acceptability	4.50±0.67[a]	4.42±0.51[a]	3.58±0.90[b]

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Values with different lowercase letters (a-b) in the same row differ significantly ($P < 0.01$).

4. Conclusion

The results showed significant effects of nitrite on the oxidative stability of kulen. However, reduction of nitrites from 110 mg/kg of $NaNO_2$ to 55 mg/kg of $NaNO_2$ did not compromise oxidative stability of kulen. Removal of nitrites from kulen formulation resulted in significantly lower values of hardness, gumminess and chewiness than in sausages with nitrites. In addition, complete removal of nitrites from the sausage formulation resulted in lower scores obtained for overall acceptability. To sum up, content of $NaNO_2$ can be reduced from 110 mg/kg of $NaNO_2$ to 55 mg/kg of $NaNO_2$ without compromising oxidative stability, chemical quality, texture or sensory parameters of traditional kulen.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the contract on research financing in 2023. No. 451-03-47/2023-01/200050 od 03.02.2023.

References

Bao, Y. & Ertbjerg, P. (2019). Effects of protein oxidation on the texture and water-holding of meat: a review. *Critical Reviews in Food Science and Nutrition,* 59(22), 3564–3578.

Berardo, A., De Maere, H., Stavropoulou, D. A., Rysman, T., Leroy, F. & De Smet, S. (2016). Effect of sodium ascorbate and sodium nitrite on protein and lipid oxidation in dry fermented sausages. *Meat Science,* 121, 359–364.

Christieans, S., Picgirard, L., Parafita, E., Lebert, A. & Gregori, T. (2018). Impact of reducing nitrate/nitrite levels on the behavior of Salmonella Typhimurium and Listeria monocytogenes in French dry fermented sausages. *Meat Science,* 137, 160–167.

Colavita, G., Piccirilli, M., Iafigliola, L. & Amadoro, C. (2014). Levels of Nitrates and Nitrites in Chili Pepper and Ventricina Salami. *Italian Journal of Food Safety,* 3(2), 1637–1637.

Djordjevic, V., Baltic, T., Parunovic, N., Simunovic, S., Tomasevic, I., Velebit, B. & Ciric, J. (2019). The nitrite content in domestic and foreign cooked sausages from the Serbian market. *IOP Conference Series: Earth and Environmental Science,* 333(1), 012058.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W. & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants,* 8(10), 429.

Flores, M. & Toldrá, F. (2021). Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products — Invited review. *Meat Science,* 171, 108272.

Gassara, F., Kouassi, A. P., Brar, S. K. & Belkacemi, K. (2016). Green Alternatives to Nitrates and Nitrites in Meat-based Products–A Review. *Critical Reviews in Food Science and Nutrition,* 56(13), 2133–2148.

Hospital, X., Hierro, E., Stringer, S. & Fernández, M. (2016). A study on the toxigenesis by Clostridium botulinum in nitrate and nitrite-reduced dry fermented sausages. *International Journal of Food Microbiology,* 218, 66–70.

Karwowska, M., Kononiuk, A. & Wójciak, K. M. (2019). Impact of Sodium Nitrite Reduction on Lipid Oxidation and Antioxidant Properties of Cooked Meat Products. *Antioxidants (Basel),* 9(1), 9, doi: 10.3390/antiox9010009

Ozaki, M. M., Munekata, P. E. S., Jacinto-Valderrama, R. A., Efraim, P., Pateiro, M., Lorenzo, J. M., & Pollonio, M. A. R. (2021). Beetroot and radish powders as natural nitrite source for fermented dry sausages. *Meat Science,* 171, 108275.

Ozaki, M. M., Santos, M. d., Ribeiro, W. O., Azambuja Ferreira, N. C. d., Picone, C. S. F., Domínguez, R. . . & Pollonio, M. A. R. (2021). Radish powder and oregano essential oil as nitrite substitutes in fermented cooked sausages. *Food Research International,* 140, 109855.

Pennisi, L., Verrocchi, E., Paludi, D. & Vergara, A. (2020). Effects of vegetable powders as nitrite alternative in Italian dry fermented sausage. *Italian Journal of Food Safety,* 9(2), 8422.

Pini, F., Aquilani, C., Giovannetti, L., Viti, C. & Pugliese, C. (2020). Characterization of the microbial community composition in Italian Cinta Senese sausages dry-fermented with natural extracts as alternatives to sodium nitrite. *Food Microbiology,* 89, 103417.

Rajic, S., Đorđević**, V., Tomasevic, I. & Djekic, I. (2021)**. The role of food systems in achieving the sustainable development goals: Environmental perspective. *Business Strategy and the Environment, n/a*(n/a).

Revilla, I. & Vivar-Quintana, A. (2004). Changes in Quality and Antioxidant Properties of Dry sausages produced by Type and Doses of Paprika. *Czech Journal of Food Sciences,* 22, 183–186.

Sebranek, J. G. & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Science,* 77(1), 136–147.

Simunovic, S., Đorđević**, V., Barba, F. J., Lorenzo, J. M., Rašeta, M., Janković, S. & Tomasevic, I. (2021)**. Characterisation of changes in physicochemical, textural and microbiological properties of Njeguška sausage during ripening. *Journal of Food Science and Technology,* 58(10), 3993–4001.

Simunovic, S., Đorđević, V. Ž., Lakićević, B., Djekic, I., Lorenzo, J. M., Barba, F. J. & Tomasevic, I. (2022). Digital Evaluation of Nitrite-Reduced "Kulen" Fermented Sausage Quality. *Journal of Food Quality,* 2480746.

Simunovic, S., Đorđević, V. Ž., Rašeta, M., Lukić, M., Lorenzo, J. M., Djekic, I. & Tomašević, I. (2022). Reformulation of Traditional Fermented Tea Sausage Utilizing Novel (Digital) Methods of Analysis. *Foods,* 11(8), 1090.

Sucu, C. & Turp, G. Y. (2018). The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. *Meat Science,* 140, 158–166.

Tang, R., Peng, J., Chen, L., Liu, D., Wang, W. & Guo, X. (2021). Combination of Flos Sophorae and chili pepper as a nitrite alternative improves the antioxidant, microbial communities and quality traits in Chinese sausages. *Food Research International,* 141, 110131.

Tomasevic, I., Simunovic, S., Đorđević**, V.,** Đekić**, I. & Tomović, V. (2022)**. Traditional pork sausages in Serbia: Manufacturing process, chemical composition and shelf life. In Paulo E. S. Munekata, Mirian Pateiro, D. Franco & J. M. Lorenzo (Eds.), *Pork* (pp. 171–195). Boca Raton: CRC Press.

Tong, M., Neusner, A., Longato, L., Lawton, M., Wands, J. R. & de la Monte, S. M. (2009). Nitrosamine exposure causes insulin resistance diseases: relevance to type 2 diabetes mellitus, non-alcoholic steatohepatitis, and Alzheimer's disease. *Journal of Alzheimers Disease,* 17(4), 827–844.

Vuković, I., Vasilev, D., Saičić, S. & Ivanković, S. (2012). Ispitivanje važnijih promena u toku zrenja tradicionalne fermentisane kobasice lemeški kulen. *Tehnologija mesa,* 53(2).

Wanjala, G. W., Onyango, A. N., Abuga, D., Onyango, C. & Makayoto, M., (2021). Does lysine drive the conversion of fatty acid hydroperoxides to aldehydes and alkyl-furans? *Scientific African,* 12, e00797.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.5.084.52
637.412.053

ID: 126633481

https://doi.org/10.18485/meattech.2023.64.2.39

Review paper

Enrichment of table eggs with selenium through designed feed for laying hens

Dragan Šefer[a], Dejan Perić[a] and Radmila Marković[a]*

[a] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, Belgrade, Serbia

ARTICLE INFO

Keywords:
Eggs
Laying hens
Organic selenium
Animal nutrition

ABSTRACT

In recent years, the foods that are used daily in human diet are not only intended to satisfy the requirements in basic nutrients, they are also expected to prevent food-related diseases and help consumers acquire a better immune status. By using specific nutritional strategies, it is possible to produce functional food that, in addition to basic nutrients, also contains components that participate in preserving health and reducing the risk of disease. Selenium, which is used as an additive in vitamin-mineral premixes in feed for laying hens, is present in one of two basic forms: organically bound to amino acids (selenocysteine and selenomethionine) or in the form of an inorganic salt (most often sodium selenite). Deposited selenium in the body is in an inactive state, and in cases of oxidative stress or selenium deficiency in feed, it changes to an active form. The source of selenium in feed mixtures for laying hens has an effect on the selenium content of eggs. By adding organic selenium to laying eggs, amounts of 20–25 μg per egg can be achieved, which is about 30% of the recommended daily intake for humans.

1. Eggs in human nutrition

Consumable chicken eggs represent an exceptional source of nutritionally valuable nutrients and are an inseparable part of a high-quality and well-balanced human diet. At the same time, edible chicken eggs are a moderate source of calories (on average 140 kcal/100g), which makes them a food with a favourable ratio of nutritional value to energy. The production and consumption of eggs in the world has been increasing in recent decades. The consumption of eggs has long been associated with negative effects on human health, mainly due to their cholesterol content. However, it is now known that the level of cholesterol in the serum is influenced by several other factors in consumers, such as genetic predisposition, hormonal status and eating habits, and not only by the cholesterol from eggs. In terms of basic chemical composition, whole egg is a mixture of water, protein, fat, carbohydrates and ash. The content of basic nutrients in eggs is mostly stable (Table 1) and depends on the ratio of egg

Table 1. The basic chemical composition of the whole edible chicken egg (*USDA*, 27; *USDA*, 23)

Nutrient	g/100
Protein (g/100g)	12.56
Fat (g/100g)	9.51
Carbohydrate (g/100g)	0.72
Moisture (g/100g)	76.15
Ash (g/100g)	1.06

***Corresponding author:** Dragan Šefer, *dsefer@vet.bg.ac.rs*

Paper received July 3rd 2023. Paper accepted July 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

white to yolk, while the presence of micronutrients is determined by the influence of several different factors, where the influence of laying hen nutrition is dominant. Water is the most abundant ingredient in the egg, followed by protein, which is evenly distributed in the egg white and yolk, while fats are mostly present in the yolk, but vitamins and minerals mostly occur in the white and yolk.

Egg protein is nutritionally complete because it contains all essential amino acids. Egg whites and yolks contain protein of high biological value and digestibility. The biological value of egg protein (a measure of the building of food protein into tissue protein) is 94 and is the standard by which the biological value of all other proteins is evaluated. One hen's egg contributes only 3% of the energy value of the recommended daily energy intake, which is 2000 kcal, and at the same time provides 11% of the daily protein needs. The contribution of the intake of essential amino acids amounts to 13–31%, depending on the type of essential amino acid. The average protein content in a fresh hen's egg is about 12.5%. Egg yolk contains about 16% protein, which is a complex of low-density lipoprotein (LDL), high-density lipoprotein (HDL), phosvitin and livetin. In the composition of egg white, the share of protein is on average 10–11%, and it consists of albumin and globulin (in the thin egg white), ovalbumin (in the thick egg white), mucin and mucoid (structural part of egg white). Ovalbumin makes up more than 50% of the protein in the egg white, it is rich in essential amino acids, which are crucial for the development of the chicken embryo, but are also an exceptional source of amino acids in human nutrition. Chicken egg white contains numerous proteins with a unique structure and functional properties, such as ovotransferrin, ovomucoid, ovomucin, ovomacroglobulin (ovostatin), ovoflavoprotein, lysozyme, ovoinhibitor, ovocystatin and avidin. Many of these proteins, as well as their breakdown products, have been proven to have biological activities significant for improving human health, such as antimicrobial, antioxidant and immunoregulatory properties.

The lipid content in the whole edible chicken egg is, on average, 10% (French Agency for Food, 2017). The entire lipids in the egg are concentrated in the yolk in the form of triglycerides (65%), phospholipids (28–30%) and cholesterol (4–5%). The composition of lipids in the yolk is determined by various factors, of which diet has the greatest influence. Unsaturated (monounsaturated and polyunsaturated) fatty acids make up approximately 50% of the fatty acid composition of egg lipids. Of the monounsaturated fatty acids, the most abundant is oleic (C18:1 n-9), and of the polyunsaturated, linoleic (C18:2 n-6) and arachidonic (C 20:4 n-6) acids. Saturated fatty acids make up 30–35% of the fatty acid composition of the egg, with the largest share being palmitic (C16:0) and stearic (C18:0) acids. Egg lipids also contain sterols, the most important of which is cholesterol. An edible hen's egg contains an average of 400 mg of cholesterol per 100 g (USDA, 27).

Table 2. Vitamins in whole egg (*Maqbool et al.*, 2017)

Vitamin	µg/100g
Vitamin A (Retinol)	193
Vitamin D (Cholecalciferol)	1.5
Vitamin E (Tocopherol)	1.3
Vitamin K (Phylloquinone)	0.3
Vitamin B1 (Thiamine)	40
Vitamin B2 (Riboflavin)	450
Vitamin B3 (Niacin)	80
Vitamin B5 (Pantothenic acid)	1700
Vitamin B6 (Pyridoxine)	170
Vitamin B9 (Folate)	47
Vitamin B12 (Cobalamin)	0.89

Chicken eggs are a nutritionally valuable source of water-soluble and fat-soluble vitamins. Yolks are primarily a source of the fat-soluble vitamins A, D, E and K, but also contain vitamins of the B complex (B1, B2, B5, B6, B9 and B12). Egg white contains a high concentration of vitamins B2, B3 and B5, but also significant amounts of vitamins B1, B6, B9 and B12 (Table 2). According to literature data, consuming two chicken eggs can satisfy 10–30% of daily vitamin needs.

Edible chicken eggs contain significant amounts of minerals, primarily potassium, sodium, calcium and phosphorus. Also, they are a source of essential microelements, copper, iron, magnesium, manganese, selenium and zinc (Table 3).

Table 3. Minerals in whole egg (*USDA*, 27)

Mineral	mg/100g
Calcium	56
Magnesium	12
Selenium	0.03
Sodium	142
Zinc	1.29
Phosphorus	198
Manganese	0.028
Iodine	0.021
Copper	0.072
Iron	1.75
Potassium	138

2. Functional food

In recent years, the foods that are used daily in human diet are not only intended to satisfy the needs in basic nutrients, they are also expected to prevent food-related diseases and help consumers acquire a better immune status. Functional food cannot be simply defined, since a large number of different food products can be classified as functional foods. That is why the European Commission proposed a working definition that implies that functional food must be composed of natural ingredients, must not be in the form of tablets, capsules or food supplements, and is important for improving health and/or reducing the risk of disease development. A functional food is consumed as part of the daily, usual diet, and its effectiveness must be scientifically proven. Functional food can be natural food, food enriched with a certain ingredient or that has had a certain ingredient removed from it, food in which the properties or bioavailability of one or more ingredients have been changed, or any combination of the above possibilities (*Roberfroid*, 2002). The development of functional products and the functional food market has increased with the development of the science of animal nutrition, as a basic condition for the creation of functional foods. The success of a new functional product on the market does not only depend on its beneficial effect on health, but also on its acceptable taste, appearance and availability to consumers (*Grčević et al.*, 2011). By using specific nutritional strategies, it is possible to produce functional food that, in addition to basic nutrients, also contains components that participate in preserving health and reducing the risk of disease.

3. Role of selenium

Selenium is an essential trace element that has multiple roles in the body due to its participation in biochemical processes. It is a component of 25 selenoproteins. It has a favourable effect on the immune system, preventing the occurrence of inflammatory processes, cancer and oxidative stress and reducing the risk of atherosclerosis and cardiovascular diseases. Selenium plays a role in the protection system of biological membranes against oxidative damage. It performs this role together with vitamin E (*Marković et al*., 2010). Of the total selenium in the body, 40% is present as an active ingredient in the enzyme glutathione peroxidase (GPx). Selenium, together with vitamin E, has the role of an antioxidant, and participates in the conversion of free radicals into inactive and less toxic compounds. Free radicals are present in tissues with intensive oxygen circulation, where they cause peroxidation of phospholipids, by acting on the double bonds of unsaturated fatty acids of phospholipids that are components of cell membranes. Free radicals are created when oxygen is added to those fatty acids, from which a hydrogen atom was previously separated. Free radicals can react with another lipid molecule, from which a hydrogen atom has been separated, and the product is hydroperoxide in the first molecule and a new free radical in the "attacked" lipid molecule. Molecules of lipid hydroperoxides are split to form dialdehydes, most often malondialdehyde (MDA). A series of such reactions leads to damage to the cell membrane structure and even to complete destruction (*Rayman*, 2000).

A series of positive effects of selenium on health resulting from the strengthening of the body's defences (strengthening of immunity, prevention of the formation and progression of arteriosclerosis, preservation of sperm fertility) have been confirmed, but with a rather narrow therapeutic range (in a ratio of 1:8) between the average needs (55 μg/day) and upper limit of safe intake — 400 μg/day (*Backović*, 2005). Relative selenium deficiency in humans is

associated with an increased incidence of cardiovascular and other diseases etiopathogenetically related to oxidative stress and immune-mediated inflammation, infertility, and thyroid dysfunction (*Lynne*, 2004). A complete deficit is observed in long-term total parenteral nutrition with preparations without selenium, and in some regions it is associated with the occurrence of endemic Keshan and Kashin-Boeck diseases (*Rayman*, 2000). The addition or restriction of selenium affects the activity and metabolism of neurotransmitters, which causes changes in mood and behaviour in humans and animals (*Backović et al.*, 2002). A low concentration of selenium in the soil, and consequently in the nutrients used in animal feed, can cause a deficiency of this microelement in animals. Deficiency symptoms also occur in humans through foods of animal origin, which significantly weakens the system of antioxidant protection in the body.

4. Production of selenium eggs

The utilization of selenium by animals depends on the chemical form in which it is found in the meal. Selenium, which is used as an additive in vitamin-mineral premixes in feed for laying hens, is present in one of two basic forms: organically bound to amino acids (selenocysteine and selenomethionine) or in the form of an inorganic salt (most often sodium selenite). After entering the body through a meal, selenium is incorporated into tissue proteins, which creates its reserve. Deposited selenium in the body is in an inactive state, and in cases of oxidative stress or selenium deficiency in food, it changes to an active form. The source of selenium in feed mixtures for laying hens has an effect on the selenium content of eggs. By adding organic selenium to laying eggs, amounts of 20–25 μg per egg can be achieved, which is about 30% of the recommended daily intake for humans. For the production of such eggs, it is necessary to add organic selenium in the amount of 0.3–0.5 mg/kg in feed for laying hens. In research carried out at the Department of Nutrition and Botany of the Faculty of Veterinary Medicine, the addition of organic selenium in mixtures for laying hens resulted in a product of a specific composition, called the selenium egg, with 42 μg of selenium in 100 g of egg mass. Based on these results, we can conclude that organic sources of selenium have better biological availability and that the content of selenium in table eggs is more stable. The use of organic forms of selenium in the nutrition of laying hens increases the content of selenium in eggs.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Backović, D., Jorga, J., Milovanović S. & Paunović, K. (2002). Essential role of selenium and central nervous system. *Engrami 2002*, 24, 39–47.

French Agency for Food, Environmental, and Occupation Health & Safety, (2017). ANES-CIQUAL French food composition table version (2017). Retrieved on01/11/2019from the Ciqual homepage https://ciqual. anses. fr.

Grčević, M., Gajčević-Kralik, Z., Kralik, G. & Ivanković, S. (2011). Kokošje jaje kao funkcionalna namirnica. Krmiva, 53(2), 93–100.

Lynne, A. D. (2004). Selenium: Essential and toxic but does selenium status have health outcomes beyond overt deficiency? (Editorial). *Medical Journal of Australia,* 180(8), 373– 374.

Maqbool, M. A., Aslam, M., Waseem, Akbar, W. & Zubair, Iqbal Z. (2017). Biological Importance of vitamins for human health: A review. *The Journal of Agricultural Science*, 2(3), 52– 58.

Marković, R., Baltić, M., Šefer, D., Radulović, S., Drljačić, A., Đorđević, V. & Ristić M. (2010). Einfluss erhöhter Mengen an organischem Selen und Vitamin E in der Broilermast auf ausgewählte Parameter der Fleischqualität. *Fleischwirtschaft*, 90, 132–136.

Rayman, M. (2004). The use of high-selenium yeast to raise selenium status: how does it measure up? *British Journal of Nutrition*, 92, 557–573.

Roberfroid, M. B. (2002). Global view on functional foods: European perspectives. British Journal of Nutrition, 88, 133–138.

USDA National Nutrient Database for Standard Reference. Release 27.

USDA National Nutrient Database for Standard Reference. Release 23.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.084.5

ID: 126636553

https://doi.org/10.18485/meattech.2023.64.2.40

Review paper

Live yeast cells in nutrition of monogastric animals

Radmila Marković[a], Dejan Perić[a], Svetlana Grdović[a], Dragoljub Jovanović[a], Dragan Šefer[a], Jelena Janjić[a] and Željko Maksimović[b]*

[a] *Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, Belgrade, Serbia*
[b] *Željko Maksimović, MSc in Agr, Feix Nutrition D.O.O., Bulevar Kralja Petra I 55/20, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Yeast
Animal nutrition
Health
Production performances

ABSTRACT

Yeast and yeast derivatives are used as nutrients and as feed additives. Live yeast cells (LYC) (*Saccharomyces cerevisiae*) belong to a group of potent microorganisms that are used as an additive in animal feed and represent an alternative to antibiotics that until recently were used in animal nutrition as growth stimulators. The role of yeast as a growth promoter is due mainly to mannanoligosaccharide and β-glucans, isolated from the outer cell wall of yeast, whose role is known in stimulating the body's immune response. Dietary supplementation with whole yeast or yeast cell wall at 1.0–1.5 g/kg can improve growth performance, improve digestion and absorption of nutrients by modulating gut structure, inhibiting pathogenic bacteria and lowering gut pH.

1. Introduction

The ban on the use of antibiotics as growth promoters in animal nutrition began in 2006 due to confirmed negative effects on human and animal health (*Kovitvadhl et al*., 2019). With the very hint of the need to stop using antibiotics as growth stimulators in intensive animal breeding, the need was to find alternative additives for animal feed, which will have a positive effect on production results and animal health, but without the negative effects associated with antibiotics (resistance to antibiotics, genotoxic and teratogenic effects, residues). As alternative solutions, additives, such as probiotics, prebiotics, phytobiotics, acidifiers and others, are of great importance. By using these additives, similar effects are achieved as when using antibiotics, with the advantages that they do not leave residues, nor do they have a withdrawal period. The positive effects of their use are based on the well-known importance of maintaining eubiotic relationships, because the balance in the micropopulation of the digestive tract enables efficient digestion and resorption of nutrients, increasing resistance to disorders caused by enteropathogenic bacteria. Diet can influence the maintenance of eubiosis in three ways: by including live microorganisms that become metabolically active after ingestion (probiotics), by including nutrients that contain indigestible ingredients and stimulate the growth and activity of desirable microbiota (prebiotics), or by using natural supplements with clearly demonstrated antibacterial properties (phytobiotics). The mentioned supplements enable the stimulation of animal growth by using their natural physiological potentials and mechanisms, providing the conditions for realizing the genetic potential of animals.

***Corresponding author:** Radmila Marković, *radmilam@vet.bg.ac.rs*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Yeasts in feed

Yeasts are unicellular eukaryotic microorganisms that are classified according to the kingdom of fungi. Yeasts are considered facultative aerobes, which means they can survive in the presence or absence of oxygen. Yeast reproduction occurs under aerobic conditions, when yeast cells convert oxygen and sugars into carbon dioxide and energy, which enables their efficient growth and reproduction, through oxidative metabolism. There are about 100 different genera of yeasts and about 2000 different species of yeasts, but the importance for animal feed is on the following species: *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Candida utilis* and *Saccharomyces* var. boulardii (*Pang et al*., 2022).

Yeast as a nutrient is a rich source of proteins of high biological value, vitamin B complex, organic acids, trace minerals, growth factors and many other useful substances. When yeast is used as a feed additive as an alternative to antibiotic growth promoters, results vary depending on several factors, including yeast species, yeast product components, feed ingredients, animal categories, and differences in the animal's rearing environment (*Pang et al*., 2022).

Saccharomyces cerevisiae is most often used of the yeasts in the nutrition of ruminants, pigs and poultry. The positive influence of this yeast on production performance, development and intestinal health, improvement of immunity and improvement of meat quality has been proven.

Kluyveromyces marxianus is an ascomycete yeast, also known as *Candida kefyr.* It has often been used in trials examining the possibility of binding and controlling mycotoxins in animal feed.

Candida utilis has been shown to be a protein-rich microorganism that improves gut microbiota balance and facilitates host growth. *C. utilis* improves growth and reduces diarrhoea in weaned piglets.

Saccharomyces var. boulardii is a subspecies of *S. cerevisiae* and has, compared to other members of this genus, a higher survival rate, higher tolerance to bile salts, and better antioxidant properties under different temperatures and gastric acid conditions. *S.* var. boulardii is used in animal feed because of its good effects in preventing diarrhoea in young animals, improving the animal's immunity, improving intestinal function and improving production performance. In the diet of monogastric animals, *S. cerevisiae* and *S.* var. bourardii are the most often used yeasts (*Pang et al*., 2022).

Yeast and yeast derivatives can be used in nutrition in intensive livestock production. They can be added to feed as live yeast cells, yeast cell wall, purified cell wall components and yeast extracts after fermentation. These forms of added yeast differ in appearance, composition of biologically active components and their application in the production system. In addition, yeast cultivation conditions or fermentation conditions, as well as yeast strains, have a significant impact on the outcome of the final product and application results. It is necessary to understand the differences in these products due to the choice of yeast supplements.

Yeast cell walls are mainly composed of glucan (35~45%), mannanoligosaccharide (40~45%), protein (5~10%), chitin (1~2%), lipid (3~8%) and inorganic salt (1~3%). *Speranda et al*. (2008) reported that dietary supplementation of *S. cerevisiae* cell wall can promote the proliferation of lymphocytes, such as neutrophils, and improve their immune response. Polysaccharides contained in yeast cell walls have many biological functions, such as strengthening immunity, improving the antigenicity of pathogenic substances, alleviating stress and promoting growth and development. At the same time, yeast products have no residues, drug resistance and do not pollute the environment. Therefore, yeast cell walls can be used as natural and safe feed additives, and the main components of this product are mannanoligosaccharide and β-glucan.

Mannanoligosaccharide (MOS) can promote the development of the gastrointestinal tract of animals, regulate intestinal flora, improve animal immunity and improve animal growth performance. In addition, mannanoligosaccharide is resistant to high temperatures and maintains stable structure and function under high temperatures and high pressure food processing and pelleting conditions, which makes it also suitable for application as an animal feed additive.

β-Glucan has biological functions in the development of the gastrointestinal mucosa, balancing intestinal microorganisms, promoting the development of immune organs, increasing the body's immunity and improving production performance.

Yeast cultures with fermented metabolites can be added to animal feed. In addition to their nutritional role, they increase the activity of intestinal digestive enzymes, promote digestion and absorption and improve metabolic activities through their metabolites. Due to the complex composition of yeast culture, different strain sources, uncertain mechanism

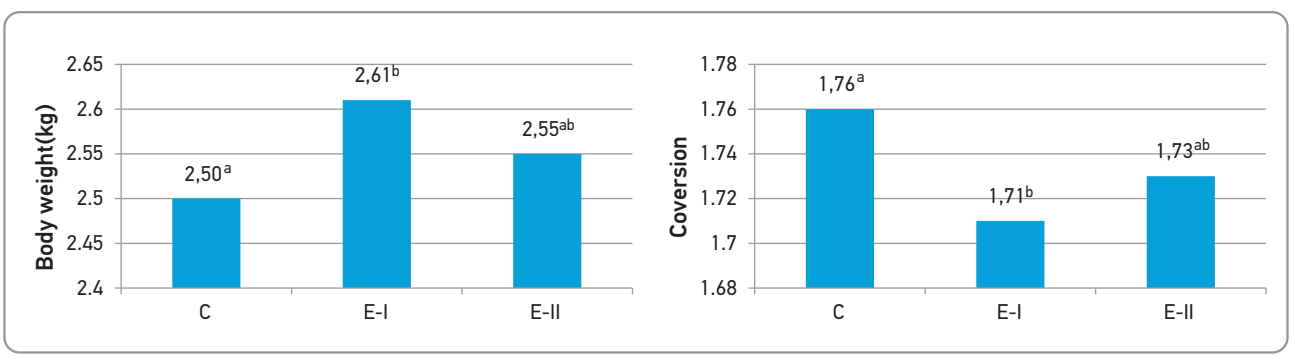
of action and different physiological environments of the gut, the effect of yeast culture produced by the same strain on different animals is different.

*Other yeast products***.** In addition to the listed main yeast products, selenized yeast and chromium yeast are also used in animal feed. Selenium-rich yeast as a highly bioavailable product has a unique role in regulating animal metabolism, improving animal health and increasing the serine content of meat and eggs compared to inorganic forms of the mineral added through feed (*Pang et al.*, 2022).

3. Live yeast cells (LYC) in feed

Live yeast cells (*S. cerevisiae*) are the source of one of the most potent microorganisms used as a supplement to animal feed (*Marković et al*., 2022; *Maksimović et al.*, 2022). They represent one of the alternatives for antibiotics that were used in animal nutrition until recently. Mannanoligosaccharide, isolated from the outer cell wall of yeast (*S. cerevisiae*), is most responsible for yeast's role as a growth promoter in animal nutrition. It has been proven that supplementing animal feed with whole yeast or yeast cell wall in the amount of 1.5–2 g/kg can improve the growth performance and meat gain in broilers (*Marković et al.*, 2022; *Maksimović et al.,* 2022). Also, the results of the research showed that the addition of yeast culture in the amount of 5 g/kg to the diet of pigs had a significant effect on the increase in body weight of pigs fed with this mixture, thanks to the improved absorption of nutrients from the digestive tract and the positive effect on the morphology of the intestinal villi. It is known that living yeast cells possess large amounts of polysaccharides, together with mannose and glucans, and their role in modulating the immune response of the body in interaction with various immunocompetent cells is recognised (*Marković et al.*, 2022; *Maksimović et al.,* 2022). In addition, the addition of live yeast cells to animal feed can improve digestion and absorption of nutrients from the intestinal tract by modulating the intestinal structure and inhibiting pathogenic bacteria in the intestines (*Trevisi et al.*, 2015). The role of living yeast cells is also reflected in the reduction of pH in the intestines, which leads to the creation of a wide range of organic acids that acidify the environment in the intestines, and thus, the consequent inhibition of pathogenic bacteria in the intestines is achieved (*Ogbuevu et al.*, 2019). It has been proven that the cell wall components of live yeast (especially β-glucan) have a role in stimulating the immune function of antibody synthesis in pigs and chickens (*Ding et al.*, 2019). Immune suppression, caused by infections that develop during intensive broiler breeding (most likely as a result of poor response to vaccines) has a huge negative economic impact on this production (*Umar et al.*, 2017). Atypical fowl disease (Newcastle disease) and infectious bursal disease are very serious broiler diseases that, apart from the fatal outcomes, have a significant negative impact on feed conversion (FCR) and lead to slow fattening of broilers (*Mahfuz et al.*, 2019). In addition to research previously conducted to examine the effect of live yeast cells on growth performance and gut microbiome of broilers, the role of *Saccharomyces cerevisiae* on antioxidant status and immune response in broilers remains a subject of further investigation. There are numerous studies on the positive impact of live yeast cells in pig feed under conditions of heat stress (*Mayorga et al.*, 2021).

Maksimović et al. (2022) examined the effect of adding different amounts (0.25 and 0.65 g/kg) of live yeast cell preparations to broiler feed (Ross 308) and determined the positive effect of the yeast



Legend: Means with the different superscript letter significantly differ at P<0.05

Figure 1. Production performances of broilers receiving diets containing different levels of yeast (*Saccharomyces cerevisiae*)

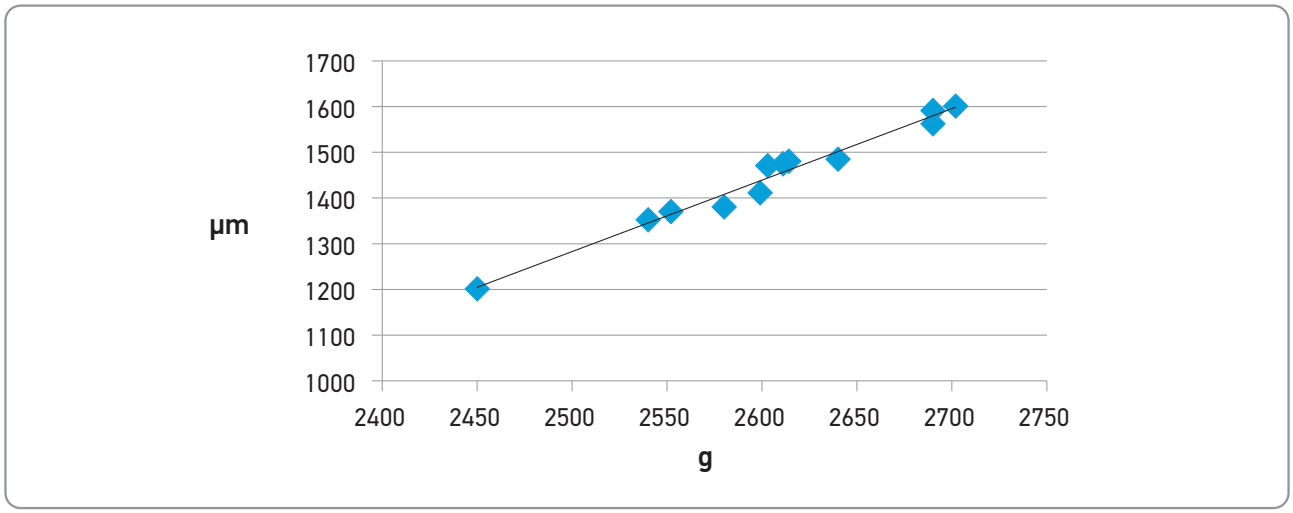
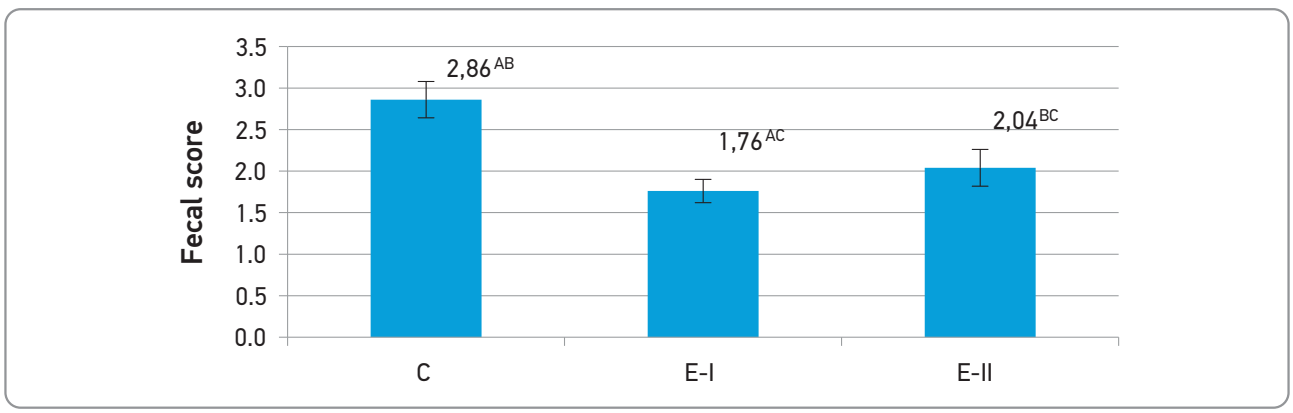


Figure 2. Correlation dependence of the final weight (g) of broilers receiving 0.25 g/kg yeast in diet (E-I) and the height of intestinal villi (µm) of the ileum



Legend: Groups with the same letters $^{A, B, C}$ are statistically different, $p<0.01$

Figure 3. Broiler faecal scores

on production performance, health status and production efficiency in broiler fattening (*Markovic et al.*, 2022). Each experimental group contained 90 animals housed in groups of 15 birds per pen in six repetitions (Figure 1).

The final weight of broilers and the height of the villi in the ileum were strongly positively correlated ($p<0.01$; $r=0.987$) statistically (Figure 2).

Assessment of excreta quality in each replicate was performed through visual faecal scoring. There were at least 2 independent evaluators and assessment was done twice a day (08.00 and 16.00 h) on days 7, 14, 21, 28 and 35. Scores ranged from 1 to 5: 1 = dry; well-formed excreta with characteristic white uric acid cover, 2 = mostly dry excreta with white uric acid cover, 3 = moist excreta with white uric acid cover, 4 = wet excreta with less white uric acid cover and droppings lose their shape, and 5 = extremely wet excreta with little to no white uric acid cover. Data were summarized for the overall excreta quality score for each treatment (Figure 3) (*Garcia et al.*, 2020). According to the results, positive effects of living yeast cells on the health condition, production results and morphometric characteristics of the broilers' intestines, and therefore on the economy of production, was confirmed. This indicates the nutritional, medical and economic justification of using living yeast cells in broiler nutrition.

4. Conclusion

Yeast and yeast products are used in animal feed with the aim of providing nutrients, having a probiotic role, stimulating animal growth and positively affecting animal immunity. A large number of scientific studies confirm that yeast and their derivatives can be good for the growth and health of animals, especially when the animals are housed in

poor animal hygiene conditions. However, there is still a need for additional information and research on the use of some yeast products. For some products and yeast derivatives, uneven product quality is apparent, and different batches of products have different nutritional values. Another possible problem is that there is no specific strain for one animal, and the same strain can act differently on different animals. Finally, there is a lack of recommendations on the application of yeast products in terms of quantity, type of yeast and preparation in different stages of production. Further research is needed to better understand the importance and possibilities of using yeast and its derivatives in animal nutrition.

Disclosure statement: No potential conflict of interest was reported by the authors.

Fundings: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Ding, B., Zheng, J., Wang, X., Zhang, L., Sun, D., Xing, Q., Pirone, A. & Fronte, B. (2019). Effects of dietary yeast beta-1,3–1,6-glucan on growth performance, intestinal morphology and chosen immunity parameters changes in Haidong chicks. *Asian Australasian Journal of Animal Sci*ence, 32,1558–1564.

Garcia, B. A., Aguirre-Reyes, A. T., Decena, K. S. & Sulabo, R. C. (2019). Effect of a performance enhancer mixture as replacement for antibiotic growth promoters on production performance, excreta quality and carcass characteristics of broilers. *Philippine Journal of Veterinary and Animal Sciences*, 45(1), 34–47.

Kovitvadhi, A., Chundang, P., Tirawattanawanich, C., Prathumpai, W., Methacanon, P. & Chokpipatpol, K. (2019). Effects of dietary supplementation with different levels and molecular weights of fungal β-glucan on performances, health and meat quality in broilers. *Asian-Australasian Journal of Animal Sciences,* 32,1548–1557.

Mahfuz, S., He, T., Liu, S., Wu, D., Long, S. & Piao, X. S. (2019). Dietary inclusion of mushroom (*Flammulina velutipes*) stem waste on growth performance, antibody response, immune status, and serum cholesterol in broiler chickens. *Animals,* 9, 692.

Maksimović, Ž., Starčević, M., Šefer, D., Janjić, J., Radovanović, A., Radulović, S., Perić, D. & Marković, R. (2022). Effects of Adding Different Dosages of *Saccharomyces cerevisiae* in Diet on Growth Performance, Carcass Characteristics, Intestinal Morphology, and Gut Microflora of Broilers. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* 28(4), 461–468.

Marković, R., Perić, D., Drljačić, A., Šefer, D., Shivani, K. & Maksimović, Ž. (2022). Addition of live yeast cells in broiler nutrition, *19th Biennial International Conference of Animal Nutrition Society of India*, Nutritional Technologies to Augment Livestock. Poultry, Canione and Fish Production for Global Competitiveness, Department of Animal Nutrition GURU Angad dev Veterinary and Animal Sciences University, Ludhiana-141004, 16–18.11.2022., Punjab, India.

Mayorga, J. E., Kvidera, K. S., Horst, A. E., Al-Qaisi, M., McCarthy, S. C., Abeyta, A. M., Lei, S., Elsasser, H. T., Kahl, S., Kiros, G. T. & Baumgard, L. H. (2021). Effects of dietary live yeast supplementation on growth performance and biomarkers of metabolism and inflammation in heat-stressed and nutrient restricted pigs. *Translational Animal Science,* 5,1–13.

Ogbuewu, I. P., Okoro, V. M., Mbajiorgu, E. F. & Mbajiorgu, C. A. (2019). Yeast (*Saccharomyces cerevisiae*) and its effect on production indices of livestock and poultry — a review. *Comparative Clinical Pathology*, 28(3), 669–677.

Trevisi, P., Colombo, M., Priori, D., Fontanesi, L., Galimberti, G., Calò, G., Motta, V., Latorre, R., Fanelli, F. & Mezzullo, M. (2015). Comparison of three patterns of feed supplementation with live *Saccharomyces cerevisiae* yeast on postweaning diarrhea, health status, and blood metabolic profile of susceptible weaning pigs orally challenged with Escherichia coli. *Journal of Animal Science,* 93, 2225–2233.

Pang, Y., Zhanget, H., Wen, H, Wan, H., Wu, H., Chen, Y., Li, S., Zhang, L., Sun, X., Li, B., Liu, X. (2022). Yeast Probiotic and Yeast Products in Enhancing Livestock Feeds Utilization and Performance: An Overview. *Journal of Fungi,* 8, 1191.

Šperanda, M., Didara, M., Šperanda, T., Domacinovic, M., Valpotic, H., Kovacevic, J. & Novoselec, J. (2008) Hydrolyzed brewery yeast product like immunomodulator in weaned piglets. *Archivos de Zootecnia,* 11, 52–60.

Umar, S., Munir, M. T., Ahsan, U., Raza, I., Chowdhury, M. R., Ahmed, Z. & Shah, M. A. A. (2017). Immunosuppressive interactions of viral diseases in poultry. *World's Poultry Science Journal,* 73,121–135.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.086/.087:635.8
636.087.2

ID: 126362633

https://doi.org/10.18485/meattech.2023.64.2.41

Review paper

Effects of spent mushroom substrate on growth performance and meat characteristics of animals

Svetlana Grdović[a], Radmila Marković[a], Dejan Perić[a] and Dragan Šefer[a]

[a] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia

ARTICLE INFO

Keywords:
Mushrooms
SMS
Animal nutrition
Environment

ABSTRACT

After the production of mushrooms for human consumption, the used substrate remains (SMS — spent mushroom substrate). As much as five kilograms of spent substrate result from the production of one kilogram of mushrooms. Considering that the global production of mushrooms in the world has increased in recent decades, the amount of spent substrate has also increased sharply, which can lead to an unfavourable impact on the environment. The assumptions are that the amount of consumed substrate will exceed the value of 6 tonnes of SMS per km^2 of global land surface. This data points to the need for thinking and scientific research on how to reuse SMS. Various studies have established that SMS can first be used as a material from which to extract enzymes that remain after the production of mushrooms. Then, SMS can be used to grow one or two more production batches of mushrooms. Finally, SMS can be used as raw material for feed, as compost for plant growth and as biofuel. Research into the use of SMS as feed is particularly interesting, where its effect in the diet of various ruminant and non-ruminant species was examined.

1. Introduction

Fungi are eukaryotic organisms, present on Earth for at least 2.4 billion years (*Bengtson et al.*, 2017). They include about 120,000 species described so far (*Mueller & Schmit*, 2006). About 2000 species of edible mushrooms are known around the world (*Chuang et al*., 2020a), and are rich in proteins, fats, minerals, vitamins and are important as probiotics (*Finimundy et al.*, 2018). Common edible mushrooms belong to various genera, *Pleurotus*, *Agaricus*, *Lentinula*, *Flammulina* and others (*Chuang et al*., 2020b).

Edible mushrooms are grown on different substrates. Crop straw (wheat, oats, barley, rye, soybeans, rice), sawdust, tree bark and branches, sugar cane, soybean husks, cotton, peanut husks, grape seeds and by-products of the brewing and coffee industries are most often used to prepare the substrate and other materials rich in lignin and cellulose. From the aspect of animal nutrition, the best substrates are based on straw and grains of wheat, oats, barley and rye. After the production of mushrooms and the removal of the fruiting bodies, what remains is a spent substrate permeated with a network of fungal hyphae, rich in enzymes (cellulase, b-glucosidase, laccase, ligninase) and decomposed lignocellulose that animals can more easily use (than uncomposed lignocellulose) in the digestion process (*Adamović et al.*, 1998). The substrate can also contain relatively high levels of nitrogen, potassium, phosphorus and calcium and traces of iron and silicon (*Ball & Jackson*, 1995). It is signif-

***Corresponding author:** Svetlana Grdović, *cecag@vet.bg.ac.rs*

Paper received July 3rd 2023. Paper accepted July 16th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

icant that the substrate does not contain mycotoxins, and the amounts of pathogenic bacteria and moulds are within the legal limits defined for the concentrations of harmful substances in feed (*Adamović et al.,* 1998). The chemical composition of SMS (73.6% neutral detergent fibre, 55.0% acid detergent fibre, 8.1% crude protein, 2.1% ether extract, 9.8% non-fibrous carbohydrate, and 6.4% crude ash) makes the substrate a potential source of feed (*Kim et al.*, 2007). SMS also contains phytogenic components, such as phenolic compounds and flavonoids, that significantly protect cells from free radical damage and can improve antioxidant capacity (*Tuzcu et al.*, 2008).

SMS contains a lot of moisture, so it is difficult to store it for a long time. The best and most cost-effective way to preserve it longer is ensiling, after combining it with other substrates that are rich in soluble sugars, primarily with the whole corn plant. Ensiled SMS, mixed with molasses and concentrate, is used by various animals in their diet (*Adamović et al.*, 1998). So far, many papers have been published on the use of SMS in the nutrition of poultry, pigs, sheep, goats, calves, cattle, heifers and cows, and its effect on the performance and characteristics of animal meat has been described.

2. Spent mushroom substrate (SMS) in animal nutrition

Traditional livestock feed includes straw and other agricultural residues that have low energy, protein and mineral content. Digestion of these feeds is hampered by the high amounts of hard-to-digest cell wall components, such as cellulose, hemicellulose and lignin (*Phillips*, 2004). Delignification of straw by chemical treatment is economically and environmentally unfavourable (*Lucio et al.*, 2020). However, fungi are very efficient decomposers of cell wall components, so these agricultural residues and lignocellulosic biomass can be degraded by biological processing using mushrooms (*Stamets*, 2000). By using mushrooms, agricultural waste is transformed into a valuable source of human food in the form of harvested mushrooms and as SMS, a source of feed. SMS is available in large quantities and its use as an alternative feed has been investigated. SMS can be included in amounts of 25–33% in the diet of adult animals, which have comparatively low nutritional needs, and in amounts of <15% in the diet of growing animals (*Van Wyk*, 2022).

The effects of SMS in the diet of calves were investigated. Spent wheat straw after the production of *Agaricus bisporus* was included in the calf diet (*Fazaeli et al.*, 2014). The results showed that this SMS, included in the pelleted diet, can be included in amounts of up to 15% and that the production performances of the calves were improved. *Kim et al.* (2011) also recommended that fermented sawdust after growing *Pleurotus ostreatus* can be used in calf diets at 10%, as it improved calf growth after weaning by 8%. Feeding dairy cows with silage prepared from green corn plants and SMS (80:20) in amounts of 4–5 kg per cow per day resulted in an increased concentration of milk fat compared to the control. This can be explained by a larger amount of cellulose, and therefore a larger amount of acetic acid, which is a precursor in the synthesis of milk fat. Studies have also shown that spent wheat straw after mushroom production can be used as a component in the diet of young heifers in amounts of 2–3 kg per heifer per day, if a smaller increase in body weight is desired (0.6–1.0 kg/ day). SMS can also be included in cattle nutrition in the amount of 2.5 kg (10% of dry matter in the meal). Growth was lower, but satisfactory, which certainly implies lower costs for food (*Adamović et al.*, 1998).

The influence of SMS in pig nutrition on growth performance, immunity and antioxidant capacity was investigated (*Boontiam et al.*, 2020). The pigs were divided into two groups, one the control, and the other supplemented with 2g/kg of SMS. The results showed that the group supplemented with 2g/kg of SMS had higher final body weight and higher daily gain, and there was no effect on daily feed intake, feed conversion rate, glucose and lipoproteins. Also, there were positive changes in immunoglobulin A, immunoglobulin G, total antioxidant capacity and glutathione peroxidase activity. The percentage of leukocytes and the concentrations of cholesterol and malondialdehyde decreased.

The nutritional value of SMS has also been investigated in the sheep diets (*Fazaeli & Talebian-Masoudi*, 2006). SMS resulting from the production of the mushroom *Agaricus bisporus* was included in different amounts (0% (control), 10%, 20%, 30%) in sheep feed. The results showed that the inclusion of 20% SMS did not affect the digestibility of dry matter, organic matter, crude fibre, acid detergent fibre or neutral detergent fibre, so the authors recommended SMS is used in this amount. Dietary supplementation with SMS in goat diets has also been studied. *Park et al.* (2012) found that in goats

fed with 15% SMS supplementation for 6 weeks, the number of white blood cells and lymphocytes significantly increased compared to the control group.

The effect of SMS was also investigated on growth performance and meat characteristics of geese (*Chang et al.*, 2016). Three groups of geese were fed with different amounts of SMS (5% SMS, 10% SMS and 15% SMS) for 8 weeks. The results showed that there were no significant effects on the relationship between feed consumption and feed conversion rate. There were no significant effects on blood biochemical parameters either. The body mass of the group with 15% SMS was significantly lower than the control group, which was explained by the increased amount of coarse fibre in the feed which reduces the rate of digestion of nutrients. However, the group with 5% SMS stood out for extremely favourable sensory properties (meat taste and acceptability) as well as color, which has a favourable effect on consumer meat choice. It was recommended that 5% of corn in goose diet can be replaced with 5% SMS, which would significantly reduce feed costs, and the meat could have better sensory characteristics. Similar results were obtained by *Foluke et al*. (2014), who investigated the use of SMS in broiler diets as a substitute for wheat bran. Five groups of broilers used wheat bran and SMS as bran replacement at different concentrations (0% (control), 25%, 50%, 75% and 100%) for eight weeks. The results showed that feed intake increased equally in all groups. Body mass and feed conversion rate were significantly higher in the control and 25% SMS groups. However, the color and quality of breast, drumstick, back, neck and wing meat were equally acceptable in all groups of broilers, so it was concluded that SMS could replace wheat bran in broiler production. Other authors (*Chuang et al., 2020c*) also believe that 5% SMS dietary supplementation can be given to broilers because it improved the composition and color of the meat.

3. Conclusion

SMS is created after the production of edible mushrooms, and its annual production has been increasing in recent decades. There is a large body of research that shows that SMS can be used in animal nutrition. There is an opportunity to develop standard feed formulations that help the farmer (by reducing feed costs) and the mushroom grower (by helping with waste disposal). The confirmed nutritional value of SMS and its economic benefits in the form of reduced feed prices could be used to a greater extent in the future. Further studies and research are certainly needed in this area.

Disclosure statement: No potential conflict of interest was reported by the authors.

Fundings: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

References

Adamović, M., Grubić, G., Protić, R. & Sretenović, L. (1998). The biodegradation of wheat straw by *Pleurotus ostreatus* mushrooms and its use in cattle feeding. *Animal Feed Science Technology*, 71, 357–362.

Ball, A. S. & Jackson, A. M. (1995). The recovery of lignocellulose degrading enzymes from spent mushroom compost. *Bio Resource Technology*, 54, 311–314.

Bengtson, S., Rasmussen, B., Ivarsson, M., Muhling, J., Broman, C., Marone, F., Stampanoni, M. & Bekker, A. (2017). Fungus-like mycelial fossils in 2.4-billion-year-old vesicular basalt. *Nature Ecology and Evolution*, 1, 141.

Boontiam, W., Wachirapakorn, C. & Wattanachai, S. (2020). Growth performance and hematological changes in growing pigs treated with *Cordyceps militaris* spent mushroom substrate. *Veterinary World*, 13(4), 768–773.

Chuang, W. Y., Hsieh, Y. C. & Lee, T. T. (2020a). The effects of fungal feed additives in animals: A review. *Animals*, 10, 805.

Chuang, W. Y., Liu, C. L., Tsai, C. F., Lin, W. C., Chang, S. C., Shih, H. D., Shy, Y. M. & Lee, T. T. (2020b). Evaluation of waste mushroom compost as a feed supplement and its effects on the fat metabolism and anti-oxidant capacity of broilers. *Animals*, 10, 445.

Chuang, W. Y., Shih, H. D., Shy, Y. M., Chang, S. C. & Lee, T. T. (2020c). Evaluation of mushroom waste compost on broiler body composition, nutrient absorption and adipose metabolism. *Italian Journal of Animal Science*, 19(1), 940–950.

Fazaeli, H., Shafyee-Varzeneh, H., Farahpoor, A. & Moayyer, A. (2014). Recycling of mushroom compost wheat straw in the diet of feedlot calves with two physical forms. *International Journal of Recycling Organic Waste in Agriculture,* 3, 65.

Fazaeli, H. & Talebian-Masoudi, A. (2006). Spent wheat straw compost of *Agaricus bisporus* mushroom as ruminant feed. *Asian-Australasian Journal of Animal Sciences,* 19(6), 845–851.

Finimundy, T. C., Barros, L., Calhelha, R. C., Alves, M. J., Prieto, M. A., Abreu, R. M. V., Dilon, A. J. P., Henriques, J. A. P., Roesch, M. & Ferreira, I. C. F. R. (2018). Multifunctions of *Pleurotus sajor-caju* (Fr.) Singer: a highly nutritious food and a source for bioactive compounds. *Food Chemistry*, 245, 150–158.

Foluke, A., Olutayo, A. & Olufem, A. (2014). Assessing spent mushroom substrate as a replacement to wheat bran in the diet of broilers. *American International Journal of Contemporary Research*, 4(4), 178–183.

Kim, M., Lee, H., Park, J., Kang, S. & Choi, Y. (2011). Recycling of fermented sawdust-based oyster mushroom spent substrate as a feed supplement for postweaning calves. *Asian-Australasian Journal of Animal Sciences,* 24, 493–499.

Kim, Y. I., Bae, J. S., Jung, S. H., Ahn, M. H. & Kwak, W. S. (2007). Yield and physicochemical characteristics of spent mushroom (*Pleurotus ryngii*, *Pleurotus osteratus* and *Ammulina velutipes*) substrates according to mushroom species and cultivation types. *Journal of Animal Science and Technology*, 49, 79–88.

Lucio, B., Hernández-Domínguez, E., Tellez, A., Martinez, M., Soto, S. & Alvarez Cervantes., J. (2020). Protein fraction, mineral profile, and chemical compositions of various fiber-based substrates degraded by *Pleurotus ostreatus*. *BioResources*, 15, 8849–8861.

Mueller, G. M. & Schmit, J. P. (2006). Fungal biodiversity: what do we know? What can we predict? *Biodiversity* and *Conservation,* 16, 1–5.

Park, J. H., Yoon, S. H., Kim, S. W., Shin, D., Jin, S. K., Yang, B. S. & Cho, Y. M. (2012). Hematological and serum biochemical parameters of Korean native goats fed with spent mushroom substrate. *Asian Journal of Animal and Veterinary Advances*, 7, 1139 –1147.

Phillips, C. J. C. (2004). In The Encyclopedia of Farm Animal Nutrition. M. F. Fuller, ed: CABI Publishing.

Chang, S. C., Lin, M. J., Chao, Y. P., Chiang, C. J., Jea, Y. S. & Lee, T. T. (2016). Effects of spent mushroom compost meal on growth performance and meat characteristics of grower geese. *Revista Brasileira de Zootecnia*, 45(6), 281–287.

Stamets, P. (2000). Growing gourmet and medicinal mushrooms. *International Journal* of Medicinal *Mushrooms,* 2.

Tuzcu, M., Sahin, N., Karatepe, M., Cikim, G., Kilinc, U. & Sahin, K. (2008). Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. *British Poultry Science*, 49, 643–648.

Van Wyk, L. (2022). Potential for use of spent substrate of Pleurotus mushrooms grown on urban waste as feed for dairy cattle. *McGill Science Undergraduate Research Journal*, 17(1), 64–69.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 613.28:595.7

ID: 126363657

https://doi.org/10.18485/meattech.2023.64.2.42

Review paper

Entomophagy — a novel option in animal and human nutrition

Ksenija Nešić[a], Radmila Marković[b] and Dragan Šefer[b]*

[a] *Scientific Institute of Veterinary Medicine of Serbia, Smolucska 11, 11077 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Edible insects
Feed
Food

ABSTRACT

Entomophagy is not a new phenomenon in the world. Moreover, it is a traditional diet in a large part of the planet. However, in the European framework it belongs to the category of novel food and although the topic is often debated, it represents a growing choice in human and animal nutrition. Edible insects have the potential to serve as a healthy, sustainable alternative to animal protein sources due to their valuable nutritional composition. They may have superior health benefits based on high levels of essential amino acids, omega-3 and omega-6 fatty acids, vitamin B12, iron, zinc, fibre and antioxidants. They could offer a myriad of environmental benefits, including overall reductions in greenhouse gas emissions and reduced use of agricultural land and water. Future research should aim to understand the beneficial effects of whole insects or insect isolates compared to traditional foods of animal and plant origin. Although insects have the potential to be used as meat substitutes or dietary supplements, leading to benefits for human health and the environment, this paper does not aim to ultimately propagate their use, but to point out their advantages and qualities, as well as potential dangers and risks, and finally to present ways of placing insects on the European market.

1. Introduction

Entomophagy, or the eating of insects, has been of great importance throughout history. There are numerous indications about the consumption of insects through various historical sources and literature and in many religious documents belonging to Christianity, Islam and Judaism. Despite the fact that entomophagy persisted in some parts of the world, in modern Western societies, through the centuries that followed, it lost its importance and presence. It is assumed that the most probable reason is the development of agriculture and livestock production. Recently, this topic has attracted the attention of the public all over the world. Due to the food security issues, new scientific research has begun on the contribution that insects make to ecosystems, nutrition, food security and livelihoods (*Nesic*, 2022).

Considering a growing world population on one side and climate change and other aggravating factors on the other, new food chain strategies are aimed at sustainable food systems that are secure, safe and environmentally friendly. Insects, which represent a new choice in the European nutrition, contribute to this concept and are favourable candidates for supplementing traditional protein sources. Insect farming is expanding in Europe and consumers are becoming increasingly receptive to this idea. To ensure future development of the insect market, it is very important to provide safe insect products.

***Corresponding author:** Ksenija Nešić, *ksenija.nesic@nivs.rs*

Paper received April 18th 2023. Paper accepted April 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Although insects as a possible choice in human and animal nutrition is a great economic and ecological opportunity, consumers must first of all be fully informed and protected (*Delgado et al.*, 2022).

2. Advantages of entomophagy

Food and Agriculture Organization of the United Nations (*FAO*, 2013; 2021) published its reports to raise awareness of the many valuable roles that insects play in sustaining nature and human life, and to document the contribution that insects already make to diversifying diets and improving food security. The *EFSA* (2015) list of insects reported to have the highest potential for use as food and feed in the EU includes the following species: *Musca domestica*, *Hermetia illucens*, *Tenebrio molitor*, *Zophobas atratus (morio)*, *Alphitobus diaperinus*, *Galleria mellonella, Achroia grisella, Bombyx mori, Acheta domesticus, Gryllodes sigillatus, Locusta migratora migratorioides, Schistocerca Americana*.

Interest in entomophagy is growing primarily due to the good nutritional composition of insects. They are a source of biologically valuable proteins (they contain over 60%) with high levels of essential amino acids (e.g. lysine, tryptophan), omega-3 and omega-6 fatty acids, vitamin B12, iron, zinc, fibre and antioxidants. A recent study comparing the nutritional characteristics of a range of insects showed that the amino acid profile of dipterous insects was superior to soybean meal and more similar to fish meal (*Barroso et al.*, 2014). The profile of unsaturated fatty acids is similar to that of poultry and white fish, but contains more polyunsaturated fatty acids (PUFA) than poultry or red meat (*Rumpold and Schluter*, 2013), while it largely depends on the species and developmental stage, but also on their diet.

The price of other protein feedstuffs also contributes to the popularization of this novel food, as animal feed makes about 70% of livestock production costs. Therefore, insects that are characterized by a favourable content of nutrients, low impact on the environment, smaller requirements for living space, and which are already part of the natural diet of pigs, poultry and fish, are an ideal alternative for feeding animals (*Rumpold and Schluter*, 2013). Feed conversion ratios in insects are good, like crickets for example, which need only 2 kg of feed for every kilogram of body mass (*Collavo et al.*, 2005). In addition to the protein precedence, insect fat as a byproduct of protein production is being considered for biodiesel refineries (*Wang et al.*, 2017).

There are several environmental benefits associated with insect farming (*FAO*, 2021). Their cultivation requires less water than domestic animal farming and also has a high land use efficiency compared to traditional protein sources (*Alexander et al.*, 2017). Greenhouse gas emissions from insects are far lower than from conventional animal husbandry (*Oonincx et al.*, 2010). The production of one kg of edible protein from insects requires less energy than a kg of beef, and is comparable to the production of pork, while a kg of chicken requires slightly more energy. Insects are considered a sustainable source of protein due to the facts that they can be grown throughout the year, that most of their body is edible, that they have a high fertility and growth rate, and that they efficiently convert the growing substrate into their own body mass (*FAO,* 2021).

Insects participate in the natural recycling of nutrients. Their contribution in maximizing the efficiency of waste management by using waste nutrients for growth is also known. About one third of the food produced for human consumption worldwide is thrown away as waste. Current waste management practices are not only expensive, but also have a negative impact on the environment. Therefore, experimental evidence, such as that provided by *Yandi et al.* (2023) on the successful bioconversion of organic waste by *Hermetia illucens* larvae, is of great importance for the creation of high-value insect-derived products with the simultaneous valorization of waste. The recycling of food waste generated in urban and suburban environments using insects such as *Hermetia illucens* to create protein sources for animal feed was also discussed by *Law and Wein* (2018). One of the potential benefits of insect farming is that the excrement they produce can be used as fertilizer to improve soil fertility. However, since the nutritional composition as well as the microbiological and toxicological profiles of insects depend on the composition of the substrate on which they are produced (*Harsányi et al.*, 2020; *Parry et al.,* 2020), more research is needed to demonstrate all safety aspects of using insects for food and even for fertilizer, after cultivation on different substrates.

There are data in the literature on the degradation of materials such as Styrofoam and other forms of polystyrene, as well as polyethylene, by worms (*Brandon et al.*, 2018; *Koh et al.*, 2020; *Nukmal et al.*, 2018). Insects can also serve in the production of biofuels, as well as chitin and lipids used in food, textiles, cosmetics, pharmaceutical products and as surfactants (*Gortari and Hours*, 2013; *Houben et al.*, 2020; *Verheyen et al.*, 2020). Another use mentioned in the literature is entomoremediation, where insects

are used to perform *in situ* remediation of various environmental pollutants from soil (*Ewuim*, 2013).

3. Risks of entomophagy

The use of insects in food and feed in Europe is a relatively new practice on a commercial scale and many questions are yet to be definitively answered. First of all, there are concerns about the risk of introducing pathogens and other safety threats into the production system. Further limitations are based on the lack of complete information on the bioavailability of all nutrients found in insects, the effect of different processing methods on nutrient composition, and the lack of evidence that insects are an acceptable substitute for meat in the quantities necessary to be nutritionally beneficial (*Payne et al.*, 2016). The problem of consumer acceptability of insects is one of the biggest obstacles to mass use. It is also necessary to adopt appropriate regulations, address welfare issues and establish adequate laboratory control of food and feed.

Regarding the presence in insects of bacteria, species of the genera *Staphylococcus*, *Streptococcus*, *Bacillus, Pseudomonas, Micrococcus, Lactobacillus, Erwinia, Clostridium* and *Acinetobacter* are mentioned, as well as members of the *Enterobacteriaceae* family (*EFSA*, 2015; *Garofalo et al.*, 2019; *Murefu et al*., 2019). Some of these species are not only pathogenic and opportunistic bacteria, but can also be responsible for reducing the shelf life of edible insects. To reduce the transmission of pathogens to humans through the consumption of insects, it is important that insect farms have strong biosecurity measures and prevent contact with other animals. Growing materials may also present a potential microbiological risk to consider. For example, if materials such as paper egg cartons are used for rearing insects, there is a risk of contamination with S*almonella* and *Campylobacter*. The risks are higher if the cartons have been in contact with poultry droppings (*Walia et al.*, 2018). Research shows that treating insects in the same way as other food of animal origin (washing and thorough heating) greatly reduces the risk of foodborne bacterial diseases (*Grabowski & Klein*, 2017). The occurrence of transmissible antimicrobial resistance (AMR) genes has also been investigated, and potential sources of AMR bacteria are linked to contamination of the substrate, water and/or insect breeding environment (*Milanović et al*., 2016).

So far, the risks associated with food-borne viruses, such as hepatitis A, hepatitis E and norovirus, which could have their source in the consumption of insects, are considered to be quite negligible, but care must still be taken to ensure that the viruses are not introduced into insect production units via substrate (*Vandeweyer et al.*, 2020). Insects can potentially serve as replicative vectors for viruses that infect vertebrates. Additional studies are needed to investigate the possibility of arboviruses transmitted by edible insects, which are transmitted by arthropods and can cause a number of diseases in humans, such as West Nile disease, Rift Valley fever and haemorrhagic fever (*EFSA*, 2015).

Some mycotoxicological risk has also been identified, given that several mycotoxins have been detected in edible insects, but not at levels of public health concern (*De Paepe et al.*, 2019). The publication by *FAO* (2021) also mentions certain types of moulds and yeasts (*Aspergillus, Penicillium* and *Fusarium*), but more research is needed to better identify the metabolic pathways, metabolites and their potential toxicological effects.

Regarding the presence of parasites, *Belluco et al.* (2013) report the finding of metacercariae in some species of edible insects in Asia, and consequently the development of intestinal fluke (trematode) infections in humans. Infection of mammals and humans with nematodes for which insects are the transitional host, as well as oral trypanosomiasis, have also been recorded. Myiasis, parasitic infestation of the body of live animals and humans with fly larvae is also possible. *Gałęcki and Sokól* (2019) warn that insect farms supplying edible insects can pose both direct and indirect parasitic risks for humans and animals, so therefore, have to be regularly monitored for parasites to guarantee the safety of food and feed sources. In the *EFSA* (2021) scientific opinion on *Tenebrio molitor* as novel food, it is stated that the applicant of this request confirms application of measures to monitor the presence of the developmental forms of tapeworms (class: *cestodes*), *Hymenolepis diminuta* and *Hymenolepis nana*, which can cause zoonotic symptoms in humans.

Studies on the level of contamination with organic and metal contaminants in both whole edible insects and insect-based food products indicate that contaminant levels are generally lower than those reported in other common animal products (*Poma et al.,* 2017). The *EFSA* (2021) opinion states that levels of heavy metals, pesticide residues, polychlorinated biphenyls and dioxins need to be monitored in feed for *Tenebrio molitor* larvae, as they can bioaccumulate such chemical agents. *Truzzi et al.* (2020) suggested that since the content of heavy metals in *Hermetia illucens* pupae depends on the growth substrate, in addition to the list

of insect species that can be used for the production of processed animal proteins, a specific list of tested growth substrates that are used safely for the production of edible insects should be formed.

Insects could contain some anti-nutritive factors (*Nishimune et al.*, 2000, *Shantibala et al.*, 2014). Considerations of allergic threats related to insects and their consumption are also ongoing. *EFSA* (2021) states that people allergic to mites are also potentially sensitive to some edible insects; allergens from feed consumed by larvae can have a prolonged allergenic effect. Edible insect allergens appear to be resistant to heat treatments and enzyme digestion (unless very specific conditions are applied), similar to the behaviour of crustacean allergens (*Ribeiro et al.*, 2021). Therefore, for safety reasons, it is necessary for food products made from insects to be clearly labelled and adequately declared in order to draw attention and warn susceptible consumers (*Nešić et al.,* 2020).

4. Placement of insects on food and feed market

In mid-2017, the European Commission adopted the amendment EU Regulation No. 2017/893 (*European Union*, 2017), allowing seven species to be reared and used in feeding aquaculture. This closed list of authorised insects includes: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*). The conditions for the production of insect processed animal protein (PAP) are strictly regulated. Insects must be fed only with category 3 material (which in principle would still be appropriate for human consumption), not allowing, for example, manure or heavy metal contaminated debris as a feed source. Furthermore, insect PAP has to be treated at least according to method no. 7, following Regulation (EU) No. 142/2011 (*European Union*, 2011), which means that bacterial contamination must be reduced in order to make a safe product. By the EU Regulation 2021/1372 (*European Union*, 2021), entered into force on September 7th 2021, insects are, besides for aquaculture animals and pets, for the first time officially approved for use in pig and poultry nutrition.

For the approval of novel food in Europe, Regulation 2015/2283 has been in force from 2018 (*European Union*, 2015). This regulation determines conditions that allow food business operators to introduce new food items to the EU market, while ensuring a high level of food safety for European consumers. Insects are explicitly mentioned, for which traditional use in the European Union is not evident. It is necessary for companies placing insects on the EU market to submit an application for review and approval to EFSA. However, in certain cases, a simplified procedure is possible for traditional food originating from third countries, if it can be proven that it has been part of the human diet for at least 25 years without any safety concerns. Considering that, it is expected that some insects or their products will enter the European market in this way.

The last authorisation of the European Commission was granted for frozen, paste, dried and powder forms of *Alphitobius diaperinus* (lesser mealworm) in January 2023. In addition to this insect, three more species were previously approved: *Acheta domesticus* (house cricket), *Tenebrio molitor* (yellow mealworm) and *Locusta migratoria*. Currently, there are eight applications for insects intended to be marketed in different forms, which are subject to a safety evaluation by EFSA (*European Commission*, 2023).

5. Conclusion

It is generally considered that insects as an ingredient in food and feed can be consumed without additional risks compared to conventional animal products. It is necessary to establish safety and quality standards from primary production to consumption, appropriate regulations and control methods for their implementation. The upcoming goal is also to help establish the profile of insects as a source of food and animal feed as recognized by national and international food agencies and to attract the attention of farmers, the media, the general public and decision makers in governments, donor agencies, investment firms, the large number of research centres and the food and feed industries. After all, consumer acceptance is crucial.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The paper is published as part of the Contract between the Scientific Institute of Veterinary Medicine of Serbia and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia No 451-03-47/2023-01/200030.

References

Alexander, P., Brown, C., Arneth, A., Dias, C., Finnigan, J., Moran, D. & Rounsevell, M. D. A. (2017). Could consumption of insects, cultured meat or imitation meat reduce global agricultural land use? *Global Food Security*, 15, 22–32, https://doi.org/10.1016/j.gfs.2017.04.001

Barroso, F. G., de Haro, C., Sánchez-Muros, M-J., Venegas, E., Martínez-Sánchez, A. & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. *Aquaculture*, 422–423, 193–201, https://doi.org/10.1016/j.aquaculture.2013.12.024

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G. & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. Comprehensive *Reviews in Food Science and Food Safety*, 12, 296–313, https://doi.org/10.1111/1541-4337.12014

Brandon, A. M., Gao, S. H., Tian, R. & Criddle, C. S. (2018). Biodegradation of polyethylene and plastic mixtures in mealworms (larvae of *Tenebrio molitor*) and effects on the gut microbiome. *Environmental Science and Technology*, 52, 6526–6533, https://doi.org/10.1021/acs.est.8b02301

Collavo, A., Glew, R. H., Huang, Y. S., Chuang, L. T., Bosse, R. & Paoletti, M. G. (2005). House cricket small-scale farming. In: Paoletti, M.G. Ecological implications of Minilivestock: Potential of insects, Rodents, Frogs and Snails. Science publisher, New Hampshire, USA.

De Paepe, E., Wauters, J., Van Der Borght, M., Claes, J., Huysman, S., Croubels, S. & Vanhaecke, L. (2019). Ultra-high-performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry for multi-residue screening of pesticides, (veterinary) drugs and mycotoxins in edible insects. *Food Chemistry*, 293, 187–196, https://doi.org/10.1016/j.foodchem.2019.04.082

Delgado, L., Garino, C., Moreno, F. J., Zagon, J. & Broll, H. (2022). Sustainable Food Systems: EU Regulatory Framework and Contribution of Insects to the Farm-To-Fork Strategy. *Food Reviews International*, http://dx.doi.org/10.1080/87559129.2022.2130354

EFSA (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, 13(10), 4257, https://doi.org/10.2903/j.efsa.2015.4257

EFSA (2021). Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/22 83, Scientific Opinion. *EFSA Journal*, 19(1), 6343, https://doi.org/10.2903/j.efsa.2021.6343

European Commission. (2023). Approval of fourth insect as a Novel Food. Retrieved from https://food.ec.europa.eu/safety/novel-food/authorisations/approval-insect-novel-food_en. Accessed April 5, 2023.

European Union (2011). European Commission. Commission regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. *Official Journal of the European Union*, L54, 1–25, http://data.europa.eu/eli/reg/2011/142/oj

European Union (2015). Regulation (EU) No 2015/2283 of 25 November 2015 on novel foods, amending Regulation (Eu) No 1169/2011 and repealing Regulation (EC) No 258/97 and Commission Regulation (EC) No 1852/2001. Official Journal of the European Union, L327, 1–22, http://data.europa.eu/eli/reg/2015/2283/oj

European Union (2017). Regulation (EU) No 2017/893 of 24 May 2017 amending annexes I and IV to regulation (EC) No 999/2001 of the European Parliament and of the Council and annexes X, XIV and XV to commission regulation (EU) No 142/2011 as regards the provisions on processed animal protein. *Official Journal of the European Union*, L138, 92–116, http://data.europa.eu/eli/reg/2017/893/oj

European Union (2021). Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals. *Official Journal of the European Union*, L295, p. 1–17, http://data.europa.eu/eli/reg/2021/1372/oj

Ewuim, S. C. (2013). Entomoremediation — A novel in-situ bioremediation approach. *Animal Research International*, 10, 1681–1684.

FAO (2013). Edible insects: future prospects for food and feed security, FAO, UN, https://www.fao.org/3/i3253e/i3253e.pdf

FAO (2021). Looking at edible insects from a food safety perspective. Challenges and opportunities for the sector. Rome, https://doi.org/10.4060/cb4094en

Gałęcki, R. & Sokół, R. (2019). A parasitological evaluation of edible insects and their role in the transmission of parasitic diseases to humans and animals. *PLoS ONE*, 14, e0219303, https://doi.org/10.1371/journal.pone.0219303

Garofalo, C., Milanović , V., Cardinali, F., Aquilanti, L., Clementi, F. & Osimani, A. (2019). Current knowledge on the microbiota of edible insects intended for human consumption: A state-of-the-art review. *Food Research International*, 125, 108527, https://doi.org/10.1016/j.foodres.2019.108527

Gortari, M. C. & Hours, R. A. (2013). Biotechnological processes for chitin recovery out of crustacean waste: a mini review. *Electronic Journal of Biotechnology*, 16, http://dx.doi.org/10.2225/vol16-issue3-fulltext-10

Grabowski, N. T. & Klein, G. (2017). Microbiology of processed edible insect products — results of a preliminary survey. *International Journal of Food Microbiology*, 243, 103–7, https://doi.org/10.1016/j.ijfoodmicro.2016.11.005

Harsányi, E., Juhász, C., Kovács, E., Huzsval, L., Pintér, R., Fekete, G., Varga, Z. I., Aleksza, L. & Gyuricza, C. (2020). Evaluation of organic wastes as substrates for rearing *Zophobas morio*, *Tenebrio molitor*, and *Acheta domesticus* larvae as alternative feed supplements. *Insects*, 11(9), 604, https://doi.org/10.3390/insects11090604

Houben, D., Daoulas, G., Faucon, M.-P. & Dulaurent, A.-M. (2020). Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Scientific Reports, 10, https://doi.org/10.1038/s41598-020-61765-x

Koh, D.W.-S., Ang, B.Y.-X., Yeo, J.Y., Xing, Z. & Gan, S.K.-E. (2020). Plastic agriculture using worms: Augmenting polystyrene consumption and using frass for plant growth towards a zero-waste circular economy. bioRxiv (preprint server), https://doi.org/10.14324/111.444%2F000048.v2

Law, Y. & Wein, L. (2018). Reversing the nutrient drain through urban insect farming — opportunities and challenges. *AIMS Bioengineering*, 5, 226–237, https://doi.org/10.3934/bioeng.2018.4.226

Milanović, V., Osimani, A., Pasquini, M., Aquilanti, L., Garofalo, C., Taccari, M., Cardinali, F., Riolo, P. & Clementi, F. (2016). Getting insight into the prevalence of antibiotic resistance genes in specimens of marketed edible insects. *International Journal of Food Microbiology*, 227, 22–28, https://doi.org/10.1016/j.ijfoodmicro.2016.03.018

Murefu, T. R., Macheka, L., Musundire, R. & Manditsera, F. A. (2019). Safety of wild harvested and reared edible insects: A review. *Food Control*, 101, 209–224, https://doi.org/10.1016/j.foodcont.2019.03.003

Nesic, K. (2022). Insekti kao izvor proteina u hrani i hrani za životinje. Naučni institut za veterinarstvo Srbije, Beograd, Srbija.

Nesic, K. & Zagon, J. (2019). Insects — a promising feed and food protein source? *Meat Technology*, 60(1), 56–67, https://doi.org/10.18485/meattech.2019.60.1.8

Nešić, K., Tasić, A. & Pavlović I. (2020). Insekti kao održivi izvori proteina u hrani i hrani za životinje. *Ecologica*, 99, 553–559.

Nishimune, T., Watanabe, Y., Okazaki, H. & Akai H. (2000). Thiamin is decomposed due to *Anaphe spp.* entomophagy in seasonal ataxia patients in Nigeria. *The Journal of Nutrition*, 130(6), 1625–1628, https://doi.org/10.1093/jn/130.6.1625

Nukmal, N., Umar, S., Amanda, S. P. & Kanedi, M. (2018). Effect of styrofoam waste feeds on the growth, development and fecundity of mealworms (*Tenebrio molitor*). *OnLine Journal of Biological Sciences*, 18, 24–28, https://doi.org/10.3844/ojbsci.2018.24.28

Oonincx, D. G . A . B., Van Itterbeeck, J., Heetkamp, M. J. W., Van Den Brand, H., Van Loon, J. J. A. & Van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*, 5, e14445, https://doi.org/10.1371/journal.pone.0014445

Parry, N. J., Pieterse, E. & Weldon, C. W. (2020). Stocking rate and organic waste type affect development of three *Chrysomya* species and *Lucilia sericata* (*Diptera: Calliphoridae*): Implications for bioconversion. *Journal of Applied Entomology*, 144, 94–108, https://doi.org/10.1111/jen.12712

Payne, C. L. R., Scarborough, P., Rayner, M. & Nonaka, K. (2016). A systematic review of nutrient composition data available for twelve commercially available edible insects and comparison with reference values. *Trends in Food Science and Technology*, 47, 69–77, https://doi.org/10.1016/j.tifs.2015.10.012

Poma, G., Cuykx, M., Amato, E., Calaprice, C., Focant, J. F. & Covaci, A. (2017). Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. *Food and Chemical*, 100, 70–9, https://doi.org/10.1016/j.fct.2016.12.006

Ribeiro, J. C., Sousa-Pinto, B., Fonseca, J., Caldas Fonseca, S. & Cunha, L. M. (2021). Edible insects and food safety: allergy. *Journal of Insects as Food and Feed*, 7(5), 833–847, https://doi.org/10.3920/JIFF2020.0065

Rumpold, B. A. & Schluter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research*, 57(5), 802–823, https://doi.org/10.1002/mnfr.201200735

Shantibala, T., Lokeshwari, R. K. & Debaraj, H. (2014). Nutritional and antinutritional composition of the five species of aquatic edible insects consumed in Manipur, India. *Journal of Insect Science*, 14, https://dx.doi.org/10.1093%2Fjis%2F14.1.14

Truzzi, C., Annibaldi, A., Girolametti, F., Giovannini, L., Riolo, P., Ruschioni, S., Olivotto, I. & Illuminati, S. (2020). A Chemically Safe Way to Produce Insect Biomass for Possible Application in Feed and Food Production. *International Journal of Environmental Research and Public Health,* 17(6), 2121, https://doi.org/10.3390/ijerph17062121

Vandeweyer, D., Lievens, B. & Van Campenhout, L. (2020). Identification of bacterial endospores and targeted detection of foodborne viruses in industrially reared insects for food. *Nature Food*, 1, 511–516, https://doi.org/10.1038/s43016-020-0120-z

Verheyen, G. R., Theunis, M., Vreysen, S., Naessens, T., Noyens, I., Ooms, T., Goossens, S., Pieters, L., Foubert, K. & Van Miert, S. (2020). Glycine-acyl surfactants prepared from black soldier fly fat, coconut oil and palm kernel oil. *Current Green Chemistry*, 7, 239–248, http://dx.doi.org/10.2174/2213346107999200424084626

Walia, K., Kapoor, A. & Farber, J. M. (2018). Qualitative risk assessment of cricket powder to be used to treat undernutrition in infants and children in Cambodia. *Food Control*, 92, 169–182, https://doi.org/10.1016/j.foodcont.2018.04.047

Wang, H., Rehman, K., Xiu Liu, Yang, Q., Zheng, L., Li, W., Cai, M., Li, Q., Zhang, J. & Yu, Z. (2017). Insect biorefinery: a green approach for conversion of crop residues into biodiesel and protein. *Biotechnology for Biofuels*, 10, 304–317, https://doi.org/10.1186/s13068-017-0986-7

Yandi, I., Öztürk, R. C., M. Kocabas, M., Kurtoglu, I. Z. & Altinok, I. (2023). Nutritional composition of black soldier fly (*Hermetia illucens*) reared on chicken waste meal, fruit & vegetable waste, and their mixture. *Journal of Insects as Food and Feed* (in press), 1–12, https://doi.org/10.3920/JIFF2022.0064

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.4.09:[616.98:579.842.1/.2

ID: 126364681

https://doi.org/10.18485/meattech.2023.64.2.43

Review paper

Yersinia enterocolitica and control measures for reducing risks in the pork production chain

Miloš Arsić[a], Ivan Vićić[b*], Miloš Petrović[c*], Marko Dmitrić[d*] and Nedjeljko Karabasil[b]*

[a] *Veterinary Specialized Institute "Niš", Dimitrija Tucovića br. 175, 18106 Niš, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia*
[c] *Ministry of Agriculture and Environment, Veterinary Directorate, Omladinskih brigada 1, 11070 Belgrade, Serbia*
[d] *Veterinary Specialized Institute "Kraljevo", Žička 34, 36000 Kraljevo, Serbia*

ARTICLE INFO

Keywords:
Yersinia
Pigs
Farm
Slaughterhouse
Hygiene

ABSTRACT

Yersiniosis caused by *Yersinia enterocolitica* is the third most common zoonosis transmitted from asymptomatic, healthy pigs to humans through raw or insufficiently cooked meat. The occurrence of *Y. enterocolitica* on a farm can vary, depending on different risk factors, including production system and biosecurity level. At the slaughterhouse, the contamination level of carcasses depends to a great extent on practices during lairage, along with the handling and processing of the head, tongue, tonsils, and rectum of slaughtered pigs. A comprehensive approach for further *Y. enterocolitica* farm/slaughterhouse categorization, improved hygiene practices, and mandatory surveillance for underestimated pathogens within the food chain is necessary for maintaining the One Health concept.

1. Essential characteristics of *Yersinia enterocolitica*

According to the latest taxonomic investigations, *Yersinia enterocolitica* belongs to the genus *Yersinia* and the family *Yersiniaceae* within the order *Enterobacterales* (*Schoch et al.*, 2020). *Y. enterocolitica* has two subspecies: *Yersinia enterocolitica* subsp. *enterocolitica* includes strains with the 16S rRNA type of American origin and *Yersinia enterocolitica* subsp. *palearctica* includes strains of European origin (BT1A, BT2, 3, 4, and 5) (*Neubauer et al.*, 2000). *Y. enterocolitica* is a heterogeneous group of strains classified into six biotypes (1A, 1B, 2, 3, 4, and 5) based on their phenotypic characteristics. Biotypes 1B, 2, 3, 4, and 5 are pathogenic to humans (EFSA, 2011), while biotype 1A is considered non-pathogenic (*Singh & Virdi*, 2004). Based on the chemical properties of the surface O antigen, *Y. enterocolitica* is divided into more than 48 serotypes. The most pathogenic serotypes for humans are biotype 4 (serotype O:3) and biotype 2 (serotype O:2) (*EFSA*, 2011; *Keet*, 1974). In many European countries, the most critical serotype of *Y. enterocolitica* is serotype O:3, followed by serotype O:9 (*Bottone*, 1997). *Y. enterocolitica* is an asporogenic, facultatively anaerobic, Gram-negative bacillus with morphological variations ranging from small cocco-bacilli with rounded ends to elongated bacilli. It is motile at 25°C, with peritrichously arranged flagella, and becomes non-motile when cultured at 37°C. Unlike other enteropathogenic bacteria, *Y. enterocolitica* is psychotropic and can grow from 0 to 44°C (*Keet*, 1974). As such, it can multiply at refrigerator temperatures and survive in frozen food for extended periods.

***Corresponding author:** Miloš Arsić, *arsicvsinis@gmail.com*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Y. enterocolitica can grow on solid nutrient media such as blood agar with sheep blood, MacConkey, and Hektoen-enteric agar. However, on these media, *Enterobacteriaceae* also grow well simultaneously (*Bottone*, 2015). The standard method for the isolation and identification of *Y. enterocolitica* (*SRPS EN ISO 10273:2017*, 2017) involves the use of a selective solid medium, CIN agar (agar supplemented with antibiotics cefsulodin, irgasan, and novobiocin), which is incubated at 30°C for 24–48 hours. Characteristic *Y. enterocolitica* colonies on CIN agar are small (<1 mm) and smooth, with a red centre (due to mannitol fermentation) and a translucent zone (*SRPS EN ISO 10273:2017*, 2017). Because of their characteristic appearance on CIN agar, they are called bull's-eye colonies, allowing easy recognition (*Schiemann*, 1979).

2. *Yersinia enterocolitica* within the food production chain

Y. enterocolitica is widely distributed in nature and can be found in the intestinal tracts of various mammals, birds, cold-blooded, and aquatic species. Isolates from the environment are primarily avirulent and do not cause human disease. However, isolates originating from pigs may contain pathogenic serotypes. Additionally, it is stated that dogs, ruminants, rodents, and water from the environment can also be reservoirs of these pathogenic bio-serotypes (*Fredriksson-Ahomaa et al*., 2006a; 2006b). The main source of infection is considered to be healthy, asymptomatic pigs. Humans are most commonly infected by consuming raw or undercooked food and contaminated water (*EFSA and ECDC*, 2019). Pigs are considered a natural reservoir of *Y. enterocolitica*, which explains its presence in slaughterhouses and the association between pork consumption and the frequency of yersiniosis cases (*Bonardi et al.*, 2013; *Vilar et al.*, 2015). It is estimated that 77.3% of yersiniosis cases in humans are attributed to consuming contaminated pork (*Fosse et al*., 2008). The most common mode of transmission is through the consumption of undercooked pork and pork products (*EFSA and ECDC*, 2018).

Production systems (conventional and organic) can influence the presence of these bacteria, with higher prevalence on farms using conventional production methods than organic production (*Von Altrock et al.*, 2006). The prevalence can vary within a farm, indicating the influence of specific factors on the farm. It has been observed that the prevalence of *Y. enterocolitica* BT4/O:3 is higher on open farms that purchase piglets (fattening farms) from external sources or small farms (*Skjerve et al.*, 1998; *Arsić et al.*, 2022; *Arsić*, 2023). Depending on the applied biosecurity measures on the farm, it has been found that the prevalence is higher on farms with lower levels of biosecurity measures (*Zdolec and Kiš*, 2021; *Arsić et al.*, 2022).

The slaughter process of pigs is an open process, during which the slaughter of infected pigs can lead to contamination and cross-contamination of carcasses and organs of slaughtered pigs (*Fredriksson-Ahomaa et al*., 2006a; 2006b). As this pathogenic microorganism remains present in the pig meat production chain, contamination of carcasses and products is possible at the early stages, especially during the handling and processing of the head, tongue, and tonsils of slaughtered pigs (*Van Damme et al.*, 2015). In a study conducted in a slaughterhouse in Brazil, the presence of *Y. enterocolitica* BT4/O:3 was detected in 10% of tonsil samples (*Martins et al.*, 2021). *Van Damme et al.* (2013) found the presence of *Y. enterocolitica* BT4/O:3 in 11.4% of swab samples from carcass surfaces and 4.9% of ground pork samples.

3. Implementation of good hygiene practices to reduce contamination

The presence of *Y. enterocolitica* cannot be detected by conventional meat inspection methods, so control measures focus on preventing or reducing faecal and other contamination starting from the farm during transportation, lairage period, and slaughter operations. The application of these measures is ensured through the implementation of good hygiene practices and the analysis of risks and critical control points at all stages of production (*Blagojevic et al.*, 2021). Slaughtering techniques and hygiene can influence the percentage of contamination on slaughter products. As pigs are asymptomatic carriers of *Y. enterocolitica*, meat inspection on the slaughter line can pose a risk of further meat contamination (*Laukkanen et al.*, 2009), since there is a possibility of transmitting this bacterium further along the meat production chain during carcass cutting and lymph node examination. Particular attention should be given to removing tonsils from the throat to reduce contamination, as incomplete removal can lead to contamination from lymphatic tissue to adjacent muscle tissue (*Borch et al.*, 1996). Implementing good hygiene practices throughout the entire slaughter process, particularly in handling procedures such as the release of the rectum and simultaneous tying with a plastic bag, can significantly reduce carcass contamination (*Andersen*, 1988). It has been found that removing the

head without prior splitting together with the carcass and washing and sterilizing knives can significantly reduce the contamination of carcasses with pathogenic *Y. enterocolitica* strains (*Van Damme et al.*, 2015).

4. *Y. enterocolitica* farm and slaughterhouse categorization

In recent years, the risk-based meat safety system has been the subject of scientific research (*Buncic et al*., 2019). The strategy to reduce the prevalence of *Y. enterocolitica* infections includes gathering epidemiological data and exchanging information within the food chain system, as well as defining risk factors and implementing measures to reduce or eliminate them to achieve and determine the priority categorization of farms/slaughterhouses, aiming to enhance food safety and public health (*Blagojevic et al*., 2021; *Zdolec and Kiš,* 2021). The presence of *Y. enterocolitica* in asymptomatic carriers poses an additional challenge to meat safety systems and interferes with hazard control in meat production. Since detecting this hazard on each carcass is practically infeasible and economically unjustifiable, applying preventive and control measures on farms and slaughterhouses is proposed as the only effective and efficient control approach (*Blagojević and Antić*, 2014). Based on risk categorization data on farms, the veterinarian overseeing the slaughter process could make decisions regarding ante-mortem meat inspection and, accordingly, approve or prohibit slaughter or implement additional measures for risk control, such as traditional post-mortem meat examination methods, meat freezing, laboratory analyses, or the application of carcass decontamination techniques (hot water, steam). This approach ensures better meat safety compared to the application of existing standard techniques (*Blagojević and Antić*, 2014; *Zdolec and Kiš*, 2021). Serological methods at the farm level are recommended for *Y. enterocolitica* farm categorization (*Bonardi et al*., 2016).

5. Monitoring and surveillance of *Y. enterocolitica*

At the level of the European Union, there is currently no obligation to monitor and report *Y. enterocolitica* findings in pigs and pork products. However, due to the increasing incidence of yersiniosis in humans caused by pathogenic strains of *Y. enterocolitica*, the European Food Safety Authority (EFSA) proposes the implementation of monitoring and reporting of *Y. enterocolitica* findings (*EFSA*, 2009). In the Republic of Serbia, a year-long epidemiological study has been conducted on *Y. enterocolitica* in pigs on the slaughter line. The test results indicate a prevalence of 10.4%, and the main risk factors for *Y. enterocolitica* infection have been identified as open-type farms, prolonged stay of pigs in slaughterhouse depots, and the winter season (*Arsić et al*., 2022; *Arsić*, 2023). Risk factor analysis revealed a twofold increased risk of infection in pigs originating from fattening farms compared to farrow-to-finish farms ($p<0.001$) (*Arsić et al.*, 2022; *Arsić,* 2023). There is also an increased risk of infection associated with prolonged stay in slaughterhouse depots (>3h) compared to shorter stay (0–3h) at the slaughterhouse level ($p<0.035$) (*Arsić et al*., 2022; *Arsić*, 2023). Regarding seasons, there is an almost fourfold higher probability of pig infection during the winter season compared to other annual periods ($p<0.001$) (*Arsić et al*., 2022; *Arsić*, 2023).

6. Conclusion

The finding of a large number of pigs infected with *Y. enterocolitica*, the possibility of further cross-contamination during slaughter and meat processing, and the ability of the bacteria to multiply at low temperatures during storage represent a risk to public health. Therefore, it is crucial to pay special attention to the hygiene conditions during the slaughter and the handling of pig parts, such as the head, tonsils, tongue, and rectum. Understanding the sources and pathways of *Y. enterocolitica* contamination is crucial in preventing foodborne illnesses. In addition to measures applied at the slaughterhouse, reducing the initial contamination on the pig farms is essential. A comprehensive approach for further *Y. enterocolitica* farm/slaughterhouse categorization and improved hygiene practices, along with mandatory surveillance for underestimated pathogens within the food chain, are necessary for maintaining the One Health concept.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The work was supported by grants from the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract no. 451-03-47/2023-01/200143).

References

Andersen, J. K. (1988). Contamination of freshly slaughtered pig carcasses with human pathogenic *Yersinia enterocolitica*. *International Journal of Food Microbiology*,7, 193–202.

Arsić, M. (2023). Izolacija i karakterizacija sojeva *Yersinia enterocolitica* kod svinja na liniji klanja (Isolation and characterization of *Yersinia enterocolitica* strains in pigs on the slaughterline). Doctoral Dissertation, Faculty of Veterinary Medicine, University of Belgrade.

Arsić, M., Vićić, I., Galić, N., Dmitrić, M., Kureljušić, J., Dimitrijević, M., Petrović, M., Šarić, LJ. & Karabasil, N., (2022). Risk factors and the overall characterization of *Yersinia enterocolitica* as an initial model of pathogen surveillance in the pig production system in Serbia. *Research in Veterinary Science*, 152, 167–174, https://doi.org/10.1016/j.rvsc.2022.08.007

Blagojevic, B. & Antic, D. (2014). Assessment of potential contribution of official meat inspection and abattoir process hygiene to biological safety assurance of final beef and pork carcasses. *Food Control*, 36(1), 174–182, https://doi.org/10.1016/j.foodcont.2013.08.018

Blagojevic, B., Nesbakken, T., Alvseike, O., Vågsholm, I., Antic, D., Johler, S., Houf, K., Meemken, D., Nastasijevic, I., Vieira Pinto, M., Antunovic, B., Georgiev, M., & Alban, L. (2021). Drivers, opportunities, and challenges of the European risk-based meat safety assurance system. *Food Control*, 124, 107870, https://doi.org/10.1016/j.foodcont.2021.107870

Bonardi, S., Bassi, L., Brindani, F., D'Incau, M., Barco, L., Carra, E. & Pongolini, S. (2013). Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. *International Journal of Food Microbiology*, 163(2), 248–257, https://doi.org/10.1016/j.ijfoodmicro.2013.02.012

Bonardi, S., Bruini, I., D'Incau, M., Van Damme, I., Carniel, E., Brémont, S., Cavallini, P., Tagliabue, S. & Brindani, F. (2016). Detection, seroprevalence and antimicrobial resistance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in pig tonsils in Northern Italy. *International Journal of Food Microbiology*, 235, 125–132, https://doi.org/10.1016/j.ijfoodmicro.2016.07.033

Borch, E., Nesbakken, T. & Christensen, H. (1996). Hazard identification in swine slaughter with respect to foodborne bacteria. *International Journal of Food Microbiology*, 30(1– 2), 9 –25, doi: 10.1016/0168-1605(96)00988-9

Bottone, E. J. (1997). *Yersinia enterocolitica*: the charisma continues. *Clinical Microbiology Review*, 10(2), 257–276, doi: 10.1128/CMR.10.2.257

Bottone, E. J. (2015). *Yersinia enterocolitica*: Revisitation of an enduring human pathogen. *Clinical Microbiology Newsletter*, 37(1), 1–8, https://doi.org/10.1016/j.clinmicnews.2014.12.003

Buncic, S., Alban, L. & Blagojevic, B. (2019). From traditional meat inspection to development of meat safety assurance programs in pig abattoirs — The European situation. *Food Control*, 106, 106705, https://doi.org/10.1016/j.foodcont.2019.06.031

EFSA, (2009). European Food Safety Authority. Technical specifications for harmonised national surveys of *Yersinia enterocolitica* in slaughter pigs on request of EFSA. *EFSA Journal,* 7(11), 1374, doi:10.2903/j.efsa.2009.1374

EFSA and ECDC, (2018). European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*, 16(12), e05500, https://doi.org/10.2903/j.efsa.2018.5500

EFSA and ECDC, (2019). European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union One Health 2018 Zoonoses Report. *EFSA Journal,*17(12), 5926, https://doi.org/10.2903/j.efsa.2019.5926

EFSA, (2011). European Food Safety Authority. Technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine. *EFSA Journal*, 9(10), 2371, https://doi.org/10.2903/j.efsa.2011.2371

Fosse, J., Seegers, H. & Magras C. (2008). Foodborne zoonoses due to meat: a quantitative approach for a comparative risk assessment applied to pig slaughtering in Europe. *Veterinary Research*, 39(1),1, doi: 10.1051/vetres:2007039

Fredriksson-Ahomaa, M., Stolle, A. & Korkeala, H. (2006a). Molecular epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunology Medicine Microbiology*, 47(3), 315 –29, doi: 10.1111/j.1574-695X.2006.00095.x

Fredriksson-Ahomaa, M., Stolle, A., Siitonen, A. & Korkeala H. (2006b). Sporadic human *Yersinia enterocolitica* infections caused by bioserotype 4/O:3 originate mainly from pigs. *Journal of Medicine Microbiology*, 55(6), 747–749, doi: 10.1099/jmm.0.46523-0

Keet, E. E. (1974). *Yersinia enterocolitica* septicemia — Source of infection and incubation period identified. *Journal of Medical Science*, 74(12), 2226–2230.

Laukkanen, R., Martínez, P. O., Siekkinen, K.-M., Ranta, J., Maijala, R. & Korkeala, H. (2009). Contamination of Carcasses with Human Pathogenic *Yersinia enterocolitica* 4/O:3 Originates from Pigs Infected on Farms. *Foodborne Pathogens and Disease*, 6(6), 681–688, https://doi.org/10.1089/fpd.2009.0265

Martins, B. T. F., Azevedo, E. C. de, Yamatogi, R. S., Call, D. R. & Nero, L. A. (2021). Persistence of *Yersinia enterocolitica* bio-serotype 4/O:3 in a pork production chain in Minas Gerais, Brazil. *Food Microbiology*, 94, 103660, https://doi.org/10.1016/j.fm.2020.103660

Neubauer, H., Aleksic, S., Hensel, A., Finke, E. J. & Meyer, H. (2000). *Yersinia enterocolitica* 16S rRNA gene types belong to the same genospecies but from three homology groups. *International Journal of Medicine Microbiology*, 290(1), 61–64, doi: 10.1016/S1438-4221(00)80107-1

Schiemann, D. A. (1979). Synthesis of a selective agar medium for *Yersinia enterocolitica*. *Canadian Journal of Microbiology*, 25(11), 1298–1304, doi: 10.1139/m79-205

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S. & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). Jan 1; 2020:baaa062, doi: 10.1093/database/baaa062

Singh, I. & Virdi, J. S. (2004). Production of *Yersinia* stable toxin (YST) and distribution of *yst* genes in biotype 1A strains of *Yersinia enterocolitica. Journal of Medicine Microbiology*, https://doi.org/10.1099/jmm.0.45527-0

Skjerve, E., Lium, B., Nielsen, B. & Nesbakken T. (1998). Control of *Yersinia enterocolitica* in pigs at herd level. *International Journal of Food Microbiology*, 45(3), 195–203, doi: 10.1016/s0168-1605(98)00162-7

SRPS EN ISO 10273:2017, (2017). Microbiology of the food chain — Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Institute for Standardization of Serbia. International Organization for Standardization, Geneva, Switzerland.

Van Damme, I., Berkvens, D., Botteldoorn, N., Dierick, K., Wits, J., Pochet, B. & De Zutter, L. (2013). Evaluation of the ISO 10273:2003 method for the isolation of human pathogenic *Yersinia enterocolitica* from pig carcasses and minced meat. *Food Microbiology*, 36(2), 170–175, https://doi.org/10.1016/j.fm.2013.05.007

Van Damme, I., Berkvens, D., Vanantwerpen, G., Baré, J., Houf, K., Wauters, G., & De Zutter, L. (2015). Contamination of freshly slaughtered pig carcasses with enteropathogenic *Yersinia* spp.: Distribution, quantification and identification of risk factors. International *Journal of Food Microbiology*, 204, 33–40. https://doi.org/10.1016/j.ijfoodmicro.2015.03.016

Vilar, M. J., Virtanen, S., Laukkanen-Ninios, R. & Korkeala H. (2015). Bayesian modelling to identify the risk factors for *Yersinia enterocolitica* contamination of pork carcasses and pluck sets in slaughterhouses. International Journal of Food Microbiology, 197, 53 – 57, doi: 10.1016/j.ijfoodmicro.2014.12.020

Von Altrock, A., Louis, A. L., Rösler, U., Alter, T., Beyerbach, M., Kreienbrock, L. & Waldmann, K. H. (2006). The bacteriological and serological prevalence of *Campylobacter* spp. and *Yersinia enterocolitica* in fattening pig herds in Lower Saxony. *Berliner und Münchener Tierärztliche Wochenschrift*, 119(9–10), 391–399.

Zdolec, N. & Kiš, M. (2021). Meat safety from farm to slaughter—risk-based control of *Yersinia enterocolitica* and *Toxoplasma gondii*. *Processes*, 9(5), 815, https://doi.org/10.3390/pr9050815

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 502.131.1:613.2
502.131.1:338.439

ID: 126393353

https://doi.org/10.18485/meattech.2023.64.2.44

Review paper

The One Health concept: a comprehensive approach to the function of a sustainable food system

Dragan Milićević[a], Radman Šelmić[b] and Zoran Petrović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *Serbian Chamber of Commerce, Resavska 13–15, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Sustainable development goals
European Green Deal
Circular economy
One Health concept

ABSTRACT

The modern world faces the challenge of rapid economic/industrial development being based on the irrational use of natural resources. Likewise, the technical-technological and civilizational progress that humanity has achieved, accompanied by the increase in the human population, has led to environmental damage, affecting climate change, intensifying global warming and causing environmental pollution. There is no doubt that due to socio-economic, political, health and environmental dimensions of sustainability, these processes are unsustainable in the long term. Moreover, if the world continues to use natural resources in accordance with the existing economic and demographic projections, by 2050, we would need three times the capacity of the Earth in terms of natural resources including energy. The Sustainable Development Goals (SDGs) are an important policy initiative, based on using clean and innovative technologies in a socially responsible manner that ensures poverty reduction, sustainable use of terrestrial ecosystems, ensures healthy life, and promotes well-being as a whole, accompanied by reduction of environmental pollution, proactive approach in the prevention of new sources and types of pollution and the protection of biodiversity. This review examines sustainable development aspects of importance from a One Health perspective, focusing on Serbia.

1. Sustainable development concept and significance

As a result of global, intensive efforts over the last decade, to apply the positive transformation of modern society, a UN Summit in New York on 25–27 September 2015 launched the resolution (A/RES/70/1) — Transforming our world: the 2030 Agenda for Sustainable Development (*UN,* 2015). Sustainable development has been defined in many ways, but the most frequently quoted definition is from The International Institute for Sustainable Development (IISD), which has defined this concept as a development that meets the needs of the present without compromising the ability of future generations to meet their own needs *(IISD*, 2022). The Sustainable Development Goals (SDGs) are a very complex and important policy initiative that cover harmonization, balance and synergy of three dimensions of human life:

- ecological — protect the planet from degradation, including through sustainable consumption and production, sustainably managing its natural resources and taking urgent action on climate change mitigation and adaptation,

***Correspondig author:** Dragan Milićević, *dragan.milicevic@inmes.rs*

Paper received March 30th 2023. Paper accepted April 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

- economic — economic, social and technological progress occurs in harmony with nature,
- social — end poverty and hunger in all their forms and dimensions, and ensure that all humans can fulfil their potential in dignity and equality and a healthy environment.

Each country commits to urgently mobilize all resources so that the 17 SDGs and 169 targets are achieved by 2030 and thereby shift the world onto a sustainable and resilient path.

Food security, as a prerequisite for the prevention of hunger and malnutrition, is a matter of priority of the Agenda for Sustainable Development 2030 (COR 2), aimed at protecting people's health and ensuring global economic development (*Linda et al.* 2020). Alongside continuing development priorities, food safety and security are integrated and indivisible with many of the SDGs, especially ending hunger and poverty (SDG 1 and 2) and promoting good health and well-being (SDG 3) (*FAO*, 2018; *Veldhuizena et al.* 2020).

The food system and sustainable nutrition are affected by several factors which interact: loss of biodiversity and deforestation, climate change, intensification of industrial production, natural resource scarcity, waste generation, the lack of fresh water and (renewable) resources, the trend of human population growth that requires ever-increasing amounts of food, nutrition-related diseases, social and economic inequalities and war conflicts (*Willett et al.* 2019) (Fig. 1). According to the World Economic Forum's Davos 2023 Global Risks Report, climate change remains the greatest long-term threat facing humanity. Climate change is one of the greatest challenges of our time, and its adverse impacts undermine the ability of all countries to achieve sustainable development. In addition, it generates new hazards and challenges not only in the least developed countries as was considered previously, but also at the global level. The Intergovernmental Panel on Climate Change released a report (*IPCC,* 2022) stating that the effects of climate change are present on all continents and in all oceans. According to a report by the Republic Hydrometeorological Service of Serbia, 15 of the 20 hottest years in Serbia were registered after 2000 (period 1951–2019) (*RHSS,* 2022). To avoid the most severe consequences for the ecosystem, the UN member states adopted the Paris Agreement (*UNFCCC,* 2015) to work on reducing greenhouse gas emissions. The Paris Agreement is the main framework for international cooperation in the fight against climate change and aims to keep the increase in the global average temperature well below 2°C above pre-industrial levels and for the signatory countries to continue their efforts to limit the increase to 1.5°C.

In addition to being an ecological problem, due to extreme weather conditions and natural disasters, climate change directly and/or indirectly negatively affects land and ocean ecosystems, water reserves and human habitats, and thus agriculture and human and animal health (*Dorward and Gille,* 2022). According to the World Bank data from 2016, the agriculture sector, including crop production and horticulture, covers almost 40% of the land area. Abiotic factors, such as air pollution, nutrient deficiencies and extreme temperatures, will affect soil quality, plant health and crop productivity. On the other hand, the impact of climate change will also be significant on the biotic factors of the environment, that is, on the spread of insects, rodents, plant pests and other vectors of infectious diseases common to humans and animals (zoonoses). If the implementation of good agricultural and good production practices does not meet the set goals, we can expect increased use of pesticides and other chemicals against plant pests (*Lam et al.* 2021). Therefore, the issue of real concern to human health due to the intake of low, but still toxic concentrations of pesticides and mycotoxins through food is raised (*Milićević et al.* 2020). The importance of the safety of animal feed should not be underestimated, as feed contaminated by environmental contaminants (toxic elements, pesticides, polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs)) jeopardizes the food chain and directly affects the presence of residues in food of animal origin (*Smulders et al.* 2019).

2. European Green Deal

The European Green Deal (*EU,* 2020a) is a direct response to the EU Agenda for Sustainable Development 2030. The European Green Deal sets out how to make Europe the first climate-neutral continent by 2050. It maps a new, sustainable and inclusive growth strategy to boost the economy, including construction, energy, transport, agriculture and food production, improve people's health and quality of life, care for nature and leave no one behind. This strategy presents the circular economy as one of the central elements for different use of raw materials, energy efficiency and substantial changes along the whole value and supply chains in the economy. In addition, it conceptualizes resource independence and resilience and keeps policy objec-

tives linked to sustainable consumption and production. Finally, the new set of policies directly related to the Green Deal also include potential trade barriers for countries that are not actively working to reduce greenhouse gas emissions.

Just one year after the adoption of this EU strategic document, the Green Agenda for the Western Balkans was also adopted in 2020, as a document that provides a strategic direction for the restructuring of economies in the region to make them compatible with the economies and societies of the EU, to which the Western Balkans converge. All these central pillars of the EU Green Deal are also incorporated into the Green Agenda for the Western Balkans.

3. Circular economy

The circular economy is a relatively new paradigm, an instrument for the realization of SDGs to replace the linear economy model, which in modern business conditions is unsustainable due to limited resources, accumulation of waste, inadequate waste management and damage to the environment (*World Resources Institute,* 2019). In the circular economy, products are produced, designed and used in a way that requires less use of natural resources, eliminating waste and pollution (landfills), more efficient management of waste as a raw material (recycling) and reduction of pollution to a minimum. Applied together, these principles can help tackle the environment and provide a better standard of living population (EU, 2020). We deem it relevant to underline that the circular economy as such, or some of its elements including, for example, carbon accounting, have become part of a regular university curricula in many countries both in the Global North and the Global South. Therefore, we are pleading for reconsideration to incorporate elements of the circular economy in the regular curricula within the whole spectrum of faculties at universities in Serbia.

Because of the expected global population growth, (8.5 billion by 2030 and 9.7 billion by 2050), an increase in production is expected from agriculture and the food industry, which will be one of the major drivers for the future consequences for the environment (*Petrovic et al.* 2015; *Petrovic et al.* 2017). The direct links between natural resources and the production processes make food systems a sector of particular importance to adopting the circular economy model, which should enable better use of resources without negative effects on biodiversity and the environment. The circular economy presents a great challenge for every national economy because it synthesizes strategies and innovative busi-

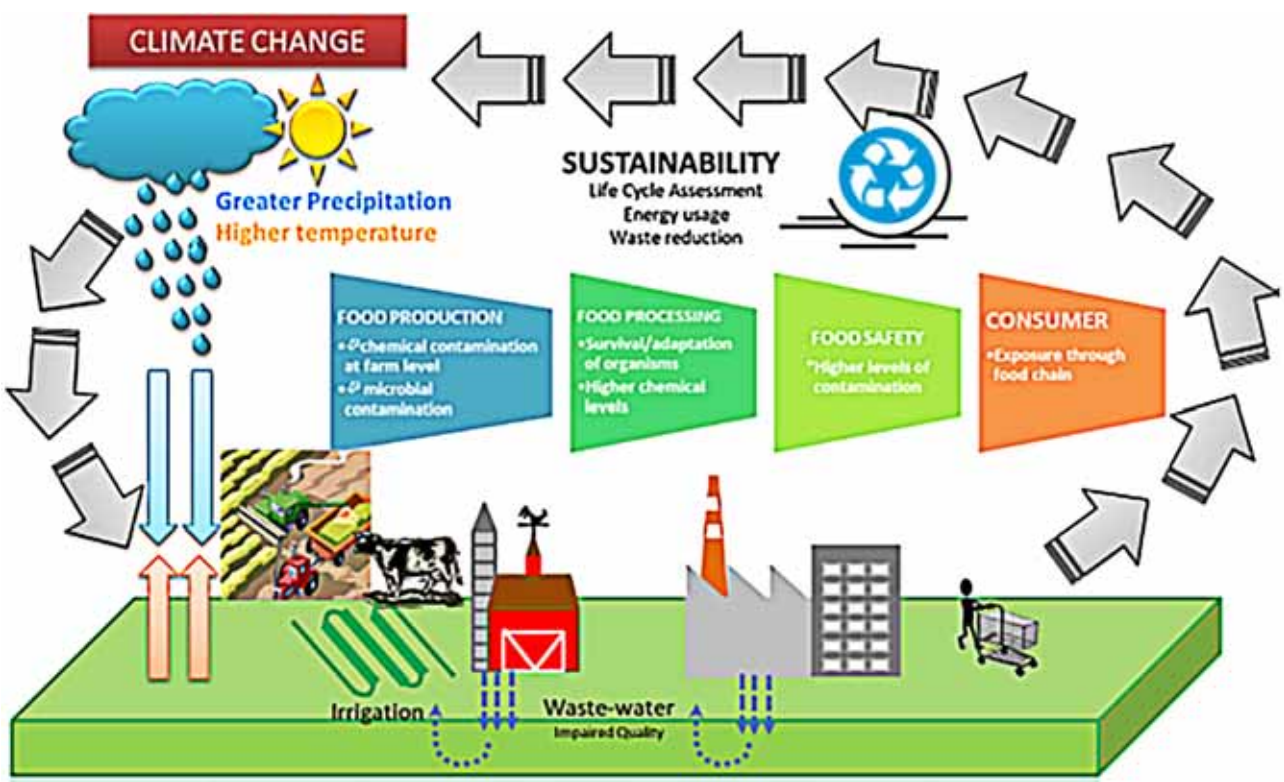


Figure 1. Illustration of climate change and food system interactions (Adopted by *Jeanne-Marie Membre*, 2022)

ness models. In the process of joining the EU, Serbia adopted the National Sustainable Development Strategy for the period from 2008 to 2017, as well as the Industrial Policy Strategy for the period 2021 to 2030, which also emphasizes the necessity of introducing the principles of the circular economy. The two strategies provide guidelines for further action in the area of sustainable development and new alternative strategies through the circular-green economy. The government of Serbia has recently adopted two documents that support this strategic commitment — the Program of Cooperation between the Ministry of the Economy and the Centre for Circular Economy within the Chamber of Commerce and Industry of Serbia; the Program for Circular Economy adopted in December 2022 by the Ministry for Environmental Protection. It must be pointed out that both these programs explicitly underline the urgent need for development of new academic programs and adaptation of existing university curricula.

4. The farm-to-fork strategy

Agriculture not only provides food and other raw materials but acts as the backbone of rural development, a sector of the economy that, with its multifunctional roles (economic, ecological, cultural and sociological), maintains the stability of society as a whole. The strategy from field to table, derived from the Common Agricultural Policy of the EU, is an innovative new strategy for the sustainability of the food system in the EU (*EC,* 2020b), which, along with the circular economy model and the biodiversity strategy, represent the very heart of the Green Deal. It comprehensively responds to the challenges of a sustainable food and nutrition chain and will enable the transition to a sustainable food system that guarantees food security and access to healthy foods originating from a healthy planet. This strategy will reduce the ecological and climatic footprint of the food system in EU countries and strengthen its resilience, protect the health of citizens, and ensure the continued operation of entities in the food system. The strategy includes specific goals for the transformation of the EU food system, including reducing the risks linked to pesticides by 50%, reducing the use of fertilizers by at least 20% by 2030, and reducing overall EU sales of antimicrobials for farmed animals and aquaculture by 50% by 2030. This approach will help to reach the objective of at least 25% of the EU's agricultural land under organic farming by 2030, and thus, to a significant increase in the share of organic production. The strategy also proposes ambitious measures to ensure that a healthy choice is also the best choice for EU citizens, which implies harmonized mandatory front-of-pack nutrition labelling so that consumers are better informed about food products.

5. One Health concept

The outset of the 21st century was characterized by the emergence of several pandemics caused by new zoonotic viruses such as severe acute respiratory syndrome (SARS), highly pathogenic avian influenza (HPAI), H5N1, pandemic H1N1, Zika and Ebola. These pandemics revealed how the health and economic systems of even developed countries can be affected and modulated by infectious diseases, pointing to the weaknesses of their healthcare concept and supply chain management (*AL-Eitan et al.* 2023). On the other hand, when evaluating the sustainability of the food chain from the perspective of consumers, all components of a sustainable food system must be taken into account. The World Trade Organization allows member countries to ban import of products from countries or companies found to be violating animal welfare standards, on the grounds of public morality. Surprisingly, food fraud, especially during the pandemic, has increased dramatically, and thus, trade chains are allowed to upgrade elements of private standards (GlobalG.A.P, FSSC 22000, BRC, IFC) to improve the market chain of agri-food products and strengthen consumer confidence. Guarantees are needed that food coming from farms is healthy, produced with sustainable production methods, with minimal impact on the environment and with a responsible approach to worker safety and animal welfare (*Bittisnich,* 2023).

The One Health concept is a joint tripartite collaborative idea from the FAO, WHO and WOAH (previously OIE) and implies cooperation, coordination and communication, across all relevant sectors and disciplines, with the ultimate goal of achieving optimal health outcomes for people, animals and the environment (*FAO, OIE, WHO,* 2019).

Areas of action of the One Health concept include:

- Control of zoonoses,
- Food safety,
- Action plan against antimicrobial resistance,
- Environmental protection

The One Health approach was primarily launched due to reduce risks of existing and potentially re-emerging infectious diseases transmissi-

ble between animals and humans, and it has been proven to be visionary in reducing pandemic risks. In addition, this approach has shown the importance of a coordinated, multisectoral approach in the prevention and control of other threats that arise as a result of the interaction of humans, animals and the environment (*Milićević and Pleadin,* 2022). Taking into account the processes of globalization and increasingly pronounced climate changes, as well as new trends in food safety and security, innovative solutions and stronger integration of all participants in the food chain will be required. Therefore, the concept of One Health is necessary as the only choice and answer to existing and future challenges related to the sustainable development of the food system (*Milićević and Nedeljković-Trailović,* 2021).

6. Conclusion

The concept of sustainable development was built from the need for economic growth to be based on clean and innovative technologies with socially responsible businesses that ensure poverty reduction, long-term better use of resources, improvement of health conditions and quality of life, accompanied by reduction of environmental pollution. Proactive approaches such as One Health in the prevention of new sources and types of pollution and in the preservation of biodiversity are in line with SDGs.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

AL-Eitan, L., Sendyani, S. & Alnemri, M. (2023). Applications of the One Health concept: Current status in the Middle East. *Journal of Biosafety and Biosecurity*, 5(1), 21–31, https://doi.org/10.1016/j.jobb.2023.01.001

Bittisnich, D. (2023). Food Safety in Global Trade: Opportunities and Challenges to Trade Harmonization, *Reference Module in Food Science*, Elsevier, 2023.

Dorward, A. & Gille, K. E. (2022). Change in the climate and other factors affecting agriculture, food or poverty: An opportunity, a threat or both? A personal perspective. *Global Food Security*, 33 100623, https://doi.org/10.1016/j.gfs.2022.100623

EC, (2020a). European Commission of Green Deal, Circular Economy Action Plan For a cleaner and more competitive Europe 2020. Retrieved from https://ec.europa. eu/environment/circular-economy/pdf/new_circular_economy_action_plan.pdf (accessed January 24, 2023).

EC, (2020b). Farm to fork strategy—for a fair, healthy and environmentally-friendly food system, COM/2020/381 final. Retrieved from https://ec.europa.eu/food/farm2fork_en (accessed January 24, 2023).

FAO, OiE, WHO, (2019). Taking a Multisectoral One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries; Food and Agriculture Organization of the United Nations, World Organization for Animal Health, World Health Organization: Rome, Italy. Retrieved from https://apps.who.int/iris/handle/10665/325620 (accessed January 24, 2023)

FAO, (2018). Sustainable Food Systems: Concept and Framework. Food and Agriculture Organization of the United Nations, Rome. Retrieved from https://www.fao.org/3/ca2079en/CA2079EN.pdf (accessed January 24, 2023).

Hospido, A. & Membré, J. M. (2022). Evaluation of climate change effects on food sustainability, safety and quality. *Trends in Food Science & Technology,* 126, 205–206.

International Institute for Sustainable Development (IISD). (2022). 20 Years of the Environmental Integrity Group in Global Climate Governance. Published by the International Institute for Sustainable Development. Website: enb.iisd.org

IPCC, (2022). Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, et al., (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844

Lam, S., Dodd, W., Berrang-Ford, L., Ford, J., Skinner, K., Papadopoulos, A. & Harpe. S. L. (2021). How are climate actions evaluated? A review of United Nations food security evaluations. *Global Food Security*, 28 100509, https://doi.org/10.1016/j.gfs.2021.100509

Milićević, D. & Nedeljković-Trailović, J. (2021). One Health approach in the food chain-the role of the veterinary service. In: One Health. Monographs of scientific conferences of AMS SMS, 10, number 1.

Milićević, D. & Pleadin J. (2022). Mycotoxins incidence in southeastern Europe countries: Implication on the food supply chain. In: Sustainable and nutrition-sensitive food systems for healthy diets and prevention of malnutrition in Europe and Central Asia, Editors: Cheng Fang and Mirjana Gurinović, Chapter: 3. Publisher: Food and Agriculture Organization of the United Nations (FAO).

Milićević, D., Udovički, B., Petrović, Z., Janković, S., Radulović, S., Gurinović, M. & Rajković. A. (2020). Current status of mycotoxin contamination of food and feeds and associated public health risk in Serbia. *Meat Technology*, 61(1), 1–36.

Petrovic Z., Milicevic D., Vranic D., Rajic S. & Simunovic S. (2021). Green Economy and Meat Processing — Future Prospects. The 61st International Meat Industry Conference 26–29 September 2021, Zlatibor, Serbia. *IOP Conf. Series: Earth and Environmental Science* 854 012074.

Petrovic, Z., Djordjevic, V., Milicevic, D., Nastasijevic, I. & Parunovic, N. (2015). Meat production and consumption: Environmental consequences (Proceedings Paper). 58. TH International Meat Industry Conference (Meatcon2015). October 4–7th 2015. in Zlatibor, Serbia. 5 pp. 235–238.

RHSS (Republic Hydrometeorological Service of Serbia), (2023). Annual bulletin for Serbia 2022. [Electronic resource: http://www. hidmet.gov.rs. Access date 24.03.2023] (In Serbian).

Smulders, F. J. M., Rietjens, I. M. C. M. & Rose, M. D. (2019). Chemical hazards in foods of animal origin and the associated risks for public health: elementary considerations. In: Chemical hazards in foods of animal origin. Wageningen Academic Publishers, The Netherlands, 672 pp, https://doi.org/10.3920/978-90-8686-877-3_01

UN, (2015). Transforming our world: the 2030 Agenda for Sustainable Development. United & UNFCCC. (2015). Paris agreement. Available from: https://unfccc.int/sites/default/file s/english_paris_agreement.pdf. Accessed 22.03 2023

Veldhuizena, L. J. L., Giller, K. E., Oosterveer, P., Brouwer, I. D., Janssen, S., van Zanten, H. H. E. & Slingerlan, M. A. (2020). The Missing middle: Connected action on agriculture and nutrition across global, national and local levels to achieve Sustainable Development Goal 2. *Global Food Security*, 24, 100336.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S. & Garnett, T., et al., **(2019).** Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet,* 393, 447–492.

World Resources Institute. (2019). World Resources Report. Creating a Sustainable Food Future: A Menu of Solutions to Feed Nearly 10 Billion People by 2050. Retrieved from https://reliefweb.int/report/world/world-resources-report-creating-sustainable-food-future-menu-solutions-feed-nearly-10. Accessed January 20. 2023

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 672.46:664.8
664.91:620.19

ID: 126439945

https://doi.org/10.18485/meattech.2023.64.2.45

Original scientific paper

Porosity and discontinuity of food can protective coatings — simple chemical tests and serious consideration

Zoran Petrović[a], Dragan Milićević[a], Saša Janković[a], Nikola Borjan[a], Danijela Vranić[a] and Dejana Trbović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Food cans
Protective lacquer
Defect
Porosity
Corrosion

ABSTRACT

In order to protect food cans against corrosion, tin coatings and lacquers are employed for internal and external protection. Coating porosity and discontinuity are practical problems in the food canning industry. The prevention of these problems lies in the timely observation and analysis of the scale that can arise in the production of food cans after filling and thermal processing. In this paper, we discuss results of two referenced simple chemical methods (sulphur dioxide and copper sulphate tests) for examining the porosity and discontinuity of food can protective coatings (tin and lacquer layers). In some specific cases, the tests showed precise locations of defects invisible to the eye.

1. Introduction

Satisfactory performance of organic coatings on tinplate depends upon the beneficial interaction of the tinplate surface and the organic formulation when they are joined by the metal roller coater to form lacquer/tinplate interface (*Barilli et al*., 2003). Metal can corrode, leading to a considerable loss of valuable food products and often making products unattractive and unacceptable, especially when food cans are intended for prolonged storage. Another form of corrosion noticed during storage is the under-film corrosion originating at lacquer pores or cracks (*FAO,* 1986). The ability to identify container defects at the start of control in food canneries is the basis for surveillance inspections. Many can defects are associated with the actual manufacturing process, and others are caused by handling of the container in the canning or can manufacturing industry. The main task of the can inspection specialist is to properly identify damaged products and to remove them from the supply system (*AMEDDC&S,* 2023). The organic coating must have satisfactory adhesion and porosity on the substrate (tinplate) and also must follow the movements of the metallic substrate during can manufacturing operations (beading, drawing, plastic deformation, manufacturing, etc).

Tests for continuity are frequently required for coatings of tin on the steel base of tinplate or organic coatings (lacquers) on the tin surface of tinplate. A distinction has to be made between clear gaps in a lacquer coating (wetting failures on the tinplate surface) or breaks made during can-making and the permeability of the film substance itself to molecules and ions (*Britton,* 1975)**.** In this paper, the porosity and discontinuity of protective lacquers was studied at laboratory scale employing copper sulphate solutions and sulphur dioxide test to reveal the loca-

***Corresponding author:** Zoran Petrović, zoran.petrovic@inmes.rs

Paper received April 24th 2023. Paper accepted May 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

tions of unsatisfactory internal end external protection against corrosion changes in food cans.

2. Materials and methods

The cans normally produced and used/or intended for packaging of fruit (pasteurised cherry) and low acid meat product (luncheon meat) were subjected to continuity and porosity tests. Cans were protected using a BADGE- free, organosol lacquer (canned fruit) or epoxyphenolic lacquer a BADGE free (luncheon meat). These types of coatings are commonly used in the food can manufacturing.

2.1. Sulphur dioxide test

A test vessel made of thick tempered glass of volume 30 L, fitted with an airtight closure of glass with a synthetic sealing compound around the rim was used. Solution was prepared using sodium thiosulphate ($Na_2S_2O_3$) crystal (Sigma Aldrich), 10 g/L and sulphuric acid (H_2SO_4; Merck) 0.1 N (4.9 g concentrated acid in 1 L). A volume of $Na_2S_2O_3$ solution equal to 1/20 of the capacity of the test vessel was introduced into the bottom of the vessel. A volume of H_2SO_4 equal to ¼ of the volume of $Na_2S_2O_3$ solution was then added to the vessel. After 5 minutes, test cans were hung on lines, fed through a little hole in the can wall previously drilled with a spike, then introduced in test container and lowered carefully into position in the test vessel so that no part of the can surface was more than 300 mm or less than 100 mm from the surface of the solution. Each can was more than 20 mm distant from other cans and from the walls of the test vessel. The vessel was closed and the cans left undisturbed for 24 h (Figure 1). Areas of exposed steel appear as dark reaction spots and the coating was assessed according to the number and distribution of these spots (Figure 2).

2.2. Copper sulphate test

Immersion test solution was prepared by dissolving 200g Copper(II)sulphate pentahydrate crystals ($CuSO_4 \cdot 5H_2O$; Merck) + 15 g sodium chloride (NaCl; Merck) in a normal vessel (1000 mL) by adding 2 ml hydrochloric acid (36%) (HCl; Merck). Test cans were introduced into a volume of solution enough for complete immersion during 30 minutes (plastic container). After this period, the cans were removed from solution with tweezers, gently rinsed with water and observed for deposited reddish spots (Figures 5 and 6).

3. Results and discussion

Typical surface spots visible after the chemical treatments described in Sections 2.1 and 2.2 are presented in Figures 1 to 6. Figures 1–4 are in relation to results of sulphur dioxide test and Figures 5–6 are in relation to copper sulphate test.

By observing the distribution of spots (Figures 2 and 3), it was established that coating damage occurs usually at the stamped end profile rings (Figure 2) or embossed beadings (can wall reinforcements) inside the can. Visual inspection of the defects present showed characteristic increases in the length of the lacquer cracks (Figure 3) inside the can. It seems that the elasto-plastic wave propagation of cracks (Figure 4) is in relation to internal stresses in the coating (*Fitzsimons & Parry,* 2011) where the surface shrinks faster than the layer of the coating film during can body fabrication and also due to the nature and acidity of content (pasteurized cherry).

The characteristics of the content can be of influence, although some types of fruit are less acidic than cherries, but also a greater corrosive potential that can undermine the lacquer and tin coating. The corrosion potential of the fruit being preserved is related to the profile of the organic fruit acids present, not the measured acidity itself (*FAO,* 1988).

On the one hand, the problem can be viewed from the point of view of the general flexibility of the coating applied, which is visible only after thermal processing and storage of the cherry cans. In this case — cracking of the protective coating is considered as a phenomenon and not a classic loss of adhesiveness (Figure 3). On the other hand, numerous factors influence the surface of the tinplate coatings, so the wetting of the organic coatings during application can be considered, as can possible contamination of the surface of the tinplate sheets before applying the coating, an unsuitably large amount of lacquer applied, high content of tin oxide on the surface of the tinplate if it has been stored for a long time, especially in warm and humid conditions, and a small amount and/or uneven distribution of the chrome passivation layer on the surface of the tinplate (*Benitez et al.,* 2006*; ITRA,* 2000). Furthermore, the topography of the surface of the tinplate, which has a high roughness, affects the distribution of the passivation layer of chrome, which is desirable for the stability of the organic coating that is applied, or could mean low deposit of tin in certain zones of the sheet, etc. (*Berk,* 2009). Can examinations using the faster copper sulphate test method by total immersion of cans (Figures 5 and 6) for 30 minutes produced visible indica-

Figure 1.

Figure 2.

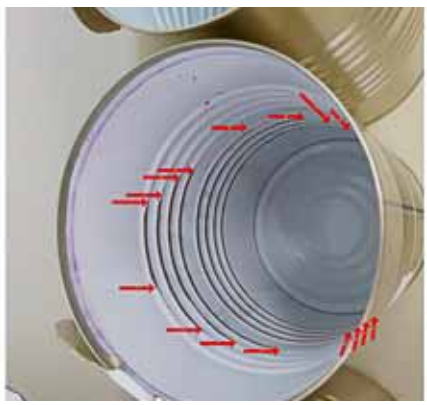


Figure 3.

Figure 4.

Figure 5.

Figure 6.

tions (deposits of copper) where lacquer porosity or discontinuity were present (internal protection of the can body welded joint and external surface of double seam around the can bottom). These spots would be the critical places where corrosive changes in subsequent food can production steps could occur. Primarily these problems can include: impurities in the processing water, longer cooling period after thermal processing, inadequate water drainage from cans, slow drying and poor storage and transportation conditions (*CAC,*1993). Electrochemical measurements (impedance measurements, polarization curves, potential measurement) can be carried out on cans, on a model solution or on the food product itself (*Montanari et al.,* 1996). These quantifiable measurements are comparable numerically in contrast to the chemical methods used in this study.

4. Conclusion

The sulphur dioxide test is very effective in revealing areas in cans where lacquer protection has been diminished by can making operations (stamping, beading, flexing, seaming), but the test is too slow for routine control measures. This method reveals the exposed steel base of tinplate used in can making process.

For lacquered tinplate intended to be used in food can fabrication for low acid products, the copper sulphate test gave a fast and effective indication all of the gaps and discontinuities in the protective lacquer film applied during can manufacture. Although this type of test does not give a numerical result (unlike instrumental electro-chemical measurements), other than an estimation of the number of discontinuities, it has the advantage that the position of the discontinuities is made clear.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, according to the provisions of the Contract on research financing 2023. No 451-03-47/2023-01/200050 dated from 03.02.2023.

Acknowledgements: We dedicate this work to our long-time colleague retired metal packaging specialist — technician Miroslav Mažibrada.

References

AMEDDC & S (2023). U.S. Army Medical Department Center and School, subcourse MD 0708, edition 100, Fort Sam Houston, Texas 78234-6100, Retrieved from https://www.scribd.com/document/13055187/food-containers#, Accessed April 18, 2023

Barilli, F., Fragni, R., Gelati,A. & Montanari A. (2003). Study on the adhesion of different types of lacquers used in food packaging. *Progress in Organic Coatings*, 46, 91−96.

Benitez, J. G., Cirillo, P. A., Gines, J. L. M. & Egli, W. A. (2006). Internal corrosion in tinplate cans, 16th, IAS Rolling Conference, San Nicolas, Argentina.

Berk, Z. (2009). Food packaging and shelflife, chapter 27, p -631, Food Process Engineering and Technology, ISBN 978-0-12-373660-4, Elsevier Academic Press, https://www.sciencedirect.com/book/9780123736604/food-process-engineering-and-technology

Britton, S. C., (1975). Tin Versus Corrosion, International Tin Research Institute, Fraser Road, Perivale, Greenford, Middlesex, England. I.T.R.I Publication No. 510, Section 4.4.1, p 94.

CAC/RCP 23–1979, Rev. 2 (1993). 1. Code of Hygienic Practice and Recommended Code of Hygienic practice for Low-Acid and Acidified Low Acid Canned Foods.

FAO, (1986). Guidelines for can manufacturers and food canners, FAO food and nutrition paper 36, Food and Agriculture Organization of the United Nations, Rome.

FAO, (1988). Quality control in fruit and vegetable processing, FAO food and nutrition paper 39, Food and Agriculture Organization of the United Nations, Rome.

Fitzsimons, B. & Parry, T. (2011). Fitz's Atlas 2 of Coating Defects, MPI Group, Surrey, U.K.

ITRA, (2000). Guide to tinplate, ITRA Ltd., Kingston Lane, Uxbridge, Middlesex, UB8 3PJ, United Kingdom.

Montanari, A., Pezzani, A., Cassara, A., Quaranta, A. & Lupi R. (1996). Quality of organic coatings for food cans: evaluation techniques and prospects of improvement. *Progress in Organic Coatings*, 29, 159−165.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664:579.86
579.86:575.111

ID: 126424841

https://doi.org/10.18485/meattech.2023.64.2.46

Review paper

Whole genome sequencing as the ultimate genomic subtyping tool for the identification and control of *Listeria monocytogenes* in the RTE food chain

Brankica Lakićević[a], Vesna Janković[a], Ariane Pietzka[b] and Werner Ruppitsch[c]*

[a] *Institute of Meat Hygiene and Technology, Department for Microbiological and Molecular-biological Testing, Belgrade, Republic of Serbia*
[b] *Institute of Medical Microbiology and Hygiene/National Reference Laboratory for Listeria Division for Public Health, Austrian Agency for Health and Food Safety, Graz, Austria*
[c] *Institute of Medical Microbiology and Hygiene Division for Public Health, Austrian Agency for Health and Food Safety, Vienna, Austria*

ARTICLE INFO

Keywords:
Listeria monocytogenes,
WGS
Food microbiology

ABSTRACT

Listeria monocytogenes, a saprophytic facultative anaerobic Gram-positive pathogen, has been involved in numerous outbreaks worldwide along with high mortality rate. Food contamination related to listeriosis occurs during primary production or more commonly, in food production facilities. The use of advanced diagnostic technologies such as whole genome sequencing (WGS) support surveillance, foodborne outbreak investigation as well as pathogen source tracking in food industry. WGS can also be useful for determining traits such as different genetic markers including virulence and antimicrobial resistance associated genes, which can be of great benefit for enhancing public health protection and for effective food safety management system. This is a very affordable, fast and powerful tool for obtaining high quality genomic data which can also be used in the regulatory field to differentiate more and less pathogenic *L. monocytogenes* clones.

1. Introduction

Listeria monocytogenes is a pathogenically heterogeneous species, comprising 14 serotypes and four evolutionary lineages that have been grouped into multiple clonal complexes (CCs) and sequence types (ST) according to multilocus sequence typing (MLST) (*Yin et al.,* 2019; *Orsi et al.,* 2011; *Ragon et al.,* 2008). *L. monocytogenes* CCs consist of: (a) infection-associated isolates, which belong to hypervirulent lineage I (e.g. CC1, CC2, CC4, and CC6), (b) food-associated isolates, which belong to hypovirulent lineage II and are mainly found within food processing environments (e.g. CC9 and CC121), and (c) intermediate-associated isolates that are greatly connected to both human and food settings (*Maury et al.,* 2016). Listeriosis is a relatively rare disease but causes a large proportion of deaths and severe cases mainly in vulnerable populations, including immunocompromised individuals, infants, pregnant women and elderly persons affecting those over 60 years of age. This is a predominantly foodborne disease where the people usually become ill after eating food contaminated by different clones of *L. monocytogenes*. In Europe, a total of 1876 listeriosis cases were reported in 2020 (*European Food Safety Authority and Euro-*

***Corresponding author:** Brankica Lakičević, *brankica.lakicevic@inmes.rs*

Paper received April 28th 2023. Paper accepted May 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

pean Centre for Disease Prevention and Control, 2021). Even low numbers of colony forming units (CFU) can be pathogenic for the susceptible people. The largest listeriosis outbreak was reported from a South African manufacturer in January 2017 lasting until July 2018 with a total of 1060 associated cases. Ready-to-eat processed meat products (polony) were pointed out as the source of infection (*Smith et al.,* 2019).

2. Transmission routes and WGS

Nastasijevic et al. (2017) illustrated a proof of concept for the practical application of WGS to provide insights into contamination pathways of *L. monocytogenes* in a pork processing factory. The authors used WGS to characterize and track 8 positive environmental isolates (out of 53 *Listeria* spp.) of *L. monocytogenes* that originated from critical sampling locations (CSLs) in pork meat production environment such as slaughter line, chilling chamber, deboning, modified atmosphere packaging (MAP) and dispatch. WGS analysis classified these isolates into three serotypes and clonal complexes (CC26, CC9 and CC1). The isolates of *L. monocytogenes* that originated from the above mentioned CSLs were either genetically close and/or identical to isolates from the slaughter line. Accordingly, *Nastasijevic et al.* (2017) deduced that the contamination derived from the slaughter line and emphasized WGS as a powerful tool of food safety management system. Furthermore, using WGS, *Demaître et al*. (2021) concluded that the persistence of *Listeria* contamination on the slaughter line was due to the presence of hypovirulent lineage II CC9 strain in the carcass splitter for longer than one year. However, *Burnett et al*. (2022) found hypervirulent I strains of *L. monocytogenes* in two meat processing facilities during 2011–2015. These strains originated from raw and environmental samples, representing serotypes 4b, 1/2b and 3b and belonged to clonal complexes: CC1, CC2, CC5 and CC288. Only a single lineage II isolate of serotype 1/2c was discovered in raw meat and belonged to CC9. The authors indicated that hypervirulent strains of *L. monocytogenes* can also well colonize, persist and predominate in food establishments. In that regard, colonization and persistent contamination of food and production establishments represents a high risk for human health.

3. Outbreak investigations and WGS

Large-scale listeriosis outbreaks investigated by WGS were reported in the US and EU.

In November 2014, WGS data showed that one restaurant in Rhode Island was the likely cause of a small outbreak. The establishment was also linked to a listeriosis case that happened in 2013 (*Berkley et al.,* 2016). In a multistate outbreak linked to Blue Bell Creamery, the ice cream was likely contaminated from the manufacturing facility (*Anonymous,* 2017). Due to the fact that ten cases from four separate states were found between 2010 and 2015, this outbreak was both complex and uncommon. Also, this product contained very low levels of contamination and did not support the growth of *L. monocytogenes* (*Chen et al.,* 2016; *Pouillot et al.* 2016). In another U.S multistate outbreak associated with the soft cheese distributed by Karoun Dairies in 2015, 30 persons in 10 states were affected and three deaths were recorded from California and Ohio (*Centers for Disease Control and Prevention*, 2015). In 2020, FDA and CDC analysed by WGS a multistate outbreak linked to contaminated fresh enoki mushrooms imported from Republic of Korea (*Centers for Disease Control and Prevention,* 2020). Some other listeriosis reports involve a 2018 multistate outbreak linked to pork products produced by Long Phung Food Products and an outbreak associated with hard-boiled eggs produced at the Almark Foods Gainesville, Georgia (*Centers for Disease Control and Prevention,* 2018, 2019). Interestingly, in the listeriosis outbreak reported in a 2020, deli meats were suspected as the cause, but the specific supplier and type of deli meat have never been confirmed (*Centers for Disease Control and Prevention,* 2020). In each of these outbreaks, WGS was utilized to depict genetic relatedness between outbreak strains and isolates suspected as an infection source. However, there are no WGS data for the latest listeriosis reports which include different RTE products such as packaged salads, cooked chicken and cheese.

In Denmark, WGS was used to solve outbreaks caused by the consumption of smoked fish, which led to deaths of seven persons and one stillborn baby (*Lassen et al.*, 2016). Also, two multi-country outbreaks caused by *L. monocytogenes* ST8 and ST1247 that affected Germany, France, Denmark, Estonia, Finland, France and Sweden were likely caused by consumption of ready-to-eat salmon products (*European Food Safety Authority European Centre for*

Disease Prevention and Control, 2018; 2019). An outbreak of listeriosis that occurred in Austria was caused by *L. monocytogenes* IVb-CC4-ST4-CT7652 strain which has not been detected anywhere before. Liver pâté was identified as the likely source of this outbreak (*Cabal et al.,* 2019). *Lüth et al.* (2020) used WGS to investigate one of the largest listeriosis outbreaks in Germany associated with consumption of meat products from a single producer. According to the *European Food Safety Authority* (2018), another multi-country outbreak of *L. monocytogenes* serogroup IVb, ST6 infection was traced to frozen corn that was produced in Hungary and packed in Poland. According to all of the aforementioned findings, WGS-based typing methods are a powerful tool for outbreak investigations and source tracking of *L. monocytogenes* in the food industry. As the amount of WGS data for *L. monocytogenes* increases, it is now possible to simultaneously identify some other genetic components, such as accessory genes associated with virulent and antibiotic-resistant phenotypes. This primarily refers to *Listeria* pathogenicity islands, (LIPI)-3 and (LIPI)-4, which carry genes conferring higher virulence (*Maury et al.,* 2016; *Tavares et al.*, 2020; *Centorotola et al.*, 2021; *Roedel et al.*, 2019) and antibiotic target-modifying enzymes. WGS analysis showed that antibiotic target-modifying enzymes (*mprF and fosX*) were detected in clonal complexes CC21 and CC121, while complexes CC8 and CC1 exclusively harboured *mprF* that changes cell wall charge (*Zuber et al*. 2019).

Overall, cooperation between epidemiologists, molecular microbiologists and bioinformaticians is essential for properly conducted outbreak investigations at both national and international level. Moreover, WGS has become an invaluable tool for discovering transmission routes and various antimicrobial resistance genes. In recent years, the cost of WGS has decreased significantly, allowing its routine use in the food industry which will benefit food safety management systems, public health, and food agencies. On the other hand, WGS can help in the revision of existing regulation because, in terms of quantitative microbiological risk assessments, not all *L. monocytogenes* CCs are considered to be equally pathogenic. In this way, CCs would be ranked according to their degree of virulence and association with different RTE foods (*Lakicevic et al*., 2022).

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, under the Agreement No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Anonymous, (2017). Blue Bell Creameries Inc. Summary of Root Cause Assessment Brenham, Texas, Facility, https://www.fda.gov/media/96744/download

Berkley, J. S., Gosciminski, M. & Miller, A. (2016). Whole-genome sequencing detection of ongoing *Listeria* contamination at a restaurant, Rhode Island, USA, 2014. *Emerging Infectious Disease*, 22, 1474–1476, doi:10.3201/eid2208.151917

Burnett, E., Kucerova, Z., Freeman, M., Kathariou, S., Chen, J. & Smikle, M. (2022). Whole-genome sequencing reveals multiple subpopulations of dominant and persistent lineage I isolates of *Listeria monocytogenes* in two meat processing facilities during 2011–2015. *Microorganisms*, 10, 1070, https://doi.org/10.3390/microorganisms10051070

Cabal, A., Allerberger, F., Huhulescu, S., Kornschober, C., Springer, B., Schlagenhaufen, C., Wassermann-Neuhold, M. & et al. (2019). Listeriosis outbreak likely due to contaminated liver pâté consumed in a Tavern, Austria, December 2018. Eurosurveillance, 24 (39), 1900274, https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900274

Centers for Disease Control and Prevention (CDC), (2015). Multistate outbreak of listeriosis linked to soft cheeses distributed by Karoun Dairies, Inc, https://www.cdc.gov/listeria/outbreaks/soft-cheeses-09-15/index.html

Centers for Disease Control and Prevention (CDC), (2018). Outbreak of *Listeria* infections linked to pork products, https://www.cdc.gov/listeria/outbreaks/porkproducts-11-18/index.html

Centers for Disease Control and Prevention (CDC), (2019). Outbreak of *Listeria* infections linked to hard-boiled eggs, https://www.cdc.gov/listeria/outbreaks/eggs-12-19/index.html

Centers for Disease Control and Prevention (CDC), (2020). Outbreak of *Listeria* infections linked to enoki mushrooms, https://www.cdc.gov/listeria/outbreaks/enoki-mushrooms-03-20/index.html

Centers for Disease Control and Prevention (CDC), (2020). Outbreak of *Listeria* infections linked to deli meats, https://www.cdc.gov/listeria/outbreaks/delimeat-10-20/index.html

Centorotola, G., Guidi, F., D'Aurizio, G., Salini, R., Di Domenico, M., Ottaviani, D., Petruzzelli, A., & et al. (2021). Intensive environmental surveillance plan for *Listeria monocytogenes* in food producing plants and retail stores of central Italy: Prevalence and genetic diversity. *Foods,* 10, 1944, https://doi.org/10.3390/foods10081944

Chen, Y. I., Burall, L. S., Macarisin, D., Pouillot, R., Strain, E., Jesus A. J. D. E., Laasri, A., & et al. (2016). Prevalence and level of *Listeria monocytogenes* in ice cream linked to a listeriosis outbreak in the United States. *Journal of Food Protection,* 79, 1828–1832, https://doi.org/10.4315/0362-028X.JFP-16-208

Demaître, N., De Reu, K., Haegeman, A., Schaumont, D., De Zutter, L., Geeraerd, A. & Rasschaert, G. (2021). Study of the transfer of *Listeria monocytogenes* during the slaughter of cattle using molecular typing. *Meat Science*, 175, 108450, https://doi.org/10.1016/j.meatsci.2021.108450

European Food Safety Authority (EFSA), (2018). Multi-country outbreak of *Listeria monocytogenes* serogroup IVb multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables — first update. EFSA Supporting Publications, https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2018.EN-1448

European Food Safety Authority European Centre for Disease Prevention and Control (EFSA and ECDC), (2018). Multi-country outbreak of *Listeria monocytogenes* sequence type 8 infections linked to consumption of salmon products. Technical Report, doi:10.2903/sp.efsa.2018.EN-1496

European Food Safety Authority European Centre for Disease Prevention and Control (EFSA and ECDC), (2019). Multi-county outbreak of *Listeria monocytogenes* clonal complex 8 infections linked to consumption of cold-smoked fish products. Technical Report, doi:10.2903/sp.efsa.2019.EN-1665

European Food Safety Authority and European Centre for Disease Prevention and Control (2021). The European Union One Health 2020 Zoonoses report. *EFSA Journal*, 19, 6971, doi: 10.2903/j.efsa.2021.6971

Lakicevic, B., den Besten, H. M. & De Biase, D. (2022). The landscape of stress response and virulence genes among *Listeria monocytogenes* strains. *Frontiers in Microbiology*, 12, https://doi.org/10.3389/fmicb.2021.738470 738470

Lassen, S. G., Ethelberg, S., Björkman, J. T., Jensen, T., Sørensen, G., Jensen, A. K., Müller, L. & et al. (2016). Two *Listeria* outbreaks caused by smoked fish consumption — using whole-genome sequencing for outbreak investigations. *Clinical Microbiology and Infection*, 22, 620–624, doi: 10.1016/j.cmi.2016.04.017

Lüth, S., Halbedel, S., Rosner, B., Wilking, H., Holzer, A., Roedel, A., Dieckmann, R. & et al. (2020). Backtracking and forward checking of human listeriosis clusters identified a multiclonal outbreak linked to *Listeria monocytogenes* in meat products of a single producer. *Emerging Microbes and Infections*, 9, 1600–1608, doi:10.1080/22221751.2020.1784044

Maury, M. M., Tsai, Y. H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A., Criscuolo, A. & et al. (2016). Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. *Nature Genetics*, 8, 308–313, doi: 10.1038/ng.3501

Nastasijevic, I., Milanov, D., Velebit, B., Djordjevic, V., Swift, C., Painset, A. & Lakicevic, B. (2017). Tracking of *Listeria monocytogenes* in meat establishment using whole genome sequencing as a food safety management tool: a proof of concept. *International Journal of Food Microbiology*, 257, 157–164, doi: 10.1016/j.ijfoodmicro.2017.06.015

Orsi, R. H., den Bakker, H. C. & Wiedmann, M. (2011). *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *International Journal of Medical Microbiology*, 301, 79–96, doi: 10.1016/j.ijmm.2010.05.002

Pouillot, R., Klontz, K. C., Chen, Yi., Chen, Yi., Burall, L. S., Macarisin, D., Doyle, M. & et al. (2016). Infectious dose of *Listeria monocytogenes* in outbreak linked to ice cream, United States, 2015. *Emerging Infectious Diseases,* 22, 2113–2119, doi:10.3201/eid2212.160165

Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le Monnier, A. & Brisse, S. (2008). A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathogens*, 4, e1000146, doi:10.1371/journal.ppat.1000146

Roedel, A., Dieckmann, R., Brendebach, H., Hammerl, J. A., Kleta, S., Noll, M., Al Dahouk, S. & et al. (2019). Biocide-tolerant *Listeria monocytogenes* isolates from German food production plants do not show cross-resistance to clinically relevant antibiotics. *Applied and Environmental Microbiology*, 85, e01253–e1319, https://doi.org/10.1128/AEM.01253-19

Smith, A. M., Tau, N. P., Smouse, S. L., Allam, M., Ismail, A., Ramalwa, N. R., Disenyeng, B. & et al. (2019). Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: laboratory activities and experiences associated with whole-genome sequencing analysis of isolates. *Foodborne Pathogens and Disease*, 16, 524–530, doi: 10.1089/fpd.2018.2586

Tavares, R. M., Silva, D. A. L. D., Camargo, A. C., Yamatogi, R. S. & Nero, L. A. (2020). Interference of the acid stress on the expression of llsX by *Listeria monocytogenes* pathogenic island 3 (LIPI-3) variants. *Food Research International*, 132, https://doi. org/10.1016/j.foodres.2020.109063 109063

Yin, Y., Yao, H., Doijad, S., Kong, S., Shen, Y., Cai, X., Tan, W. & et al. (2019). A hybrid sub-lineage of *Listeria monocytogenes* comprising hypervirulent isolates. *Nature Communications*, 10, 4283, https://doi.org/10.1038/s41467-019-12072-1

Zuber, I., Lakicevic, B., Pietzka, A., Milanov, D., Djordjevic, V., Karabasil, N., Teodorovic, & et al. (2019). Molecular characterization of *Listeria monocytogenes* isolates from a small-scale meat processor in Montenegro 2011–2014. *Food Microbiology*, 79, 116–122, doi:10.1016/j.fm.2018.12.005

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 613.281-056.24:616.12-008

ID: 126465289

https://doi.org/10.18485/meattech.2023.64.2.47

Original scientific paper

Development of functional meat cutlets with improved nutritional value and antioxidant properties to correct the diet of patients with cardiovascular disease

Marietta A. Aslanova[a], Olga K. Derevitskaya[a], Andrey S. Dydykin[a], Anna L. Bero[a] and Nataliya E. Soldatova[a]*

[a] *Federal State Budgetary Scientific Institution "V. M. Gorbatov Federal Research Center for Food Systems" of Russian Academy of Sciences,26, Talalikhina str., 109316, Moscow, Russia*

ARTICLE INFO

Keywords:
Cardiovascular diseases
Fatty acid composition
Meat cutlets
Oxidative stability
Antioxidant capacity

ABSTRACT

Taking into account the modern recommendations for patients to reduce cardiac risk, a recipe for functional meat cutlets was developed. The product features the substitution of saturated fatty acids with polyunsaturated ones, the reduction of the level of table salt and adding biologically active substances with pronounced antioxidant properties. The introduction of rapeseed oil instead of beef fat made it possible to significantly reduce the content of saturated fatty acids and increase the ratio of unsaturated fatty acids, thus providing a given ratio of PUFA:SFA in the range of 2.3–1.6. The test sample did not contain *trans*-fats. The results of studies of the antioxidant capacity of cutlet samples showed that the total background antioxidant capacity of the test sample was 9 times higher than in the control sample. The introduction of the antioxidant complex contributed to the preservation of the high antioxidant capacity of this product during its storage. It is possible to recommend inclusion of this product into the diet designed for cardiovascular disease prevention as a source of complete protein, vitamins and minerals.

1. Introduction

Cardiovascular diseases (CVD) are currently the main cause of deaths worldwide (*Smetneva et al.*, 2020). The contribution of dietary correction to progression of CVD ranges from 10% to 40%, which is quite comparable to the effect of drug therapy (*Petrikov et al.*, 2023). Diets enriched with ω3 fatty acids (≥ 0.85 g/day), containing an increased level of polyunsaturated fatty acids (PUFAs), and a reduced level of fat in the daily diet (less than 30%), have a significant capacity to reduce the risk of general mortality and mortality caused by CVD (*Petrikov et al.*, 2023).

Meat is the main source of complete animal protein (*Dydykin et al.*, 2022). Recently, however, several meta-analyses have linked high rates of red meat and processed meat consumption to various health conditions, including CVD (*Leroy et al.*, 2020, *Johnston et al.*, 2019; *Vernooij et al.*, 2019; *Zeraatkar et al.*, 2019). The lipid fraction of some kinds of meat is characterized by a relatively high content of saturated fatty acids (SFAs) and cholesterol, dietary intake of which is traditionally associated with an increased risk of CVD.

It has been shown that the characteristics of the meat, along with the overall chemical composition of the diet, are to be rather considered as important

***Corresponding author:** Anna L. Bero, *a.bero@fncps.ru*

Paper received April 28th 2023. Paper accepted May 16th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

factors. For example, the addition of 500 g/week of unprocessed lean (<10 g total fat, <5 g saturated fat, and <95 mg cholesterol per 100 g product) red meat to a cardioprotective diet did not result in increased CVD risk or adverse effects on blood lipid levels (*Vernooij et al.*, 2019).

In addition to the role of SFA and low-density cholesterol in atherogenesis, the role of oxidized lipoproteins in vascular disorder aetiology is also important (*Ahotupa et al.*, 2017, *Del gado et al.*, 2021; *Dehghan et al.*, 2017, *Mente et al.*, 2017). Some types of lipids are easily oxidized, forming a complex mixture of oxidation products that are actively involved in inflammatory reactions in atherosclerosis (*Testa et al.*, 2018). Oxidized lipids can be generated internally as well as supplied in the diet (*Otaegui-Arrazola et al.*, 2010). Lipid oxidation also occurs during the passage of meat through the various parts of the gastrointestinal tract, as the oxidation is facilitated by haeme iron, high levels of PUFAs, and the absence of suitable antioxidants (*Van Hecke et al.*, 2017). Vitamins A, C, E and D serve as components with antioxidant properties; the same is true for polyphenols, whose anti-radical properties are associated with the availability of several hydroxyl groups (*Bobrysheva et al.*, 2023).

Provided it is managed to level the existing negative factors, the introduction of functional meat products into common practice will help to adjust preventive diets. Based on relevance presented above, the aim of the research was to develop the nutrient composition of functional meat cutlets to correct the diet of patients with CVD, and to investigate the nutritional value and antioxidant properties of the functional cutlets.

2. Materials and methods

The object of the study were: green tea extract (polyphenol content 95%), (LLC Kazan Plant of Extracts); composition of vitamins and minerals (B6, B12, C, D3, E, calcium, magnesium); ready-to-eat meat cutlets – test sample and control sample.

To prepare the control samples of cutlets, minced meat (beef and chicken, in a 1:3 ratio) was used. Also, oatmeal flour, beef fat, chicken eggs, milk protein, salt and water were added. The content of table salt in minced meat was 2.0 g/100 g. The test samples were prepared according to a similar recipe, but with the replacement of beef fat with rapeseed oil and enriched with a vitamin-mineral complex and green tea extract. The content of table salt in minced meat was 0.9 g/100 g.

The prepared semi-finished cutlets were brought to culinary readiness (up to 85°C inside the product) in a Unox XVC 304 Chef Top LI1615AO steam convection oven (UNOX S.r.J, Padova, Italy) on the "steaming" mode for 100% of the cooking time, at a temperature of 110°C.

The prepared cutlet samples were tested in the Testing Center of the V.M. Gorbatov's Federal Research Center for Food Systems of the Russian Academy of Sciences (Accreditation Certificate No. RA.RU21PP69) according to the following research methods: The mass fraction of protein was determined according to the method of Kjeldahl (*GOST 25011-2016,* 2017). The mass fraction of fat was determined according to the major method of Soxhlet (*GOST 23042-2015,* 2017). The mass fraction of fat-soluble vitamins D_3 and E was determined by a method based on alkaline hydrolysis of a product sample and extraction with diethyl ether (*GOST 32307-2013,* 2013). Quantitative determination of water-soluble vitamins B_{12}, B_6 and C was carried out by high performance liquid chromatography in the ultraviolet range of the spectrum with a specified wavelength (*GOST R 55482-2013,* 2013). The mass fraction of magnesium (Mg) was determined by the flame atomic absorption method (*GOST R 55484-2013,* 2013). Determination of the mass fraction of calcium (Ca) was carried out by the atomic absorption method (*GOST R 55573-2013,* 2013). The acid value of the samples was determined by the titration of free fatty acids with a potassium hydroxide solution according to method *GOST R 55480-2013* (2013). The peroxide value of the samples was determined by titration with sodium thiosulfate solution and the quantitative determination of released iodine (*GOST 34118-2017,* 2017). The composition of fatty acids was determined by gas chromatography (*GOST R 55483-2013,* 2013) on Agilent 7890A automatic gas chromatograph (Agilent Tech., USA) with a flame ionization detector. A Supelco SP 2560 100m x 0.25mm x 0.2mkm chromatography column (Supelco, USA) was used to determine fatty acids. Total antioxidant capacity (TAC) was determined according to the FRAP (Ferric Reducing/Antioxidant Power) method.

All experiments were performed in triplicate. The data obtained were expressed as the mean value of triplicates ± standard deviation. Significance was determined by two-way analysis of variance (ANOVA) with Duncan's multiple range tests using SPSS Statistics 19.0 software (IBM Corp., USA). The

selected significance level for all statistical tests was equal to 5% ($p < 0.05$).

3. Results and discussion

Taking into account the current recommendations of the World Health Organization, the main marker indicators of functional meat cutlets for the prevention of CVD and their values are determined (Table 1) (*WHO*, 2017; *Starodubova et al*., 2020; *MR 2.3.1.0253-21*; *Petrikov et al.*, 2023).

The concentrations of functional ingredients in the specialized products should be between 15% and 50% of the average daily requirement of the respective nutrient. At the same time, functional ingredients should not deteriorate the nutritional value of the product and its consumer properties (*Kodentsova et al.*, 2020). Table 2 shows the list and the required level of application of functional ingredients that provide a positive effect on cardiovascular pathology.

Along with cardioprotective effects, vitamins C, E and D have antioxidant effects on free radicals

Table 1. Guidelines for food composition to reduce cardiac risk

Nutrients	Recommended daily intake	Recommended content per 100 g of product	Guidelines for intake level to reduce cardiac risk
Protein, g	80.0	Not less than 15.0	Consume an adequate amount of protein
Fat, g	70.0	Not more than 7.0	Reducing fat content to 30% of dietary energy, substitution of animal fats with vegetable fats
SFA, g	23.0	Not more than 1.5	Less than 10% of energy
MUFA, g	25.0	1.5–2.5	10–15% of energy
PUFA, g	22.0	Not less than 1.5	7–10% of energy
Ratio of PUFA:SFA	2.3–1.6	2.3–1.6	
Cholesterol, mg	less than 300	6–30	
Trans fats	→ 0	0	Less than 1% of energy
Table salt, g	less than 5.0	Not more than 1.2	Limited sodium intake

Table 2. Composition and recommended levels of functional ingredients

Nutrients	Recommended daily intake (MR 2.3.1.0253-21)	Recommended content per 100 g of product	Known cardioprotective effects (*Smetneva*, 2020; *Yardim-Akaydin*, 2003)
Flavonoids Flavan-3-ols, mg	200	not less than 100	Prevention of the development of atherosclerosis, normalization of tissue respiration
Vitamin B_6, mg	2.0	0.3–1.0	Improves the condition of blood vessels, regulates blood pressure
Vitamin B_{12}, μg	3.0	0.45–1.5	Protects vessel walls from compaction
Vitamin C, mg	100.0	15.0–50.0	prevents the processes of lipid peroxidation
Vitamin D_3, μg	15	2.25–7.5	Affects DNA methylation processes by altering the expression of many CVD-associated genes
Vitamin E, mg	15	2.25–7.5	Protects heart muscle from free radicals damage
Magnesium, mg	400.0	60.0–200.0	Restores heart rhythm
Calcium, mg	1,000.0	150.0–500.0	Contributes to lowering blood cholesterol levels

(*Smetneva et al.*, 2020; *Yardim-Akaydin et al.*, 2003). The same effects are found in flavonoids (including isoflavones), an effective source of which is green tea.

To confirm the efficiency of the green tea extract containing 95% polyphenols and the composition of vitamins and minerals, their TAC was evaluated. In the tested samples of green tea extract and composition of vitamins and minerals TAC was 442.424±0.398 and 147.204±2.777 μmol eq quercetin/g, respectively. The analyzed green tea extract containing 95% polyphenols proved to have a high antioxidant capacity (*Yang et al.,* 2016).

The fat composition of cutlets was modified by replacing beef fat with rapeseed oil, which is a source of ω-3 PUFAs. In rapeseed oil, oleic acid (OA: C18:1 n-9) is the most abundant (its share is 59%), followed by linoleic acid (LA: C18:2 n-6) with 19% and alpha-linolenic acid (ALA: C18:3 n-3) with 9%, but it lacks LC-PUFAs such as EPA and DHA *ω*-3 fatty acids (*Willora et al.,* 2021).

Several authors have added ω-3 PUFAs to enrich meat food. *Solomando et al.* (2020) added encapsulated fish oil to model meat systems to increase ω-3 content. *Lorenzo et al.* (2016) enriched the Spanish salchichon sausage to obtain better lipid formulations. Due to this enrichment, the total amount of PUFA in modified sausages was increased by 2.3%.

The results of the chemical composition of the test and control samples of cutlets are presented in Table 3.

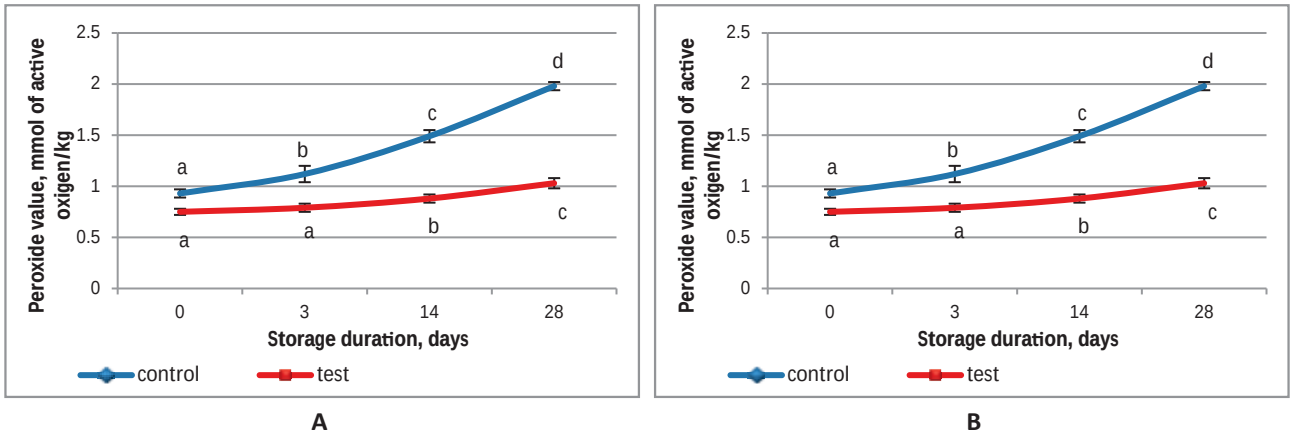
As can be seen from the above data, the use of rapeseed oil instead of beef fat significantly reduced the SFA content and increased the unsaturated fatty acid content, resulting a specific PUFA:SFA ratio

Table 3. Results of the analysis of the chemical composition of the samples

Parameter	Content per 100 g of cutlet	
	Control cutlet	Test cutlet
Protein, g	16.9±2.5	17.1±2.6
Fat, g	6.5±1.0	6.3±1.0
ΣSFA, g	3.37±0.31	1.36±0.14
ΣMUFA, g	2.68±0.10	2.47±0.17
ΣPUFA, g	0.44±0.04	2.53±0.19
PUFA:SFA ratio	0.13±0.03	1.86±0.05
Cholesterol, mg	39.02±0.91	30.5±0.79
Trans fats, g	0.18±0.03	not found
Table salt, g	2.50±0.50	0.98±0.19
Vitamin B_6, mg	0.32±0.06	1.38±0.35
Vitamin B_{12}, μg	1.44±0.11	1.98±0.67
Vitamin C, mg	0.30±0.07	45.50±10.47
Vitamin D_3, μg	0.08±0.03	8.57±1.71
Vitamin E, mg	0.24±0.01	2.75±0.41
Magnesium, mg	28.423±2.71	145.258±29.052
Calcium, mg	13.87±1.14	185.535±31.541

Table 4. Antioxidant capacity of the cutlet samples

Sample	OAE_{FRAP}, μmol eq quercetin/100 g of sample			
	0 days	3 days	14 days	28 days
Control cutlet	25.866 ± 0.330	25.228 ± 0.153	31.467 ± 0.554	33.268 ± 2.473
Test cutlet	232.754 ± 0.603	239.783 ± 0.726	228.058 ± 1.060	232.870 ± 0.385



Different letters (a, b, c, d) denote statistically significant differences between the corresponding samples during storage at p <0.05.

Figure 1. Change in peroxide value (A) and acid value (B) during storage of control and test cutlets

in the range of 2.3–1.6. The test sample did not contain *trans* fats.

To determine the efficiency of the selected composition of vitamins and green tea extract in the product composition, we analyzed the antioxidant capacity of the test sample during its storage in comparison with the control sample (Table 4).

The results of the analysis of the antioxidant capacity of the test and control samples of the cutlets showed that the TAC of the test sample was 9 times higher than that of the control sample. No significant differences in the TAC were observed during the storage of the test sample. The addition of the antioxidant complex helped to maintain the high antioxidant capacity of the test sample during its storage.

The effect of the antioxidant complex on the inhibition of oxidative processes during the storage of the food is shown in Figure 1. The inhibition of oxidative processes is expressed by the changes in peroxide value (Fig. 1 (A)) and acid value (Fig. 1 (B)).

The data in Fig. 1 (A) showed that the peroxide values in the control sample were higher than in the test sample during the storage period. The increase in peroxide value at day 28 was 112% for control samples and 37.3% for the test samples.

The same tendency was detected for the acid value (Fig. 1 (B)), as an accumulation of low molecular weight fatty acids. At the end of the storage period, the acid value increased by 3.5 and 2.6 times in the control samples and test samples, respectively.

The different dynamics of changes in peroxide and acid values in the test and control samples is probably associated with a different fatty acid composition of the samples and the presence of antioxidants with high antioxidant capacity.

4. Conclusion

Taking into account modern recommendations for patients to reduce cardiac risk, a recipe for functional meat cutlets was developed. In order to prevent CVD and correct risk factors, SFAs in the developed food were substituted with PUFAs, the level of table salt was reduced, and biologically active substances with pronounced antioxidant properties were added. The introduction of the antioxidant complex also reduced the intensity of oxidative processes in cutlets. The food can be recommended for inclusion into diet as a source of complete protein, vitamins and minerals for the prevention of CVD.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was carried out as part of the research topic No. FNEN-2019-0008 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS.

References

Ahotupa, M. (2017). Oxidized lipoprotein lipids and atherosclerosis. *Free Radical Research*, 51(4), 439–447, https://doi.org/10.1080/10715762.2017.1319944

Bobrysheva, T. N., Anisimov, G. S., Zolotoreva, M. S., Bobryshev, D. V., Budkevich, R. O. & Moskalev, A. A. (2023). Polyphenols as promising bioactive compounds. *Voprosy pitaniia [Problems of Nutrition]*, 92(1), 92–107, https://doi.org/10.33029/0042-8833-2023-92-1-92-107

Dehghan, M., Mente, A., Zhang, X., Swaminathan, S., Li, W., Mohan, V.... & Garcia, R. (2017). Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *The Lancet*, 390(10107), 2050–2062, https://doi.org/10.1016/S0140-6736(17)32252-3

Delgado, J., Ansorena, D., Van Hecke, T., Astiasarán, I., De Smet, S. & Estévez, M. (2021). Meat lipids, NaCl and carnitine: Do they unveil the conundrum of the association between red and processed meat intake and cardiovascular diseases? _Invited Review. *Meat Science*, 171, 108278, https://doi.org/10.1016/j.meatsci.2020.108278

Dydykin, A. S., Aslanova, M. A., Derevitskaya, O. K., Zubarev, Yu. N. & Logunova, E. I. (2022). Meat — a unique resource for creating functional food products. *Vsyo o Myase*, 3, 34–39, https://doi.org/10.21323/2071-2499-2022-3-34-39

GOST 23042-2015, (2015) . "Meat and meat products. Methods of fat determination" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/ 1200133107. Accessed April 05, 2023.

GOST 25011-2016, (2016). "Meat and meat products. Protein determination methods" Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146783. Accessed April 05, 2023.

GOST 32307-2013, (2013). "Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/ 1200107182. Accessed April 05, 2023.

GOST 34118-2017, (2017). "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146654 Accessed April 05, 2023.

GOST R 55480-2013, (2013). "Meat and meat products. Method for determination of acid value". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200103311 Accessed April 05, 2023.

GOST R 55482-2013, (2013). "Meat and meat products. Method for determination of water-soluble vitamins" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/ 1200104685. Accessed April 05, 2023.

GOST R 55483-2013, (2013). "Meat and meat products. Determination of fatty acids composition by gas chromatography". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200103852 Accessed April 05, 2023.

GOST R 55484-2013, (2013). "Meat and meat products. Determination of sodium, potassium, magnesium and manganese by flame atomic absorption" Moscow: Standartinform, 2014. Retrieved from https://docs.cntd.ru/document/ 1200103312. Accessed April 05, 2023.

GOST R 55573-2013, (2013). "Meat and meat products. Determination of calcium by atomic absorption and titrimetric methods" Moscow: Standartinform, 2020. Retrieved from https://docs.cntd.ru/document/ 1200105941. Accessed April 05, 2023.

ISO/IEC 17025:2017, (2017). "General requirements for the competence of testing and calibration laboratories". Technical Committee: ISO/CASCO Committee on conformity assessment, 2018. Retrieved from https://www.iso.org/standard/66912.html Accessed April 05, 2023.

Johnston, B. C., Zeraatkar, D., Han, M. A., Vernooij, R. W. M., Valli, C., El Dib, R. & Guyatt, G. H. (2019). Unprocessed red meat and processed meat consumption: dietary guideline recommendations from the Nutritional Recommendations (NutriRECS) Consortium. *Annals of Internal Medicine*, 171(10), 756–764, https://doi.org/10.7326/M19-1621.

Kodentsova, V. M., Zhilinskaya, N. V. & Shpigel, B. I. (2020). Vitaminology: from molecular aspects to improving technology of vitamin status children and adults. *Voprosy pitaniia [Problems of Nutrition]*, 89(4), 89-99, https://doi.org/10.24411/0042-8833-2020-10045

Leroy, F. & Cofnas, N. (2020). Should dietary guidelines recommend low red meat intake? *Critical Reviews in Food Science and Nutrition*, 60(16), 2763–2772, https://doi.org/10.1080/10408398.2019.1657063

Lorenzo, J. M., Munekata, P. E. S., Pateiro, M., Campagnol, P. C. B. & Domínguez, R. (2016). Healthy Spanish salchichón enriched with encapsulated n−3 long chain fatty acids in konjac glucomannan matrix. *Food Research International*, 89, 289–295, https://doi.org/10.1016/j.foodres.2016.08.012

Mente, A., Dehghan, M., Rangarajan, S., McQueen, M., Dagenais, G., Wielgosz, A. & Bo, H. (2017). Association of dietary nutrients with blood lipids and blood pressure in 18 countries: a cross-sectional analysis from the PURE study. *The Lancet Diabetes & Endocrinology*, 5(10), 774–787, https://doi.org/10.1016/S2213-8587(17)30283-8

Methodical recommendations MR 2.3.1.0253-21. "Norms of physiological requirements in energy and nutrients of various groups of the population of the Russian Federation", approved by the Head of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing Chief State Sanitary Physician of the Russian Federation 22.07.2021.

Otaegui-Arrazola, A., Menendez-Carreño, M., Ansorena, D. & Astiasarán, I. (2010). Oxysterols: A world to explore. *Food and Chemical Toxicology*, 48(12), 3289–3303, https://doi.org/10.1016/j.fct.2010.09.023

Petrikov, S. S., Khubutia, M. S. & Popova T. S. (2023). Parenteral and enteral nutrition: National Guidelines (2nd ed.), (Series "National Guidelines"), https://doi.org/10.33029/9704-7277-4-PAR-2023-1-1168

Smetneva, N. S., Pogozheva, A. V., Vasil'ev, Yu. L., Dydykin, S. S., Dydykina, I. S. & Kovalenko, A. A. (2020). The role of optimal nutrition in the prevention of cardiovascular diseases. *Voprosy pitaniia [Problems of Nutrition]*, 89(3), 114–124, https://doi.org/10.24411/0042-8833-2020-10035

Solomando, J. C., Antequera, T. & Perez-Palacios, T. (2020). Lipid digestion and oxidative stability in ω-3-enriched meat model systems: Effect of fish oil microcapsules and processing or culinary cooking. *Food Chemistry*, 328, 127125, https://doi.org/10.1016/j.foodchem.2020.127125/

Starodubova, A. V., Livantsova, E. N., Derbeneva, S. A., Kosyura, S. D., Polenova, N. V. & Varaeva, Yu. R. (2020). Cardiovascular nutrition: disease management and prevention as major public health problem nowadays. *Voprosy pitaniia [Problems of Nutrition*], 89(4), 146–160, https://doi.org/10.24411/0042-8833-2020-1004

Testa, G., Rossin, D., Poli, G., Biasi, F. & Leonarduzzi, G. (2018). Implication of oxysterols in chronic inflammatory human diseases. *Biochimie*, 153, 220–231, https://doi.org/10.1016/j.biochi.2018.06.006

Van Hecke, T., Van Camp, J. & De Smet, S. (2017). Oxidation during digestion of meat: interactions with the diet and helicobacter pylori gastritis, and implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, 16(2), 21–233, https://doi.org/10.1111/1541-4337.12248

Vernooij, R. W., Zeraatkar, D., Han, M. A., El Dib, R., Zworth, M., Milio, K., ... & Johnston, B. C. (2019). Patterns of red and processed meat consumption and risk for cardiometabolic and cancer outcomes: a systematic review and meta-analysis of cohort studies. *Annals of Internal Medicine*, 171(10), 732–741, https://doi.org/10.7326/M19-1583

WHO, (2017). Cardiovascular Diseases (CVDs). Geneva, Switzerland : World Health Organization. Retrieved from https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed April 3, 2023.

Willora, F. P., Grønevik, B., Liu, C., Palihawadana, A., Sørensen, M. & Hagen, Ø. (2021). Total replacement of marine oil by rapeseed oil in plant protein rich diets of juvenile lumpfish (Cyclopterus lumpus): Effects on growth performance, chemical and fatty acid composition. *Aquaculture Reports*, 19, Article 100560, https://doi.org/10.1016/j.aqrep.2020.100560

Yang, X. & Kong, F. (2016). Evaluation of the in vitro α-glucosidase inhibitory activity of green tea polyphenols and different tea types. *Journal of the Science of Food and Agriculture*, 96(3), 777–782, https://doi.org/10.1002/jsfa.7147

Yardim-Akaydin, S., Özkan, Y., Özkan, E., Torun, M. & Şimşek, B. (2003). The role of plasma thiol compounds and antioxidant vitamins in patients with cardiovascular diseases. *Clinica Chimica Acta*, 338(1–2), 99–105, https://doi.org/10.1016/j.cccn.2003.07.021

Zeraatkar, D., Han, M. A., Guyatt, G. H., Vernooij, R. W., El Dib, R., Cheung, K. ... & Johnston, B. C. (2019). Red and processed meat consumption and risk for all-cause mortality and cardiometabolic outcomes: a systematic review and meta-analysis of cohort studies. *Annals of Internal medicine*, 171(10), 703–710, https://doi.org/10.7326/M19-0655

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.521.238.4

ID: 126487817

https://doi.org/10.18485/meattech.2023.64.2.48

Original scientific paper

Monitoring of sulfites in kebabs and grilled meat

Jasna Kureljušić[a], Nikola Roković[a], Marija Pavlović[a], Aleksandra Tasić[a], Jelena Maletić[a], Dragana Ljubojević-Pelić[b] and Tanja Bijelić[a]*

[a] *Scientific Institute of Veterinary Medicine of Serbia, 11107, Janisa Janulisa, 14, Belgrade, Serbia*
[b] *Scientific Veterinary Institute "Novi Sad", Rumenacki put 20, 21113 Novi Sad*

ARTICLE INFO

Keywords:
Sulfites
Kebabs
Meat for grilling

ABSTRACT

Food additives are substances of known chemical composition, which are not consumed as food, nor are they a typical ingredient of food, regardless of nutritional value, but are added to food with the purpose of improving technological performance and retaining certain sensory properties. Additives widely used in the food industry include sulfur dioxide (SO_2) and sulfites (E 220 – E 228). Sulfur dioxide and its derivatives are added to food with the purpose of inhibiting and controlling the growth of microorganisms, preventing non-enzymatic browning, inhibiting reactions catalyzed by enzymes, and as antioxidants and reducing agents. The harmful effects of sulfur dioxide and sulfites are most often associated with allergic reactions from food, so it is necessary to provide consumers with information about their presence in food, even when they are found in very small amounts, because even then the possibility of an allergic reaction is not excluded. This research was conducted with the aim of determining the amount of sulfites in meat products in the period from 2019 to 2022. Altogether, 128 meat product samples were analyzed of which 53 were kebabs and 75 were meat for grilling. After testing, the mean levels of sulfur dioxide and sulfites in positive samples expressed in mg/kg were 210.0 mg/kg in kebabs and 110.6 mg/kg in meat for grilling. In conclusion, in most of the tested meat products, the sulfite concentration was below the established maximum permissible values according to national and European regulations.

1. Introduction

Sulfur dioxide and sulfites (SO_2) are additives that have been used for their disinfecting and purifying capabilities for at least 2,000 years. Sulfites are used in various technologies as preservatives, bleaching agents, antioxidants and flour treatment agents. They are allowed in many different foods, including wine, desserts, dried fruits and vegetables. They are employed in the food business to preserve the product's color, increase shelf life, and stop the development of bacteria. The majority of the time, they are added as additives during the production, processing, and storage of food products. However, they can also be naturally occurring components of foods, byproducts of the fungal metabolism in fermented beverages (beer, wine), or breakdown products of secondary metabolites containing sulfur (*Konić-Ristić and Šobajić*, 2005).

Different types of sulfites are used as additives. Because they are efficient antibacterial agents in acidic or acidified foods, inhibiting lactate dehydrogenase and other bacterial dehydrogenases, they are most frequently utilized as preservatives. Sulfites are additionally used as browning inhibitors since they

***Corresponding author:** Jasna Kureljušić, *jasnakureljusic@yahoo.com*

Paper received May 10th 2023. Paper accepted May 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

prevent both enzymatic and non-enzymatic browning. Sulfite stabilizes vitamins A and C in food by its facile oxidation to sulfate anion, which underlies its antioxidant activity. In the past, they were also employed to stop fresh food from browning, but most nations now forbid this practice. Sulfites have been used as additives for a very long time; records of their use extend back to the time of ancient Greece and Rome (*Queensland Government*, 2021).

Sulfites have negative impacts despite being crucial to the production of food. Since only 1% of people are sensitive to sulfites, the US Food and Drug Administration (FDA) mandates that sulfites be disclosed if they are used as a food ingredient, a processing aid, or a component of an ingredient used in food. Sulfites can lead to allergic reactions, which most frequently manifest as asthma symptoms in people who already have the allergic condition, occasionally as allergic rhinitis-like reactions, infrequently as urticaria (hives), and extremely rarely as anaphylaxis (severe allergic reaction) (*Leclercq et al*., 2000; *Warner et al*., 1986). Since SO_2 gas irritates, one possibility is that inhaling it causes the airways to reflexively contract. This mechanism might account for the symptoms' sudden onset. The enzyme sulfite oxidase, which aids in the breakdown of SO_2, is partially deficient in some asthmatics that react to sulfites. Skin tests for sulfite allergies rarely reveal real allergies in persons. The degree of exposure to SO_2 or sulfites from all sources determines a person's susceptibility to sulfites in food. Although the biochemical mechanisms underlying the onset and progression of adverse reactions to sulfites are poorly understood, it is unlikely that these reactions would be allergic, immune-mediated, or result in anaphylactic shock (*EFSA* 2004).

Although the threshold for sensitive reactions may be considerably lower, the European Union mandates the labeling of goods containing sulfites at concentrations of 10 mg/kg or greater. Depending on the kind of food, sulfur dioxide (SO_2) concentrations are given in mg/kg or mg/L and relate to the total amount from all sources in various foods. Where total SO_2 concentration is equal to or higher than 10 mg/kg (ppm), this is applicable. Given that this is the detection threshold, sulfites below this level are regarded as inconsequential and the food as sulfite-free.

In order to rationalize the range of additives that are already in use and to make the identification of additives as easy as possible, the E-numbering system (so-called E-numbers) was introduced in the EU. The prefix E indicates that these are additives that are applicable in Europe, which is shown in Table 1 (*Commission Decision*, 2021; *Rulebook of the Republic of Serbia*, 2018). This study's objective was to establish the levels of sulfites present in Serbian kebabs and grilled meat at stores.

Table 1. E-numbers for sulfites

Sulfites	E number
Sulfur dioxide	E220
Sodium sulfite	E221
Sodium hydrogen sulfite	E222
Sodium metabisulfite	E223
Potassium metabisulfite	E224
Calcium sulphite	E226
Calcium hydrogen sulphite	E227
Potassium hydrogen sulphite	E228

2. Materials and methods

2.1. Materials

The tested samples were sampled in Serbia in the period from 2019 to 2022. The meat products examined were kebabs and meat for grilling. The samples were sampled in plastic bags in amounts of 0.5 kg per sample. Following the cold chain, they were delivered to the laboratory and stored at a temperature of 2°C to 8°C in a refrigerator until the start of the analysis. Before analysis, they were taken out of the refrigerator and homogenized.

2.2. Method

To determine sulfites in kebabs and meat for grilling, the *AOAC* (2005) method was used. The method measures in food free sulfites and the fraction of bound sulfites, such as carbonyl addition products. Each test sample was heated under reflux with HCl (approximately 4 M) to convert the sulfites to SO_2. The stream of nitrogen introduced below the surface of the refluxed solution pushes SO_2 through the water-cooled condenser and through the drain

connected to the condenser into a 3% H_2O_2 solution, where SO_2 was oxidized to H_2SO_4. The sulfite content is directly related to the H_2SO_4 content, which was determined by titration with a standard NaOH solution. For verification, sulfates can be determined gravimetrically as $BaSO_4$ (*AOAC*, 2005).

3. Results and discussion

In the period from 2019 to 2022, the sulfite concentrations were determined in 53 samples of kebabs and 75 samples of meat for grilling, as shown in Table 2.

Within the group of 14 kebabs examined in 2019, sulfites were found in two of them. In 2020, 8 kebab samples were analyzed, of which sulfites were found in three. One of the seven samples tested in 2021 was positive for sulfites. In 2022, 24 samples were analyzed, three of which were found to contain sulfites, as shown in Figure 1.

Within 22 samples of meat for grilling inspected in 2019, sulfites were found in two of them. In 2020, 26 samples were analyzed, of which sulfites were found in four. Two of the 11 samples tested in 2021 tested positive for sulfites. In 2022, 16 samples were analyzed, one of which was found to contain sulfites, as shown in Figure 2.

In Serbia, the Rulebook on the quality of chopped meat, semi-finished products and meat products (*Official Gazette of the Republic of Serbia*, 2019; 2023) that contain nitrites and nitrates, sulfur dioxide, sulfites and phosphates cannot be used in the production of semi-finished meat products.

Table 2. Average sulfite level (mg/kg) in positive samples of kebabs and meat for grilling in the period from 2019 to 2022.

		Kebabs	Meat for grilling
2019	No. of samples	14	22
	No. of positive samples	2	2
	Average sulfite concentration in positive samples	101.95	87.90
2020	No. of samples	8	26
	No. of positive samples	3	4
	Average sulfite concentration in positive samples	265.20	232.73
2021	No. of samples	7	11
	No. of positive samples	1	2
	Average sulfite concentration in positive samples.	196.70	56.65
2022	No. of samples	24	16
	No. of positive samples	3	1
	Average sulfite concentration in positive samples	276.07	65.10

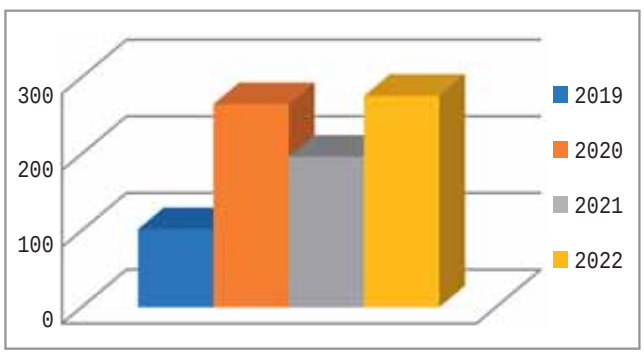


Figure 1. Mean values of sulfites mg/kg in kebabs in the period from 2019 to 2022

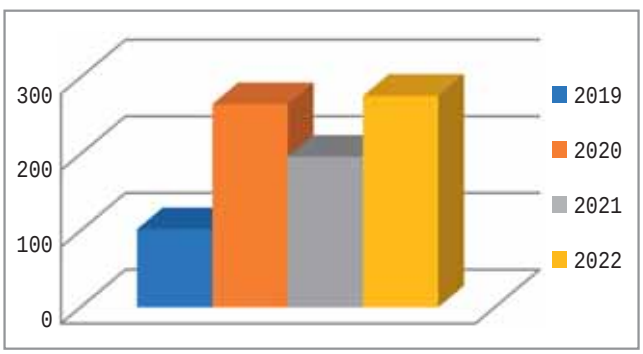


Figure 2. Mean values of sulfites (mg/kg) in meat for grilling in the period from 2019 to 2022

4. Conclusion

As a result of control activities carried out by an accredited laboratory to determine levels of sulphites in meat products, 128 meat product samples were analysed. A non-negligible percentage (14%) of these meat products contained sulphites, which confirmed that control of sulphite addition in meat preparations is still an important task for the food safety authorities. In particular, 18 samples resulted positive at a screening test. In any case, the mandatory labelling for sulfite concentrations higher than 10 mg/kg was established by the Regulation (EC) N. 1169/2011; *European Commission*, 2011). Also, as early as 1986, the US Food and Drug Administration (FDA) began to require that all sulfites be declared on the label of each product in which the concentration exceeded 10 mg/kg (ppm) (measured as sulfur dioxide (SO_2)), and that no sulphites are added to any food product intended for serving raw or presented as fresh food. Finally, even small amounts of sulfites can trigger food allergies in sensitive people, which is why the declaration is mandatory.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract 451-03-47/2023-01/200030).

References

AOAC, (2005). AOAC Official Method 990.28 — Sulfites in Foods — Optimized Monier-Williams Method.

Commission Decision, (EU) 2021/741, (2021). *Official Journal of the European Union*, 159.

European Commission, (2011). Council Regulation of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004, 1169/2011/EC. In: Official Journal, L 304/18, 22.11.2011.

European Food Safety Authority, (2004). Opinion of the Scientific Panel on dietetic products, nutrition and allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. *EFSA Journal*, 32, 1–197.

Konić-Ristić, A. & Šobajić, S. (2005). Rezidualni sumpor-dioksid u uzorcima suvog voća sa beogradskog tržišta. Institut za bromatologiju, 46(3–4), 51–55.

Leclercq, C., Molinaro, M. G., Piccinelli, R., Baldini, M., Arcella, D. & Stacchini P. (2000). Dietary intake exposure to sulphites in Italy-analytical determination of sulphite-containing foods and their combination into standard meals for adults and children. *Food Additives & Contaminants*, 17, 979–989.

Queensland Government, (2021). Sulphur dioxide in meat from https://www.qld.gov.au/health/staying-healthy/food-pantry/food-safety-for-consumers/food-warnings-and-advice/sulphur-dioxide-in-meat.

Rulebook of the Republic of Serbia, (2018). Known as the Rulebook on Food Additives, Official Gazette of the RS No. 53/18.

Warner, C. R., Daniels, D. H., Joe, F. L. & Fazio T., (1986). Reevaluation of Monier-Williams method for determining sulfite in food. *Journal Association of Official Analytical Chemists*, 69, 3–5.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664:658.56(470)

ID: 126495753

https://doi.org/10.18485/meattech.2023.64.2.49

Original scientific paper

Qualimetric assessment as a tool of the quality and safety management system of meat products with undeclared components

Elizaveta Kryuchenko[a], Yulia Kuzlyakina[a] and Valentina Zamula[a]*

[a] *V.M. Gorbatov Federal Research Center for Food Systems, Quality Management and Conformity Assessment Department, 109316, Moscow, Russia*

ARTICLE INFO

Keywords:
Meat products
Food components
Food allergen
Methods
Qualimetric assessment

ABSTRACT

The range of food components used in food production is constantly growing. The presence of food components is determined by the recipe, but however, contamination or unscrupulous use of them is possible. The presence of undeclared food components is fraught with, in addition to falsification, the risk of harm to the health of the consumer. It is important to determine the exact content of food components in a food product. This is especially true for substances that can cause food intolerance. It is possible to detect trace amounts of food components using various methods. Regulatory authorities in many countries, realizing the seriousness of the problem, are adopting laws, regulations and standards requiring food labelling to indicate the possible presence of food components that can lead to a state of hypersensitivity, thereby ensuring their exclusion. In the Russian Federation, such requirements are contained in TR CU 022/2011. The control of food components, including allergens, is required by all voluntary standards that set requirements for food safety management systems such as BRC, IFS, ISO 22000, etc. However, the scope of control measures is much wider than just food labelling and these measures are impossible without modern methodology and analytical methods. To control the presence of trace amounts of food components in food products, various methods are used — both qualitative and quantitative. The concentration of an additive (e.g., allergen, food component) that can cause a serious health hazard can be in the micro- and nanogram levels, and approaches are constantly being developed to increase the sensitivity of allergen detection methods in food products. These are immunoanalytical, mass spectrometric, chromatographic, histological methods, methods based on nucleic acid amplification, proteomic analysis, methods using biosensors. This article discusses such methods, and their advantages and disadvantages, and presents a qualimetric assessment of these methods in order to determine the most effective one, which will provide consumers with high-quality and safe products.

1. Introduction

Food hypersensitivity is a serious problem that affects approximately 3 to 10% of adults and 8% of children worldwide (*Osborne*, 2011). Reducing the risk of adverse food hypersensitivity reactions in food consumers requires the elimination of certain substances from the diet. However, it will not be effective without clear information about the presence of undeclared food components, in particular allergens, in products. This is especially true for those components that are not included in the recipe,

***Corresponding author:** Elizaveta Kryuchenko, *l.kryuchenko@fncps.ru*

Paper received May 4th 2023. Paper accepted May 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

but may potentially appear in the product in small quantities. EU labelling rules require clear labelling of 14 substances or products causing allergies or intolerances, while the US requires eight.

In the Russian Federation, the requirements for the labelling of food products containing the most common components, the use of which may cause allergic reactions or is contraindicated in certain types of diseases, are contained in Technical regulation of the Eurasian Customs Union № 022 "Food products in terms of their labelling" (TR CU 022/2011). The list of these food components is harmonized with the EU.

An analysis of publication activity for the period from 1990 to 2023 in the Scopus, PubMed and E-library databases revealed a sharp increase, approximately since 2005, in the number of scientific publications dealing with this topic. Interestingly, the e-library found 89 publications in 2023 for the keyword "food allergen" and 189 for "food intolerance". At the same time, 18840 and 15532 publications were issued by Scopus and PubMed, respectively. This indicates the lag of domestic science in this area, although since 2015 there has been a significant increase in interest in this topic, which is reflected in the growth of publication activity.

At present, worldwide there are no uniform established values for the minimum concentration of a particular food component that can cause an undesirable effect on the health of the consumer, the threshold dose. On the other hand, fast and sensitive methods for detecting food components are needed, especially when it comes to substances that are not included in the formulation of the product and contained in trace amounts. A mini-review of modern methods and approaches for the detection of food components in meat products, as well as their qualimetric assessment is presented.

2. Materials and methods

Qualimetric assessment of methods for determining food components in meat products includes several successive stages (*Bazrova*, 2015).

Table 1. List of potentially effective research methods for determining the presence of food components, including allergens, in meat products

№	Method name	The essence of the method
1.	Enzyme immunoassay (ELISA)	The method allows determination of the presence of hidden proteins in meat products that can lead to a hypersensitivity reaction.
2.	Lateral Flow Device (LFD)	The LFD is a test strip (rapid test). The method is based on an immunochromatographic approach in which proteins in a sample interact with antibodies and are conjugated simultaneously and held for a single short period.
3.	Polymerase chain reaction (PCR)	The method is characterized by amplification of DNA segments. PCR analysis can be viewed as a series of sequential steps: sampling, sample preparation, DNA extraction, DNA quantification, titration, PCR setup, equipment operation, software analysis, manual analysis.
4.	Mass spectrometry	The method allows protein identification, determines the primary amino acid sequences of the protein, identifies post-translational modifications and quantifies proteins in meat products.
5.	Histological method	The method is based on the identification of plant components of protein origin in various types of meat raw materials and finished meat products in accordance with their microstructural features using histological preparations.
6.	Liquid chromatography high-resolution mass spectrometry (LC-HRMS)	The method directly analyses the cleaved peptide fragments of the desired proteins by their differences in molecular weights.
7.	Surface plasmon resonance (SPR) biosensors	The method allows real-time detection of compounds interacting with an immobilized target molecule: an antibody against the desired protein or a single-stranded DNA molecule capable of hybridizing with a specific DNA fragment of food components.

At the first stage, a literature search of methods was carried out and for further assessment, those for which there was not found enough data on their effective use were excluded. As a result, a list of potentially effective research methods was formed that effectively and accurately determines the presence of undeclared protein-containing food components in meat products (Table 1).

Next, a qualimetric assessment was carried out to determine the current and expected (required) parameters in order to determine and predict changes in the compared characteristics (*Yankovskaya*, 2019; *Novak*, 2020).

The values of the weighting coefficients were calculated by the direct assessment method, which involves assigning points (C_{ij}) to each of the assessed indicators using scales. The average score C_i was calculated for each indicator using the formula:

$$C_i = \frac{\sum_{j=1}^{N} C_{ij}}{N}, \quad (1)$$

where C_{ij} is the score attributed to the i-th indicator by each expert;

N is the number of experts.

The weight coefficient of the i-th indicator was determined by the formula:

$$M_i = \frac{C_i}{\sum_{i=1}^{n} C_i}, \quad (2)$$

where C_i is the average score attributed to the i-th indicator; n is the number of evaluated indicators.

2. Results and discussion

Taking into account the principle of decomposition, a property tree diagram of quality indicators of the most appropriate method for determining food components in meat products was developed (Figure 1). The developed property tree made it possible to establish a range of indicators that determine the quality of methods for determining food components in meat products, build a hierarchical structure of the concept of quality of methods for determining food components in meat products, establish weight coefficients and conduct a comparative assessment (*Dunchenko*, 2019).

The histological method is defined as the most established, standardized method applicable to the study of food components, including allergens. Comprehensive studies of the structural features of vegetable protein components used in the production of meat products have been carried out in the V. M. Gorbatov Federal Research Center for Food Systems for fifteen years. The features of changes in vegetable protein components' microstructures during technological processing have been studied, and histological methods for their identification in any type of meat raw materials, semi-finished products and finished products have been developed by center experts. Based on the results of the research, the center developed the methodology GOST 31474-2012 "Meat and meat products. Histological method of identification of plant protein additives".

However, histological studies of vegetable protein preparations used in the meat industry have shown that similar technological variants of different proteins have similar microstructural identification indicators, which makes it difficult to analyse and interpret the results. So, for example, the structure and tinctorial properties of soy and wheat texturates are the same. Particles of some versions of the albumin preparation have microstructural characteristics similar to soy isolated proteins. Thus, the use of traditional histological methods for the detection of such proteins cannot always provide reliable information about their content in the composition of meat products.

ELISA remains the most widely used method for detecting food components and quantifying their presence. One reason for this is that the ELISA targets proteins in the food components that are the main causes of the hypersensitivity reaction. ELISA is relatively simple and can be performed by qualified personnel in a laboratory using relatively inexpensive equipment. The analysis can be completed within a few hours. ELISA test kits can detect the presence of gluten, lupine, soy and other protease inhibitors. There are currently no approved standards for the ELISA method for determining food components, including food allergens, in meat products (*Koeberl*, 2018).

Important advantages of ELISA include the presence of an express method (test strips). The method is well developed, has high sensitivity, which makes it possible to detect concentrations of up to 0.05 ng/ml of food components in meat products, the ability to use minimal volumes of the test material, the simplicity of the reaction, and the low

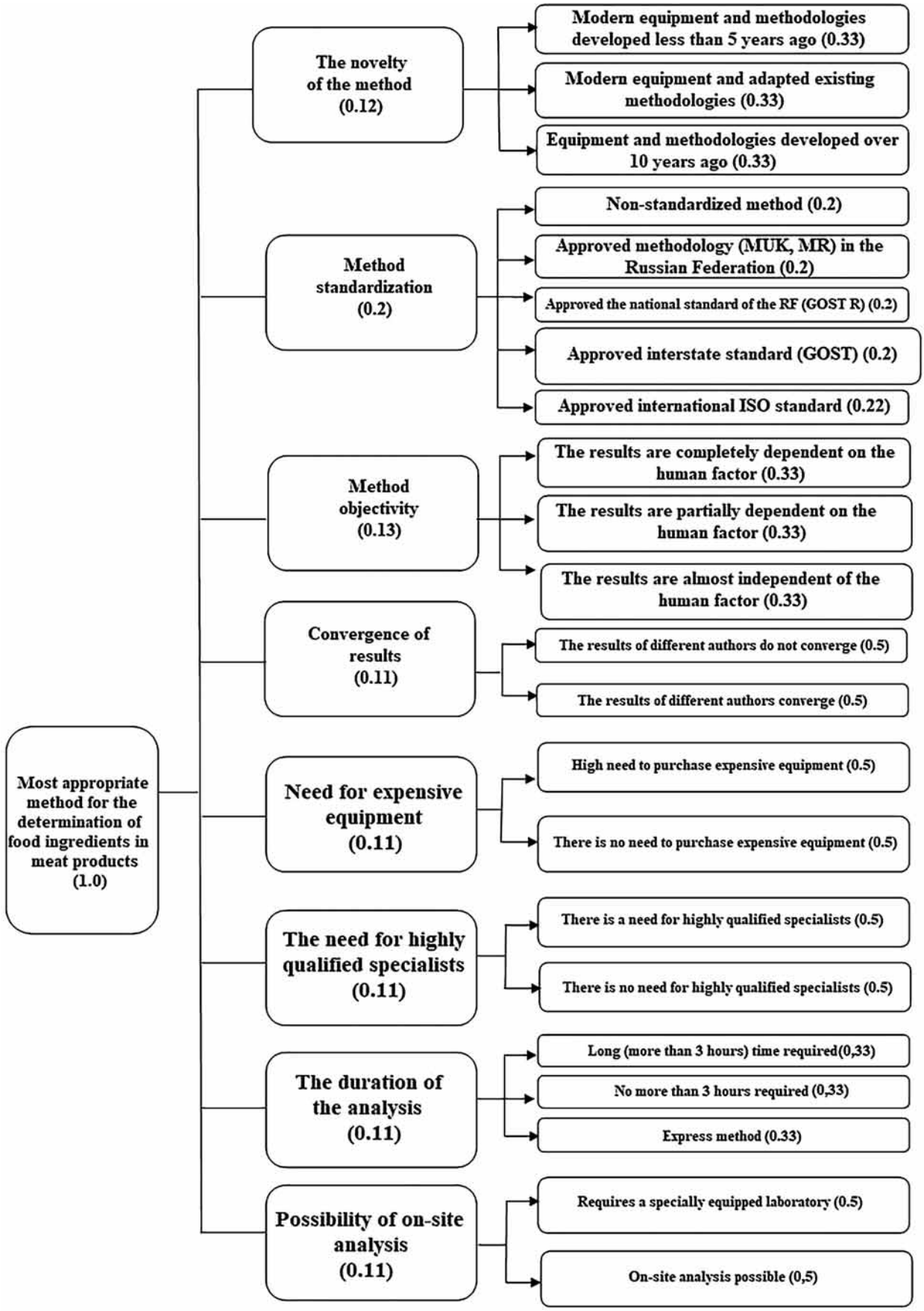


Figure 1. Property tree of quality indicators of the most appropriate method for determining food components in meat products

cost of diagnostic kits. ELISA test kits allow you to determine the target protein even in products that have undergone deep processing.

Immunohistochemical methods are currently being developed. They are highly specific and combine the advantages of traditional histological methods with the sensitivity of immunological ones.

Another method most commonly used is the polymerase chain reaction (PCR). The general principle of PCR is sequential and exponential amplification of the target DNA using a thermostable DNA polymerase. PCR can be applied to most food components containing DNA, and this method is required by law in many countries, for example, in the EU in accordance with Annex 3a of the European Directive 2003/89/EC. The specificity of the method allows, in most cases, unambiguous identification of the food component, which is especially important in the case of the presence of a protein in the product that can cause a hypersensitivity reaction. This makes the PCR method an important analytical tool. In addition, its high specificity makes it possible to check the results obtained by ELISA, which cannot be absolutely accurate due to the nature of the antibody-based ELISA method. Moreover, PCR is able to distinguish several protein targets that cannot be separated from other food components using ELISA. PCR requires a special laboratory room and trained personnel (*Cheng*, 2016).

A key advantage of the lateral flow device for detection is the rapidity and simplicity of the test, typically requiring little or no sample or reagent preparation. A huge advantage is that the method does not require expensive equipment. Also, the lateral flow device is an express method for determining the presence of food components in food products such as: lupine, soy, mustard, celery, gluten (*Dzantiev*, 2014).

The LC-HRMS/MS method is an analytical chemistry method that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). The advantage of the LC-HRMS/MS method is that it is able to detect the presence of several food components in meat products. However, the method has significant drawbacks: the need for expensive equipment, the complexity and duration of the analysis. These disadvantages complicate the method's widespread introduction into laboratory practice under production conditions at industry enterprises (*Stella*, 2020). Currently, there is an active standardization of methods based on LC-HRMS/MS.

The method of proteomic analysis, coupled with mass spectrometry, is a fairly new technology in relation to the detection of the presence of food components in products. The method requires expensive equipment and qualified personnel.

The use of SPR biosensors is a new tool for identifying food components. The use of biosensors makes it possible to identify food components in real time. Main disadvantages: expensive equipment, takes up a lot of space in the laboratory, it is difficult to simultaneously analyse several food components in one sample. At the same time, SPR biosensors have great potential for manufacturers and regulatory authorities (*Vasilescu*, 2020).

3. Conclusion

The conducted qualimetric evaluation of the main methods used to detect food components allows us to compare methods based on common characteristics.

The results of assessing the potential effectiveness of methods for determining the presence of food components in meat products allowed us to build the following sequence of effectiveness: histological method; ELISA; PCR; 2DE-MS; LFD; SPR.

The widespread use of many methods is complicated by the need to purchase expensive equipment, insufficient development of express method modifications, and the complexity of implementing most methods in production laboratories.

Considering the ever-growing range of food additives, the temptation for unscrupulous manufacturers to include unreasonable amounts of additives in formulations, the importance of in situ monitoring and control of food components, and the obligation of manufacturers to include food component control in production control programs, a qualimetric approach to choosing an appropriate method can serve as a reliable tool for the manufacturer.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was supported by the state assignment for V.M. Gorbatov Federal Research Center for Food Systems of RAS, project No. FNEN-2019-0007.

References

Bazrova, F. S., Bessonova, L. P. & Antipova, L.V. (2015). Qualimetric quality assessment of iodine supplements. Proceedings of the Voronezh State University of Engineering Technologies, 1, 143–149, https://doi.org/10.20914/2310-1202-2015-1-143-149

Cheng, F., Wu, J., Zhang, J, Pan, A., Quan, S, Zhang, D, Kim, H. Y., Li, X., Zhou, S. & Yang, L. (2016). Development and inter-laboratory transfer of a decaplex polymerase chain reaction assay combined with capillary electrophoresis for the simultaneous detection of ten food allergens. *Food Chemistry*, 199, 799–808, https://doi.org/10.1016/j.foodchem.2015.12.058

Dunchenko, N. I., Hadgu M. S., Voloshina E. S., Yankovskaya V. S., Kuptsova S.V. & Ginzburg M. A. (2019). Identification of health and safety management system components in the fish patty production. Proceedings of the Voronezh State University of Engineering Technologies, 81(1), 105–111, https://doi.org/10.20914/2310-1202-2019-1-105-111

Dzantiev, B. B., Byzova, N. A., Urusov, A. E. & Zherdev, A. V. (2014). Immunochromatographic methods in food analysis. *Trends in Analytical Chemistry*, 55, 81–93, https://doi.org/10.1016/j.trac.2013.11.007

Koeberl, M., Sharp, M. F., Tian, R., Buddhadasa, S., Clarke, D. & Roberts, J. (2018). Lupine allergen detecting capability and cross-reactivity of related legumes by ELISA. *Food Chemistry*, 256, 105–112, https://doi.org/10.1016/j.foodchem.2018.02.043

Novak, A. I., Lyashchuk, Y. O., Ivanishchev, K. A. & Platonova, O. V. (2020). Analysis of quality and safety indicators in the production of halal meat products. Proceedings of the Voronezh State University of Engineering Technologies, 82(4), 69–76, https://doi.org/10.20914/2310-1202-2020-4-69-76

Osborne, N. J., Koplin, J. J., Martin, P. E., Gurrin, L. C., Lowe, A. J., Matheson, M. C., Ponsonby, A.-L., Wake, M, Tang, M. L. K, Dharmage, S. C. & Allen, K. J. (2011). Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *Journal of Allergy and Clinical Immunology*, 127(3), 668–676, e2, https://doi.org/10.1016/j.jaci.2011.01.039

Stella, R., Sette, G., Moressa, A., Gallina, A., Aloisi, A. M., Angeletti, R. & Biancotto, G. (2020). LC-HRMS/MS for the simultaneous determination of four allergens in fish and swine food products. *Food Chemistry*, 331, 127276, https://doi.org/10.1016/j.foodchem.2020.127276

Vasilescu, A., Polonschii, C., Titoiu, A. M., Mishra, R., Peteu, S. & Marty, J.-L. (2020). Bioassays and biosensors for food analysis: focus on allergens and food packaging. *Commercial Biosensors and Their Applications,* 217–58, https://doi.org/10.1016/B978-0-12-818592-6.00009-8

Yankovskaya, V., Dunchenko, N, Artykova, D, Ginzburg, M, Mikhaylova, K. & Voloshina, E. (2019). Food quality management based on qualimetric methods. Proceedings of the 9th International Scientific Conference Rural Development, 93–97, https://doi.org/10.15544/RD.2019.005

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 504.5:638.138

ID: 126496521

https://doi.org/10.18485/meattech.2023.64.2.50

Review paper

Honeybee pollen as a bioindicator of contamination: an overview

Jelena Ćirić[a], Nils Haneklaus[b,c], Tatjana Baltić[a], Sara Simunović[a], Nenad Parunović[a], Dejana Trbović[a] and Boris Mrdović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *Universität für Weiterbildung Krems, 3500, Krems an der Donau, Austria*
[c] *Technische Universität Bergakademie Freiberg, 09599, Freiberg, Germany*

ARTICLE INFO

Keywords:
Apiculture
Bee pollen Pesticide
Heavy metal
Residue

ABSTRACT

Honeybees and honeybee products (honey, bee wax, bee pollen and bee bread) are potential bioindicators of contaminants (pesticides, mycotoxins, pyrrolizidine alkaloids, toxic elements, radionuclides etc.) in the environment. In this study, recent results on the food safety risks of bee pollen and data about the concentration of toxic substances detected in bee pollen are summarized. Based on different studies, a risk assessment was conducted for the most common pesticide active substances (chlorpyrifos, fluvalinate, carbendazim, thiacloprid), heavy metals (arsenic, cadmium, mercury, lead) and common mycotoxins (aflatoxin-B1, ochratoxin-A, fumonisins, zearalenone, deoxynivalenol, T-2 toxin).

1. Introduction

Bee pollen contains essential nutrients. According to *Campos et al*., (2008), carbohydrates (13–55%), proteins (10–40%), lipids (1–13%) and fibre (0.3–20%) all contribute to the composition of bee pollen. In addition, bee pollens are rich in biologically active micronutrients like minerals, polyphenols and vitamins. Based on a report by *Habryka et al*., (2016), the product is used in apitherapy mainly for its antioxidant, anti-inflammatory, antibiotic and antiallergic effects. Bee pollen improves blood supply to the nerve tissue, thereby increasing mental performance and eliminating the state of fatigue. Research works have also shown a positive effect of bee pollen on some diseases of the liver, heart and prostate. The main consumers of bee pollen are the followers of health- and environmentally-conscious lifestyles, as well as the elderly, who use it due to its antioxidant and other therapeutic effects (*Végh et al*., 2021).

Besides all of these positive effects and high biological and nutritive value, bee pollen can contain hazardous trace elements, pesticide residues (*Ambrus et al*., 2020; *Bostan et al*., 2019; *Toselli and Sgolastra*, 2020), toxic metals and metalloids (*Spirić et al*., 2019; *Ćirić et al*., 2021; *Murashova et al*., 2020; *Roman*, 2009), moulds and mycotoxins (*Alarcón et al*., 2019), pyrrolizidine alkaloids (*Botías et al*., 2015), allergens (*Pitsios et al*., 2006; *Nonotte-Varly*, 2016) and GM (genetically modified) foods (*Malone*, 2002). To ensure the safety and quality of bee pollen, some counties issued national legislations, decisions and guidelines which correlate with European and International standards. In Serbia for instance, honeybee

***Corresponding author:** Jelena Ćirić, *jelena.ciric@inmes.rs*

Paper received Jun 6th 2023. Paper accepted Jun 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Table 1. Pesticide residue contents of bee pollen from different studies

Pesticide Concentration Mean value	Active ingredients	Country of origin	Reference
30 µg/kg 61 µg/kg 16 µg/kg	Tebuconazole Thiacloprid Chlorpyrifos	Poland	*Roszko et al*. (2016)
470 µg/kg 79 µg/kg	Thiacloprid Prothioconazol-desthio	Germany	*Böhme et al*. (2018)
133 µg/kg 40 µg/kg	Thiacloprid Permethrin-cis	Luxembourg	*Beyer et al*. (2018)
24 µg/kg 7 µg/kg	Carbendazim Amitraz II	France	*Lambert et al*. (2013)
915 µg/kg 128 µg/kg 83 µg/kg	Fluvalinate Chlorpyrifos Carbaryl	Taiwan	*Nai et al*. (2017)
6 µg/kg 28 µg/kg 227 µg/kg 50 µg/kg 3 µg/kg	Coumaphos Carbaryl Phosmet Carbendazim Atrazine	USA	*Stoner and Eitzer* (2013)
1 µg/kg 3 µg/kg <1 µg/kg	Azoxystrobin Carbendazim Carbaryl	Uruguay	*Niell et al*. (2015)

products must meet legal criteria (*Republic of Serbia*, 2015). Maximum Residue Level (MRL) values for honey vary between 0.01 and 1 mg/kg, but for other honeybee products, no MRLs are applicable until individual products have been identified and listed (*EU Pesticides Database*, 2021). The website of the International Honey Commission (IHC) is often quoted in scientific research in which maximum limits have been proposed for Pb (500 µg/kg), Cd (30 µg/kg) and Hg (10 µg/kg) in honey. Applying the data of Table 1, risk assessments for pesticide residues in bee pollen were performed in different studies. The results indicate that a major pesticide is thiacloprid.

Table 2 summarizes the literature data on the concentration of toxicologically important elements (As, Cd, Hg, Pb) in bee pollen. The mean values for Cd concentration of bee pollen samples exceeded 30 µg/kg in most studies, except in Brazil (*de Oliveira et al*., 2017). In some bee pollens from Europe, the Pb concentrations exceeded the 200 µg/kg limit (*Lambert et al*. 2012; *Adaškevičiūtė et al*., 2019).

Table 2. Toxic metal contamination of bee pollen from different studies

Mean concentration of toxic elements (µg/kg)					
Lead (Pb)	Arsenic (As)	Mercury (Hg)	Cadmium (Cd)	Country of origin	Reference
112	/	/	30	Italy	*Conti and Botré* (2001)
237	/	/	/	France	*Lambert et al*. (2012)
247	/	/	88	Europe	*Adaškevičiūtė et al*. (2019)
20	/	/	20	Chile	*Mejías et al*. (2018)
148	169	/	2	Brazil	*de Oliveira et al*. (2017)

Table 3. Mould contamination of bee pollen from different studies

Isolated mould	Country of origin	Reference
Alternaria Aspergillus Fusarium Mucor Penicillium Rhizopus	Serbia	*Kostić et al*. (2017)
Alternaria Aspergillus Cladosporium Penicillium Rhizopus	Ukraine, Slovakia	*Shevtsova et al*. (2014)
Alternaria Aspergillus Cladosporium Fusarium Mucor Paecilomyces Penicillium Rhizopus	Slovakia	*Kačániová et al*. (2011)

Over the past decades, several studies have been conducted in Europe on the mycotoxin content of bee pollen samples. The results of the reviewed studies are presented in Table 3. *Kostić et al*. (2017) reported that the average concentrations of aflatoxin-B1 exceeded the legal limit by more than four times in multifloral pollen samples from Serbia. The same authors isolated different moulds from bee pollen in Serbia: *Alternaria*, *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus*. Also, similar moulds were isolated in a study from Slovakia and Ukraine (*Shevtsova et al*., 2014).

2. Conclusions

Bee pollen is a very popular bee product that is presently not defined in most national regulations. In Europe, many studies have been conducted on this bee product, but little data is available from other continents. According to different studies, bee pollen is characterized by heterogenous food safety risks and could also be used as a potential environmental bioindicator. The common contaminants of bee pollen are pesticides, heavy metals, metalloids and mycotoxins. In this short overview, recent findings on the above-mentioned substances and data on concentrations determined in bee pollen were summarized, from different countries. A number of scientific works on the topic of bee pollen is associated with biomonitoring systems, and bee pollen can indeed be used as an environmental bioindicator, similar to the way other bee products (bee wax, honey and bee bread) are already in use.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023 and the German Federal Ministry of Education and Research (BMBF) (Bridge2ERA2021: 100579052).

References

Adaškevičiūtė, V., Kaškonienė, V., Kaškonas, P., Barčauskaitė, K. & Maruška, A. (2019). Comparison of physicochemical properties of bee pollen with other bee products. *Biomolecules*, 9(12), 819.

Alarcón, D. C. M., Cáceres, E. X. U. & Becerra, A. M. A. (2019). Mycotoxins in foods that cause damage to humans. *Journal of Applied Biotechnology & Bioengineering*, 6, 259–263.

Ambrus, Á., Szenczi-Cseh, J., Griff, T., Kerekes, K., Miklós, G., Vásárhelyi A. & Szigeti, T. J. (2020). Food safety assessment of the mycotoxin and pesticide residue contamination of our foods, Part 2. Mycotoxins. *Journal of Food Investigation*, 66, 2938–2949.

Beyer, M., Lenouvel, A., Guignard, C., Eickermann, M., Clermont, A., Kraus, F. & Hoffmann, L. (2018). Pesticide residue profiles in bee bread and pollen samples and the survival of honeybee colonies—a case study from Luxembourg. *Environmental Science and Pollution Research*, 25, 32163–32177.

Böhme, F., Bischoff, G., Zebitz, C. P., Rosenkranz, P. & Wallner, K. (2018). Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. *PLoS One*, 13(7), e0199995.

Bostan, I., Onofrei, M., Gavriluță, A. F., Toderașcu, C. & Lazăr, C. M. (2019). An integrated approach to current trends in organic food in the EU. *Foods*, 8(5), 144.

Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E. & Goulson, D. (2015). Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. *Environmental Science & Technology*, 49(21), 12731–12740.

Campos, M. G., Bogdanov, S., de Almeida-Muradian, L. B., Szczesna, T., Mancebo, Y., Frigerio, C. & Ferreira, F. (2008). Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research*, 47(2), 154–161.

Conti, M. E. & Botrè, F. (2001). Honeybees and their products as potential bioindicators of heavy metals contamination. *Environmental Monitoring and Assessment*, 69, 267–282.

Ćirić, J., Spirić, D., Baltić, T., Lazić, I. B, Trbović, D., Parunović, N., Petronijević, R. & Đorđević V., 2020. Honey bees and their products as indicators of environmental element deposition. *Biological Trace Element Research*, 199, 2312–2319, doi: 10.1007/s12011-020-02321-6. Epub 2020 Aug 5. PMID: 32757175

de Oliveira, F. A., de Abreu, A. T., de Oliveira Nascimento, N., Froes-Silva, R. E. S., Antonini, Y., Nalini Jr, H. A. & de Lena, J. C. (2017). Evaluation of matrix effect on the determination of rare earth elements and As, Bi, Cd, Pb, Se and In in honey and pollen of native Brazilian bees (*Tetragonisca angustula*–Jataí) by Q-ICP-MS. Talanta, 162, 488–494.

EU Pesticides Database, (2021), https://ec.europa.eu/food/plant/pesticides/eu-pesticides database/public/?event=activesubstance.selection&language=EN/ (2021), Accessed 19th Mar 2021.

Habryka, C., Kruczek, M. & Drygaś, B. (2016). Bee products used in apitherapy. *World Scientific News*, 48, 254–258.

Kačániová, M., Juráček, M., Chlebo, R., Kňazovická, V., Kadasi-Horáková, M., Kunová, S., ... & Šimko, M. (2011). Mycobiota and mycotoxins in bee pollen collected from different areas of Slovakia. Journal of Environmental Science and Health, Part B, 46(7), 623– 629.

Kostić, A. Ž., Petrović, T. S., Krnjaja, V. S., Nedić, N. M., Tešić, Ž. L., Milojković-Opsenica, D. M., ... & Pešić, M. B. (2017). Mold/aflatoxin contamination of honey bee collected pollen from different Serbian regions. *Journal of Apicultural Research*, 56(1), 13–20.

Lambert, O., Piroux, M., Puyo, S., Thorin, C., L'Hostis, M., Wiest, L., ... & Pouliquen, H. (2013). Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of Western France. *PloS one*, 8(6), e67007.

Malone, L. A. (2002). Literature review on genetically modified plants and bee products. HortResearch.

Mejías, E., Gómez, C. J., Gareil, P., Delaunay, N. & Montenegro, G. (2018). Characterization of phenolic profile alterations in metal-polluted bee pollen via capillary electrophoresis. *Ciencia e Investigación Agraria: Revista Latinoamericana de Ciencias de la Agricultura*, 45(1), 51– 63.

Murashova, E. A., Tunikov, G. M., Nefedova, S. A., Karelina, O. A., Byshova, N. G. & Serebryakova, O. V. (2020). Major factors determining accumulation of toxic elements by bees and honey products. *International Transaction Journal of Engineering, Management and Applied Sciences and Technologies*, 11(3), 11A03N.

Nai, Y. S., Chen, T. Y., Chen, Y. C., Chen, C. T., Chen, B. Y. & Chen, Y. W. (2017). Revealing pesticide residues under high pesticide stress in Taiwan's agricultural environment probed by fresh honey bee (Hymenoptera: Apidae) Pollen. *Journal of Economic Entomology*, 110(5), 1947–1958.

Niell, S., Jesus, F., Pérez, C., Mendoza, Y., Diaz, R., Franco, J., ... & Heinzen, H. (2015). QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. *Journal of Agricultural and Food Chemistry*, 63(18), 4484–4492.

Nonotte-Varly, C. (2016). Why not evaluate the allergenic potential of bee pollen with a skin testing method? *Alergologia Polska* [Polish Journal of Allergology], 3(3), 114–122.

Pitsios, C., Chliva, C., Mikos, N., Kompoti, E., Nowak-Wegrzyn, A. & Kontou-Fili, K. (2006). Bee pollen sensitivity in airborne pollen allergic individuals. *Annals of Allergy, Asthma & Immunology*, 97(5), 703–706.

Republic of Serbia, (2015). Rulebook on Quality of Honey and Other Bee Products. *Official Gazette of the Republic of Serbia*, 101.

Roman, A. (2009). Concentration of chosen trace elements of toxic properties in bee pollen loads. *Polish Journal of Environmental Studies*, 18(2).

Roszko, M. Ł., Kamińska, M., Szymczyk, K. & Jędrzejczak, R. (2016). Levels of selected persistent organic pollutants (PCB, PBDE) and pesticides in honey bee pollen sampled in Poland. *PLoS One*, 11(12), e0167487.

Shevtsova, T., Kačániová, M., Garkava, K., Brindza, J. & Petrova, J. (2014). Contamination of *Betula verrucosa* Ehrh. pollen by microorganisms, mycotoxins and heavy metals, https://dspace.nau.edu.ua/bitstream/NAU/10121/1/jmbfs_xxs1_shevtsova.pdf

Spirić, D., Ćirić, J., Đorđević, V., Nikolić, D., Janković, S., Nikolić, A... & Teodorović, V. (2019). Toxic and essential element concentrations in different honey types. *International Journal of Environmental Analytical Chemistry*, 99(5), 474 –485.

Stoner, K. A. & Eitzer, B. D. (2013). Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. *PLoS One*, 8(10), e77550.

Toselli, G. & Sgolastra, F. (2020). Seek and you shall find: An assessment of the influence of the analytical methodologies on pesticide occurrences in honey bee-collected pollen with a systematic review. *Chemosphere*, 258, 127358.

Végh, R., Csóka, M., Sörös, C. & Sipos, L. (2021). Food safety hazards of bee pollen–A review. *Trends in Food Science & Technology*, 114, 490–509.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 613.291:616.12-008.331.1

ID: 126357257

https://doi.org/10.18485/meattech.2023.64.2.51

Review paper

Reduction of salt content in meat products

Milenko Babić[a], Danijela Vranić[a], Branka Borović[a], Jelena Babić Milijašević[a], Tamara Gerić[a] and Slobodan Lilić[a]*

[a] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

ARTICLE INFO

Keywords:
Salt reduction
Meat products
Strategy
Recommendations

ABSTRACT

Excessive salt/sodium intake is recognized as a main cause of essential hypertension and it is linked to several health disorders. The World Health Organization (WHO) made a recommendation to Member States to reduce population salt intake by 30%, as part of the nine global targets to reduce premature mortality from non-communicable diseases by 25% by 2025. WHO recommends that adults consume less than 5 g of salt per day (less than 2000 mg sodium).

WHO adopted key broad strategies for salt reduction: (1) government policies — including appropriate fiscal policies and regulation to ensure food manufacturers and retailers produce healthier foods or make healthy products available and affordable; (2) working with the private sector to improve the availability and accessibility of low-salt products; (3) consumer awareness and empowerment of populations through social marketing and mobilization to raise awareness of the need to reduce salt intake consumption; (4) creating an enabling environment for salt reduction through local policy interventions and the promotion of "healthy food" settings such as schools, workplaces, communities, and cities; (5) monitoring of population salt intake, sources of salt in the diet and consumer knowledge, attitudes and behaviours relating to salt to inform policy decisions; (6) salt reduction programmes and programmes that promote fortification with micronutrients of salt, condiments or seasonings high in salt (bouillon cubes, soy and fish sauce) can complement each other.

1. Introduction

Sodium chloride (salt) has been used from ancient times in daily food preparation as well as in fermenting processes. Its use was important primarily for taste and shelf life of food. At the end of 19th century, use of salt was rapidly increased. Recently, salt production was valued at 28.5 billion US$ in 2020, and it is projected to reach a value of over 32 billion US$ by 2026 (*Shahbandeh*, 2022).

Nowadays, dietary sodium intake above 2 g/day is in a positive correlation with average blood pressure and prevalence of hypertension (*Cappuccio et al*., 2022).

There are some controversies about the relation between dietary sodium intake and blood pressure (*Sullivan*, 1991), and some authors described the term "sodium sensitivity" linked with variations in blood pressure due to amounts of sodium in food (*Kawasaki et al*., 1978).

Excessive sodium intake can be associated with some other health problems. *Du Cailar et al.* (2002) mentioned the increasing left ventricular mass and microalbinuria in normotensive patients. Salt intake, in patients with essential hypertension, is an independent determinant of left ventricular hypertrophy, besides blood pressure and obesity (Schmieder and

***Corresponding author:** Slobodan Lilić, *slobodan.lilic@inmes.rs*

Paper received July 13th 2023. Paper accepted July 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Messerli, 2000). *Avolio et al.* (1986) cited that lower sodium intake reduces arterial stiffness, and that has a beneficial effect on distensibility of the central aorta and large peripheral arteries.

After a large load of sodium from food, renin and aldosterone levels are raised that decrease water excretion, which consequently leads to the appearance of idiopathic oedema (*Streeten et al.*, 1973). *Yatabe et al.* (2010) cited salt sensitivity as being linked with insulin resistance in essential hypertensive persons.

Excessive salt intake enhances airway inflammation in asthmatics following exercise (*Mickleborough et al.*, 2005), it is associated with the risk of gastric cancer (*Tsugane et al.,* 2004), with urinary calcium excretion and bone density reduction in adolescence (*Matkovic et al.*, 1995), bone mineral loss in post-menopausal women (*Devine et al.*, 1995), and with excessive urinary sodium excretion that consequently leads to kidney stones (*Cirillo et a*l., 1994).

2. Strategy for salt reduction

In 2013, the WHO made a recommendation to all Member States to reduce population salt intake by 30%, as part of the nine global targets to reduce premature mortality from non-communicable diseases (NCDs) by 25% by 2025 (World Health Organization.(2013):

1. A 25% relative reduction in the overall mortality from cardiovascular diseases, cancer, diabetes, or chronic respiratory diseases
2. At least 10% relative reduction in the harmful use of alcohol, as appropriate, within the national context
3. A 10% relative reduction in prevalence of insufficient physical activity
4. A 30% relative reduction in mean population intake of salt/sodium
5. A 30% relative reduction in prevalence of current tobacco use in persons aged 15+ years
6. A 25% relative reduction in the prevalence of raised blood pressure or contain the prevalence of raised blood pressure, according to national circumstances
7. Halt the rise in diabetes and obesity
8. At least 50% of eligible people receive drug therapy and counselling (including glycaemic control) to prevent heart attacks and strokes
9. An 80% availability of the affordable basic technologies and essential medicines, including generics, required to treat major noncommunicable diseases in both public and private facilities

The World Health Assembly (WHA) adopted in 2004 “Global Strategy on Diet, Physical Activity and Health”, including World Health Organization, international partners, the private sector and civil society to take action to support healthy diets and physical activity.

There are the key facts about salt consumption:

- High sodium consumption (>2 grams/day, equivalent to 5 g salt/day) and insufficient potassium intake (< 3.5 grams/day) contribute to high blood pressure and increase the risk of heart disease and stroke.
- The main source of sodium in our diet is salt, although it can come from sodium glutamate, used as a condiment in many parts of the world.
- Most people consume too much salt — on average 9 to 12 grams per day, or around twice the recommended maximum level of intake.
- Salt intake of less than 5 grams per day for adults helps to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart attack. The principal benefit of lowering salt intake is a corresponding reduction in high blood pressure.
- WHO Member States have agreed to reduce the global population’s intake of salt by a relative 30% by 2025.
- Reducing salt intake has been identified as one of the most cost-effective measures countries can take to improve population health outcomes. Key salt reduction measures will generate an extra year of healthy life for a cost that falls below the average annual income or gross domestic product per person.
- An estimated 2.5 million deaths could be prevented each year if global salt consumption were reduced to the recommended level.

Recommendations for salt reduction are:

- For adults: WHO recommends that adults consume less than 5 g (just under a teaspoon) of salt per day.
- For children: WHO recommends that the recommended maximum intake of salt for adults be adjusted downward for children aged two to 15 years based on their energy requirements relative to those of adults. This recommenda-

tion for children does not address the period of exclusive breastfeeding (0–6 months) or the period of complementary feeding with continued breastfeeding (6–24 months).

- All salt that is consumed should be iodized or "fortified" with iodine, which is essential for healthy brain development in the foetus and young child and optimizing people's mental function in general.

WHO adopted key broad strategies for salt reduction:

- government policies — including appropriate fiscal policies and regulation to ensure food manufacturers and retailers produce healthier foods or make healthy products available and affordable,
- working with the private sector to improve the availability and accessibility of low-salt products,
- consumer awareness and empowerment of populations through social marketing and mobilization to raise awareness of the need to reduce salt intake consumption,
- creating an enabling environment for salt reduction through local policy interventions and the promotion of "healthy food" settings such as schools, workplaces, communities, and cities,
- monitoring of population salt intake, sources of salt in the diet and consumer knowledge, attitudes and behaviours relating to salt to inform policy decisions.
- salt reduction programmes and programmes that promote fortification with micronutrients of salt, condiments or seasonings high in salt (bouillon cubes, soy and fish sauce) can complement each other.

3. Salt reduction in meat products

Salt reduction in meat products can be achieved by reducing added sodium chloride (*Sofos et al.*, 1983), by replacement of sodium chloride with other salts (*Sofos et al.*, 1983; *Terrell*, 1983; *Guàrdia et al*, 2006), by use of flavour enhancers and masking agents (*Desmond*, 2006), by combinations of the aforementioned *(Sofos et al.*, 1983; *Terrell*, 1983), by optimization of physical form of salt (*Angus et al.,* 2005), and by alternative processing techniques (*Claus and Sørheim*, 2006).

Potassium chloride is the most common replacers for sodium chloride, but total replacement is not possible due to its bitter and metallic taste (*Gou et al*., 1996). The use of potassium salts is disputed because some parts of the population are sensitive to them, i.e. people with diabetes mellitus type I, as well as people with kidney and adrenal insufficiency (*Food Safety*, 2003)

With the aim of the improvement, flavour enhancers can be used that activate receptors in the mouth that help to compensate reduced salt content (Brandsma, 2006) and masking agents like yeast extract, lactates, monosodium glutamate and nucleotides. Adenosine 5'-monophorphate, which blocks the activation of gustducin in receptor cells, can be used for taste and to prevent stimulation of nerves that innervates taste receptors (*McGregor*, 2004).

Some salts are used due to their technological characteristics, such as phosphates (*Ruusunen*, 2002). Also physical forms of salt, like salt flakes, can be used for better water holding capacity and protein solubility (*Campbel*l, 1980). Besides the aforementioned, due to technological properties, phosphates can be used in pre-rigor meat or high pressure technology (*Claus and Sørheim*, 2006).

Disclosure Statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, according to the provisions of the Contract on research financing in 2023 (No 451-03-47/2023-01/200050 dated 03.02.2023).

References

Angus, F., Phelps, T., Clegg, S., Narain, C., Den Ridder, C. & Kilcast, D. (2005). Salt in Processed Foods: Collaborative Research Project. Leatherhead Food International.

Avolio, A. P., Clyde, K. M., Beard, T. C., Cooke, H. M., Ho, K. K. L. & O'Rourke, M. F. (1986). Improved Arterial Distensibility in Normotensive Subjects on a Low Salt Diet. *Arteriosclerosis,* 6, 166–169, doi:10.1161/01.atv.6.2.166

Brandsma, I. (2006). Reducing Sodium — A European Perspective. *Food Technology*, 60, 24–29.

Cailar, G. Du, Ribstein, J. & Mimran, A. (2002). Dietary Sodium and Target Organ Damage in Essential Hypertension. *American Journal of Hypertension,* 15(3), 111–229, doi: 10.1016/s0895-7061(01)02287-7

Campbell, J. F. (1980). Binding Properties of Meat Blends Effects of Salt (Sodium Chloride) Type, Blending Time and Post-Blending Storage. doi:10.3/JQUERY-UI.JS.

Cappuccio, F. P., Campbell, N. R. C., He, F. J., Jacobson, M. F., MacGregor, G. A., Antman, E., Appel, L. J., Arcand, J. A., Blanco-Metzler, A., Cook, N. R. et al. (2022). Sodium and Health: Old Myths and a Controversy Based on Denial. *Current Nutrition Reports*. 11, 172–184, doi:10.1007/s13668-021-00383-z

Cirillo, M., Laurenzi, M., Panarelli, W. & Stamler, J. (1994). Urinary Sodium to Potassium Ratio and Urinary Stone Disease. *Kidney International*, 46, 1133–1139, doi:10.1038/KI.1994.376

Claus, J. R. & Sørheim, O. (2006). Preserving Pre-Rigor Meat Functionality for Beef Patty Production. *Meat Science*, 73, 287–294, doi:10.1016/J.MEATSCI.2005.12.004

Desmond, E. (2006). Reducing Salt: A Challenge for the Meat Industry. *Meat Science*, 74, 188–196, doi:10.1016/J.MEATSCI.2006.04.014

Devine, A., Criddle, R. A., Dick, I. M., Kerr, D. A. & Prince, R. L. (1995). A Longitudinal Study of the Effect of Sodium and Calcium Intakes on Regional Bone Density in Postmenopausal Women. *American Journal of Clinical Nutrition,* 62, 740–745, doi:10.1093/ajcn/62.4.740

Food Safety Authority of Ireland Salt and Health: Review of the Scientific Evidence and Recommendations for Public Policy in Ireland, Dublin (2005). ISBN 1904465234, https://www.fsai.ie/getattachment/ab9a196e-258a-40d5-b857-7f0308bb96de/10507_fsai_salt_report_fa1_accessible.pdf?lang=en-IE

Gou, P., Guerrero, L., Gelabert, J. & Arnau, J. (1996). Potassium Chloride, Potassium Lactate and Glycine as Sodium Chloride Substitutes in Fermented Sausages and in Dry-Cured Pork Loin. *Meat Science*, 42, 37–48, doi:10.1016/0309-1740(95)00017-8

Guàrdia, M. D., Guerrero, L., Gelabert, J., Gou, P. & Arnau, J. (2006). Consumer Attitude towards Sodium Reduction in Meat Products and Acceptability of Fermented Sausages with Reduced Sodium Content. *Meat Science*, 73, 484–490, doi:10.1016/J.MEATSCI.2006.01.009

Kawasaki, T., Delea, C. S., Bartter, B. C. F. & Bethesda, H. S. (1978). The Effect of High-Sodium and Low-Sodium Intakes on Blood Pressure and Other Related Variables in Human Subjects with Idiopathic Hypertension. *The American Journal of Medicine*. 64, 193–198, doi: 10.1016/0002-9343(78)90045-1

Matkovic, V., Ilich, J. Z., Andon, M. B., Hsieh, L. C., Tzagournis, M. A., Lagger, B. J. & Goel, P. K. (1995). Urinary Calcium, Sodium, and Bone Mass of Young Females. American *Journal of Clinical Nutrition*, 62, 417–425, doi:10.1093/AJCN/62.2.417

McGregor, R. (2004). Taste Modification in the Biotech Era. *Food Technology Magazine,* 58, 5.

Mickleborough, T. D., Lindley, M. R. & Ray, S. (2005). Dietary Salt, Airway Inflammation, and Diffusion Capacity in Exercise-Induced Asthma. *Medicine and Science in Sports and Exercise*. 37, 904–914, doi:10.1249/01.mss.0000166949.11296.2b

Schmieder, R. E. & Messerli, F. H. (2000). Hypertension and the Heart. *Journal of Human Hypertension*, 14, 597–604.

Shahbandeh, M. (2022). https://www.statista.com/statistics/237162/worldwide-salt-production.

Sofos, J. N. (1983). Effects of Reduced Salt (NaCI) Levels on Sensory and Instrumental Evaluation of Frankfurters. *Journal of Food Science*, 48, 1692–1696, doi:10.1111/J.1365-2621.1983.TB05062.X

Streeten, D. H. P., Dalakos, T. G., Souma, M., Fellerman, H., Clift, G. V., Schletter, F. E., Stevenson, C. T. & Speller, P. J. (1973). Studies of the Pathogenesis of Idiopathic Oedema: The Roles of Postural Changes in Plasma Volume, Plasma Renin Activity, Aldosterone Secretion Rate and Glomerular Filtration Rate in the Retention of Sodium and Water. *Clinical Science and Molecular Medicine,* 45, 347–373, doi:10.1042/CS0450347

Sullivan, J. M. (1991). Session III. Salt Sensitivity Salt Sensitivity Definition, Conception, Methodology, and Long-Term Issues. *Hypertension*. 17, 161, doi: 10.1161/01.hyp.17.1_suppl.i61

Terrell, R. N. (1983). Reducing the Sodium Content of Processed Meats. *Food Technology,* 37, 66–71.

Tsugane, S., Sasazuki, S., Kobayashi, M. & Sasaki, S. (2004). Salt and Salted Food Intake and Subsequent Risk of Gastric Cancer among Middle-Aged Japanese Men and Women. *British Journal of Cancer*, 90, 128–134, doi:10.1038/sj.bjc.6601511

World Health Organization, (2013). Global action plan for the prevention and control of noncommunicable diseases 2013–2020. WHO: Geneva, Switzerland, ISBN 9789241506236

World Health Organization, (2004). Global strategy on diet, physical activity and health. https://www.who.int/publications/i/item/9241592222

Yatabe, M. S., Yatabe, J., Yoneda, M. (2010). Salt Sensitivity Is Associated with Insulin Resistance, Sympathetic Overactivity, and Decreased Suppression of Circulating Renin Activity in Lean Patients with Essential Hypertension. *American Journal of Clinical Nutrition*, 92, 77–82, doi: 10.3945/ajcn.2009.29028

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.055:636.2
579.67

ID: 126361097

https://doi.org/10.18485/meattech.2023.64.2.52

Original scientific paper

The influence of different gas mixtures on the shelf life of fresh beef

Milan Milijašević[a], Jelena Babić Milijašević[a], Ivan Nastasijević[a] and Slavica Vesković Moračanin[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Modified atmosphere
Beef round primal cuts
Microbiological status
Gas mixture

ABSTRACT

The paper presents the results of testing the influence of different gas mixtures on the shelf life of different beef round primal cuts. A total of five gas mixtures were used, and the change in microbiological status (total number of bacteria, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Proteus* spp., sulphite-reducing clostridia), change in pH and sensory characteristics of the meat were monitored. The results of this study showed that beef packaged in a gas mixture consisting of 70% O_2 and 30% CO_2 was the most acceptable in terms of sensory characteristics and microbiological status.

1. Introduction

Nowadays, food must be healthy, minimally processed and attractively packaged, as consumer expectations are constantly growing (*Martinez et al.*, 2006). As a consequence, the demands to be met by manufacturers of food and packaging materials have also increased (*McMillin et al.*, 1999; *Brody*, 2003). Today's consumers are also very sensitive when it comes to the use of additives in the food industry. Public demands to be able to get fresh food at any time are increasing, and food safety and easy availability of all kinds of foods are very important. That is why it is becoming increasingly difficult to meet consumer expectations. It is clear that time, as a factor in food production, plays an important role. From the moment when the fruit is harvested, the cattle are slaughtered, or the fish is caught, the race against time begins. From that moment on, natural degradation and deterioration threaten the quality and sustainability of the product. However, external factors such as the hygiene of the production process, temperature, etc., also pose dangers to the usability of the product. Therefore, the ways in which the food will be handled during the production, packaging, or cold chain processes are very important. Special emphasis should be placed on the packaging stage, because the packaging method can enable greater sustainability of the product (*Phillips et al.*, 2001).

Over the past two decades, modified atmosphere packaging (MAP) has become the dominant form of packaging in the meat industry (*Robertson*, 1993). The main reasons that stimulated the development of this technology are the constant increase in the consumption of fresh meat, the increase in the population in cities and the reduction of natural food sources. MAP can be defined as the removal of air from the package and its replacement with a specific gas or mixture of gases. The purpose of this technology is to extend the sustainability of food by preventing or slowing biochemical processes (oxidation of fats, formation of metmyoglobin), the growth of spoilage bacteria and the degree of product respiration. Numerous literature data indicate that several gases, such as carbon dioxide (CO_2), nitro-

***Corresponding author:** Milan Milijašević, *milan.milijasevic@inmes.rs*

Paper received July 22nd 2023. Paper accepted May 31st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

gen (N_2), oxygen (O_2) and carbon monoxide (CO), are used individually or in different combinations in MAP technology (*Xiong*, 1999). CO_2 shows antimicrobial activity, mostly against Gram-negative bacteria and psychrotrophic pathogens such as *Yersinia enterocolitica* and *Aeromonas hydrophila* (*Brody*, 1989), but this gas does not significantly inhibit the growth of *Listeria monocytogenes*. CO_2 also does not affect the growth of *Clostridium botulinum* (*Sinell*, 1988). $O_2$is used to preserve the bright red color of the meat and prevent the growth of anaerobic bacteria (*Martinez et al.*, 2006). $N_2$is an inert gas that replaces O_2 in the mixture and prolongs the product's shelf life, and also prevents rancidity and the growth of ae robic bacteria *(Blakistone*, 1998). N_2, due to its low solubility, has a role to prevent the collapse of the package. CO is very effective in preserving the red color of fresh meat, as it has a 20-fold greater affinity for binding to myoglobin, compared to O_2 (*Boeckman*, 2006).

The aim of this experiment was to investigate the effect of different gas mixtures on the color, pH value and microbiological status of beef packaged in a modified atmosphere.

2. Materials and methods

The study was carried out in a medium-sized meat processing plant. Beef round parts were used as the matrix. The average temperature of the pieces of meat during packaging was 3.1°C, and the pH was 5.75. Twenty-four hours after packaging, the pieces of meat were cut into smaller pieces, where the average temperature of the meat was 4.2°C, and the pH was 5.54. Temperature and pH values were measured every three days using a Testo 205 apparatus (Testo AG, Germany). Pieces of meat, 1–2 cm thick, were placed in Cryovac LidSys polystyrene containers with a depth of 50 mm (Sealed Air, USA), in which there were papers for absorbing meat juice (Sealed Air, USA). The pieces of meat were then packed in a modified protective atmospheres and stored for 28 days at 4°C. A total of five different gas mixtures (Messer AG, Germany) were used in the study (Table 1).

The microbiological status and pH were examined 2, 6, 13, 17 and 28 days after setting up the study. The color of the meat was evaluated organoleptically, at the beginning and at the end of the study, without instrumental measurement. Microbiological tests were performed according to validated ISO methods. These tests were the total number of viable aerobic bacteria (TVA*), Escherichia coli, Salmonella* spp., *Listeria monocytogenes*, *Proteus* spp. and sulphite-reducing clostridia.

The change in the total number of bacteria is presented in Figure 1.

In cuts of meat packed in a gas mixture consisting of 30% CO_2 and 70% N_2, and in 100% CO_2,

Table 1. Composition of gas mixtures used in the modified atmosphere packaging for beef.

	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
CO_2	30%	100%	50%	30%	20%
O_2	-	-	-	70%	20%
N_2	70%	-	50%	-	60%

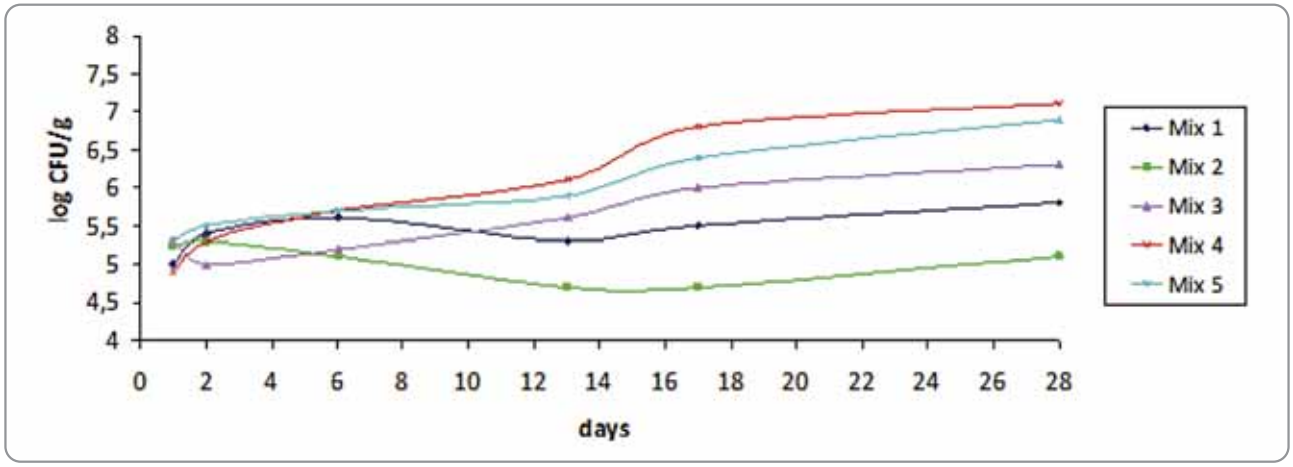


Figure 1. Total viable aerobic counts measured in beef packed in different gas mixtures (see Table 1) during the study.

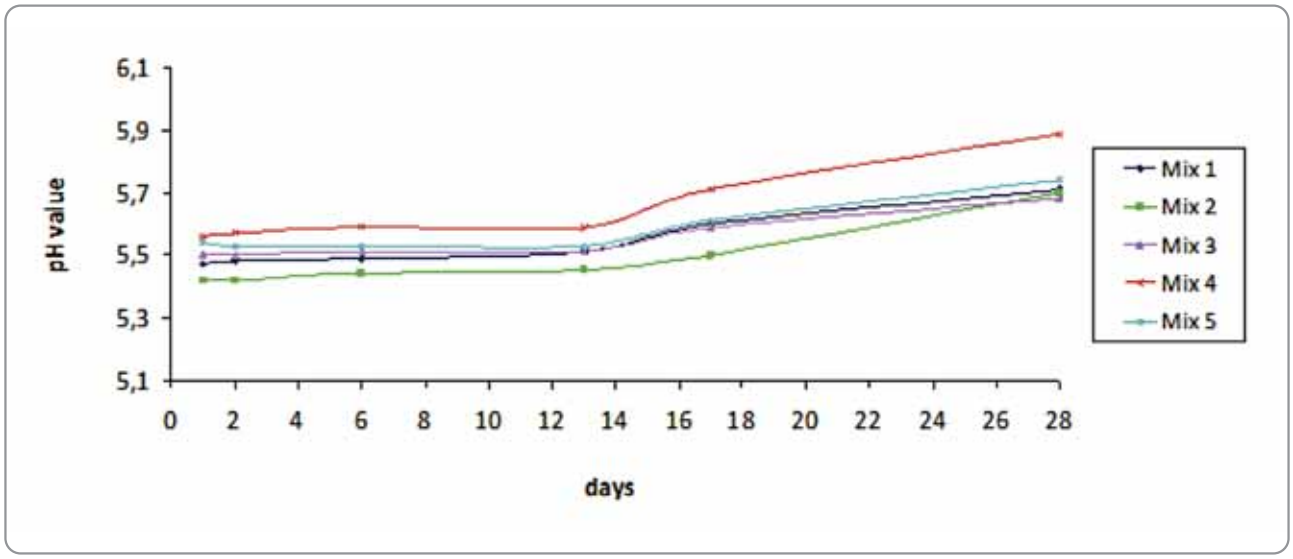


Figure 2. pH measured in beef packed in different gas mixtures (see Table 1) during the study.

the total number of bacteria remained low almost throughout the study. In beef pieces packed in gas mixture containing 20% and 70% O_2, during the entire study, there was a gradual increase in TVA, with a sudden increase between days 13 and 17.

The presence of pathogenic bacteria during the entire experiment (*E. coli*, *Salmonella* spp., *L. monocytogenes*, sulphite-reducing clostridia) was not recorded in any sample of beef packed in MAP.

The results of monitoring the pH change are shown in Figure 2. A significant increase in the pH value occurred in beef packaged in a gas mixture containing 70% O_2. Other mixtures showed a slow but steady increase in pH. A significant increase in pH value was noted around day 15, for all gas mixtures.

Pieces of beef packaged in gas mixtures containing a high percentage of CO_2 acquired a dark green color, and meat packaged in a mixture consisting of 30% CO_2 and 70% O_2 retained the most acceptable red color.

3. Discussion and results

Increasing the concentration of oxygen from 20% to 70% in the packaging has a positive effect on the color stability of the micro-fabricated parts of the beef leg. These results were also confirmed in research by other authors (*Seyfert et al.*, 2007; *Djenane et al.*, 2001; *Brody*, 1989; *Martinez et al.*, 2006). This is probably due to the formation of a larger amount of oxymyoglobin in the meat in proportion to the higher concentration of oxygen in the gas mixture. Pieces of beef packed in mixtures containing a high percentage of CO_2 acquired an unacceptable dark green color due to the formation of metmyoglobin.

Beef that was packed in gas mixtures with a higher percentage of CO_2 showed lower pH values. This is explained by the dissolution of CO_2 in the aqueous phase of the meat, whereby carbonic acid is formed, which leads to a decrease in pH. These statements were made by other authors in their works (*Phillips*, 1996; *Antoniewski et al.*, 2007; *Arnold et al.*, 1993; *Bendall et al.*, 1972; *Klettner*, 2004; *Hess et al*., 1980).

The TVA was higher in beef packed in the gas mixture with a higher (70%) O_2 than in the 20% O_2 mixture. This was due to the aerobic conditions that prevailed in the packaging and the slightly higher pH of the beef in the high-O_2 mixture that favoured the development of microorganisms.

4. Conclusion

The TVA was higher in beef packed in a mixture with a higher percentage of O_2, due to the aerobic conditions that prevailed in the packaging and slightly higher pH values that favoured the development of microorganisms.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, according to the provisions of the Contract on research financing in 2023 (No 451-03-47/2023-01/200050 dated 03.02.2023).

References

Antoniewski, N., Barringer, A., Knipe, L. & Zerby, N., (2007). Effect of a gelatin coating on the shelf life of fresh meat. *Journal of Food Science*, 72(6), E382–E387.

Arnold, N., Arp, C., Scheller, K., Williams, N. & Schaefer, M., (1993). Tissue equilibrium and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science*, 71, 105–118.

Bendall, R. & Taylor, A., (1972). Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscles. *Journal of the Science of Food and Agriculture*, 23, 707–719.

Blakistone, A., (1998). Introduction. In B. A. Blakistone (Ed.), Principles and applications of modified atmosphere packaging of foods (pp. 1–13). Gaithersburg, Maryland: Aspen Publishers.

Boeckman, M., (2006). Regulatory status of carbon monoxide for meat packaging. In Proceedings 59th reciprocal meat conference (pp. 59–60), 18–21 June 2006, Champaign-Urbana, Illinois, USA.

Brody, A. L. (2003). *"Nano, Nano*" *Food Packaging Technology*. *Journal of Food Technology*, 12, 52–54.

Brody, L., (1989). Modified atmosphere/vacuum packaging of meat. In A. L. Brody (Ed.), Controlled/modified atmosphere/vacuum packaging of foods (pp. 17–37). Trumbull, Connecticut: Food & Nutrition Press.

Djenane, D., Sánchez-Escalante, A., Beltrá, A. & Roncalés, P., (2001). Extension of the Retail Display Life of Fresh Beef Packaged in Modified Atmosphere by Varying Lighting Conditions. *Journal of Food Science*, 66(1), 181–185.

Hess, E., Ruosch, W. & Breer, C., (1980). Extending the shelf-life of prepacked fresh meat. *Fleischwirtschaft*, 60 (8), 1513–1516.

Klettner, P.-G., (2004). Schutzgasverpackung bei Frischfleisch (Rückenmuskulatur von Schwein und Rind) unter besonderer Berücksichtigung von Kohlenmonoxid, Mitteilungsblatt der Fleischforschung, Kulmbach 43. Nr. 166, 331–342.

Martínez, L., Djenane, D., Cilla, I., Beltrán, A. & Roncalés, P., (2006). Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Food Chemistry*, 94, 219–225.

McMillin, W., Huang, Y., Ho, P. & Smith, S., (1999). Quality and shelf-life of meat in case-ready modified atmosphere packaging. In Y. L. Xiong, F. Shahidi, & C. T. Ho (Eds.), Quality attributes in muscle foods (pp. 73–93). New York: ACS Symposium Series, Plenum Publishing Corporation.

Phillips, C., (1996). Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*, 31, 463–479.

Phillips, L., Faustman, C., Lynch, P., Govoni, E., Hoagland, A. & Zinn, A., (2001). Effect of dietary a-tocopherol supplementation on color and lipid stability in pork. *Meat Science*, 58, 389–393.

Robertson, G., (1993). Food packaging principles and practice. New York: Marcel Dekker 431–469.

Seyfert, M., Mancini, A., Hunt, C., Tang, J. & Faustman, C., (2007). Influence of carbon monoxide in package atmospheres containing oxygen on colour, reducing activity, and oxygen consumption of five bovine muscles. *Meat Science*, 75, 432–442.

Sinell, H.-J., (1988). Mikrobiologie des Fleisches. In Prändl-Fischer-Schnidhofer-Sinell, 1988, Fleisch, Ulmer, 1988, Stuttgart.

Xiong, Li, (1999). Modified Atmosphere Packaging. Dept. of Food Science, Penn State University.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.12.043
613.24

ID: 126365705

https://doi.org/10.18485/meattech.2023.64.2.53

Original scientific paper

Detection of milk fat in dairy products — an alternative approach

Aleksandar Bajčić[a], Dejana Trbović[a], Dragan Vasilev[b], Vesna Đorđević[a], Ivana Branković Lazić[a], Čaba Silađi[a] and Radivoj Petronijević[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Dairy products
Butyric acid
Milk fat

ABSTRACT

Milk fat is a highly valuable product, which is why accurate determination of its content in milk and milk products is very important. The use of the GC-FID method in our study proved to be very precise, as in the case of other authors, which signifies the importance of using this method to quantify milk fat. A total of 51 samples of dairy products were analyzed for fatty acid composition with particular attention to butyric acid. Butyric acid contents were in the range from 3.4 ± 0.73 in yogurt to 4.60 ± 0.08 in butter. Milk fat was in the range from 98.5 ± 4.77 in yogurt to 115.0 ± 1.73 in butter. Our results were in accordance with those of many other authors. Development of butyric acid and milk fat analyses in dairy products by GC-FID is essential for laboratories that must conduct analyses for food production, quality control during production, and inspection tasks for the import and export of these food products.

1. Introduction

Milk and milk products have great importance in human nutrition, especially for children and the elderly, due to the products' unique composition of macro and micronutrients. Essential nutrients such as high-value proteins, easily digestible lipids, sugars, minerals and vitamins, especially vitamins A, D, K, vitamins B2, B6, B12 (*Dons et al.*, 2023), are all components that make milk and milk products very healthy for consumption. From a health perspective, the importance of regular consumption of milk products is reflected in the improvement of digestion and the health status of the gastrointestinal tract, reduction in the risk of cardiovascular diseases and of the occurrence of cancer, and prevention of type 2 diabetes (*Hasegawa and Bolling*, 2023). The milk product industries have a leading role in the production of functional food, as these products strengthen the immune system, kill pathogenic microorganisms and lower blood pressure (*Korhonen*, 2006). Also, milk and its products that are available on the market do not require additional preparation before consumption, but are ready for use, which adds to the popularity of these products. According to FAO data, world production of milk and milk products has reached 927,800 million tons with a tendency of further growth (*FAO*, 2022).

Milk products include: products of sour-milk fermentation (yogurt, sour milk, acidophilic milk, kefir), milk drinks (chocolate milk, white coffee, milk cocktails with fruit juices etc.), condensed milk (condensed and evaporated), milk powder, butter, cheeses, cream, sour cream, ice cream etc. Milk fat

***Corresponding author:** Aleksandar Bajčić, *aleksandar.bajcic@inmes.rs*

Paper received May 23rd 2023. Paper accepted July 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

plays a very important role in producing the aforementioned foods. In human nutrition, milk fat is the most complex of all fats, having a wide range of lipid types with different molecular configurations, fatty acid chain lengths, and degrees of saturation (*Rico and Razzaghi*, 2023). This chemical composition of milk fat has important roles from an organoleptic point of view in milk products, realizing an adequate texture and enabling an attractive taste for consumers. However, the human population is increasing, which increases the consumption of food and, therefore, the consumption of milk products.

As a consequence, milk and milk product industries strive to improve production methods, in order to increase their capacities, and at the same time maintain their product quality (*Genis et al*., 2021). However, some misusage of new methods is most often reflected in the partial or complete replacement of milk fat with vegetable oils, which are significantly cheaper than milk fat on the market. Mostly, palm oil, soybean oil and sunflower oil are used (*Kim et al*., 2015). The misuse of these products as counterfeit milk and milk products, has expected economic importance, apropos profit, so it also has great ethical significance, because these fraudulent foods are not declared, and they can cause specific health problems for consumers who are subjected to this deception (*Gutiérez et al.*, 2008). According to the literature, milk and milk products are among the leading categories of foods that are counterfeited (*Johnson*, 2014).

The aim of the present study is to analyse butyric acid and milk fat contents in various dairy products and to compare the butyric acid content with experimentally determined milk fat content according to the Roese-Gottlieb method.

2. Materials and methods

Samples — A total of 51 samples of dairy products were analysed for fatty acid composition with particular attention to butyric acid. Dairy samples were „kačkavalj", a type of Serbian cheese, cream, sour cream, butter, cream cheese, yogurt, sour milk, kefir, semi-hard cheese and fresh cheese. The analysed samples are sold on the Serbian market.

2.1. Fatty acid analysis by gas chromatography

The total fat content was determined according to Roese-Gottlieb method (AOAC,1995). Fatty acids methyl esters (FAMEs) were analysed with a gas-liquid chromatograph (GLC, Shimadzu 2010, Japan) combined with flame ionization detector and capillary HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperatures were maintained at 250°C and 280°C, respectively. Nitrogen was used as the carrier gas at flow rate of 1.87 mL min^{-1}. The injector split ratio was set at 1:50. Injector syringe volume was 10 μL and injection volume was 1 μL. The oven with column was programmed: temperature starting at 50°C and ending at 230°C was applied. Total analysis time was 63.12 min. The chromatographic peaks in the samples were identified by comparing FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, PA).

2.2. Statistical analysis

All values are expressed as mean ± standard deviation. The statistical analysis was performed with software XLSTAT2023 (trial version for Microsoft Excel, Addinsoft, NY, USA). Prior to statistical analyses, all data sets were grouped according to similar production methods.

3. Results and discussion

After the tests carried out on the samples grouped in Table 1, the butyric acid contents ranged from 3.94 ± 0.73 to 4.60 ± 0.08 respectively. The results show that the percentage C4:0 in the milk products was satisfactory in all samples, which was confirmed by the obtained calculated results of milk fat, which ranged from 98.5 ± 4.77 to 115.0 ± 1.73. The importance of using this method, which determines the percentage of butyric acid in milk products, through which we calculated the percentage of milk fat, is becoming increasingly important.

This study showed the capability of GC-FID to analyse butyric acid and milk fat in dairy products with reliable results. According to the Regulation (EC) No 900/2008 (2008), milk fat content is calculated based on butyric acid content.

Comparison of the butyric acid content against Roese-Gottlieb fat for the industrially-produced milk products are presented in Figure 1.

In Figure 1, it can be seen that analysed dairy products were grouped according to their milk fat content vs. butyric acid content in corresponding milk fat. Other authors stated that analytical errors of ± 10% should be allowed for when calculating butyric acid (C4:0) content (*Molkentin and Precht*, 2000). Our results for milk fat content and butyr-

Table 1. Butyric acid content (% of total fat), milk fat (calculated based on butyric acid) and milk fat measured by the Roese-Gottlieb method

	Butyric acid (C4:0) (% of fat)	Milk fat (calculated based on butyric acid)	Milk fat by Roese-Gottlieb method (%)
Kačkavalj (n=6)	4.38 ± 0.81	109.5 ± 4.16	26.08 ± 0.14
Cream (n=3)	4.47 ± 0.19	111.7 ± 4.61	45.50 ± 0.41
Sour cream (n=3)	4.21 ± 0.35	105.2 ± 4.72	23.15 ± 0.17
Butter (n=3)	4.60 ± 0.08	115.0 ± 1.73	82.50 ± 0.18
Cream cheese (n=3)	4.29 ± 0.50	107.2 ± 4.31	15.75 ± 0.16
Yogurt, sour milk, kefir (n=8)	3.94 ± 0.73	98.5 ± 4.77	2.80 ± 0.33
Cheese — hard, semi-hard, fresh (n=25)	4.47 ± 0.33	111.7 ± 6.09	26.36 ± 2.95

ic acid content were in accordance with the studies by *Molkentin and Precht* (2000) (3.43–3.59%) and *Molkentin and Precht* (1998) (3.06–3.42%). The butyric acid and milk fat contents for butter were in accordance with published results obtained by *Danudol and Judprasong* (2022). They reported butyric acid 3.6% and milk fat 100.02% in butter. The butyric acid content in bovine milk was consistent with the data of *Mänsson* (2008) (4.4%), *Salamon et al.* (2006) (2.8–3.6%) and *Adamska et al.* (2014) (3.79–4.2%), but which can depend on the season. In a report by *Sacchi et al*. (2018), the GC/FID method was compared with ^{13}C NMR spectroscopy for analysis of butyric acid to evaluate the content of milk fat. They concluded that both methods can be used successfully, even though they stated that the ^{13}C NMR method has a lower detection level. Therefore, those authors concluded that correlation between the two independent methods was excellent. They reported butyric acid in the range from 1.91 to 8.44% for cow dairy products from the literature and milk fat contents ranging between about 22 and 26%. High variability in the composition of milk fat even from cows fed the same diet has been previously reported (*Bobe et al.*, 2003).

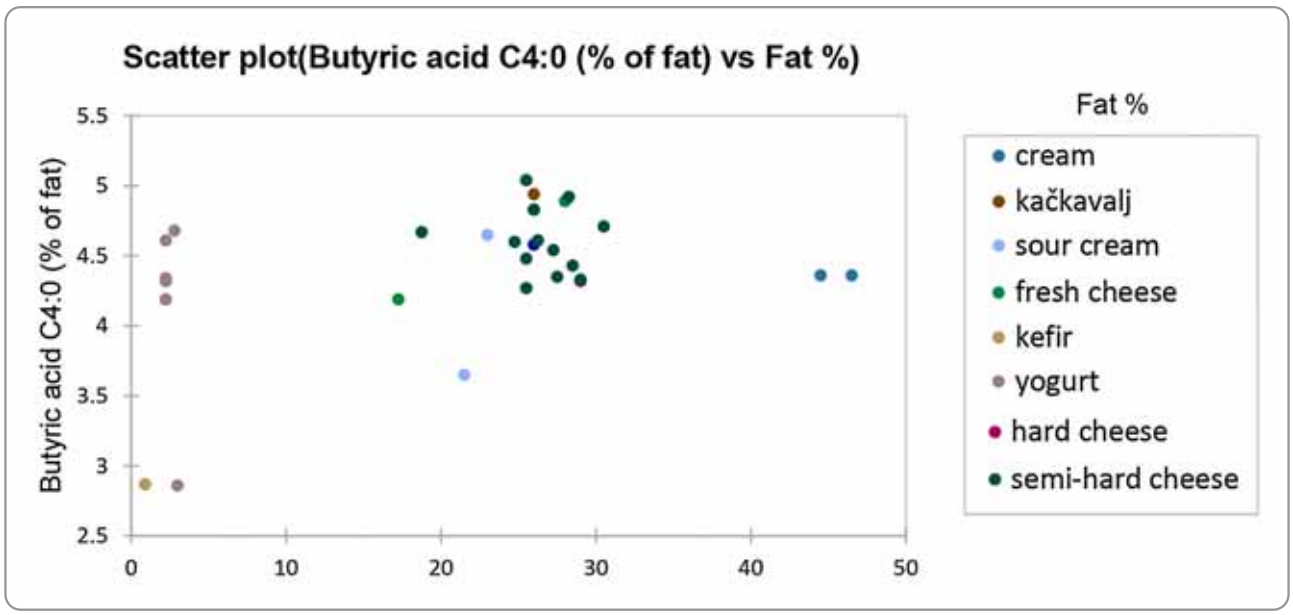


Figure 1. Butyric acid content in different dairy products (g/100 g fat) on the Serbian market

4. Conclusion

This study revealed that determination of the milk fat based on butyric acid content in extracted fat from dairy products gave significant information on quality, and indirect, on authenticity of product. Milk fat is a highly valuable nutrient, so the exact determination of its content in milk and milk products is very important. Development of butyric acid and milk fat analysis in dairy products by GC-FID is essential for laboratories that analysed dairy, and in general, food products, considering quality control during production, and inspection tasks for the import and export of these food products.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, under the Agreement No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Adamska, A., Rutkowska, J., Tabaszewska M. & Białek, M. (2014). Milk of Polish Red and White cows as a source of nutritionally valuable fatty acids. *Archiv Tierzucht*, 57 (10), 1–10.

AOAC, (1995). **Official Methods of Analysis.** 16th edition, Volume II, Arlington, Virginia, USA, method 905.02.

Bobe, G., Hammond, E. G., Freeman, A. E., Lindberg, G. L. & Beitz, D. C. (2003). Texture of butter from cows with different milk fatty acid compositions. *Journal of Dairy Science*, 86, 3122e3127.

Danudol, A. & Judprasong, K. (2022). Development and method validation of butyric acid and milk fat analysis in butter blends and blended milk products by GC-FID. *Foods*, 11, 3606, https://doi.org/10.3390/foods11223606

Dons, T., Candelario, V., Andersen, U. & Ahrné, L. M. (2023). Gentle milk fat separation using silicon carbide ceramic membranes. *Innovative Food Science & Emerging Technologies*, 84, 103299.

European Union, (2008). Commission Regulation (EC) No 900/2008 of 16 September 2008 laying down the methods of analysis for imports of certain goods resulting from the processing of agricultural products. *O.J. L* 2, 488.

FAO, 2022. Dairy Market Review: Overview of global dairy market and policy developments in 2021. Rome.

Genis, D. O., Sezer, B., Durna, S. & Boyaci, I. H. (2021). Determination of milk fat authenticity in ultra-filtered white cheese by using Raman spectroscopy with multivariate data analysis. *Food Chemistry*, 336, 127699.

Gutiérez, R., Vega, S. Diaz, G., Sánchez, J., Coronado, M., Ramírez, A., Pérez, J. & Gonzáles, M. (2008). Detection of non-milk fat in milk fat by gas chromatography and linear discriminant analysis. *Journal of Dairy Science*, 92, 1846–1855.

Hasegawa, Y. & Bolling, B. W. (2023). Yogurt consumption for improving immune health. *Current Opinions in Food Science*, 101017.

Johnson, R. (2014). Food Fraud and "Economically Motivated Adulteration" of Food and Food Ingredients (Washington D.C, Congressional Research Service, Library of Congress, USA) pp 1–40.

Kim, J. M., Kim, H. J. & Park, J. M. (2015). Determination of milk fat adulteration with vegetable oils and animal fats by gas chromatographic analysis. *Journal of Food Science*, 80, C1945-C1951.

Korhonen, H. J. (2006). Technological and health aspects of bioactive components of milk. *International Dairy Journal*, 11(16), 1227–1228.

Mänsson, (2008). Fatty acids in bovine milk fat. Food & Nutrition Research DOI: 10.3402/fnr.v52i0.1821.

Molkentin, J. & Precht, D. (2000). Validation of a gas-chromatographic method for the determination of milk fat contents in mixed fats by butyric acid analysis. *European Journal of Lipid Science and Technology*, 194–201.

Rico, D. E. & Razzaghi, A. (2023). Animal board invited review: The contribution of adipose stores to milk fat: Implications on optimal nutritional strategies to increase milk fat synthesis in dairy cows. *Animal*, 100735.

Sacchi, R., Paduano, A., Caporaso, N., Picariello, G., Romano, R. & Addeo F. (2018). Assessment of milk fat content in fat blends by 13C NMR spectroscopy analysis of butyrate. *Food Control*, 91, 231e236.

Salamon, R., Varga-Visi, É., Sára, P., Csapó-Kiss, Zs. & Csapó, J. (2006). The influence of the season on the fatty acid composition and conjugated linolic acid content of the milk. *Krmiva*, 48(4), 193–200.

Wen, C., Shen, M., Liu, G., Liu, X., Liang, L., Li, Y. ... & Xu, X. (2022). Edible vegetable oils from oil crops: Preparation, refining, authenticity identification and application. *Process Biochemistry*, 124, 168–179.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 615.279:635.49
637.5.055:636.2

ID: 126368777

https://doi.org/10.18485/meattech.2023.64.2.54

Original scientific paper

Fennel (*Foeniculum vulgare*) extracts as potential antioxidants in beef burgers

Milo Mujović[a,b,], Branislav Šojić[a], Branimir Pavlić[a], Predrag Ikonić[c], Snežana Škaljac[a] and Danijela Bursać Kovačević[d]*

[a] *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[b] *GOMBIT d.o.o. Kralja Petra I 194c, Inđija, Serbia*
[c] *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[d] *University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000, Zagreb, Croatia*

ARTICLE INFO

Keywords:
Fennel
Essential oil
Supercritical fluid extracts
Beef burgers
Natural antioxidants

ABSTRACT

The effect of fennel (*Foeniculum vulgare*) essential oil (EO) and fennel supercritical fluid extracts (SFE1 and SFE2) on lipid oxidation of beef burgers was investigated. The basic formulation of beef burgers was obtained by manually mixing fresh minced beef meat with the table salt (2%). From the obtained basic formulation, eight treatments were produced with the addition of the following ingredients extracted from fennel: essential oil (EO), supercritical fluid extract 1 (SFE1), SFE2, and also the standard compound, anethole (A). The ingredients were used at two levels: 0.075 and 0.150 µL/g. The basic formulation of the beef burger was marked as a control (without antioxidants). Lipid oxidative reactions in beef burgers were determined by a spectrophotometric method – the TBARS test. All ingredients reduced lipid oxidation in beef burgers. SFE1 and SFE2 had a higher ($p<0.05$) antioxidative potential than EO and anethole. Therefore, the results of this study displayed the significant antioxidative potential of fennel EO, and especially fennel SFEs, as novel natural antioxidants in beef burger processing.

1. Introduction

Minced meat products, including burgers, meatballs, and fresh sausages, possess significant nutritional value, excellent sensory qualities (e.g., odour, flavour, and texture), availability, and relatively low cost (*Salter*, 2018). According to Serbian regulations, minced meat products are produced by grinding and mixing meat and fatty tissue with table salt and spices. Then the obtained meat mixtures are shaped (manually or mechanically) (*Regulation No 50/19*, 2019).

The high level of grinding, a significant percentage of nutritional compounds (proteins, fats, and vitamins), deficiency of food additives (preservatives and synthetic antioxidants), and absence of thermal treatments lead to microbial and chemical spoilage and, consequently, relatively short shelf-life of these meat products (*Schilling et al.*, 2018; *Bantawa et al.*, 2018). Lipid and protein oxidation are the leading causes of chemical spoilage and reduced shelf-life for minced meat products (*Šojić et al.*, 2014). Concerning the valuable content of bioactive compounds (e.g., phenolics, terpenoids, carotenoids) with strong protective effects against microbial growth and oxidative reactions, different extracts isolated from aromatic and medicinal

***Corresponding author:** Milo Mujović, *mujovic.9.21.d@uns.ac.rs*

Paper received May 26th 2023. Paper accepted Jun 4th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

plants could be used as natural additives and quality enhancers for minced meat products (*Danilović et al.*, 2021; *Kocić-Tanackov et al.*, 2017; *Šojić et al.*, 2023; *Šuput et al.*, 2012; *Tomović et al.*, 2017).

Fennel (*Foeniculum vulgare*) is one of the significant aromatic and medicinal plants in the Mediterranean region, with strong preservative (antioxidative and antimicrobial) potential in the food sector. Fennel's antioxidative and antimicrobial potential is associated with its essential oil (EO) level and chemical profile, particularly the contents of phenolics, terpenoids, and carotenoids (*Badgujar et al.*, 2014).

According to current scientific knowledge, there needs to be more investigation regarding the application of fennel and its extracts as novel additives in meat processing. Thus, the goal of this study was to evaluate the antioxidative potential of fennel EO, fennel SFE1, and fennel SFE2 in the beef burger as a real system.

2. Materials and methods

2.1. Beef burger processing

Initially, fresh beef chucks were manually deboned and minced by an industrial meat grinder (Mado, Germany) until 4 mm meat particles were achieved (Gombit d.o.o., Inđija, Serbia). In the next step, the basic formulation of beef burgers was obtained by manually mixing fresh minced beef meat with table salt (2%). From the obtained basic formulation, eight treatments were produced with the addition of the following ingredients: EO, SFE1, SFE2, and the standard compound, anethole (A). The ingredients were used at two levels: 0.075 and 0.150 µL/g, labelled in the results as 75 and 150, respectively. The basic formulation of the beef burger was used as a control (without antioxidants). All treatments and control were manually shaped, packed in polypropylene trays and overwrapped with an oxygen-permeable polyvinyl chloride film. Finally, beef burgers (approximately 0.1 kg each) were stored in a cooling chamber at 3 ± 1°C for three days. Samples were taken at different periods, after 0, 1, 2, and 3 days of refrigerated storage, consisting of three randomly selected beef burgers from each treatment and control. TBARS test was conducted on three samples from each group of beef burgers in duplicates.

2.2. Plant materials and extracts

Conventional (hydrodistillation) and novel (supercritical fluid extraction – SFE) extraction techniques were used for recovery of fennel essential oil. The official procedure from Ph. Jug. IV (1984) was applied for hydrodistillation and EO recovery. The SFE was performed using a laboratory-scale high pressure extraction plant (HPEP, NOVA, Swiss, Efferikon, Switzerland) described in detail by *Pekić et al*. (1995). Fennel was placed in an extractor vessel, and the extraction process was carried out for 4 h under the following conditions: the first extract (SFE1) was obtained at 100 bar and 40°C, while the second extract (SFE2) was obtained at 300 bar and 40°C. All other parameters were the same for both types of extraction. *Trans*-anethole and estragole were the most dominant compounds in the chemical profile of EO (466 mg/g) and SFEs (SFE1 = 117.84 mg/g; SFE2 = 40.30 mg/g), respectively.

2.3. TBARS test

Lipid oxidative reactions in beef burgers were determined by a spectrophotometric method – the TBARS test described in *Šojić et al.* (2014). Results were expressed as mg malondialdehyde (MDA) per kg of beef burgers.

Table 1. TBARS values (mg malondialdehyde/kg) in fresh beef burger during cold storage

Storage day	Treatments at 0.075 and 0.150 µL/g								
	Control	A-75	A-150	EO-75	EO-150	SFE1-75	SFE1-150	SFE2-75	SFE2-150
0	0.14 ± 0.00^{BCd}	0.13 ± 0.00^{CDd}	0.12 ± 0.00^{Dd}	0.18 ± 0.00^{Ad}	0.14 ± 0.00^{BCd}	0.14 ± 0.01^{Bd}	0.13 ± 0.01^{CDd}	0.17 ± 0.01^{Ad}	0.12 ± 0.00^{Dd}
1	0.27 ± 0.02^{Ac}	0.17 ± 0.01^{Dc}	0.17 ± 0.00^{Dc}	0.26 ± 0.01^{Ac}	0.23 ± 0.00^{Bc}	0.20 ± 0.01^{Cc}	0.20 ± 0.01^{Cc}	0.23 ± 0.00^{Bc}	0.19 ± 0.01^{Cc}
2	0.41 ± 0.00^{Ab}	0.28 ± 0.01^{Eb}	0.30 ± 0.00^{Db}	0.39 ± 0.01^{Bb}	0.31 ± 0.00^{Db}	0.30 ± 0.01^{Db}	0.28 ± 0.00^{Eb}	0.34 ± 0.01^{Cb}	0.35 ± 0.01^{Cb}
3	0.58 ± 0.02^{Aa}	0.41 ± 0.00^{Da}	0.39 ± 0.00^{DEa}	0.59 ± 0.01^{Aa}	0.47 ± 0.01^{Ca}	0.37 ± 0.01^{EFa}	0.35 ± 0.01^{Ga}	0.47 ± 0.02^{Ca}	0.50 ± 0.00^{Ba}

Values with different letters $^{(A-C)}$ in the same row are significantly different ($p<0.05$); Values with different letters $^{(a-c)}$ in the same column are significantly different ($p<0.05$). A, anethole; EO, essential oil; SFE, supercritical fluid extract. 75 = 0.075 µL/g and 150 = 0.150 µL/g.

3. Results

TBARS values of beef burgers produced with different ingredients (EO, SFE1, SFE2 and anethole) are presented in Table 1. On the initial day of storage (day 0), TBARS values ranged in narrow intervals from 0.12 μL/g (A-150, SFE2-150) to 0.18 μL/g (EO-75). As expected, during three days of storage, TBARS values significantly ($p<0.05$) increased in all eight treatments and the control. At the end of storage, the TBARS values among the treatments were in the order: C ≤ EO-75 < SFE2-150 < EO-150; SFE2-75 < A-75 ≤ A-150 ≤ SFE1-75 < SFE1-150.

4. Discussion

The obtained results suggest the strong antioxidative potential of fennel EO and SFEs. This antioxidative potential could be related to the chemical activity of terpenoids contained in these fennel products. Similar to our results, terpenoid-rich extracts obtained from different plant materials (raspberry pomace, sage herbal dust, chokeberry extract) also efficiently reduced lipid oxidation in minced meat products (*Kryževičūtė et. al*., 2017; *Šojić et al*., 2018; *Tamkutė et al*., 2021).

Also, it should be noticed that EO at a concentration of 0.150 μL/g had a similar antioxidative potential as the other natural ingredients at half the concentration. Moreover, TBARS values in all treatments, except control and EO-75 throughout the storage, were less than the upper limit (≤ 0.5 mg MDA/kg), set as a marker of oxidative rancidity for meat and processed meat products (*Jin et al*., 2018). The standard compound, anethole, showed a significantly greater ($p<0.05$) reduction of TBARS values compared to EO and SFE2. These results suggest the significant antioxidative potential of this compound.

The TBARS test is one of the primary methods for detecting the lipid oxidative status of meat and meat products. The lowest TBARS values were determined in treatment SFE1. This could probably be related to the relatively high estragole content in SFE1 (117.84 mg/g). It is well known that the antioxidant potential of anethole and, primarily, estragole are related to the hydroxyl group connected to the aromatic ring, accomplished by donating hydrogen atoms with electrons and neutralizing free radicals (*Falowo et al.*, 2019). When comparing the chemical composition of EO, SFE1, and SFE2, it should be noted that the estragole significantly impacted the neutralizing free radicals and increasing oxidative stability of beef burgers.

Also, it is possible that SFE1 possesses the highest liposolubility and, consequently, has the most intensive interaction with the main constituents of muscle lipids, including free fatty acids, sterols, phospholipids, etc. Similarly, in our previous studies in minced meat products, we determined that SFEs showed better antioxidant potential than did conventional EOs (*Šojić et al.*, 2018; *Šojić et al.*, 2019).

5. Conclusion

Trans-anethole and estragole were the most dominant compounds in the chemical profile of fennel EO and SFEs, respectively. EO and SFEs isolated from fennel efficiently decreased lipid oxidation in beef burgers. The lowest antioxidative potential was determined in SFE1 at 0.150 μL/g. Hence, fennel EO and SFEs with evident antioxidant activity (in this case, SE2), could be used in beef burger processing to improve the meat products' quality and safety.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The research in this paper was financed by the Science Fund of the Republic Serbia: "Novel extracts and bioactive compounds from under-utilized resources for high-value applications"—BioUtilize, No: 7750168.

Acknowledgements: The authors would like to thank meat industrial plant (Gombit d.o.o, Inđija) for sponsoring this research.

References

Badgujar, S. B., Patel, V. V. & Bandivdekar, A. H. (2014). *Foeniculum vulgare* Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed Research International*, https://doi.org/10.1155/2014/842674

Bantawa, K., Rai, K., Subba Limbu, D. & Khanal, H. (2018). Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. *BMC Research Notes*, 11, 1–5, https://doi.org/10.1186/s13104-018-3722-x

Danilović, B., Đorđević, N., Milićević, B., Šojić, B., Pavlić, B., Tomović, V. & Savić, D. (2021). Application of sage herbal dust essential oils and supercritical fluid extract for the growth control of *Escherichia coli* in minced pork during storage. *LWT*, 141, 110935, https://doi.org/10.1016/j.lwt.2021.110935

Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Afolayan, A. J. & Muchenje, V. (2019). Phytochemical constituents and antioxidant activity of sweet basil (*Ocimum basilicum* L.) essential oil on ground beef from boran and nguni cattle. *International Journal of Food Science*, 2628747, https://doi.org/10.1155/2019/2628747

Jin, S. K., Choi, J. S., Yang, H. S., Park, T. S. & Yim, D. G. (2018). Natural curing agents as nitrite alternatives and their effects on the physicochemical, microbiological properties and sensory evaluation of sausages during storage. *Meat Science*, 146, 34–40, https://doi.org/10.1016/j.meatsci.2018.07.032

Kocić-Tanackov, S., Dimić, G., Mojović, L., Gvozdanović-Varga, J., Djukić-Vuković, A., Tomović, V. & Pejin, J. (2017). Antifungal activity of the onion (*Allium cepa* L.) essential oil against Aspergillus, Fusarium and Penicillium species isolated from food. *Journal of Food Processing and Preservation*, 41, e13050. https://doi.org/10.1111/jfpp.13050

Kryževičūtė, N., Jaime, I., Diez, A. M., Rovira, J. & Venskutonis, P. R. (2017). Effect of raspberry pomace extracts isolated by high pressure extraction on the quality and shelf-life of beef burgers. *International Journal of Food Science and Technology*, 52, 1852–1861, https://doi.org/10.1111/ijfs.13460

Pekić, B., Zeković, Z., Petrović, L. & Tolić, A. (1995). Behavior of (–)-α-Bisabolol and (–)-α-Bisabololoxides A and B in camomile flower extraction with supercritical carbon dioxide. *Separation science and technology*, 30, 3567–3576, https://doi.org/10.1080/01496399508015137

Regulation on the Quality of Ground Meat, Meat Preparations and Meat Products. UNEP Law and Environment assistance platform. Available online: https://leap.unep.org/countries/rs/national-legislation/regulation-quality-ground-meat-meat-preparations-and-meat.

Salter, A. M. (2018). The effects of meat consumption on global health. *Revue scientifique et technique (International Office of Epizootics)*, 37, 47–55, doi: 10.20506/rst.37.1.2739

Schilling, M. W., Pham, A. J., Williams, J. B., Xiong, Y. L., Dhowlaghar, N., Tolentino, A. C. & Kin, S. (2018). Changes in the physiochemical, microbial, and sensory characteristics of fresh pork sausage containing rosemary and green tea extracts during retail display. *Meat Science*, 143, 199–209, https://doi.org/10.1016/j.meatsci.2018.05.009

Šojić, B. V., Petrović, L. S., Mandić, A. I., Sedej, I. J., Džinić, N. R., Tomović, V. M. & Ikonić, P. M. (2014). Lipid oxidative changes in traditional dry fermented sausage *Petrovská klobása* during storage. *Hemijska industrija*, 68, 27–34.

Šojić, B., Ikonić, P., Kocić-Tanackov, S., Peulić, T., Teslić, N., Županjac, M. & Pavlić, B. (2023). Antibacterial activity of selected essential oils against foodborne pathogens and their Application in fresh turkey sausages. *Antibiotics*, 12, 182, https://doi.org/10.3390/antibiotics12010182

Šojić, B., Pavlić, B., Tomović, V., Ikonić, P., Zeković, Z., Kocić-Tanackov, S. & Ivić, M. (2019). Essential oil versus supercritical fluid extracts of winter savory (*Satureja montana* L.) — Assessment of the oxidative, microbiological and sensory quality of fresh pork sausages. *Food Chemistry*, 287, 280–286, https://doi.org/10.1016/j.foodchem.2018.12.137

Šojić, B., Pavlić, B., Zeković, Z., Tomović, V., Ikonić, P., Kocić-Tanackov, S. & Džinić, N. (2018). The effect of essential oil and extract from sage (*Salvia officinalis* L.) herbal dust (food industry by-product) on the oxidative and microbiological stability of fresh pork sausages. *LWT*, 89, 749–755, https://doi.org/10.1016/j.lwt.2017.11.055

Šuput, D., Petrović, L., Šojić, B., Savatić, S., Lazić, V. & Krkić, N. (2012). Application of chitosan coating with oregano essential oil on dry fermented sausage. *Journal of Food and Nutrition Research*, 51, 60–68.

Tamkutė, L., Vaicekauskaitė, R., Melero, B., Jaime, I., Rovira, J. & Venskutonis, P. R. (2021). Effects of chokeberry extract isolated with pressurized ethanol from defatted pomace on oxidative stability, quality and sensory characteristics of pork meat products. *LWT,* 150, 111943, https://doi.org/10.1016/j.foodchem.2022.133978

Tomović, V., Jokanović, M., Šojić, B., Škaljac, S. & Ivić, M. (2017). Plants as natural antioxidants for meat products. In IOP conference series: earth and environmental science, vol. 85, No. 1, p. 012030, IOP Publishing.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 628.473.042

ID: 126425353

https://doi.org/10.18485/meattech.2023.64.2.55

Review paper

Food loss and waste: a global problem

Slavica Vesković Moračanin[a], Milan Milijašević[a], Branka Borović[a] and Jasna Kureljušić[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, 11107 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Food loss
Food waste
Food spoilage
Damage

ABSTRACT

The world produces an enormous amount of food to sustain its population, yet a substantial portion of it goes to waste or is lost throughout the supply chain. Food loss refers to the reduction in the quantity or quality of food throughout the production, post-harvest, and processing stages, primarily in developing countries with inadequate infrastructure and storage facilities. It is estimated that up to one-third of all food produced globally is lost before it even reaches consumers. Food waste occurs mainly in developed countries, primarily at the consumer and retail levels. It refers to the discarding of edible food that is still fit for consumption.

Addressing food waste and food loss requires a multifaceted approach involving governments, businesses, communities, and individuals. By tackling food waste and food loss on a global scale, we can work towards achieving food security, reducing environmental degradation, and fostering a more equitable and sustainable future for all.

1. Introduction

Providing a sufficient amount of safe food with defined desirable quality parameters is a crucial responsibility of every producer, but also a basic human right guaranteed by the Universal Declaration of Human Rights in 1948 (Article 25) (*Beuchelt et al*., 2022). The attitude of people towards food has changed over time. Today's consumers are more aware and informed about food safety and quality, and they expect the food they consume to be safe, nutritious, and of high quality. This has led to a growing demand for food products that meet specific quality parameters, such as minimally processed foods, organic food, non-GMO foods, and food with reduced content of sugar, salt, unhealthy fats, etc (*Alcorta et al.*, 2021). However, in parallel with modern trends in the food industry, significant increases in the food losses and food waste, as well as risk of food spoilage in the food chain are now being seen. These questions are becoming even more significant as the global population continues to increase. According to the United Nations' "World Population Prospects 2019" report (*UN*, 2019), the global population is projected to reach 9.7 billion by 2050 and 10.9 billion by 2100.

By minimizing these risks we can better meet the increasing global food demand and ensure a more sustainable future for all.

2. Food losses and waste in the world

In the 21st century, food loss, with its consequent losses, is of great influence for economic, ecological and sociological aspects (*Seberini*, 2020). In addition to direct material damage, a significant loss

***Corresponding author:** Slavica Vesković Moračanin, *slavica.veskovic@inmes.rs*

Paper received May 26th 2023. Paper accepted Jun 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

is also reflected in lost work, used water, energy, land, and other resources that enter the food production chain (*Lipinski et al.*, 2013). Food loss occurs at all stages of food production starting from primary stage (field or farm), through processing, storage and transportation to end-users, excluding retail stores (*Vågsholm et al*., 2020). Inefficient farming practices, storage and transportation losses, and inadequate infrastructure contribute to significant food losses even before food reaches consumers.

In contrast to food loss at the producer and market interface, food wastage is linked to the reduction of its quantity due to specific decrees and activities within retail systems, food service providers and from consumers themselves. In developed countries, consumers often discard edible food due to various factors, such as purchasing excess quantities, improper storage, confusion over expiration dates, or aesthetic preferences for visually appealing produce. It is necessary to recognize that food wastage comprises rejected safe and nutritious foods (*FAO*, 2011).

Estimating the exact amount of food wasted globally, or in specific countries, is challenging due to the lack of comprehensive data and variations in measurement methodologies. According to the Food and Agriculture Organization (*FAO*, 2011) of the United Nations, it is estimated that approximately one-third of all food produced for human consumption is lost or wasted globally each year in the first decade of the 21st century. This amounts to about 1.3 billion metric tons of food, or approximately $1 trillion in economic losses. However, food losses and waste varies across regions and countries due to various factors, such as, the level of economic development, cultural practices, infrastructure and food supply chain management practices. Generally, higher levels of food waste are observed in more economically developed countries, while in developing nations, food losses often occur at earlier stages of the supply chain (during production, storage, and transportation).

The United States is one of the countries with significant food waste. It is estimated that around 30–40% of the food supply is wasted, amounting to approximately 63 million tons of food annually with an estimated value of around $161 billion (*FDA*, 2023). In the UK, it is estimated that around 9.5 million tons of food is wasted annually, with households accounting for the majority of the waste. Australia wastes around 7.3 million tons of food each year, with consumers and households contributing to about 34% of the total food waste. China is the world's largest producer and consumer of food, and food waste is a growing concern. While specific figures are challenging to ascertain, it is estimated that China wastes around 17–18 million tons of food annually (it is important to note that these figures are approximate and may vary based on different data sources and reporting methods).

In Europe, the economic costs of food waste and loss are also substantial. Is considered that Europe is responsible for 22% of food waste on the global level (*Searchinger et al*., 2014). According to a study by the European Commission, food waste in the EU alone costs around €143 billion per year. This includes the costs of producing, processing, and transporting food that is ultimately wasted, as well as the environmental and social costs of food waste. It is an interesting fact that the level of food wastage in Europe is quite uniform in the countries with high-, middle- and lower middle-income (*UNEP*, 2021).

Serbia still does not have a dedicated national food waste study, nor comprehensive data on food waste. However, according to estimates (*NALED*, 2019), about 247 thousand tons of edible food are wasted in Serbia annually, about 30–40 kg per capita, the estimated value of which is about €240 million.

Table 1. Annual wastage quantity (kg/capita/year) depending on the country's economic development level (FAO, 2021)

Level of development	Average food waste (kg/capita/year)		
	Household	Food service	Retail
High-income countries	79	26	13
Upper middle-income countries	76	Insufficient data	
Lower middle-income countries	91	Insufficient data	
Low-income countries	Insufficient data		

In Table 1, the average annual quantities of food wastage in countries of different levels of development according to the World Bank research data (*FAO*, 2021) are shown.

Research has shown that in developing countries, due to the lack of modern processing and preservation technologies, as well as adequate storage methods, much more food is lost during the production/processing phase and immediately afterwards. In contrast, in industrialized countries one-third of food spoilage and write-offs occur at the retail or consumer level (*FAO*, 2011). The worrying data is that in the countries with medium and high incomes, food is to a large extent wasted, even if it is still suitable for human consumption, but also due to its increased production in relation to the actual needs of end users.

Despite global progress in the development of human society, access to food remains limited in many regions of the world, which is contributed to by poverty, conflict, climate change, inadequate infrastructure, and unequal distribution of resources. It was estimated in 2020 that from 720 to 811 million people worldwide experienced hunger, which is as much as 161 million people more than the year before (*FAO*, 2021 ; *Tomaszewska et al.*, 2022) and in 2021, between 702 and 828 million were hungry. Hunger affected 46 million more people in 2021 compared to 2020, and a total of 150 million more people since 2019, before the COVID-19 pandemic. Also, an estimated 45 million children under the age of five were suffering from wasting, the deadliest form of malnutrition, while 149 million children (of the same age) had stunted growth and development due to a chronic lack of essential nutrients in their diets. The listed figures highlight the stark contrast between the prevalence of hunger and food waste in the world today.

3. Food spoilage

Food spoilage is a natural, metabolic process that leads to sensory changes in texture, odour, taste, or appearance of food that becomes undesirable or unacceptable for human consumption (*Doyle,* 2007 ; *Nychas and Panagou*, 2011). Irrespective of its origin (vegetable or animal), due to its composition (moisture, proteins, lipids, carbohydrates and other organic and mineral substances) food can be an ideal environment for the development of undesired microbiological, chemical and physical processes that lead to the emergence of unpleasant sensory changes, i.e., spoilage. Numerous factors are involved in the aetiology of food spoilage occurrence. These are, in the first place, activities of microorganisms (primarily, bacteria, yeast and moulds), insects, rodents and other pests, then action of food enzymes themselves, effects of storage temperature, air composition (especially oxygen level), light, air humidity level and other factors. These aforementioned factors rarely act separately, isolated and independent from each other. In most cases, their action is interconnected and simultaneous.

Although microorganisms are the most common causes of the food spoilage process, this process does not always lead to consumer illness, given that pathogenic microorganisms or their toxins need not be present in spoiled food. However, the resulting changes in sensory properties mean that this type of food must not be used further in human diets. The highest percentage of food loss was found in root vegetables (40–50%), fruits and vegetables (35%), fish and seafood (30%), cereals (20%), meat, oilseeds and dairy products (20%) (*FAO*, 2019).

Food spoilage is a major concern of the entire world population, with developing parts of the world particularly affected. At the same time, the research results indicate that there is a global lack of information about this problem, especially in quantifying food loss in relation to the aetiology of the cause, as well as the extent of economic damage that accompanies such loss (*FAO*, 2011). In parallel with these unfamiliarities, there are also no appropriate assumptions about potential costs that could be spent on needed activities aimed at reducing or preventing food loss due to food spoilage.

4. What are ways to minimize waste and food loss?

Regardless of the level of economic development and maturity of the system in the country, it is necessary to strive to reduce food losses to a minimum. Considering the importance of this problem, many countries in the EU have, as part of their national policies in the area of food production, and as an important part of their strategies, raised the issue of reducing food loss and wastage, requesting that by legally binding statutes. However, in most countries, legislation is designed in such a way that food safety is treated separately from the issue of food spoilage. Despite such a prevailing approach, the aforementioned areas cannot be separated or considered individually. The occurrence of diseases

with accompanying health problems, as well as economic losses due to foodborne diseases, are directly related and complementary to economic losses due to food spoilage (*Di Renzo et al.*, 2015).

Looking at the food chain as a whole, based on the many analyses carried out so far, we think that the following approaches (or a combination thereof) could help reduce food losses (*FAO*, 2019 ;*Veskovic Moračanin & Đukuć*, 2022): implementing better post-harvest handling practices (including proper harvesting techniques, efficient transportation, and storage facilities that maintain optimal temperature and humidity levels); improving infrastructure (enabling more efficient transportation of food to markets and storing it in appropriate conditions, thus reducing spoilage and losses along the supply chain); developing efficient and well-coordinated supply chains (establishing effective linkages between farmers, processors, distributors, and retailers to ensure a smooth flow of food products); implementing quality control measures and product standards (ensuring that only high-quality produce enters the market, minimizing potential losses due to rejection or spoilage through inspections, certifications, and adherence to food safety standards throughout the supply chain); educating farmers, processors, retailers, and consumers about proper handling, storage, and utilization of food; and promoting the development and implementation of new technologies and innovations in the food chain. Additionally, government interventions through the implementation of policies and regulations in the food sector can create an enabling environment for stakeholders to prioritize and invest in reducing food losses.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, according to the provisions of the Contract on research funding in 2023 (No. 451-03-47/2023-01/200050, dated 03.02.2023).

References

Alcorta, A., Porta, A., Tárrega, A., Alvarez, M. D. & Vaquero, M. P. (2021). Foods for Plant-Based Diets : Challenges and Innovations. *Foods*, 10(2), 293, https://doi:10.3390/foods10020293

Beuchelt, T. D., Schneider, R. & Gamba, L. (2022). Integrating the Right to Food in Sustainability Standards: A Theory of Change to Move Global Supply Chains from Responsibilities to Impacts. *Applied Economic Perspectives and Policy*, 44(4), 1864–1889, https://doi.org/10.1002/aepp.13308

Di Renzo, L., Colica, C., Carraro, A., Beniamino, C. G., Luigi T. M., Botta, R., Colombo, M. L., Gratteri, S., Chang, T. F. M., Droli, M., Sarlo F. & De Lorenzo, A. (2015). Food safety and nutritional quality for the prevention of non communicable diseases: the Nutrient, hazard Analysis and Critical Control Point process (NACCP). *Journal of Translational Medicine*, 13, 128, https://doi.org/10.1186/s12967-015-0484-2

Doyle, J. J. (2007). Child Protection and Child Outcomes: Measuring the Effects of Foster Care. *American Economic Review*, 97(5), 1583-1610, https://www.aeaweb.org/articles/pdf/doi/10.1257/aer.97.5.1583.

Food and Agriculture Organization of the United Nations FAO, (2011). Global food losses and food waste – Extent, causes and prevention. Rome. Retrieved from http://www.fao.org/food-loss-and-food-waste/flw-data.

Food and Agriculture Organization of the United Nations FAO, (2021). The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. Rome, Italy, https://doi.org/10.4060/cb4474en

Food and Agriculture Organization of the United Nations FAO, (2019). The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction. Rome. Italy. Retrieved from: https://www.fao.org/3/ca6030en/ca6030en.pdf.

Food and Drug Administration FDA, (2023). Food Loss and Waste. Retrieved from: https://www.fda.gov/food/consumers/food-loss-and-waste.

Lipinski, B., Hanson, C., Lomax, J., Kitinoja, L., Waite, R. & Searchinger, T. (2013). Reducing Food Loss and Waste: Working Paper. World Resources Institute. Washington, DC, http://pdf.wri.org/reducing_food_loss_and_waste.pdf.

Nacionalna alijansa za lokalni ekonomski razvoj (NALED), (2019). Analiza sistema upravljanja otpadom od hrane u Republici Srbiji. (in Serbian). Retrieved from: https://naled.rs/htdocs/Files/04672/Analiza_Otpada_od_hrane.pdf.

Nychas, G-J. E. & Panagou, E. Z. (2011). Microbiological spoilage of foods and beverages. In D. Kilcast, & P. Subramaniam (Eds.), Woodhead Publishing Series in Food Science, Technology and Nutrition, Food and Beverage Stability and Shelf Life (pp.3–28), https://doi.org/10.1533/9780857092540.1.3

Searchinger, T., Hanson, C., Ranganathan, J., Lipinski, B., Waite, R., Winterbottom, R., Dinshaw, A. & Heimlich, R. (2014). Creating a Sustainable Food Future. A Menu of Solutions to Sustainably Feed More than 9 Billion People by 2050. World Resources Institute. Washington, DC, USA. ISBN 978-1-56973-817-7

Seberini, A. (2020). Economic, social and environmental world impacts of food waste on society and Zero waste as a global approach to their elimination. SHS Web of Conferences 74, 03010, https://doi.org/10.1051/shsconf/20207403010

Tomaszewska, M., Bilska, B. & Kołożyn-Krajewska, D. (2022). The Influence of Selected Food Safety Practices of Consumers on Food Waste Due to Its Spoilage. *International Journal of Environmental Research and Public Health*, 19(13), 8144, https://doi.org/10.3390/ijerph19138144

United Nations Environment Programme UNEP, (2021). Food Waste Index Report 2021. Nairobi, Kenya. Retrieved from: file:///C:/Users/User/Downloads/Food-Waste%20(4).pdf.

United Nations, Department of Economic and Social Affairs, Population Division, (2019). World Population Prospects 2019: Highlights, (ST/ESA/SER.A/423). Retrieved from: https://reliefweb.int/report/world/world-population-prospects-2019-highlights?

Vågsholm I., Arzoomand N. S. & Boqvist S. (2020). Food security, safety, and sustainability — getting the trade-offs right. *Frontiers in Sustainable Food Systems*, 4, 16, https://doi.org/10.3389/fsufs.2020.00016

Vesković Moračanin, S. & Đukić, D. (2022). Food control systems and protection of human health. Introductory presentation at the XXVI International Eco-conference 2022, Novi Sad, Serbia.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 639.371.057:597.351.214
637.56:[639.21:597.351.214

ID: 126431753

https://doi.org/10.18485/meattech.2023.64.2.56

Original scientific paper

Evaluation of sensory characteristics of common carp reared in purified wastewater from a slaughterhouse

Miloš Pelić[a], Milica Živkov Baloš[a], Nikolina Novakov[b], Nikola Puvača[c], Jasna Kureljušić[d], Ana Gavrilović[e] and Dragana Ljubojević Pelić[a]*

[a] *Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia*
[b] *University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*
[c] *University Business Academy, Faculty of Economics and Engineering Management, Department of Engineering Management in Biotechnology, Cvecarska 2, 21000 Novi Sad, Serbia*
[d] *Scientific Veterinary Institute of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia*
[e] *University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

ARTICLE INFO

Keywords:
Fish Production
Integrated system
Sustainability
Wastewater

ABSTRACT

The aim of the present research was to evaluate the sensory characteristics of common carp as well as their correlation with the season of sampling in the common carp reared in integrated production system. Fish were collected in spring and autumn from fish pond which received purified water from slaughterhouse. The sensory quality of common carp fillets shows very high scores for all examined parameters and overall impression.

1. Introduction

Consumption of fish meat is encouraged because of its high protein content, presence of essential amino acids, minerals, vitamin A, and other nutrients (*Hussain et al.*, 2011). Furthermore, fish meat is one of the most important sources of n-3 highly unsaturated fatty acids (HUFAs). These fatty acids can lower the blood cholesterol and triglyceride levels and can prevent cardiovascular and neurological diseases (*Morris et al.*, 2003), thus making fish meat valuable in the human diet.

Although aquaculture is increasing worldwide, it is encountering an unused potential in Serbia. In some regions of the country, there are vast unused areas which are not cultivated or suitable for other agricultural activities. However, these areas are in the immediate vicinity of slaughterhouses, and could be used for aquaculture. Furthermore, fish production in Serbia mostly consists of the traditional rearing system, which is a semi-intensive culture system, and the fish diet is based on a combination of natural food and supplementary feed (cereals, such as wheat, maize and barley). A similar situation is found in many countries worldwide. To improve and intensify carp production, in the last few years, cereals have been replaced by extruded feed (*Steffens and Wirth*, 2007; *Ljubojević et al.*, 2013). Rural areas present ideal hotspots for developing integrated production, since the land is far away from human settlements, purified wastewater can be used for filling ponds, thus significantly reducing the release of harmful agents into rivers and the environment, and applying good agricultural practices could lead to favourable natural food composition.

***Corresponding author:** Miloš Pelić, *milosp@niv.ns.ac.rs*

Paper received May 26th 2023. Paper accepted Jun 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Wastewater from slaughterhouses in developing countries is mostly discharged into rivers, lakes and seas without being adequately treated to removed impurities. Such wastewater contains plenty of organic matter which is ideal source of nutrients for fish, but also for development of different microorganisms. However, usage of slaughterhouse wastewater in aquaculture could be a health risk for humans and fish and other aquatic organisms due to possible introduction of pathogenic bacteria into the aquatic environment (*Sapkota et al*., 2008). Therefore, an integrated production system could present a significant risk for public health. It is already known that fish and other aquaculture products can be vehicles for various pathogens (*Ljubojević et al.*, 2016), and the health advantages arising from consuming products from wastewater-fed fish ponds are questionable. *Lan et al.* (2007) underlined that the safety of consuming fish from wastewater fed ponds is questionable. *Scallan et al.* (2011) stated that more foodborne outbreaks are caused by pathogens than by chemical or physical contaminants.

In the available literature, there are insufficient data on meat safety and quality regarding the common carp reared in purified wastewater-fed earthen ponds, which makes this investigation a novelty in the field of integrated fish production systems. Since there are also scarce data about the sensory quality of fish reared in integrated production systems with purified slaughterhouse wastewater in the literature, the aim of this study was to assess the meat quality of freshwater fishes cultivated in an integrated fish pond and compare them with other cultivation systems.

2. Materials and methods

2.1. Fish ponds and waters

An earthen pond with an area of 1 ha and average depth of 1.2 m was built in Pećinci village (N 44°54′19″, E 19°57′35″), Srem District, Serbia, in the vicinity of a slaughterhouse facility. Fish ponds were filled directly with purified water from the slaughterhouse and with added well water, and aeration of the pond was secured. A channel, 2 m wide, was dug around the pond for filtering the water from the pond and thus significantly reducing the required amount of water. The fish pond was aerated by continuous use of an aerator. The water flow was approximately 3.5 L s^{-1}, which secured the absence of harmful effects of carbon dioxide and ammonia on the fish.

The wastewater treatment system for slaughterhouse wastewater consisted of a pump station and tanks. The dimensions of the system were 25.50 × 16.15 m, and it contained: accumulation tank, fat separation system, denitrification treatment system, equalization tank and a biological oxidation tank for biological wastewater treatment.

2.2. Fish species and supplementary feed

Two year old fingerlings of common carp (*Cyprinus carpio*) obtained from a commercial fish farm were stocked at density 2500 individuals/ha. The fish pond was stocked in March 2017. The mean of initial live weight of fish was 200 g. Carp were grown under variable natural atmospheric conditions. Relatively low cost, locally available nutrients were used as additional feed and industrial feed for fish was supplied to the fish.

2.3. Sampling

Fresh reared common carp were collected from the fish pond that was fed with purified wastewater from slaughterhouse using the same protocols in spring (April 2018) and autumn (October 2018). Seven fish at each sampling time were sacrificed by a quick blow to the head, and the average weight was 820 g and 1950 g in April and October, respectively. Each fish was placed in a sterile plastic bag. All fish were kept at refrigerator temperature during transportation to the laboratory and were analysed and assessed for sensory characteristics and quality within four hours of harvesting.

2.4. Sensory analysis

Sensory assessment was conducted at the laboratory of Scientific Veterinary Institute Novi Sad in Novi Sad, Serbia, equipped in accordance with the ISO standard (*SRPS*, 2015). Five trained panellists with expertise in sensory evaluation of fish and fish products evaluated selected properties for fillets (colour, odour, taste, softness, chewiness and juiciness) of the common carp. Sensory analyses were evaluated using a point system of analytical descriptive tests with a scale from 1 to 5, for the colour (1=very bad; 2=bad, 3=good; 4=very good; 5=extremely good), for the odour and taste (1=extremely unpleasant; 2=unpleasant; 3=insufficiently pleasant; 4=pleasant, good; 5=extremely pleasant, excellent), for softness (1=moderately ten-

der; 2=tender; 3=moderately tender; 4=moderately soft; 5=soft), for chewiness (1=moderately tough; 2=slightly tough; 3=chewable; 4=moderately soft; 5=soft), for juiciness (1=dry; 2=moderately dry; 3=moderately juicy; 4=juicy; 5=extremely juicy) and for overall impression (1=sufficient; 2=suitable; 3=good; 4=very good; 5=excellent). Panellists were randomly supplied with thermally processed samples of fillets of common carp. Between each sample evaluation, panellists cleansed their palates with distilled water, bread or apple. Between each repetition period of sample evaluation, one hour was allowed for the panellists to rest their senses.

2.5. Statistical analysis

The Student's t-test was used to compare the geometric mean sensory results from the common carp that were harvested in spring and in autumn. The data were analysed in Excel 2013. The average of duplicate scores for all responses were averaged. All data are presented as means ± S. D. and the differences were regarded as significant when $p<0.05$. Significant effects of type of analysed sample were further evaluated using analysis of variance (ANOVA). A significance level of $P<0.05$ was used.

3. Results

Results for sensory characteristics (colour, odour, taste, softness, chewiness, juiciness and overall impression) of fillets of the common carp collected in spring and autumn are presented in Table 1. No significant differences ($P>0.05$) in meat quality characteristics were obtained, with exception of chewiness (fish were more chewy in autumn than in spring), which could be attributed to the fish size, as fish harvested in October were bigger in comparison with fish harvested in April. The fillets from both seasons had almost the same visual colour scores. The visual colour score of fillets (4.4) was very high. For the fish fillets' odour, significant differences ($P>0.05$) were not seen, but a numerically higher score of 4.0 was recorded in autumn, while the score in spring was 3.8, mainly due to an earthy/musty odour. However, wastewater off-flavours and odours were not reported by panellists. A similar tendency (numerically higher score in autumn than in spring) was observed for the taste of the fillets. As was the case with the fillets' odour, a higher score for meat softness was observed in autumn than in spring, so autumn fillets were moderately soft. A lower chewiness score was recorded in spring than in autumn, but the mean score was 4.0, i.e., fillets were moderately soft, which is very acceptable to consumers. The fillets received higher juiciness scores of 4.2 in autumn and lower scores of 4.0 in spring. For overall impression, average scores were 4.0 and 4.4 in spring and autumn, respectively.

4. Discussion

Sensory analysis is one of the oldest means of fish quality control that allows manufacturers to identify, understand and respond to consumer preferences more effectively, and consequently, helps manufacturers to be more competitive on the market (*Özogul et al.*, 2005). Off-flavours are one of the most economically significant problems encountered in freshwater fish production (*Vallod et al.*, 2007). In our study, the sensory quality of common carp fillets received very high mean scores for all

Table 1. Sensory quality (mean scores*) of fillets from common carp reared in purified slaughterhouse wastewater-fed fishpond in spring and in autumn

Sensory characteristics	Spring	Autumn	p-value
Color	4.4±0.8	4.4±0.48	1
Odour	3.8±0.74	4.0±0.63	0.6938
Taste	3.6±0.49	4.0±0.63	0.346594
Softness	3.8±0.4	4.0±0.63	0.607511
Chewiness	3.6±0.49	4.4±0.49	0.049736
Juiciness	4.0±0.63	4.2±0.75	0.6938
Overall impression	4.0±0.63	4.4±0.49	0.346594

* Seven fish fillets from each group were assessed by five trained panellists

examined parameters and overall impression, which leads to the conclusion that the rearing of these fish in purified slaughterhouse wastewater had no adverse effects on the sensory properties of the fillets. We believe that this rearing system should be compared with conventional systems of carp rearing in earthen ponds or with wild carp originated from rivers. Contrary to our results, *Mahmoud and Buettner* (2016) evaluated the sensory quality of fish fillets and showed the characteristic taste and off-odours of mud were transferred to the fish meat. The results obtained by *Vallod et al*. (2007) show that strongly off-flavour carp were found in ponds colonized by cyanobacteria, mainly *Anabaena* spp. in summer. The same authors reported that sensory evaluation confirmed the existence of off-flavours in the carp tested, and that the most commonly used descriptors associated with geosmin, a wastewater, earthy/musty odour and taste, were identified by the sensory panellists. According to *Houle et al.* (2011) geosmin causes off-flavour in fish, and *Yarnpakdee et al.* (2014) stated that the term off-flavour in fish meat refers to earthy/musty odour and taste.

Despite the fact that common carp rearing has a long tradition in European countries, the economic value of carp is still at relatively low level, mainly due to prejudices that common carp is a fatty fish with numerous bones and muddy taste. *Vallod et al.* (2007) reported that the intensity of off-flavour was the highest in July and August, and that it was lower at the end of the season, which is in accordance with our study. The lower level off-flavour in autumn is also significant from the economical point due to fact that the common carp are mainly harvested and sold during autumn and winter.

Although the option of expanding the existing area of fish ponds is not an acceptable global approach to increase food production, it can be applied in Serbia and likely in surrounding countries too, since there are vast amounts of unused land. It is necessary to introduce appropriate incentives in the form of the cession of the land in the vicinity of slaughterhouses which would be used to set up primarily small-scale fish ponds, providing incentive loans with reasonable interest rates and repayment periods that would allow this type of aquaculture to be established. Moreover, it is necessary to establish the market for products from integrated aquaculture, which would stimulate this type of production. With proper marketing and medical advice, people's awareness and nutritional habits could be changed, leading to an increase in the consumption of fish. In addition to ensuring a highly valuable and safe food for the local market and for export, this type of production could provide jobs for people and facilitate the development of rural areas.

5. Conclusion

Fish reared in this integrated production system produce fillets with satisfactory sensory properties. Comparison with common carp from conventional production systems requires further study. The presented results could be helpful to common carp processors for developing a quality-assurance program for common carp from integrated production systems.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This research was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

References

Houle, S., Schrader, K. K., Le François, N. R., Comeau, Y., Kharoune, M., Summerfelt, S. T., Savoie, A. & Vandenberg, G.W. (2011). Geosmin causes off-flavour in arctic charr in recirculating aquaculture systems. *Aquaculture Research*, 42(3), 360–365, https://doi.org/10.1111/j.1365-2109.2010.02630.x

Hussain, M., Hussain, S. M., Afzal, M., Raza, S. A., Hussain, N. & Mubarik, M. S. (2011). Comparative study of replacement of maize gluten with rice bran (3:1 and 1:3) feed supplement: Effects on fish growth in composite culture. *Pakistan Journal of Agricultural Science*, 48(4), 321–326.

Lan, N. T. P, Dalsgaard, A., Cam, P. D. & Mara, D. (2007). Microbiological quality of fish grown in wastewater-fed and non-wastewater-fed fishponds in Hanoi, Vietnam: influence of hygiene practices in local retail markets. *Journal of Water and Health*, 5(2), 209–218, https://doi.org/10.2166/wh.2007.014b

Ljubojević, D., Ćirković, M., Novakov, N., Jovanović, R., Janković, S., Đorđević, V. & Mašić Z. (2013). Productivity and Meat Nutrient in Fish: The Diet Effect. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 19(1), 43–49, https://doi.org/10.9775/kvfd.2012.7088

Ljubojević, D., Pelić, M., Đorđević, V., Milojević, L. & Ćirković, M. (2016). Bacterial hazards in fish meat: The aetiologic agents of foodborne diseases. *Meat Technology*, 57(1), 31–42.

Mahmoud, M. A. A. & Buettner, A. (2016). Characterisation of aroma-active and off-odour compounds in German rainbow trout (*Oncorhynchus mykiss*). Part I: Case of aquaculture water from earthen-ponds farming. *Food Chemistry*, 210, 623–630, https://doi.org/10.1016/j.foodchem.2016.05.030

Morris, M. C., Evans, D. A., Bienias, J. L., Tangney, C. C., Bennett, D. A., Wilson, R. S., Aggarwal, N. & Schneider, J. (2003). Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Archives of Neurology*, 60(7), 940–946, https://doi.org/10.1001/archneur.60.7.940

Özogul, Y., Özyurt, G., Özogul, F., Kuley, E. & Polat, A. (2005). Freshness assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological methods. *Food Chemistry*, 92(4), 745–751, https://doi.org/10.1016/j.foodchem.2004.08.035

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P. & Lawrence, R. (2008). Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environment International*, 34(8), 1215–1226, https://doi.org/10.1016/j.envint.2008.04.009

Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V. & Hoekstra, R. M. (2011). Foodborne illness acquired in the United States-Unspecified agents. *Emerging Infectious Diseases*, 17(1), 16–22, https://doi.org/10.3201/eid1701.P21101

SRPS, (2015). EN ISO 8589:2015. Sensory analysis — General guidance for the design of test rooms.

Steffens, W. & Wirth, M. (2007). Influence of nutrition on the lipid quality of pond fish: common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). *Aquaculture International*, 15(3), 313 –319, https://doi.org/10.1007/s10499-007-9088-z

Vallod, D., Cravedi, J.P., Hillenweck, A. & Robin, J. (2007). Analysis of the off-flavor risk in carp production in ponds in Dombes and Forez (France). *Aquaculture International*, 15(3), 287–298, https://doi.org/10.1007/s 10499-007-9080-7.

Yarnpakdee, S., Benjakul, S., Penjamras, P. & Kristinsson, H. G. (2014). Chemical compositions and muddy flavour/odour of protein hydrolysate from Nile tilapia and broadhead catfish mince and protein isolate. *Food Chemistry*, 142, 210–216, https://doi.org/10.1016/j.foodchem.2013.07.043

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 613.262:[664.8.037.5:635.1/.8

ID: 126436873

https://doi.org/10.18485/meattech.2023.64.2.57

Original scientific paper

Dietary fibre and carbohydrates in frozen vegetables

Dejana Trbović[a], Danijela Vranić[a] and Vladimir Korićanac[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade , Serbia*

ARTICLE INFO

Keywords:
Total fibre
Vegetables
Carbohydrates

ABSTRACT

Vegetables are an important source of dietary fibre. Diets high in fibre, such as fruits and vegetables, are very beneficial to human health. The EU Regulation on nutrition and health claims for food determines the requirement for the claim that a food is a source of fibre (the food must have 3g/100 g or at least 1.5 g/100 kcal). The fibre content of peas, carrots, potatoes, corn and green beans was analysed. Our results confirmed that peas are the leader in content of dietary fibre; peas contained 6.00% dietary fibre, corn 2.53%, potatoes 2.45%, carrots 2.02% and green beans 1.23%. As far as carbohydrates are concerned, they were far below the recommended daily amount in these vegetables. Vegetables were low in calories and high in fibre. Vegetables can be considered healthy food for human consumption. Studies have indicated that healthy diet including fibre has an important role in the prevention of many diseases.

1. Introduction

Vegetables are an important source of dietary fibre, but also vitamin A, vitamin E, vitamin C, vitamin B6, folate, thiamine, niacin, choline, potassium, copper, magnesium and iron. Select dark-green vegetables contain the most vitamin K, red and orange vegetables contain the most vitamin A and dietary fibre and starchy vegetables contain the most potassium. Dietary fibre is often divided into oligosaccharides and polysaccharides. Oligosaccharides are mainly present in vegetables (*U.S. Department of Health and Human Services and U.S. Department of Agriculture*, 2015–2020; *EFSA*, 2010). The dietary fibre of vegetables includes that found in fresh, frozen and cooked vegetables and vegetable juices. In studies by *Kunzmann et al.* (2015) and *Xiaosheng et al.* (2019), a high dietary fibre intake, particularly from cereals, grains and fruit, is associated with a reduced risk of colorectal cancer. A high intake of dietary fibre was associated with a reduced risk of inflammatory bowel diseases (Crohn's disease and ulcerative colitis) (*Ananthakrishnan et al.,* 2013; *Fritsch et al.,* 2021; *Yusuf et al.,* 2022.) Dietary fibre can be categorized as soluble fibre (β-glucans, mucilage, pectin, and gum) and insoluble fibre (cellulose, hemicellulose, lignin) (*Yusuf et al.,* 2022). Soluble fibre dissolves in water and forms a gel-like material. Soluble fibre is found in oats, peas, beans, apples, citrus fruits, carrots and barley. Insoluble fibre promotes the movement of material through the digestive system. Insoluble fibre is found in whole-wheat flour, wheat bran, nuts, beans and potatoes (*Mayo Clinic,* 2022). Dietary fibre intake was inversely associated with the risks of dementia (*Yamagaishi et al.,* 2022), stroke and coronary heart disease (*Threapleton et al.,* 2015; *Thearpleton et al.,* 2013).

Carbohydrates can be classified as simple or complex. Simple carbohydrates are monosaccharides (e.g., glucose and fructose) and disaccharides

***Corresponding author:** Dejana Trbović, *dejana.trbovic@inmes.rs*

Paper received May 30th 2023. Paper accepted Jun 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

(e.g., sucrose and lactose). Complex carbohydrates are starch and fibre. Dietary recommendations suggest moderate carbohydrate intake (45% or 65% kcal) in the diet (*Griel et al.,* 2006). Dietary carbohydrates can influence human disease (obesity, type 2 diabetes mellitus, cardiovascular disease, cancer, gastrointestinal diseases, dental caries and other conditions) (*EFSA,* 2010, *FAO,* 1997). The EU Regulation (*EC No 1924/2006*) on nutrition and health claims for food determines the requirement for food to be labelled as a source of fibre (3g/100 g or at least 1.5 g/100 kcal) or high in fibre (6g/100 g or at least 3g/100kcal).

Diets high in fibre, such as fruits and vegetables, are very beneficial to human health, so the current daily recommended consumption of dietary fibre is between 14–20 g for children, 22–30 g for adolescents and 25–38 g for elderly (*FAO/WHO,* 2003). Dietary fibre needs to be considered as a part of the carbohydrates in foods.

The method of measuring total dietary fibre includes correction of the residue for undigested protein and mineral contamination (*AOAC,* 1995). Total dietary fibre includes lignin, resident starch and other indigestible carbohydrates. The calculation of carbohydrates is by difference (sugars — mono and disaccharides), oligosaccharides and polysaccharides. Polysaccharides fall into two significant categories, the α-glucans (starch, starch hydrolysis product and glycogen) and non-α-glucans (non-starch polysaccharides — NSPs, which are constituent of dietary fibre) (*Greenfield and Southgate,* 2003). This paper aimed to estimate the contents of dietary carbohydrates and dietary fibre in frozen vegetables, because these are of great importance for human nutrition.

2. Materials and methods

2.1. Samples

Peas, carrots, potatoes, corn and green beans were taken for analysis. The vegetables were frozen and defrosted and three samples of each were taken for analysis. Samples were homogenized in a laboratory blender (CombiMax 600, Braun, Germany).

2.2. Methods

Total carbohydrates were calculated by difference: (proteins, total fat moisture, ash) minus the sum of dietary fibre. Total dietary fibre was determined according to the standard method (*AOAC,* 1995). Dietary fibre in g/100 kcal was calculated according to the estimated energy value of the tested vegetables. The amount of carbohydrates derived from the vegetables was calculated concerning the reference intake of 2,000 kcal. The rules on labelling and advertising in Serbia (*Official Gazette of the Republic of Serbia*, 2017–2022) recommend a daily intake of carbohydrates of 260 g day^{-1} (per 2000 kcal). Calculation of total carbohydrate daily intake was performed by dividing carbohydrate content, expressed in 100 g of product.

2.3. Statistical analysis

Analysis of variance was used to compare different vegetables. Mean values of vegetables for fibre and carbohydrates content were compared using Tukey-Kramer HSD test. Statistical analyses were conducted using JMP 10.0 software (SAS Institute Inc., NY, USA).

Table 1. Dietary fibre and carbohydrates in vegetables

	Dietary fibre, %	Dietary fibre g/100 kcal	Carbohydrates, %	Reference intake of carbohydrates, %
Peas	6.00±0.16^{A}	5.71	15.50±0.59^{B}	5.96
Carrots	2.02±0.02^{B}	4.28	9.54±0.26^{C}	3.67
Potatoes	2.45±0.29^{B}	3.43	14.75±0.91^{B}	5.67
Corn	2.53±0.74^{B}	3.12	21.37±0.30^{A}	8.23
Green beans	1.23±0.58^{B}	3.92	4.64±0.11^{D}	1.78

Means in the same column sharing different superscript letters were statistically significant ($P < 0.05$)

3. Results and discussion

Table 1 shows the mean dietary fibre and carbohydrate (percent with standard deviation) in the different vegetables.

According to *European Commission Regulation* (2006), vegetables can be labelled as a source of fibre if they contain at least 3g of fibre per 100 kcal. Our results confirmed that peas are the leader in content of dietary fibre. However, all the vegetables were low in calories and high in fibre. Therefore, these vegetables can be considered healthy food for human consumption. In the vegetable types listed in Table 1, the proportions of carbohydrates ranged from 4.64±0.11% in green beans to 15.50±0.59 in peas. These amounts of carbohydrates are far below the recommended amount. Adding fibre to breakfast cereals, breads, cakes, cookies and meat products was reported with favourable results (*Bajčić et al.,* 2019). Our results were similar to other data reported in vegetables by *Selvendran and Robertson* (1994) (1.5–2.5 g/100 g of dry weight). Total dietary fibre was, in green peas 1.9%, in peas 3.5% and in potatoes 1.30% (*Dhingra et al.* 2012), while in carrots, it was 1.5% and in corn, 0.3% (*Cho and Dreher,* 2001). In potatoes after cooking, the soluble fibre content was 2.8% (*Khanum et al.,* 2000).

4. Conclusion

Vegetables can be considered healthy foods for human consumption. Dietary intake of fibre was inversely associated with risk of many diseases including colorectal cancer, inflammatory bowel disease, dementia etc. Studies have indicated that healthy diet including fibre has an important role in the prevention of these diseases. Vegetables, i.e., peas, carrots, potatoes, corn and green beans, were analysed for the presence of dietary fibre. Our results confirmed that peas were the leader in content of dietary fibre.

Funding: The research results shown in this paper are financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, and based on the Agreement on the implementation and financing of scientific research work by the Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Ananthakrishnan, A. N., Khalili, H., Konijeti, G. G., Higuchi, L.M., de Silva P., Korzenik J. R., Fuchs C. S., Willett W. C., Richter J. M. & Chan A. T. (2013). A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and Ulcerative Colitis. *Gastroenterology,* 145(5), 970–977, https://doi.org/10.1053/j.gastro.2013.07.050

AOAC, (1995). Official Methods of Analysis of AOAC International. 16 ed, Vol II, Ch. 45. method 985(29), 70.

Bajčić, A., Petronijević, R., Vranić, D., Trbović, D., Betić, N., Nikolić, A. & Milojević, L., (2019). Validation and application of a total dietary fiber determination method to meat products. 60th International Meat Industry Conference MEATCON2019. 22–25 September 2019, Kopaonik, Serbia. IOP Publishing IOP Conf. Series: Earth and Environmental Science 333, (2019) 012043, https://doi.org/10.1088/1755-1315/333/1/012043

Cho, S. S. & Dreher, M. L. (2001). Handbook of Dietary Fiber**,** Marcel Dekker, Inc, New York, USA.

Dhingra, D., Michael, M., Rajput, H. & Patil, R. T. (2012). Dietary fibre in foods: a review. *Journal of Food Science and Technology,* 49(3), 255–266, https://doi.org/10.1007/s13197-011-0365-5

EFSA, (2010). Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal,* 8(3), 1462 (77 pp.), https://doi.org/10.2903/j.efsa.2010.1462. Retrieved from www.efsa.europa.eu

European Commission Regulation. (2006). Regulation No 1924/2006 of the European Parliament and of the Council. *Official Journal of European Union* L404/23–25.

FAO, (1997). Carbohydrates in human nutrition. (FAO Food and Nutrition Paper – 66) Report of a Joint FAO/WHO Expert Consultation Rome, 14–18 April 1997. Retrieved from https://www.fao.org

FAO/WHO, (2003). Food and Agriculture Organization/ World Health Organization, Report of Join FAO/WHO Expert Consultation. Diet, nutrition and the prevention of chronic diseases. Technical Report Series 916, Geneva, Switzerland. Retrieved from https://www.fao.org

Fritsch, J., Garces, L., Quintero, M. A., Pignac-Kobinger, J., Santader, A. M., Fernández, I., Ban, Y. J., Kwon, D., Phillips, M. C., Knight, K., Mao, Q., Santaolalla, R., Chen, X. S., Maruthamuthu, M., Solis, N., Damas, O. M., Kerman, D. H., Deshpande, A. R., Lewis, J. E., Chen, C. & Abreu, M. T. (2021). Low-fat, high-fiber diet reduces markers of inflammation and dysbiosis and improves quality of life in patients with ulcerative colitis. *Clinical Gastroenterology and Hepatology,* 19, 1189–1199, https//doi.org/10.1016/j.cgh.2020.05.026

Greenfield, H. & Southgate D. A. T. (2003). Food composition data — production, management and use, Food and Agriculture Organization of the United Nations, Rome.

Griel, A. E., Ruder, E. H. & Kris-Etherton, P. M. (2006). The changing roles of dietary carbohydrates from simple to complex. *Arteriosclerosis, Thrombosis and Vascular Biology,* 26, 1958–1965, https://doi.org/10.1161/01.ATV.0000233384.97125.bd

Khanum, F., Swamy, M. S., Krishna, K. R. S., Santhanam, K. & Viswanathan, K. R. (2000). Dietary fiber content of commonly fresh and cooked vegetables consumed in India. *Plant Foods for Human Nutrition,* 55, 207–218, https://doi.org/10.1023/a:1008155732404

Kunzmann, A. T., Coleman, H. G., Huang, W., Kitihara, C. M., Cantwel, M. M. & Berndt, S. I. (2015). Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *The American Journal of Clinical Nutrition,* 102, 881–890, https://doi.org/10.3945/ajcn.115.113282

Mayo Clinic, (2022). Dietary fiber: Essential for a healthy diet. Mayo Clinic, Rochester, Minnesota, USA, Retrieved from https://www.mayoclinic.org

Official Gazette of the Republic of Serbia. (2017–2022). Nos 19/2017, 16/2018, 17/2020, 118/2020, 17/2022, 23/2022 and 30/2022. Regulations relating to the declarations, labelling and advertising of food.

Selvendran, R. R. & Robertson J. A. (1994). Dietary fibre in foods: amount and type. In: Amado, R, Barry, J.L. (Eds.), Metabolic and physiological aspects of dietary fibre in food (pp. 11–20). Commission of the European Communities, Luxembourg.

Threapleton, D. E., Burley, V. J., Greenwood, D. C. & Cade J. E. (2015). Dietary fibre intake and risk of ischaemic and haemorrhagic stroke in the UK Women's Cohort Study. *European Journal of Clinical Nutrition,* 69(4), 467–474, https://doi.org/10.1038/ejcn.2014.260

Threapleton, D. E., Greenwood, D., Evans, C. E. L., Cleghorn, C. L., Nykjaer, C., Woodhead, C., Cade, J. E., Gale, C. P. & Burley V. J. (2013). Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *British Medical Journal,* 19, 347, https://doi.org/10.1136/bmj.f6879

U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020. Dietary Guidelines for Americans. 8th Edition. Washington, DC: U.S. Government Printing Office, Retrieved from http://health.gov/dietaryguidelines/2015/guidelines/

Xiaosheng, H., Wu, K., Zhang, X., Nishihara, R., Cao, Y., Fuchs, C. S., Giovannucci, E. L., Ogino, S., Chan, A. T. & Song, M. (2019). Dietary intake of fiber, whole grains and risk of colorectal cancer: an updated analysis according to food sources, tumor location and molecular subtypes in two large US cohorts. *International Journal of Cancer,* 145(11), 3040–3051, https://doi.org/10.1002/ijc.32382

Yamagishi, K., Maruyama, K., Ikeda, A., Nagao, M., Noda, H., Umesawa, M., Hayama-Terada, M., Muraki, I., Okada, C., Tanaka, M., Kishida, R., Kihara, T., Ohira, T., Imano, H., Brunner, E. J. Sankai, T., Okada, T., Tanigawa, T., Kitamura, A., Kiyama, M. & Iso H. (2022). Dietary fiber intake and risk of incident disabling dementia: the circulatory risk in communities' study. *Nutritional Neuroscience*, 26, 148–155, https://doi.org/10.1080/1028415X.2022.2027592

Yusuf, K., Saha, S. & Umar S. (2022). Health benefits of dietary fiber for the management of inflammatory bowel disease. *Biomedicines,* 10, 1242, https://doi.org/10.3390/biomedicines10061242

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.7/.8.045.084.5

ID: 126448393

https://doi.org/10.18485/meattech.2023.64.2.58

Original scientific paper

Evaluation of content and ratio of calcium and phosphorus in commercially available pet food for dogs and cats

Danijela Vranić[a], Vladimir Korićanac[a], Dejana Trbović[a], Dragan Milićević[a], Tamara Gerić[a], Jasna Đinović-Stojanović[a] and Zoran Petrović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Pet food
Calcium
Phosphorus

ABSTRACT

Calcium, phosphorus and vitamin D are essential nutrients for dogs and cats, and therefore, they need to be provided in the diet in adequate amounts and in bioavailable forms. Generally, calcium and phosphorus are discussed together because of their close relationship, particularly in bone health. The objective of this study was to determine calcium and phosphorus contents in commercially available pet food for dogs and cats marketed in Serbia, during 2022. The contents of calcium and phosphorus in the examined animal feeds were determined by using standard ISO procedures. According to national and EU regulations, all calcium and phosphorous contents were in permitted content ranges. The quality and safety of pet food are of great importance to pet health and welfare over their prolonged lives. These results could be discussed from a nutritional, ecological and economic point of view in order to meet optimal formulation of diets.

1. Introduction

Calcium, phosphorus and vitamin D are essential nutrients for dogs and cats, and therefore, they need to be provided in the diet in adequate amounts and in bioavailable forms. Generally, calcium and phosphorus are discussed together because of their close relationship, particularly in bone health. As with calcium, the majority of the body phosphate (approximately 85%) is present in the mineral phase of bone (*Cline*, 2012; *Stockman et al*., 2021). The remainder of body phosphate is present in a variety of inorganic and organic compounds distributed within both intracellular and extracellular compartments. Calcium has many diverse roles in the body including those related to blood clotting, blood pressure, cellular communication, brain function and signal transduction as well as muscle contraction (*Slatopolsky et al.*, 1989). Bone metabolism and calcium and phosphorus absorption and retention are influenced by vitamin D as well as the relative dietary concentrations of these and other minerals. For dogs and cats, vitamin D is also an essential nutrient because its synthesis from sunlight exposure seems to be limited (*Morris*,1999: *How et al.*, 1995).

Bioavailability and digestibility of calcium is variable depending on calcium source, physiological status of the animal, and absorption rates in the lumen of the gut. Digestibility of calcium (and phosphorus) is generally low from grain sources. Most grains contain an endogenous mineral binder called phytase, which complexes with phosphorus and calcium as well as other minerals, rendering them poor-

***Corresponding author:** Danijela Vranić, *danijela.vranic@inmes.rs*

Paper received May 31st 2023. Paper accepted Jun 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ly absorbed calcium and phosphorus sources (*Cline*, 2012). Calcium and phosphorus in pet food can be provided by bony raw materials (from meat and fish). Meat sources are typically high in phosphorus but low in calcium, so homemade or raw-type diets must be evaluated closely for calcium and phosphorus content. Many cases of hyperparathyroidism have resulted from a calcium-to-phosphorous imbalance, which can result from feeding high-protein meat products (*Kantorosinski and Morrison*,1988; *Stockman et al.*, 2021; *Baker and Czarnecki-Maulden*, 1991). Conversely, increased calcium absorption can take place when presented with a high ratio of calcium to phosphorus. So, it is vitally important that the correct amount of calcium and phosphorus be supplied in the diet and in the correct ratios (relative proportions) to each other (*Stockman et al.*, 2021). Most commercial pet foods incorporate supplemental calcium into their rations to ensure the correct calcium-to-phosphorous (C/P) ratio is obtained for the targeted life stage and lifestyle. Chronic calcium imbalance had become uncommon in developed countries with the introduction of modern pet foods, but with the resurgence of homemade and raw-type diets for dogs and cats, there is an increased incidence of calcium, phosphorus, and vitamin D imbalance (*Schlesinger and Joffe*, 2011). Nutritional factors suspected to increase the risk of osteochondrosis are rapid growth from *ad libitum* feeding of high-energy foods, high calcium intake and deficiencies in vitamins or trace elements (*Ralphs*, 2005; *Richardson and Zentek*, 1998).

The National Research Council gives recommended allowances for calcium and phosphorus for different life stages of dogs and cats (*NRC*, 2006). It also defines a safe upper limit for calcium of 4.5 g/1000 kcal of metabolizable energy for puppies, specifically those of large and giant breeds, where excess can result in skeletal abnormalities (*Nap and Hazewinkel*, 1994; *Dobenecker et al*., 2006). According to Association of American Feed Control Officials (*AAFCO*, 2022), cats and dogs have specific dietary nutrient requirements, and cats notably having more specialized nutrient needs than dogs. Because of the close relationship of calcium and phosphorus, the Association of American Feed Control Officials (*AAFCO*, 2018) guidelines recommend that commercial dog food not only meets the individual requirements, but also provides a minimum Ca/P ratio of 1:1 and a maximum ratio of 2:1.

Therefore, the objective of this study was to determine calcium and phosphorus contents in commercially available pet food for dogs and cats marketed in Serbia, during 2022. Based on the analysis results, relationship Ca/P was then estimated and discussed.

2. Materials and methods

2.1. Pet food samples

In the present study, 23 samples of commercially available pet food obtained from the Serbian retail market during 2022 were analyzed for calcium and phosphorus contents. These included 13 samples of pet food for dogs and 10 samples of pet food for cats.

2.2. Analytical procedure

The content of calcium in the examined animal feeds was determined according to the standard

ISO procedure (ISO 6449-1, 1985). The titrimetric method is applicable to all animal feeding stuffs having calcium contents greater than 1 g/kg.

The content of phosphorus in the examined animal feeds was determined according to the standard ISO procedure (ISO 6491, 1998). This specifies a spectrometric method for the determination of the phosphorus content of animal feeding stuffs, and this method is applicable to animal feeding stuffs with low phosphorus content (less than 50 g/kg).

2.3. Statistical analysis

Calcium and phosphorus contents in examined samples were determined in duplicate and were presented as mean values with standard deviation (±SD) for cat feed and min and maximum values for dog feed. The results obtained were analyzed using Microsoft Excel software (Windows 11 pro).

3. Results and discussion

Tables 1 and 2 present the calcium and phosphorous contents of the analyzed dog and cat foods compared with the manufacturer's declarations on the labels. In all of the analyzed pet foods, the calcium and phosphorus levels matched the manufacturer's declared values. According to national (*Official Gazette of RS,* No 4/2010, 113/2012, 27/2014, 25/2015, 39/2016, 54/2017) and European Union regulation (*Commission Regulation* No 939/2010), all results were in permitted content ranges, which

were defined in these regulations for the both of analyzed minerals (for calcium ±20% and ±15% of relative value; and for phosphorus: ±0.2% and ±0.15%, respectively, in comparaison with declared values). The determined calcium contents in dog food ranged from the lowest of 0.96% (complete food for medium and large breeds) to 1.57% (complete food for adult dogs) as the maximum value. Regarding the phosphorus content in dog food, the results of the analyses were very similar to the to the producers' declarations on the label, ranging from 0.92 (complete food for medium and large breed puppies) to 1.11% (complete food for medium and large breeds). Calcium and phosphorus contents of the investigated commercial cat foods were the most similar of the two food types to the manufacturer's declarations on the label, and they met the minimum recommended values from *NRC*, (2006), *AAFCO* (2014) and *FEDIAF* (2020). We found that foods marketed for adult cats had a higher calcium content compared to content of this nutrient in feed for dogs, in general. This is attributed to the lack of defined nutritional guidelines for cats (*NRC*, 2006; *AAFCO*, 2022). Some published findings (*Morino et al*., 2014; *Elliot and Barber*, 1998; *Markovich et al*., 2015), indicating that excess phosphorous can cause chronic kidney disease and decreased renal function which is common in geriatric cats, along with our finding raise concern regarding the typical intake of phosphorous and calcium in cats. A change in existing regulatory guidelines with regard to maximum phosphorous in foods formulated for cats should be considered in light of potential safety issues. For both calcium and phosphorous, the analyzed content often exceeded the minimum concentration declared on the food label claim, for both feeds, for cats and dogs.

This study showed that the contents of calcium and phosphorous in dog food are in accordance with the nutritional recommendations for dogs established by National Research Council of Unit-

Table 1. Calcium (%) and phosphorus (%) content in pet food for dogs

Sample	Calcium (%)		Phosphorous (%)		Ca/P ratio
	Declared (min-max)	Determined (min-max)	Declared (min-max)	Determined (min-max)	
Complete food for adult dogs (n=2)	-	1.28–1.57	-	1.07–1.16	1.10–1.46
Complete food for medium and large breed puppies (n=4)	1.20–1.30	1.33–1.45	0.90	0.92–1.04	1.32–1.44
Complete food for medium and large breed puppies and pregnant or lactating dogs (n=2)	1.20–1.25	1.38–1.42	0.90	0.94–1.02	1.35–1.51
Complete food for medium and large breeds. adult dog (n=5)	1.20–1.25	0.96–1.35	0.90	0.96–1.11	0.91–1.33
Total (n=13)	**1.20–1.30**	**0.96–1.57**	**0.90**	**0.92–1.11**	**0.91–1.51**

Table 2. Calcium (%) and phosphorus (%) content in pet food for adult cats

Sample	Calcium (%)				Phosphorous (%)				Ca/P ratio	
	Declared		Determined		Declared		Determined			
	Mean ± Sd	min-max	Mean ± Sd	min-max	Mean ± Sd	min-max	Mean± Sd	min-max	Mean ± Sd	min-max
Complete feed for adult cat (n=10)	1.16 ± 0.90	1.05–1.25	1.45 ± 0.40	1.16–2.38	0.78 ± 0.34	0.0–0.95	1.07±0.11	0.93–1.31	1.33 ± 0.25	1.13–1.98

ed States (*NRC*, 2006), *AAFCO* (2014) and the *FEDIAF* (2020). Our results were similar to FEDIAF (2020) recommendations that the calcium level in a pet food for early growth should be at least 1 g/100 g DM. During late growth, it is recommended that large breed and giant breed puppies continue to be fed a pet food containing at least 1 % calcium until about 6 months of age. During the whole late growth phase, pet foods for puppies of small and medium size breeds can contain less calcium (minimum 0.8 % DM) and the Ca/P ratio can be increased to 1.8/1 (*Lauten et al*. 2002). However, this level has been reported to be marginal for some breeds, particularly during the fast growing phase.

Similar to results for cat food, the results obtained shown that calcium contents (%) (1.45±0.40) as well as the Ca/P ratio (1.33±0.25) were slightly higher compared to results for foods intended for adult maintenance and those for all life stages, for dogs (0.96–1.57% calcium and 0.91–1.51, Ca/P). According to *Greco*, (2008) feeds designed for small, large- and giant-breed puppies have varying amounts of calcium and phosphorus and in appropriate ratios to control growth and development of bones and cartilage, and our results were in accordance with this observation, particularly the calcium content (0.96–1.35% for complete food for medium and large breeds to maximum 1.33–1.45% for medium and large breed puppies and 1.28–1.57%, for adult dogs).

In pet diets, it is necessary to take into account the Ca/P ratio, because it has important consequences for bone development, which can be adversely affected when this ratio increases, resulting in aberrations in bone mineral homeostasis and bone metabolism (*Kumar et al*., 2011). Furthermore, dietary Ca/P ratios are crucial to phytase efficacy and activity (*Angel et al*., 2002). Thus, most commercial pet foods incorporate supplemental calcium into their rations to ensure the correct Ca/P ratio is obtained for the targeted life stage and lifestyle. In this study, Ca/P ratio ranged from 0.91 to 1.1 in dog food, while in cat food, it was slightly higher, ranging from 1.13 to 1.98. Also, our general Ca/P ratio (1.33±0.25) for pet food for adult cats was similar to those reported in study by *Summers et al*. (2020) in cat foods labeled for senior and adult cats (1.3 and 0.8–1.7, respectively). The Ca/P ratios in pet food for dogs (0.91–1.51) were in accordance, in general, with the recommended (*AAFCO*, 2018) guidelines, in which a minimum Ca/P ratio of 1:1 and a maximum ratio of 2:1 are advised. Only in complete foods for medium and large breeds was a slightly lower Ca/P ratio (0.91) obtained compared to the recommended minimum value (1:1).

Our study had several limitations. The sample population represented the products within the Serbian market that are available for the local cat and dog owners; we could not evaluate the digestibility or bioavailability of dietary nutrients, the source or form of phosphorous, calcium and the content of vitamin D, or evaluate any possible clinical consequences of our findings. We cannot conclude with certainty that any of the tested foods would cause kidney injury to healthy cats, even if fed long term. Additional research into the nutritional requirements of cats and dogs at different life stage is also is required, and it may inform future nutritional guidelines.

5. Conclusion

The quality and safety of pet food are of great importance to pet health and welfare over their prolonged lives. In this study of pet food sold in Serbian markets, the contents of calcium and phosphorous were more or less adequate, and mostly matched the manufacturer's declared values. Despite some deviations from declared values, these variabilities were in the range of permitted tolerances for the compositional labeling of feed materials. Likewise, these results could be discussed from a nutritional, ecological and economic point of view in order to meet optimal formulation of diets.

Disclosure Statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

AAFCO, (2014). Association of American Feed control Officials, July 25–27.2014. Sacramento, California, USA, https://www.aafco.org

AAFCO, (2018). Official Publication. Atlanta (GA): Association of American Feed Control Officials. AAFCO Publications: Champaign, IL, USA, https://www.aafco.org

AAFCO, (2022). American Association of Feed Control Officials. AAFCO Publications: Champaign, IL, USA, https://www.aafco.org

Angel, R., Tamim, N. M., Applegate, T. J., Dhandu, A. S. & Ellestad, L. E. (2002). Phytic acid chemistry: Influence on phytin-phosphorus availability and phytase efficacy. *Journal of Applied Poultry Research,* 11, 471–480, https://doi.org/10.1093/japr/11.4.471

Baker, D. H. & Czarnecki-Maulden, G. L. (1991). Comparative nutrition of cats and dogs. *Annual Review of Nutrition,* 11, 239–263, https://doi.org/10.1146/annurev.nu.11.070191.001323

Commission Regulation (EU) No 939/2010 of 20 October 2010. amending Annex IV to Regulation (EC) No 767/2009 on permitted tolerances for the compositional labelling of feed materials or compound feed as referred to in Article 11(5), Text with EEA relevance, http://data.europa.eu/eli/reg/2010/939/oj

Cline, J. (2012). Calcium and vitamin d metabolism, deficiency, and excess. *Topics in Companion Animal Medicine,* 27, 159–164, DOI: 10.1053/j.tcam.2012.09.004

Dobenecker, B., Kasbeitzer, N., Flinspach, S., Kostlin, R., Matis, U. & Kienzle, E. (2006). Calcium-excess causes subclinical changes of bone growth in beagles but not in foxhound-crossbred dogs, as measured in X-rays. *Journal of Animal Physiology and Animal Nutrition,* 90, 394–401, DOI: 10.1111/j.1439-0396.2006.00618.x

Elliot J. & Barber P. J. (1998). Feline chronic renal failure: clinical findings in 80 cases diagnosed between 1992 and 1995. *Journal of Small Animal Practice*, 39(2), 78–85, DOI: 10.1111/j.1748-5827.1998.tb03598.x

FEDIAF, (2020). Nutritional guidelines for complete and complementary pet food for cats and dogs, https://www.europeanpetfood.org.

Greco, D. S. (2008). Nutritional supplements for pregnant and lactating bitches, *Theriogenology*, 70, 393–396, DOI: 10.1016/j.theriogenology.2008.04.013

How, K. L., Hazewinkel, H. A. & Mol, J. A. (1995). Photosynthesis of vitamin D in the skin of dogs cats and rats. *Veterinary Quarterly*, 17(1), S29, DOI: 10.1080/01652176.1995.9694579

ISO 6490-1:1985, (1985). Animal feeding stuffs — Determination of calcium content –Part 1. Titrimetric method. ISO: Geneva, https://www.iso.org

ISO 6491:1998, (1998). Animal feeding stuffs- Determination of phosphorus content. Spectrometric method. ISO: Geneva, https://www.iso.org

Kantorosinski, S. & Morrison, W. B. (1988). A review of feline nutrition. *The Veterinary Student,* 50, 95–106, https://www.iastate.edu

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G. & Becker, K. (2011). Phytate and phytase in fish nutrition. *Journal of Animal Physiology and Animal Nutrition*, 96, 335–340, DOI: 10.1111/j.1439-0396.2011.01169.x

Lauten, S. D., Cox, N. R., William, R., Brawner, J. R., Goodman, S. A., Hathcock, J. T., Montgomery, R. D., Kincaid, S. A., Morrison, N. E., Spano, J. S., Lepine, A. J., Reinhart, G. A. & Baker, H. J. (2002). Influence of dietary calcium and phosphorus content in a fixed ratio on growth and development in Great Danes. *American Journal of Veterinary Research,* 63(7), 1036 –1047, DOI: 10.2460/ajvr.2002.63.1036

Markovich, J. E., Freeman, L. M, Labato, M. A. & Heinze, C. R. (2015). Survey of dietary and medication practices of owners of cats with chronic kidney disease. *Journal of Feline Medicine and Surgery*, 17(12), 979–983, DOI: 10.1177/1098612X14563097

Morino, C. L, Lascelles B. D. X., Vaden S. L., Gruen M. E. & Marks S. L. (2014). Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. *Journal of Feline Medicine and Surgery*, 16(6), 465–472, DOI: 10.1177/1098612X13511446

Morris, J. G. (1999). Ineffective vitamin D synthesis in cats is reversed by an inhibitor of 7- dehydrocholestrol--delta7-reductase. *Journal of Nutrition,* 129, 903–908, DOI:10.1093/jn/129.4.903

Nap, R. C. & Hazewinkel, H. A. (1994). Growth and skeletal development in the dog in relation to nutrition; a review. *Veterinary Quarterly,* 16, 50–59, DOI: 10.1080/01652176.1994.9694417

NRC, (2006). Nutrient requirements of dogs and cats. Washington, DC: National Academies Press, https://www.nrc.gov

Official Gazette of the Republic of Serbia, No 4/2010, 113/2012, 27/2014, 25/2015, 39/2016, 54/2017, Rulebook on the quality of feed for animals.

Ralphs, C. (2005). Bilateral stifle osteochondritis dissecans in a cat. *Journal of the American Animal Hospital Association*, 41(1), 78–80, DOI: 10.5326/0410078

Richardson, D. C. & Zentek J. (1998). Nutrition and Osteochondrosis. *Veterinary Clinics of North America: Small Animal Practice,* 28, 115–135, DOI: 10.1016/s0195-5616(98)50008-3

Schlesinger, D. P. & Joffe, D. J. (2011). Raw food diets in companion animals: A critical review. *Canadian Veterinary Journal,* 52, 50–54, PMCID: PMC3003575

Slatopolsky, E., Weerts, C,, Norwood, K,, Giles, K., Fryer, P., Finch, J., Windus, D. & Delmez J. (1989). Long term effects of calcium carbonate and 2.5 mEq/liter calcium dialysate on mineral metabolism. *Kidney International,* 36(5), 897–903, DOI: 10.1038/ki.1989.277

Stockman, J., Villaverde, C. & Corbee, R. J. (2021). Calcium, Phosphorus, and Vitamin D in Dogs and Cats: Beyond the Bones. Veterinary Clinics of North America, *Small Animal Practice,* 51, 623–634, DOI: 10.1016/j.cvsm.2021.01.003

Summers, C., Stacie, S., Jonathan, L. A., Jennifer, R., Anais, S. & Zhang. L. (2020). Evaluation of nutrient content and caloric density in commercially available foods formulated for senior cats. *Journal of Veterinary International Medicine,* 34, 2029–2035, DOI:10.1111/jvim.15858

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.524.056

ID: 126454281

https://doi.org/10.18485/meattech.2023.64.2.59

Original scientific paper

Polycyclic aromatic hydrocarbons in dry fermented sausages from the Serbian market

Jasna Đinović-Stojanović[a], Danijela Vranić[a], Munevera Begić[b], Jelena Babić Milijašević[a], Milan Milijašević[a], Srdjan Stefanović[a] and Saša Janković[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *Faculty of Agriculture and Food Science, University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina*

ARTICLE INFO

Keywords:
Benzo(a)pyrene
Benzo(a)anthracene
Chrysene
Benzo(b)fluoranthene
Dry fermented sausage
Serbia

ABSTRACT

In this study, levels of benzo(a)pyrene and sum of PAH4 compounds (benzo(a)pyrene benzo(a)anthracene, chrysene and benzo(b)fluoranthene) in four types of dry fermented sausages (n=126) collected from market in Belgrade, Serbia, were analysed. The levels of benzo(a)pyrene and PAH4 compounds ranged < 0.2–0.6 µg/kg and < 0.2–2.7 µg/kg, respectively. The levels of benzo[a]pyrene and sum of PAH4 in all samples were < 2 µg/kg and < 12 µg/kg, respectively, which is the MRL in smoked meat and smoked meat products regulated both by Serbian and EU legislation. PC analysis showed that kulen and sremska sausage have a higher capacity for absorption of the analysed compounds during the process of meat smoking than do the other investigated sausages.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are numerous groups of compounds consisting of two or more condensed aromatic rings. According to the number of aromatic rings, they can be classified as light (2–3 rings) or heavy (4–6 rings) compounds (*Purcaro et al.,* 2013). PAHs are formed the during incomplete combustion of organic matter which is result of a pyrolytic process and involves high temperature and reduced oxygen levels. They are slightly water-soluble compounds, have a lipophilic character, and consequently, they tend to accumulate in the food chain. Food contamination by PAHs can originate from environmental pollution as well as during food preparation and processing. High-protein food like meat and fish which are smoked, dried or cooked are the main sources of PAHs (*Rose et al.,* 2015). Some of them are mutagenic and carcinogenic compounds. The main chemical and physical properties of 16 EU PAHs as well as their International Agency for Research on Cancer (IARC) carcinogenicity classification are summarized in Table 1 (*IARC,* 2010). In addition to IARC, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO), Scientific Committee on Food (SCF), and European Food Safety Authority (EFSA) also classify and evaluate the carcinogenicity of PAHs according to their occurrence (*Zelinkova & Wenzl,* 2015). These organizations classify BaA, CHR, BbF and BaP as mutagenic, genotoxic and carcinogenic compounds.

Smoked meat and meat products are widely distributed in the diet among the population in Serbia (*SORS,* 2022). Across the country, there are traditional smokehouses as parts of individual house-

***Corresponding author:** Jasna Đinović-Stojanović, *jasna.djinovic@inmes.rs*

Paper received May 31st 2023. Paper accepted Jun 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Table 1. List of the 16 EU priority PAHs frequently analysed in food samples with their abbreviation, chemical formula, molecular weight and IARC toxicity classification (*IARC,* 2010).

Compound Name	Abbreviation	Chemical formula	Molecular weight (g mol^{-1})	Melting Point (°C)	Boiling Point (°C)	Toxicity (IARC)*
Benzo[a]pyrene	BaP	$C_{20}H_{12}$	252.3	179	495	1
Cyclopenta[cd]pyrene	CPP	$C_{18}H_{10}$	226.3	170	439	2A
Dibenz[a,h]anthracene	DhA	$C_{22}H_{14}$	278.3	262	535	2A
Dibenzo[a,l]pyrene	DlP	$C_{24}H_{14}$	302.4	162	595	2A
Benzo[b]fluoranthene	BbF	$C_{20}H_{12}$	252.3	168	481	2B
Benzo[k]fluoranthene	BkF	$C_{20}H_{12}$	252.3	216	480	2B
Benzo[j]fluoranthene	BjF	$C_{20}H_{12}$	252.3	165–166	480	2B
Benz[a]anthracene	BaA	$C_{18}H_{12}$	228.3	158	438	2B
Chrysene	CHR	$C_{18}H_{12}$	228.3	254	448	2B
5-Methylchrysene	5MC	$C_{19}H_{14}$	242.3	118	458	2B
Dibenzo[a,i]pyrene	DiP	$C_{24}H_{14}$	302.4	281–284	594	2B
Dibenzo[a,h]pyrene	DhP	$C_{24}H_{14}$	302.4	308	596	2B
Indeno[1,2,3-cd]pyrene	IcP	$C_{22}H_{12}$	276.3	164	530	2B
Benzo[ghi]perylene	BgP	$C_{22}H_{12}$	276.3	273	550	3
Dibenzo[a,e]pyrene	DeP	$C_{24}H_{14}$	302.4	233–244	592	3
Benzo[c]fluorene	BcF	$C_{17}H_{12}$	216.3	125–127	398	3

*IARC, International Agency for Research on Cancer carcinogenicity classification [1 – The agent (mixture) is carcinogenic to humans; 2A – The agent (mixture) is probably carcinogenic to humans; 2B – The agent (mixture) is possibly carcinogenic to humans; 3 – The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans]

holds. Also, industrial meat companies produce different types of smoked meat and meat products both in traditional and industrial smokehouses (*Đinović et al.,* 2008*; Jira & Djinovic,* 2008). Presently, though, there is very little authentic and scientific information available on the PAH content of dry fermented sausages from the Serbian market (*Skrbic et al.,* 2014*; Skaljac et al.,* 2023). As production practices change with time, periodic surveillance and studies on PAH contents in smoked meat and meat products are needed to assess the safety of such products with respect to humans. Hence, the main objectives of this study were to determine the levels of BaP as well as sum of PAH4 (BaP, BaA, CHR, BbF) in different dry fermented sausages obtained from the Serbian market during 2022 and to assess the safety of the products in the line both with the Serbian and EU legislation on PAHs. In addition, the distribution of the analysed PAH compounds in the analysed samples was analysed by applying PCA analysis.

2. Materials and methods

A total of 126 dry fermented sausages (budimska, n= 14; cajna, n= 40; kulen, n = 36; sremska, n = 8 and homemade salami, n = 28) were collected in Serbian markets during 2022. After collection, samples were labelled and stored in polyethylene bags and frozen at −18°C prior to analysis. Sam-

ples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. Briefly, 2.5 g of homogenized sample was weighed into a 50 mL centrifuge tube; 10 mL of acetonitrile were added and the mixture was shaken vigorously for 1 min; after that, 1 g NaCl and 4 g MgSO4 were added, with the tube being shaken immediately after addition of the salt. Then each sample was shaken and centrifuged. Supernatant was cleaned up by solid phase extraction (transferring into a 15 mL centrifuge tube containing 150 mg primary/secondary amine, 150 mg C18 and 900 mg $MgSO_4$,) with the aim of eliminating the possible interfering compounds from the sausage extract. After centrifugation, extracts were evaporated under a stream of N_2 at 40°C to dryness and then dissolved in 1 mL of hexane. The extracts were then analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS). Determination of PAH compounds was performed using gas chromatography with a triple quadrupole mass detector.

Principal components analysis (PCA) was used to group the observed samples and to discover the distribution of polycyclic aromatic hydrocarbons in dry fermented sausages. PCA analysis and result visualization were performed using software Past 3.15 (*Hammer et al.,* 2001).

3. Results and discussion

Levels of BaP and sum of PAH4 compounds in 126 samples of dry fermented sausages are shown in Table 1. BaP and PAH4 levels were in the range from < 0.2 to 0.6 µg/kg and < 0.2 to 2.7 µg/kg, respectively. The obtained PAHs levels did not exceed maximum residue limits currently in force. The legal maximum contents of BaP (2 µg/kg) and of the sum of all four compounds (PAH4; 12 µg/kg) were established by European Commission Regulation No. 835/2011 (*EC,* 2011). The maximum residue limits (MRL) both for BaP and the sum of PAH4 compounds in smoked meat and meat products were defined by the legislation of Serbia (*SGRS,* 2014), as well, and are in accordance with the EU regulation.

Measured BaP and PAH4 levels of all analysed dry fermented sausages from this study were in the range with data reported by *Skrbic et al*. (2014) for dry fermented sausages with protected designation of origin, *Petrovska klobasa* from Serbia (BaP: < 0.17–0.51 µg/kg; PAH4: < 0.17–1.68 µg/kg). However, *Skaljac et al.* (2023) determined much higher PAH4 content (1.48–7.42 µg/kg) in dry fermented beef sausage from Serbia (*Sjenicki sudzuk*) in comparation to results from our study.

PCA analysis

Principal component analysis (PCA) was applied to establish the possible correlations among the measured BaP and PAH4 levels and the different types of dry fermented sausages. In PCA plots, points which are geometrically close to each other indicate the similarity of patterns that represent these points. The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles corresponding to high correlations). To visu-

Table 1. Levels (max – min, µg/kg) of BaP and sum of PAH4 compounds in dry fermented sausages purchased in Serbian markets

Dry fermented sausages	Levels, [µg/kg]	
	BaP	PAH4
Budimska sausage, n=14	< 0.2–0.3	< 0.2–1.8
Cajna sausage, n=40	< 0.2–0.3	< 0.2–1.3
Kulen, n=36	< 0.2–0.6	< 0.2–2.6
Sremska sausage, n=8	< 0.2–0.4	< 0.2–2.7
Homemade salami, n=28	< 0.2–0.3	< 0.2–1.6

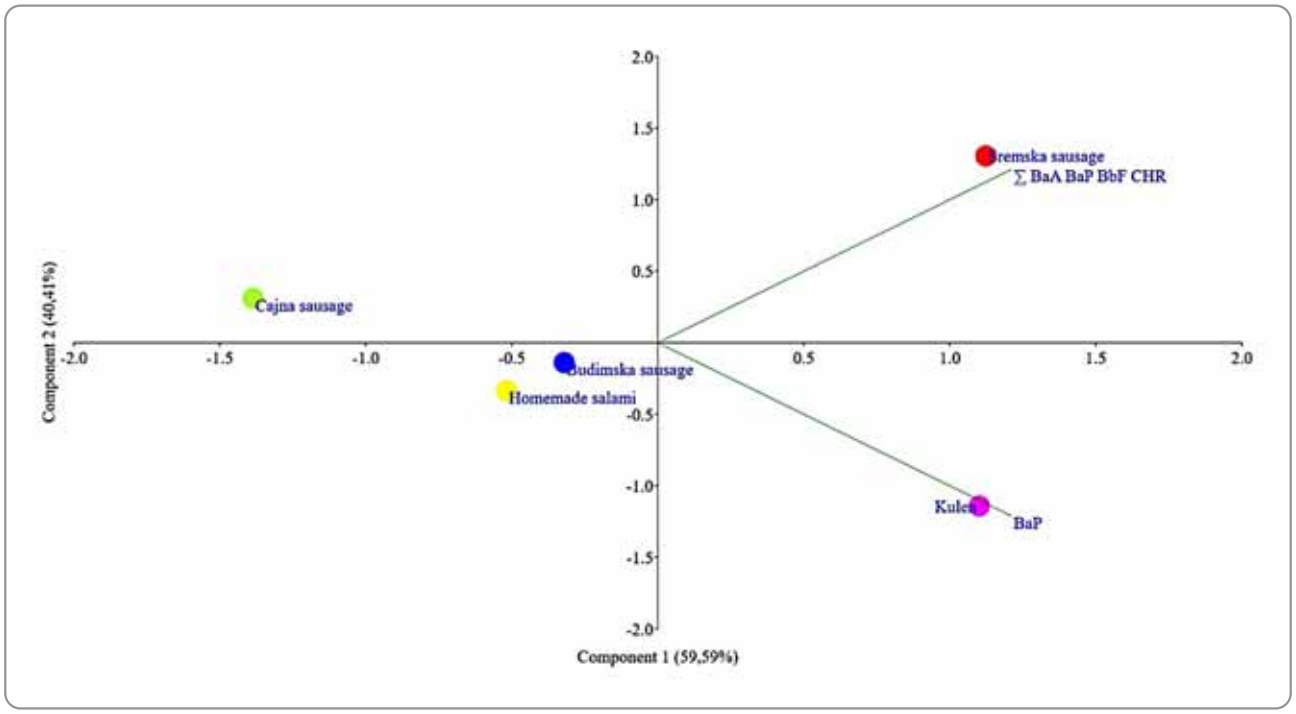


Figure 1. Biplot graph for BaP and PAH4 levels in dry fermented sausages

alise the data trends and the discriminating efficiency of the used descriptors, a scatter plot of the results from the samples using the first two principal components (PCs) from PCA of the data matrix for dry fermented sausages was obtained (Figure 1).

The influence of different parameters that describes the dry fermented sausages studied can be evaluated from the scatter plot (Figure 1), in which the sausages with higher BaP and PAH4 levels are located on the right side of the figure (sremska sausage and kulen). The lowest BaP and PAH4 levels were observed in cajna sausage, while higher levels were found in homemade salami and budimska sausage.

4. Conclusion

This study aimed to provide information on polycyclic aromatic hydrocarbons (BaP, PAH4) in five different types of dry fermented sausages from the Serbian market. PC analysis showed that high correlation exists in the levels of BaP in kulen sausage and sum of PAH4 compounds in sremska sausage. The levels of benzo[a]pyrene and sum of PAH4 in all samples were < 2 μg/kg and < 12 μg/kg, respectively, which are the maximum limits in smoked meat and smoked meat products regulated both by Serbian and European Union legislation.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Đinović, J., Popović, A., Spirić, A., Turubatović, L. & Jira, W. (2008). 16 EU prioritetnih policikličnih aromatičnih ugljovodonika (PAH jedinjenja) u dimu drveta i dimljenoj pršuti. *Tehnologija mesa*, 49(5–6), 181–183.

European Commission, (2011). Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *The Official Journal of the European Union*, 215, 4–8.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1– 9.

IARC, (2010). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; International Agency for Research

on Cancer: Lyon, France, 2010; No. 92. Available online: https://www.ncbi.nlm.nih.gov/books/NBK321712/ (accessed on 9 May 2023).

Purcaro, G., Moret, S. & Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. *Talanta*, 105C (7), 292– 305.

Rose, M., Holland, J., Dowding, A., Petch, S, White, S., Fernandes, A. & Mortimer, D. (2015). Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. *Food and Chemical Toxicology*, 78, 1–9.

SGRS, (2014). Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja. *Službeni Glasnik Republike Srbije*, 29.

Skaljac, S., Jokanovic, M., Tomovic, V., Kartalovic, B., Ikonic, P., Cucevic, N., Vranesevic, J., Ivic, M., Sojic, B. & Peulic, T. (2023). Influence of traditional smoking on the content of polycyclic aromatic hydrocarbons in dry fermented beef sausage from Serbia. *Food Control*, 150, 109766.

Skrbic, B., Durisic-Mladenovic, N., Macvanin, N., Tjapkin, A. & Skaljac, S. (2014). Polycyclic aromatic hydrocarbons in smoked dry fermented sausages with protected designation of origin Petrovska klobasa from Serbia. *Macedonian Journal of Chemistry and Chemical Engineering*, 33(2), 227–236.

SORS, Statistical Office of the Republic of Serbia, (2022). Household Budget Survey, 2021. 687, 2022. (https://www.stat.gov.rs/ accessed 08.05.22).

Zelinkova, Z. & Wenzl, T. (2015). The occurrence of 16 EPA PAHs in food — A review. *Polycyclic Aromatic Compounds*, 35, 248–284.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.052/.054:636.4

ID: 126475017

https://doi.org/10.18485/meattech.2023.64.2.60

Original scientific paper

Principal component analysis and cluster analysis for fatty acid assessment of backfat in three pig breeds

Irina Chernukha[a], Marina Nikitina[a], Elena Kotenkova[a] and Liliya Fedulova[a]*

[a] *V. M. Gorbatov Federal Research Center for Food Systems of RAS, Talalikhina st., 26, Moscow 109316, Russia*

ARTICLE INFO

Keywords:
Pig breed
Duroc
Altai
Livni
Cluster analysis
PCA

ABSTRACT

Local pig breeds are bred by crossing pig breeds, and their carcass traits, technological, chemical and physical compositions differ. The fatty acid composition and content depend on both biological and handling specifity that can be assessed using statistical tools. Backfat composition of three pig breeds was studied: two were local (Livni and Altai), one was commercial (Duroc). The calculations were performed based on the results of fatty acids analysis using small datasets in the software R Studio 2022.07.2 Build 576. Principal component and cluster analysis revealed that the fatty acid composition of the two local pig breeds differed from each other and from the commercial breed, which resulted in clustering of the individual pigs studied into separate groups. Results of cluster analysis demonstrated similarity with PCA, but all breeds formed distinct groups; Altai pigs formed the most tight and distinct group, while Duroc and Livni pigs clustered in more expanded groups with overlapping regions. Non-parametric statistics (PCA and cluster analysis) were applicable to this small dataset.

1. Introduction

Biotechnological, biological and technological processes depend on numerous parameters that affect them the and cause difficulties associated with identifying the structure of the relationships of these parameters. Therefore, the researcher often must deal with stochastic, incomplete information. In this case, the use of multivariate statistical analysis is not only justified, but also essential (*Lin et al.*, 2009). Multivariate statistical methods, among a variety of possible probabilistic and statistical models, allow selection of the best model that corresponds to the initial statistical data, thereby characterizing the real behaviour of the studied set of objects. In turn, this allows us to assess the reliability and accuracy of conclusions made on the basis of limited statistical data. Methods of multivariate classification are designed to divide the considered sets of objects, subjects or phenomena into groups, in a certain sense homogeneous. It should be taken into account that each of the objects is characterized by a large number of different and stochastically related features. Problems of complex classification can be solved by cluster analysis (*Röttger*, 2016). The presence of many initial variables characterizing objects' functional processes makes it necessary to select just the most significant variables and study a smaller set of indicators. Most often, the initial variables undergo transformation, which ensures minimal loss of information. Such a solution can be provided by dimensionality reduction methods, which include factor and principal component analysis (PCA) (*Anowar*

***Corresponding author:** Irina Chernukha, *imcher@inbox.ru*

Paper received May 30th 2023. Paper accepted Jun 11th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

et al., 2021; *Costello & Osborne*, 2019; *Everitt & Hothorn*, 2011; *Jolliffe & Cadima*, 2016).

Factor analysis is a set of methods that, based on objectively existing correlational relationships of features, make it possible to identify latent (hidden) generalizing characteristics of the structure of the studied objects and their properties (*Granato et al.*, 2018). The suitability assessment, i.e, how well the constructed model describes the structure of the initial variables, is carried out using the Kaiser–Meyer–Olkin criterion. Bartlett's sphericity criterion makes it possible to assess the correlation of the initial variables: with insufficient correlation, the use of factor analysis is impractical (*Abdi & Williams*, 2010). Usually, factor analysis PCA and/or cluster analysis are used in technological, biological, medical and biotechnological research (*Dong et al.*, 2014; *Hasan et al.*, 2021; *Kemsley et al.*, 2019; *Macchiato et al.*, 1992). In one study (*Ros-Freixedes et al.,* 2014), PCA was used to determine the relationship between meat quality traits, feeding patterns, scale activity, and number of conflict–avoidance interactions.

Pork is characterized by different quality parameters specific for different pig breeds. The pig herd in Russia has been steadily growing in recent years and reached the number of 28.3 million animals by March 2023. Russian consumers have a positive attitude towards pork, despite its high fat content (*Chernukha et al.,* 2023). Data about the negative impacts of cholesterol on the cardiovascular system and of red meat on the risk of morbid obesity and related diseases has led to a significant change in consumer requirements for food in general and pork in particular.

We have chosen and compared the backfat fatty acid content of three pig breeds raised in Russia nowadays: Livni breed (registered in 1949), Altai (2015) and Duroc — a globally known breed, that was brought to Russia and used to improve other local pig breeds. The empirical assessment of the fatty acid composition of the backfat of Livni, Altai and Duroc pigs was carried out on the basis of actual data obtained in laboratory studies using multidimensional statistical methods.

2. Materials and methods

2.1. Sampling and fatty acids analysis

Three pig breeds were used: Livni (n=6); Duroc (n=7); Altai meat breed (n=5). Pigs were kept under the conditions of a commercial pig farm and consumed complete feed, were slaughtered at the live weight 110±10kg without electrical stimulation and the carcasses cooled at 0°C for 24 h in cold storage. Backfat (BF) in between 10^{th} and 11^{th} rib samples were removed and sent to the laboratory. Pieces of backfat 5×5 cm, with a depth from the subcutaneous fat surface to the muscle layer, and weighing approximately 70–150 g (±5 g), were cut from each carcass. The samples were obtained from at least three replicates from each carcass; average values of three replicates of fatty acid composition from each carcass were used for further data processing. Determination of the fatty acid composition was performed according to the reported method in the literature (*Ivankin et al.*, 2016), with the author's modification. Results are available at (*Chernukha et al.*, 2023).

2.2. Statistical analysis of fatty acids composition

The calculations were performed in the software R Studio 2022.07.2 Build 576. The R software is a freely distributed cross-platform software tool used for statistical calculations and data visualization. R distributions are available on the websites The Comprehensive R Archive Network (https://cran.r-project.org).

3. Results

We previously reported that backfat fatty acid composition was strongly differ between Livni, Altai and Duroc breeds, that affected on nutritional indices for assessing of fatty acids composition as ΣSFA, ΣUFA, ΣMUFA, ΣPUFA, ΣHUFA, ΣPUFA/ΣSFA, Σn–3 PUFA, Σn–6 PUFA, Σn–3 PUFA/Σn–6 PUFA, C 18:2/C 14, C 18:1/C 14, ΣFA short (from C4 to C 10), ΣFA medium (from C11 to C 16), ΣFA long (>C 17), and ΣC4-C16/ΣC17-C24, and the atherogenic index (IA) and thrombogenicity (IT). In brief, the highest UFA content, in particular omega 3 and omega 6 PUFA, was found in the backfat of Altai pigs with a minimum SFA content. The backfat of Livni pigs was characterized by the highest monounsaturated and medium chain fatty acid contents and the lowest short-chain fatty acid content; the atherogenicity index in was close to this of Duroc. On contrary, thrombogenicity index in Livni backfat was even lower than the Duroc one (*Chernukha et al.*, 2023).

PC1 described 39.34% of the variance of the original dataset, while PC1 and PC2 together explained approximately 72.1% of the variance of the original dataset. The eight main components of PC1-PC6 described about 93.79% of the initial dataset. The weighting coefficients by which the original variables were included in the PCA were calculated by d_pca$rotation and are presented in Table 1.

The resulting matrix shows how the principal component axes were shifted relative to the original ones. Each component was calculated as the sum of the multiplication of the weighting coefficients with the corresponding fatty acid value. The most significant variables (fatty acids) in PC1 were C20:2w6, C14:1, C16:1 and C18:1 with weighing coefficients of −0.321, 0.315, 0.300 and 0.292, respectively, while C22:1w9 was included in PC1 with a very small weighing coefficient of −0.017. The most significant variables in PC2 were C18:0, C20:0, C15:0, C20:3w6, C20:3w3 and C20:4w6 with weighing coefficients of −0.351, 0.309, −0.306, −0.285, −0.281 and −0.275, respectively, while C18:1 was included in PC2 with a weighing coefficient only of −0.068. The most significant variables in PC3 were C20:1w9, C22:1w9, C20:3w3, C12:0 and C18:1 with weighing coefficients of −0.506, −0.418, −0.313, 0.305 and −0.300, respectively, while C17:0 was included in PC3 with a very small weighing coefficient of −0.003. Interestingly, the weighing coefficients of C21:0 and C22:1w9 in PC4 were very high and averaged −0.715 and −0.459, respectively. The highest weighing coefficients for the major fatty acids, C18:0 and C18:1, were included in PC2 and PC1, respectively, and averaged 0.351 and 0.292, respectively, while for C16:0 and C18:2w6, weighing coefficients were approximately equal in PC1 and PC2 and

Table 1. The weighing coefficients of variables for principal component analysis of fatty acids in pig backfat

Fatty acid	PC1	PC2	PC3	PC4	PC5	PC6
C12:0	0.25254	−0.15083	0.30451	−0.15960	−0.02860	0.05781
C14:0	0.28244	−0.13437	0.22284	−0.09715	−0.07601	−0.07513
C14:1	0.31499	−0.11740	−0.01659	0.06955	−0.09928	0.14156
C15:0	0.14525	−0.30626	0.08470	0.13551	−0.23892	0.27058
C16:0	0.21224	0.22819	0.26237	−0.00315	0.01579	−0.17230
C16:1	0.30046	−0.13484	0.08210	−0.11432	0.23817	−0.10021
C17:0	0.20505	−0.26119	−0.00272	0.08141	−0.03581	0.38339
C17:1	0.25919	−0.15177	−0.17790	0.06388	0.12658	0.38071
C18:0	−0.07992	0.35111	0.01583	0.15142	−0.16620	0.13819
C18:1	0.29187	0.06843	−0.30043	0.07666	0.11940	−0.05156
C18:2w6	−0.27045	−0.20982	0.16991	−0.11273	−0.02205	0.00950
C18:3w3	−0.19321	−0.26028	0.01163	0.11557	−0.34489	0.17560
C20:0	−0.11828	0.30860	−0.08270	−0.03140	0.00115	0.47631
C20:4w6	−0.21027	−0.27504	−0.00589	−0.04711	0.26284	−0.16111
C20:3w6	−0.21367	−0.28545	−0.01876	−0.11578	0.07526	0.00053
C20:2w6	−0.32092	−0.11952	−0.07649	−0.00566	−0.10877	−0.00028
C20:3w3	−0.10897	−0.28113	−0.31268	0.16291	−0.26812	−0.10250
C20:1w9	0.11012	0.17256	−0.50606	0.22402	−0.06591	−0.04578
C21:0	0.12679	0.10449	−0.18884	−0.71457	−0.57225	−0.06937
C22:0	0.20575	−0.20635	−0.21556	0.22062	−0.15703	−0.47643
C22:1w9	−0.01658	−0.14635	−0.41755	−0.45889	0.41945	0.12727

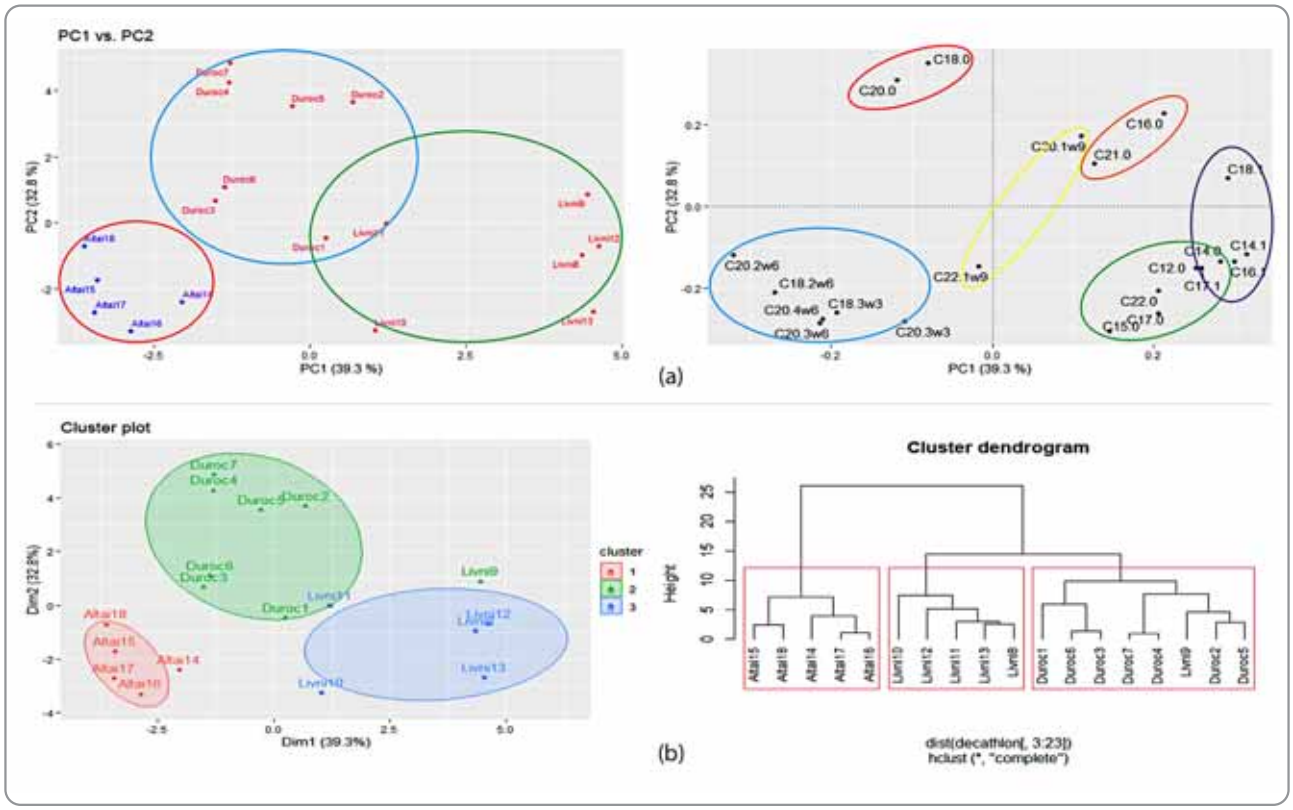


Figure 1. Results of PCA (a) and cluster (b) analysis.

in sum averaged 0.440 and −0.480, respectively. For most part, the highest weighing coefficients for the minor fatty acids were located in PC1 and PC2, while C12:0 and C20:3w3 were in PC3, but the weighting coefficients were approximately equal to those in PC1 and PC2, respectively. The highest weighting coefficients of C21:0 and C22:1w9 were in PC4, C20:0 was in PC6, C18:3w3 was in PC5, but the sum of C18:3w3 coefficients in PC1 and PC2 exceeded the weighting coefficient for this fatty acid in PC5. Figure 1 shows the results of PCA (a) and cluster (b) analysis. PCA visualization of PC1 and PC2 demonstrated that according to fatty acid composition, the pig breeds divided in three groups. Altai pigs gathered in a more tight and distinct group, while Duroc and Livni pigs clustered in more expanded groups which have an overlapping regions. Interestingly, the **ω**3 and **ω**6 fatty acids formed a tight and distinct group, while **ω**9 fatty acids were located in two quadrants. Saturated fatty acids were divided in three groups and located in different quadrants: C18:0 and C20:0; C16:0 and C21:0 pairs were each groups of one major and one minor fatty acid. The certain saturated fatty acids (C12:0, C14:0, C15:0, C17:0 and C22:0) formed a tight and distinct group, which was overlapped with the group of monounsaturated fatty acids and located in two quadrants. Results of cluster analysis demonstrated similarity with PCA, but all pig breeds formed more tight and distinct groups. With cluster analysis, Altai pigs gathered in one tight and distinct group, while Duroc and Livni pigs were in separate, more expanded groups, although one Livni pig was joined to the Duroc group.

4. Discussion

Pig breeds have different fat deposition, fat-specific metabolic characteristics, fatty acid compositions and various other different properties (*Poklukar et al.*, 2020; *Popova & Nakev*, 2019). *Zappaterra et al.* used PCA to study fatty acids composition in BF of 798 individuals Italian Large White heavy pig in order to assess environmental factors and carcass features associated with changes in fatty acids composition (*Zappaterra et al.,* 2022). *Piasentier et al.* used smaller dataset sizes that averaged 24–50 individuals in each group, where diet effect on the lard composition was weighted using the PCs scores as covariates in a tri-factorial (genotype, carcass leanness, sex) covariance design (*Piasentier et al.,* 2009). *Rocchetti et al.* investigated the impact of different diets on the lipidomic profile of pork using 36 animals and applied both PCA and cluster analysis (*Rocchetti et al.,* 2022). *Petroman et al.* applied

gas chromatography-mass spectrometry and PCA to evaluate the dissimilarity of Mangalitza lipid fractions in different layers of backfat with or without heat treatment (*Petroman et al.*, 2021). In that study, a small sample size was used, but clear discrimination was obtained between the raw and thermally processed fat as well as for fat layers (*Petroman et al.*, 2021). *Nizar et al.* revealed the clustering of lard, chicken fat, beef fat into four subclasses using PCA and only three replicates (*Nizar et al.,* 2013). We used Livni (n=6), Duroc (n=7) and Altai (n=5) meat breed pigs, and, despite the small sample, also obtained a clear, breed-specific distribution based on the results of backfat fatty acid composition. The application of both cluster analysis (*Ahn et al.*, 2012; *Dalmaijer et al.*, 2022; *McNeish & Harring*, 2017) and PCA (*Björklund,* 2019; *Shaukat et al.*, 2016; *Yata & Aoshima*, 2010) for small datasets revealed both advantages and disadvantages. Cluster analysis assumes, instead of grouping the initial variables by their correlation into factors (PCA), clustering of animal breeds, in accordance with their fatty acid characteristics, thereby contributing to the overall assessment of the backfat. Thus, the purpose of this method is to distribute pig breeds into groups (clusters) in which fatty acids are relatively homogeneous. Results of cluster analysis demonstrated similarity with PCA, but all breeds formed relatively tight and distinct groups. However, Altai pigs gathered in a very tight and distinct group, while Duroc and Livni pigs grouped in more expanded groups, and one Livni individual pig was joined to the Duroc group.

5. Conclusion

Backfat fatty acid contents of three pig breeds raised in Russia nowadays (Livni, Altai and Duroc) were compared. According to PCA, PC1 and PC2 explain approximately 72.1% of the original data. The highest weighted coefficients for the major and most of the minor fatty acids were presented in PC2 and PC1 separately or in sum, but some minor fatty acids were described by PC4 and PC6, i.e., C21:0, C22:1w9 and C20:0. Fatty acids ω3 and ω6 gathered in a common group, while ω9 were located in two quadrants. Saturated fatty acids were divided in three groups and located in different quadrants: two groups were small and wide, and were overlapped by the monounsaturated fatty acid group. Cluster analysis and PCA demonstrated similarities: all breeds formed groups, although the Altai pigs formed the most tight and distinct group, while Duroc and Livni pigs were in more expanded groups with overlapping regions. Non-parametric statistics (PCA and cluster analysis) were shown to be applicable to this small dataset.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was carried out within project No. 21-76-20032, supported by the Russian Science Foundation, https://rscf.ru/en/project/21-76-20032/.

References

Abdi, H. & Williams, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistics*, 2(4), 433–459, https://doi.org/10.1002/wics.101

Ahn, J., Lee, M. H. & Yoon, Y. J. (2012). Clustering high dimension, low sample size data using the maximal data piling distance. *Statistica Sinica*, 22, 443–464, http://dx.doi.org/10.5705/ss.2010.148

Anowar, F., Sadaoui, S. & Selim, B. (2021). Conceptual and empirical comparison of dimensionality reduction algorithms (PCA, KPCA, LDA, MDS, SVD, LLE, ISOMAP, LE, ICA, t-SNE). *Computer Science Review*, 40, Article 100378 https://doi.org/10.1016/j.cosrev.2021.100378

Björklund, M. (2019). Be careful with your principal components. *Evolution*, 73(10), 2151–2158, https://doi.org/10.1111/evo.13835

Chernukha, I., Kotenkova, E., Pchelkina, V., Ilyin, N., Utyanov, D., Kasimova, T., Surzhik, A. & Fedulova, L. (2023). Pork Fat and Meat: A Balance between Consumer Expectations and Nutrient Composition of Four Pig Breeds. *Foods,* 12, Article 690, https://doi.org/10.3390/foods12040690

Costello, A. B. & Osborne, J. W. (2019). Best practices in exploratory factor analysis: four recommendations for getting the most from your analysis. *Practical Assessment, Research, and Evaluation,* 10, Article 7, https://doi.org/10.7275/jyj1-4868)

Dalmaijer, E. S., Nord, C. L. & Astle, D. E. (2022). Statistical power for cluster analysis. *BMC Bioinformatics*, 23, Article 205, https://doi.org/10.1186/s12859-022-04675-1

Dong, J.-J., Li, Q.-L., Yin , H., Zhong, C., Hao, J.-G., Yang, P.-F., Tian, Y.-H. & Jia, S.-R. (2014). Predictive analysis of beer quality by correlating sensory evaluation with higher alcohol and ester production using multivariate statistics methods. *Food Chemistry*, 161, 376–382, https://doi.org/10.1016/j.foodchem.2014.04.006

Everitt, B. & Hothorn, T. (2011). An Introduction to Applied Multivariate Analysis with R. New York: Springer, https://doi.org/10.1007/978-1-4419-9650-3

Granato, D., Santos, J. S., Escher, G. B., Ferreira, B. L. & Maggio R. M. (2018). Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. *Trends in Food Science and Technology*, 72, 83–90, https://doi.org/10.1016/j.tifs.2017.12.006

Hasan, M. M., Sood, V., Erkinbaev, C., Paliwal, J., Suman, S. & Rodas-Gonzalez, A. (2021). Principal component analysis of lipid and protein oxidation products and their impact on color stability in bison *longissimus lumborum* and *psoas major* muscles. *Meat Science*, 178, Article 108523, https://doi.org/10.1016/j.meatsci.2021.108523

Ivankin, A. N., Oliferenko, G. L., Kulikovskii, A. V., Chernukha, I. M., Semenova, A., Spiridonov, K. I. & Nasonova, V. (2016). Determination of unsaturated fatty acids with a migrating double bond in complex biological matrices by gas chromatography with flame ionization and mass spectrometry detection. *Journal of Analytical Chemistry*, 71, 1131–1137, https://doi.org/10.1134/s1061934816110046

Jolliffe, I. T. & Cadima, J. (2016). Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A*, 374, 20150202, https://doi.org/10.1098/rsta.2015.0202

Kemsley, E. K., Defernez, M. & Marini, F. (2019). Multivariate statistics: considerations and confidences in food authenticity problems. *Food Control*, 105, 102–112, https://doi.org/10.1016/j.foodcont.2019.05.021

Lin, T. I., Ho, H. J. & Chen, C. L. (2009). Analysis of multivariate skew normal models with incomplete data. *Journal of Multivariate Analysis*, 100 (10), 2337–2351, https://doi.org/10.1016/j.jmva.2009.07.005

Macchiato, M. F., Ragosta, M., Cosmi, C. & Lo Porto, A. (1992). A method in multivariate statistics to analyze ecosystems starting from their species composition. *Ecological Modelling*, 62(4), 295–310, https://doi.org/10.1016/0304-3800(92)90004-x

McNeish, D. M. & Harring, J. R. (2017). Clustered data with small sample sizes: Comparing the performance of model-based and design-based approaches. *Communications in Statistics — Simulation and Computation*, 46(2), 855–869, https://doi.org/10.1080/03610918.2014.983648

Microsoft R Application Network (2023). Retrieved from https://mran.microsoft.com/download. Accessed May 10, 2023.

Nizar, N. N. A., Marikkar, J. M. N. & Hashim, D. M. (2013). Differentiation of Lard, Chicken Fat, Beef Fat and Mutton Fat by GCMS and EA-IRMS Techniques. *Journal of Oleo Science*, 62(7), 459–464, https://doi.org/10.5650/jos.62.459

Petroman, C., Popescu, G., Szakal, R.-N., Păunescu, V., Drăghia, L. P., Bujancă, G. S., Chirilă, C. A., Hădărugă, D. I., Văduva, L., Hădărugă, N. G. & Petroman, I. (2021). Fatty Acid Profile of Lipid Fractions of Mangalitza (*Sus scrofa domesticus*) from Northern Romania: A GC-MS-PCA Approach. *Foods*, 10, 242, https://doi.org/10.3390/foods10020242

Piasentier, E., Di Bernardo, N., Morgante, M., Sepulcri, A. & Vitale, M. (2009). Fatty acid composition of heavy pig back fat in relationship to some animal factors. *Italian Journal of Animal Science*, 8(sup2), 531–533, https://doi.org/10.4081/ijas.2009.s2.531

Poklukar, K., Čandek-Potokar, M., Batorek Lukač, N., Tomažin, U. & Škrlep, M. (2020). Lipid Deposition and Metabolism in Local and Modern Pig Breeds: A Review. *Animals*, 10, 424, https://doi.org/10.3390/ani10030424

Popova, T. & Nakev, J. (2019). Fatty acid profile of the backfat layers in four pig breeds. *Food Science and Applied Biotechnology*, 2(1), 24–29, https://doi.org/10.30721/fsab2019.v2.i1.66

Rocchetti, G., Vitali, M., Zappaterra, M., Righetti, L., Sirri, R., Lucini, L., Dall'Asta, C., Davoli, R. & Galaverna, G. (2022). A molecular insight into the lipid changes of pig Longissimus thoracis muscle following dietary supplementation with functional ingredients. *PLoS One*, 17(3), e0264953, https://doi.org/10.1371/journal.pone.0264953

Ros-Freixedes, R., Sadler, L. J., Onteru, S. K., Smith, R. M., Young, J. M., Johnson, A. K., Lonergan, S. M., Huff-Lonergan, E., Dekkers, J. C. M. & Rothschild, M. F. (2014). Relationship between gilt behavior and meat quality using principal component analysis. *Meat Science*, 96(1), 264–269, https://doi.org/10.1016/j.meatsci.2013.07.004

Röttger, R. (2016). Clustering of Biological Datasets in the Era of Big Data. *Journal of Integrative Bioinformatics*, 13(1), 300, https://doi.org/10.1515/jib-2016-300

Shaukat, S. S., Rao, T. A. & Khan, M. A. (2016). Impact of sample size on principal component analysis ordination of an environmental data set: effects on eigenstructure. *Ekológia* (Bratislava), 35(2), 173–190, https://doi.org/10.1515/eko-2016-0014

The Comprehensive R Archive Network. Retrieved from https://cran.r-project.org. Accessed May 10, 2023.

Yata, K. & Aoshima, M. (2010). Effective PCA for high-dimension, low-sample-size data with singular value decomposition of cross data matrix. *Journal of Multivariate Analysis*, 101(9), 2060–2077, https://doi.org/10.1016/j.jmva.2010.04.006

Zappaterra, M., Catillo, G., Lo Fiego, D. P., Belmonte, A. M., Padalino, B. & Davoli, R. (2022). Describing backfat and *Semimembranosus* muscle fatty acid variability in heavy pigs: Analysis of non–genetic factors. *Meat Science*, 183, 108645, https://doi.org/10.1016/j.meatsci.2021.108645

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.514.5.041(497.6 PC)

ID: 126482697

https://doi.org/10.18485/meattech.2023.64.2.61

Original scientific paper

Evaluation of the quality of minced meat and minced formed meat on the market of the Republic of Srpska

Biljana Pećanac[a], Bojan Golić[a] and Dragan Knežević[a]*

[a] Veterinary Institute Republic of Srpska "Dr Vaso Butozan" Banja Luka, Branka Radičevića 18, 78 000 Banja Luka, Bosnia and Herzegovina.

ARTICLE INFO

Keywords:
Minced meat
Quality
Collagen
Protein
Fat
Health

ABSTRACT

Minced meat products are very common and popular meat semi-products today due to their simple way of preparing various meals that does not require a lot of time. If, due to commercial carelessness, cheaper meat is fully or partially incorporated into minced meat and its products, such products will not only be of lower quality and fail to meet regulatory requirements but may also be risky for human health.

The aim of the study was to assess the quality of minced meat products as well as minced formed meat available on the market in the Republic of Srpska and, based on the results of 120 samples, evaluate the degree to which these products comply with the regulations concerning their protein content, collagen content in the meat protein (collagen/meat protein ratio), and fat content.

The tests were carried out with reference-accredited methods to determine the contents of hydroxyproline (*BAS ISO*, *2007*), nitrogen (*BAS ISO, 1978*) and fat (*BAS ISO, 2007b*).

The largest number of non-compliances with the requirements of the regulation was found in the case of an increased proportion of collagen in meat proteins, which indicates the use of a larger amount of low-quality meat in production. According to the requirements of the regulation, consumers must be provided with accurate information about food from the Republic of Srpska market because otherwise, low-quality food poses a risk to people's health. More regular and comprehensive control of meat producers and processors is necessary.

1. Introduction

Minced meat products are today very common and popular meat semi-products that are produced from minced meat of lower value to produce higher-value products (*Hudson et al*., 1986; *Hassan, 2009; Witte et al*., 2022), and their acceptability is also influenced by the simple way of preparing various meals that does not require a lot of time. If, due to commercial carelessness, cheaper meat is fully or partially incorporated into minced meat and its products, such products will not only be of lower quality and fail to meet regulatory requirements but may also be risky for human health (*Cozzolino and Murray*, *2004*). The relative proportion of connective tissue proteins in meat proteins (collagen protein ratio) is one of the quality indicators for meat and minced meat products (minced meat, minced meat prepared for forming, and minced formed meat), which is a requirement established in the *Regulation (2015).* The quality of the meat used to prepare minced meat products is assessed based on the measured content of collagen or hydroxyproline and the relative content of connective tissue proteins in meat proteins (*Messia et al.*, *2008*; *Zarkadas et al.*, *1988*; *Aćimović et al.*,

***Corresponding author:** Biljana Pećanac, *biljana.pecanac@virs-vb.com*

Paper received Jun 3rd 2023. Paper accepted Jun 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2017). The muscle connective tissue contains approximately 12.5% hydroxyproline (*Etherington & Sims, 1981*). Collagen provides skeletal resistance, whereas mineral content mostly impacts strength and stiffness (*Daneault et al.*, *2015*). The Rulebook on providing information to consumers about food («*Official Gazette of the Republic of Srpska*») establishes the requirements for the proportion of fat and the collagen/meat protein ratio in minced meat products, and the data should be listed on the product declaration to protect the interests of consumers. Minced-formed meat can be produced and marketed as ćevap, patty/burger, hamburger, and other types of related products**.**

The aim of the study was to assess the quality of minced meat products as well as minced formed meat available on the market in the Republic of Srpska and, based on the results of 120 samples, evaluate the degree to which these products comply with the regulations concerning their protein content, collagen content in the meat protein (collagen/meat protein ratio), and fat content.

2. Materials and methods

2.1. Materials

In the period from March 2020 to April 2023, tests were conducted on the content of total protein, hydroxyproline, collagen, the collagen/protein ratio, and fat. The paper analyzed a total of 120 samples of minced meat and formed minced meat, The samples were homogenized and stored at +4°C until analysis.

2.2. Methods

Testing of selected quality parameters was carried out using reference-accredited methods as follows: *BAS ISO (2007)* for hydroxyproline content, *BAS ISO (1978)* for nitrogen content and *BAS ISO (2007b)* for free fat content. The quality conditions in relation to the label "minced meat" are specified in the Rulebook (*Republic of Srpska*, *2015*).

2.3. Statistical analysis

Protein, hydroxyproline, collagen, collagen/meat or total protein ratio and fat content in examined samples of minced meat products were performed in duplicate and were presented as mean values with standard deviation (±SD). The results obtained were analyzed using Microsoft Excel software (Windows 10 pro).

3. Results and discussion

Results of quality parameters for the groups of products: minced meat and minced formed meat are in Table 1. Out of a total of 37 minced meat samples, six samples had a protein content of less than 18% and belong to the II category. Of the 28 samples tested, six had a higher collagen/meat protein ratio than allowed. The ratio of collagen and protein in other types of minced meat is satisfactory. The lowest fat was found in mixed minced meat pork and beef (4,73%). The obtained results for fat in

Table 1. Results of quality parameters for minced meat and minced formed meat

Minced formed meat	Number of analyses	Interval Min-max (%)	(%)	SD (%)
Total protein %	83	13.83–21.90	17.59	1.851
Hydroxyproline %	83	0.06–0.47	0.29	0.094
Collagen %	83	0.48 –3.76	2.32	0.752
Relative connective tissue protein in total proteins % (collagen/meat protein ratio)	83	2.35–21.34	13.41	4.420
Fat %	37	2.78–25.88	12.47	6.254
Minced meat				
Total protein %	37	16.37–22.41	19.75	1.510
Hydroxyproline %	37	0.08–0.64	0.24	0.139
Collagen %	37	0.64–5.12	1.90	1.109
Relative proportion of connective tissue proteins in total proteins	37	3.11–23.99	9.723	5.62
Fat	30	1.68–12.24	7.02	3.231

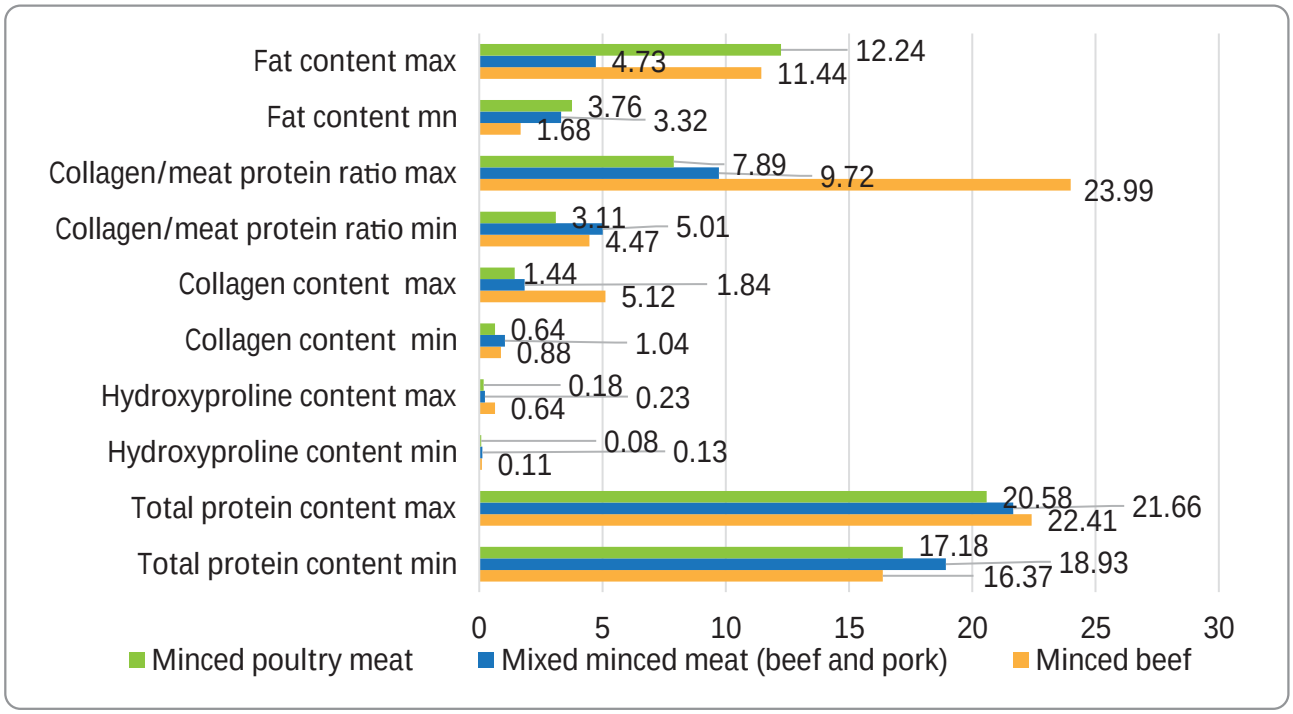


Figure 1. Min and max content of protein %, hydroxyproline, collagen %, collagen/meat or total protein ratio % and fat % in different types of minced meat

ground beef were acceptable, which is not in agreement with the results of *Hudson et al. (1986)*. Also, they determined that the collagen content in retail minced beef is in the range of 1.4% to 4.4%. Nearly similar results were reported by *Hassan, M. Gad Elrab, et al. (2009)*. We found that the content of fat with a large deviation in minced poultry meat, declared as low-fat minced meat, in three samples out of five examined was higher than the allowed 7% (9.88%, 12.24%, and 8.40%), which agrees with *Fayet-Moore et al. (2014)*.

Results of quality parameters for the groups of products: minced meat and minced formed meat are in Table 1.

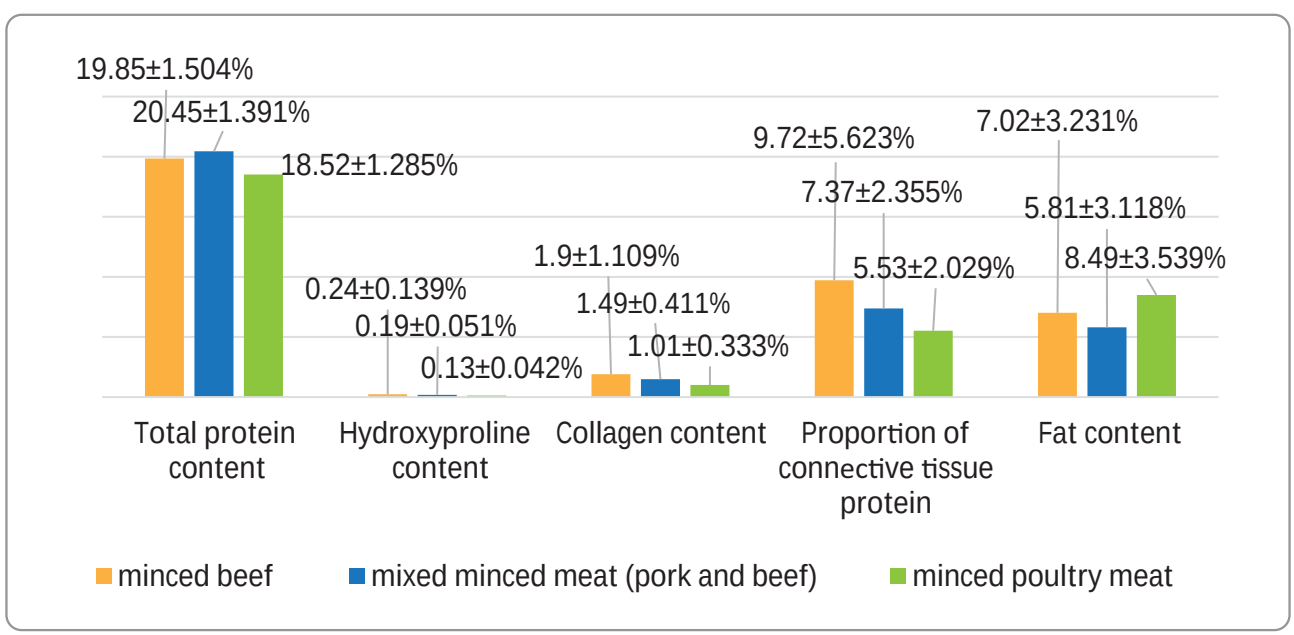


Figure 2. Protein, hydroxyproline, collagen, fet content and collagen/meat protein ratio in in different types of minced meat

In minced beef and ground poultry by-products, *Monago-Maraña, O. et al. (2021)* determined the collagen (measured by hydroxyproline) in the range of 0.1–3.3%, respectively 0.4–1.5%, and our results are in agreement with the results for minced poultry meat but higher than the results for minced beef. The fat in ground beef sold in Australia is <10 g/100 g *(Fayet-Moore et al., 2014)*, which is consistent with our results. Our results are not in accordance with the results of *Kalinova et al. (2017)* who found 23.30 ± 0.67% fat, 16.26 ± 0.73% protein, 0.12 ± 0.01% hydroxyproline, 0.96 ± 0.10 collagen, and a collagen-to-protein ratio of 6 in the mixed minced meat of pork, beef, and veal.

The minimum and maximum values of the content of fat, collagen/meat or total protein ratio, collagen, hydoxyprolin, and protein (%) in minced meat are shown in Figure 1.

The mean values of proten, hydoxyproline, collagen, and collagen/meat or total protein ratio and fat content in minced beef (29 samples), mixed minced (3), and minced poultry (5 samples) are shown in Figure 2.

The minimum and maximum values of the content of fat, hydoxyproline, collagen, and the collagen/meat ratio or total protein (%) in different types of minced formed meat are shown in Figure 3.

The mean values of fat. hydoxyproline. collagen content. and collagen/meat protein ratio (%) in beef ćevap (43 samples). mixed beef and pork ćevap (12 samples). patty (23 samples). and related products (5 samples) are shown in Figure 4.

From the group of formed minced meat. the highest collagen and collagen protein ratio was found in the ćevap. Out of a total of 55 ćevap samples tested. 16 samples (29%) did not comply with the requirements of the regulation regarding the collagen/meat protein ratio. of which non-compliance was found in 28% of beef ćevap samples. Incomplying collagen content test results were also found in six patties samples. so that 23% of the samples had a collagen/meat protein ratio higher than the prescribed limit. The highest fat content was found in the patty/burger (25.88%). while the lowest was recorded in the beef minced meat. One beef ćevap has a lower protein content (13.82%) than the prescribed 14%. and in the patty/burger more fat than the prescribed values (24.88%). Our results agree with those obtained by *Ljutić et al. (2019), Saad et al. (2018)* and *Muftić et al. (2020). Salim and Abou El-Roos (2013)* determined the average value of

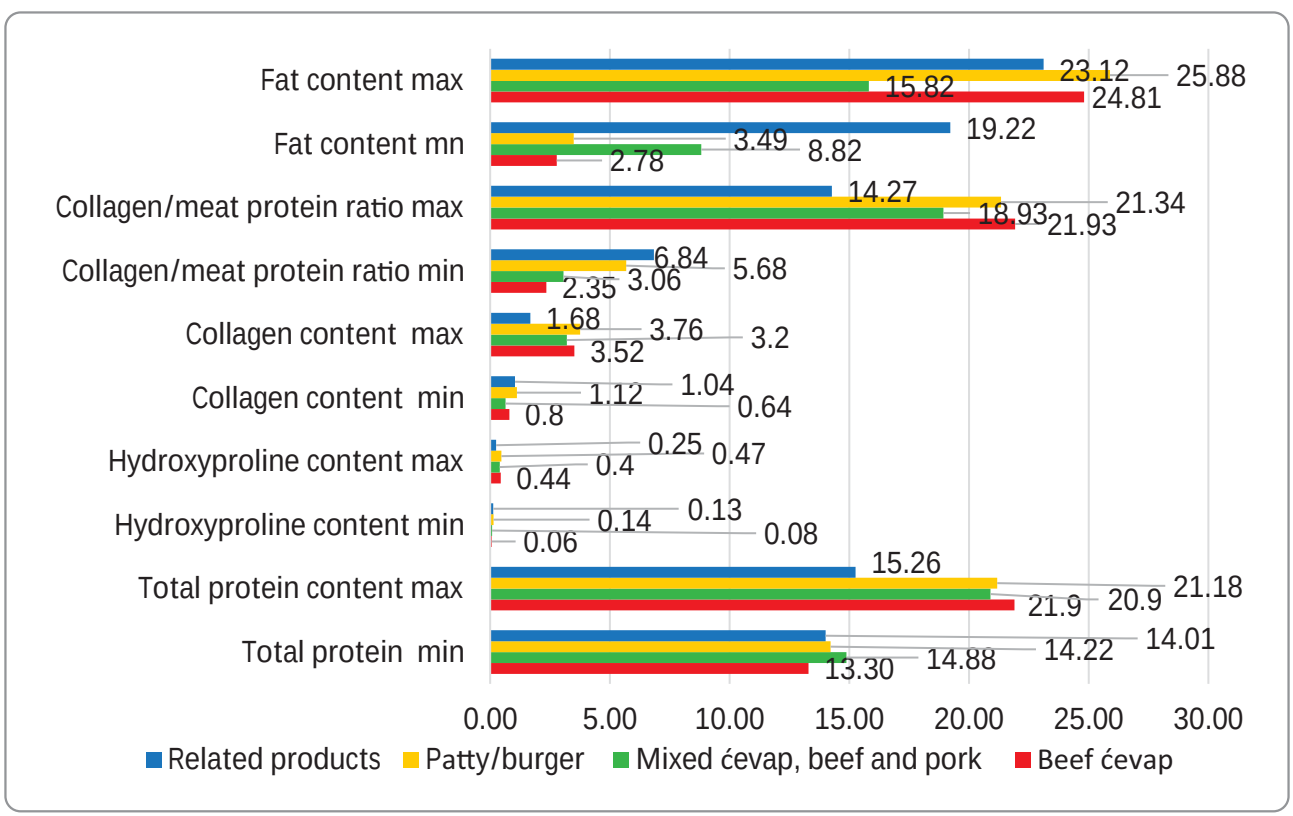


Figure 3. Min and max content of protein. hydroxyproline. collagen. fat and collagen/meat protein ratio in minced formed meat

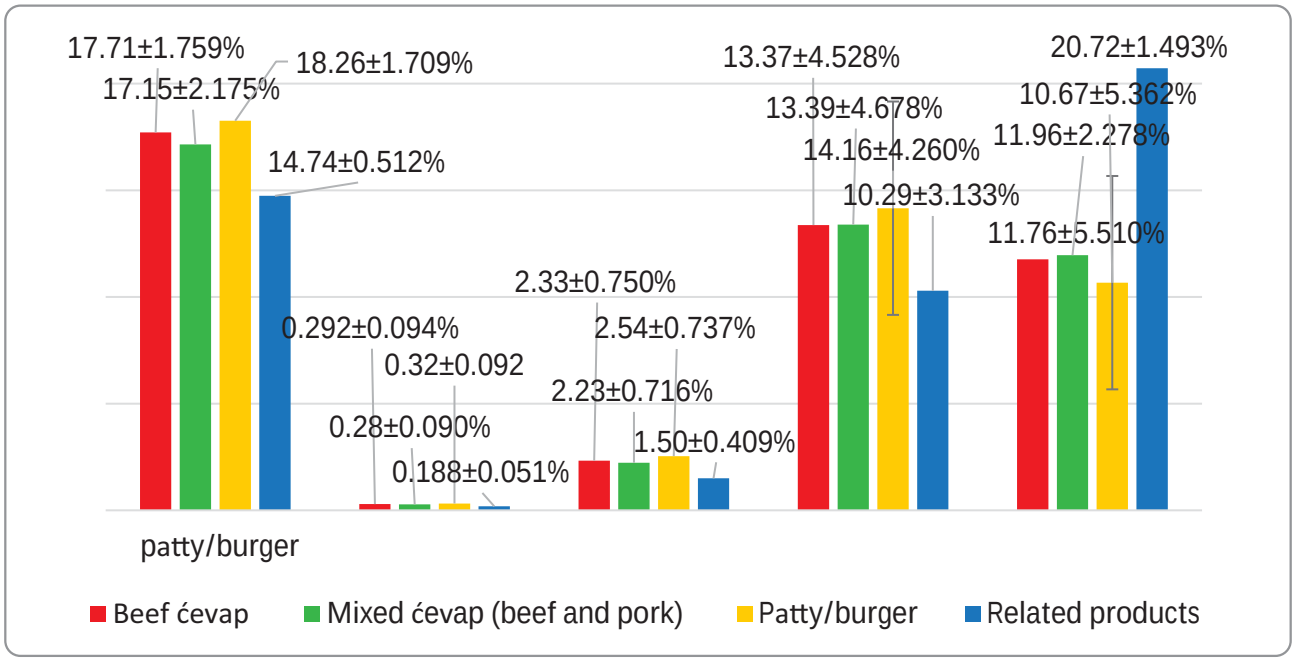


Figure 4. Fat. collagen. hydroxyproline content and collagen/meat protein ratio (%) in minced formed meat

hydroxyproline in ground beef. which is significantly lower than ours.

4. Conclusion

The largest number of non-compliances with the requirements of the regulation was the unacceptably high proportion of collagen in total meat protein, which indicates the use of a large amount of low-quality meat in production. According to the requirements of the regulation, consumers must be provided with accurate information about food on the Republic of Srpska market because otherwise, low-quality food poses a risk to people's health. More regular and comprehensive control of meat producers and processors is necessary.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Aćimović, M., Kozačinski, I., Njari, B. & Cvrtila, Ž. (2014). Comparative view of regulations on quality of meat products. *Meat*, 16(4), 342–345.

BAS ISO, (2007). 3496:2007, Meat and meat products — Determination of hydroxyproline content.

BAS ISO, (1978). 937:1978, Meat and meat products — Determination of nitrogen content.

BAS ISO, (2007b). 1444:2007, Meat and meat products — Determination of free fat content.

Cozzolinoa, D. & Murrayb, I. (2004). Identification of animal meat muscles byvisible and near infrared reflectance spectroscopy. *Lebensmittel-Wissenschaft und -Technologie,*37, 447–452. DOI:10.1016/j.lwt.2003.10.013

Daneault, A., Prawitt, J., Fabien Soule, V., Coxam, V. & Wittrant, Y. (2017). Biological effect of hydrolyzed collagen on bone metabolism. *Critical Reviews in Food Science and Nutrition*, 57, 1922–1937, DOI: 10.1080/10408398.2015.1038377

Etherington, D. J. & Sims, T. J. (1981). Detection and estimation of collagen. *Journal of the Science of Food and Agriculture*, 32, 539–546, https://doi.org/10.1002/jsfa.2740320603

Fayet-Moore, F., Cunningham, J., Stobaus, T. & Droulez, V. (2014). Fat content and composition in retail samples of Australian beef mince. *Nutrients,* 6(6), 2217–2228, https://doi.org/10.3390/nu6062217

Hassan, M., EL-Lawendy, H. & Raslan, M. (2009). The bacteriological quality. fat and collagen content of minced meat. *Kafrelsheikh Veterinary Medical Journal,* 7(1), 564–574, doi: 10.21608/kvmj.2009.108702

Hudson, W. R., Roberts, T. A., Crosland, A. R. & Casey, J. C. (1986). The bacteriological quality. fat and collagen content of minced beef at retail level. *Meat Science,* 17(2), 139–152. https://doi.org/10.1016/0309-1740(86)90060-4

Kalinova, G., Marinova, M., Mechkarova, P., Mladenova, D. & Grigorova, E. (2017). Determination and assessment of the content of collagen in minced meat and meat

products offered. *Bulgarian Journal* of *Veterinary Medicine*, 20(1), 437–441.

Lawrie, A. R. (1998). Lawri's meat science.6th Ed.Wood head publishing Ltd .USA.

Ljutić, D., Malenica, M., Krešić, G., Leštć, T., Kolarić, S., Kravar, L., Dergestin, B. & Pleadin, J. (2019). The quality of minced meat from the area of the city of Zagreb. *Meso,* 21(69), 586–593.

Messia, M. C., Di Falco, T., Panfili, G. & Marconi, E. (2008). Rapid determination of collagen in meat-based foods by microwave hydrolysis of proteins and HPAEC-PAD analysis of 4-hydroxyproline. *Meat Science,* 80(2), 401–409, https://doi.org/10.1016/j.meatsci.2008.01.003

Monago-Maraña, O., Petter Wold, J., Rødbotten, R., Riiser Dankel, K. & Kristian Afseth, N. (2021). Raman. near-infrared and fluorescence spectroscopy for determination of collagen content in minced meat and poultry by-products. *LWT*, 140, 110592, https://doi.org/10.1016/j.lwt.2020.110592.

Muftić, E., Čaklovica, K., Tahirović, D., Muftić, A. & Čaklovica, F. (2020). Quality parameters of minced meat and raw formed products on Bosnian and Herzegovinian market. *Meso: The First Croatian Magazine about Meat,* 22(2), 142–148, https://doi.org/10.31727/m.22.2.3

Rulebook on minced meat. semi-finished products and meat products, (2015). Official Gazette of the Republic of Srpska no 46/15.

Saad, M. S., Hassan, M. A., Reham1, A., Amin, E., Shater, M. A. & Shanab, M. S. (2018). Detection of starch and hydroxyproline content in some meat products. *BVMJ,* 35(2), 195–201.

Zarkadas, C. G.. Constantinos. N.. Karatzas. A. D. K.. Shahrokh. K. & Guylaine. M. (1988). Quantitative determination of the myofibrillar proteins and connective tissue content in selected porcine skeletal muscles. *Journal of Agricultural and Food Chemistry,* 36(6),1131–1146, doi: 10.1021/jf00084a004; UR — https://doi.org/10.1021/jf00084a004

Witte, F., Sawas, E., Berger, L. M., Gibis, M., Weiss, J., Röser, A., Upmann, M., Joeres, E., Juadjur, A. & Bindrich, U. (2022). Influence of Finely Chopped Meat Addition on Quality Parameters of Minced Meat. *Applied Sciences,* 12(20), 10590, https://doi.org/10.3390/app122010590

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.057:639.381(497.11)"2012/2021"

ID: 126497289

https://doi.org/10.18485/meattech.2023.64.2.62

Original scientific paper

Investigation of the volume of fish production and catch in Serbia from 2012 to 2021

Branislav Baltić[a], Aksentijević Ksenija[b], Danica Bogunović[c], Marija Starčević[d], Radmila Mitrović[e], Boris Mrdović[f] and Jelena Janjić[g]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia*
[c] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia*
[d] *Serbian Army, Belgrade, Serbia*
[e] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[f] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[g] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Fishpond
Carp
Trout
Consumption

ABSTRACT

In the Serbian market, fish is available from aquaculture (such as carp and trout) and from fishing (commercial and recreational catch). In the past ten years, from 2012 to 2021, there has been a decrease in the production of carp due to reduced farming areas, but trout production has increased. On average, during this period, aquaculture production yielded approximately 5,491 tons of carp and 2,977 tons of trout, while the fish catch averaged around 2,979 tons. The yield per hectare in carp ponds was 800 kg, while in trout ponds, it was 20 kg per square meter. As the demand for fish exceeds the domestic supply, the market is supplemented with imported fish.

1. Introduction

The world's fish for human consumption comes from two sources. One source is the capture of fish from open waters (oceans, seas, rivers, lakes), while the other source is farmed fish, i.e., fish from aquaculture. Fish in aquaculture can be farmed in marine, brackish, and mostly freshwater (artificial and natural lakes) environments. Aquaculture has been known for over 2,000 years, but its full significance was recognized in the early 1990s when the demand for fish increased due to population growth worldwide and because the capture of fish from open waters was between 90 and 100 million tons. Since then, the capture volume has not been increased as it was observed that it would disrupt the biological balance in marine ecosystems. Fish production in aquaculture was the only solution to meet the demand for fish in the global market (*Baltić & Teodorović*, 1997; *Ivanović et al*., 2015). In the 2020s, fish production in aquaculture exceeded the capture of fish from natural resources (*Boyd et al.*, 2022). Thanks to this, there were 178 million tons of fish on the global market in 2020, and it is expected to reach 196 million tons by 2025 (*Pedro & Nunes*, 2019). Some opinions suggest that in a few decades, the amount of food derived from water (fish and algae) will be equal to the amount of meat from land animals (*Baltić et al*., 2022). The Serbian market is supplied with fish from aquaculture and from capture (rivers, lakes), with

***Corresponding author:** Branislav Baltić, *branislav.baltic@inmes.rs*

Paper received Jun 6th 2023. Paper accepted Jun 16th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

the majority of fish being imported. The aim of this study is to examine the volume of fish production and capture in Serbia from 2012 to 2021.

2. Materials and methods

Data on the fish market supply in Serbia from aquaculture and capture were collected from the Statistical Yearbooks of Serbia from 2012 to 2021 (https://www.stat.gov.rs/). The Statistical Yearbooks provide information on the surface area of carp ponds expressed in hectares and the production of consumable carp expressed in tons. Data on the surface area of trout ponds (expressed in square meters) and the production of consumable trout (expressed in tons) are also presented. Separate data are provided for the total fish catch in lakes, rivers, and canals (expressed in tons) for commercial and recreational fishing separately. The Statistical Yearbooks also include data on the catch of the four most commonly caught fish species (carp, white bighead, bream, goldfish) from both commercial and recreational fishing. The results obtained were compared by statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The mean values and measures of variation of fish production and catch were calculated. Trends in fishery areas and catches were computed. All results are presented graphically.

3. Results

Carp ponds in Serbia are located in Vojvodina. During the observed period (2012–2021), the average surface area of carp ponds was 7,439.4 ± 1,055.07 ha (the largest being 8,724 ha in 2014, and the small-

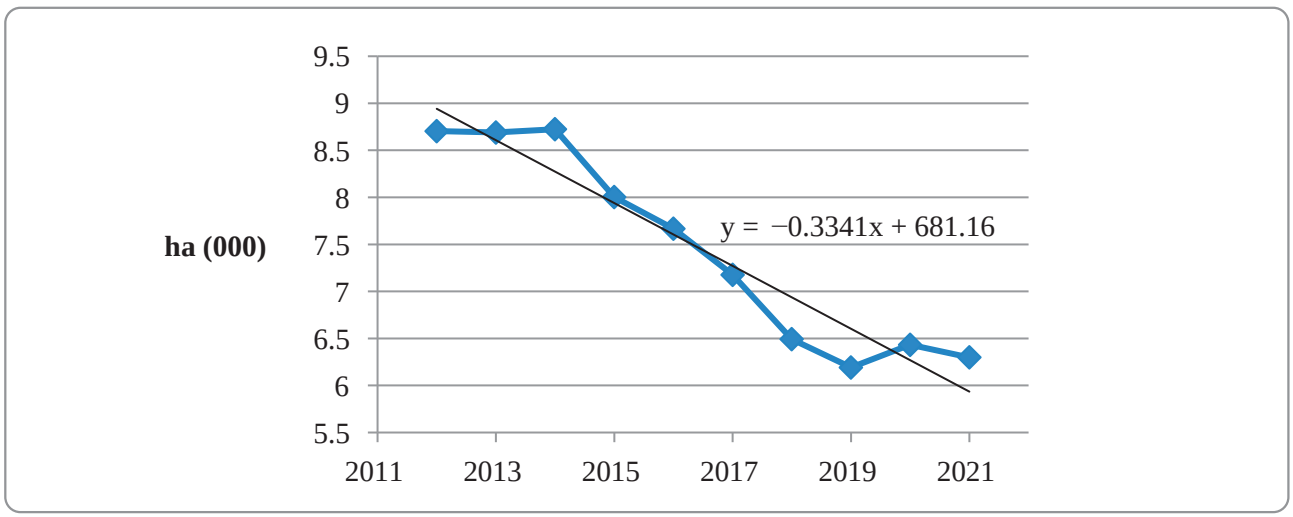


Figure 1. The trend of changes in the surface area of carp ponds from 2012 to 2021.

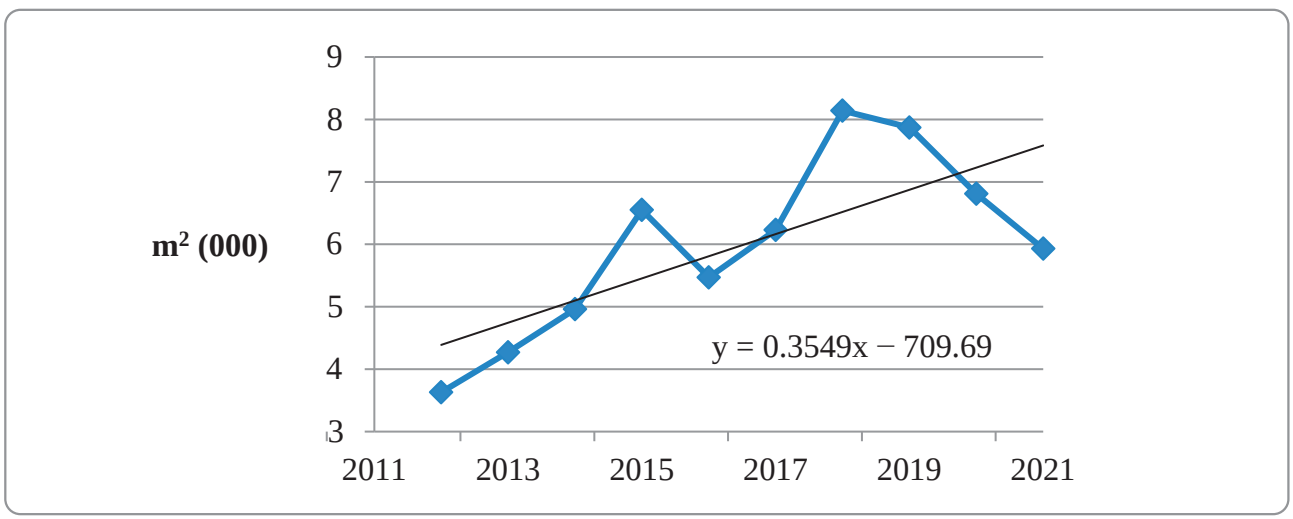


Figure 2. The trend of changes in the surface area of trout ponds from 2012 to 2021.

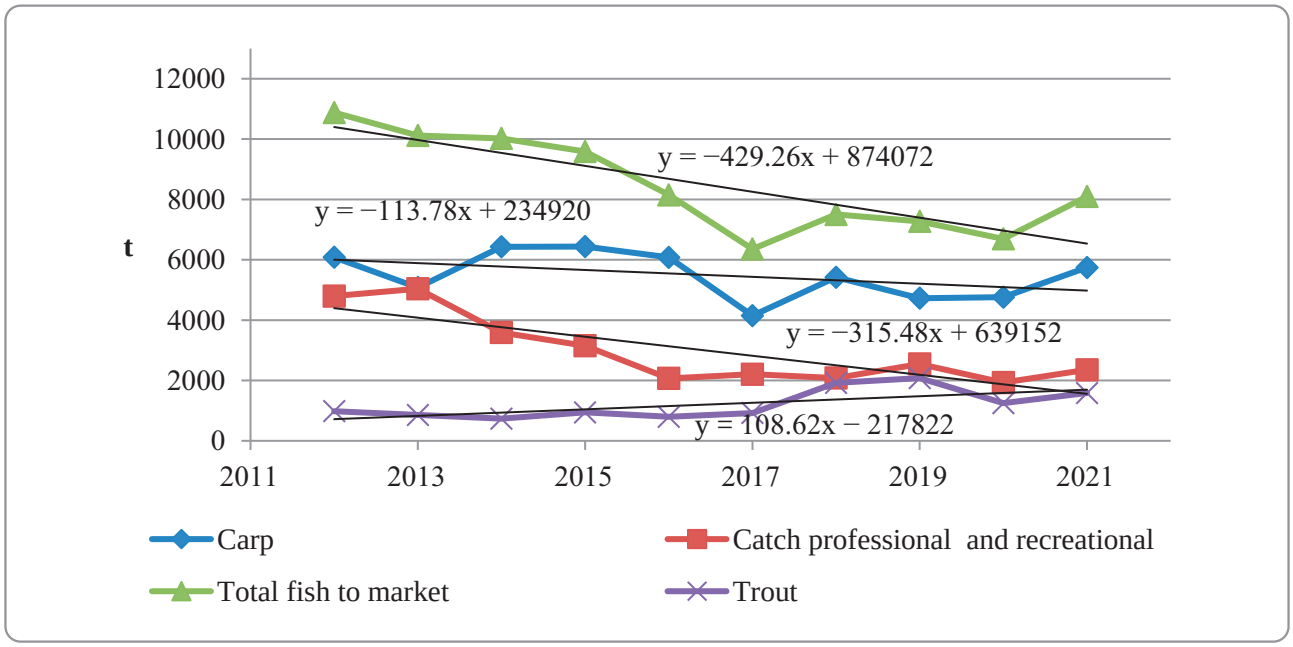


Figure 3. Trends in fish production, fish catch, and total fish supply in the Serbian market from 2012 to 2021.

est being 6,192 ha in 2019). The change in the surface area of carp ponds from 2012 to 2021 is shown in Figure 1 and can be represented by the equation y = –0.3341x + 681.16.

The surface area of trout ponds has varied to a much greater extent compared to the changes in the surface area of carp ponds. From 2012 to 2018, the surface area of trout ponds increased, but then sharply decreased by 2021. Overall, during this period, the surface area of trout ponds increased (y = 0.3459x + 709.69). The surface area of trout ponds was the smallest in 2012 (36,302 m^2), and the largest in 2018 (81,411 m^2), with an average of 59,864.6 ± 1,455.38 m^2 over the ten-year period (Figure 2).

The average catch of carp from 2012 to 2021 was 5,491.5 ± 0.791 t, and for trout it was 1,206.6 ± 0.486 t, making a total of 6,698.1 t. The highest production of consumable carp was 6,438 t in 2015, while the lowest was 4,728 t in 2020. The highest catch of consumable trout was in 2019 (2,079 t), and the lowest was in 2014 (736 t). In the total pro-

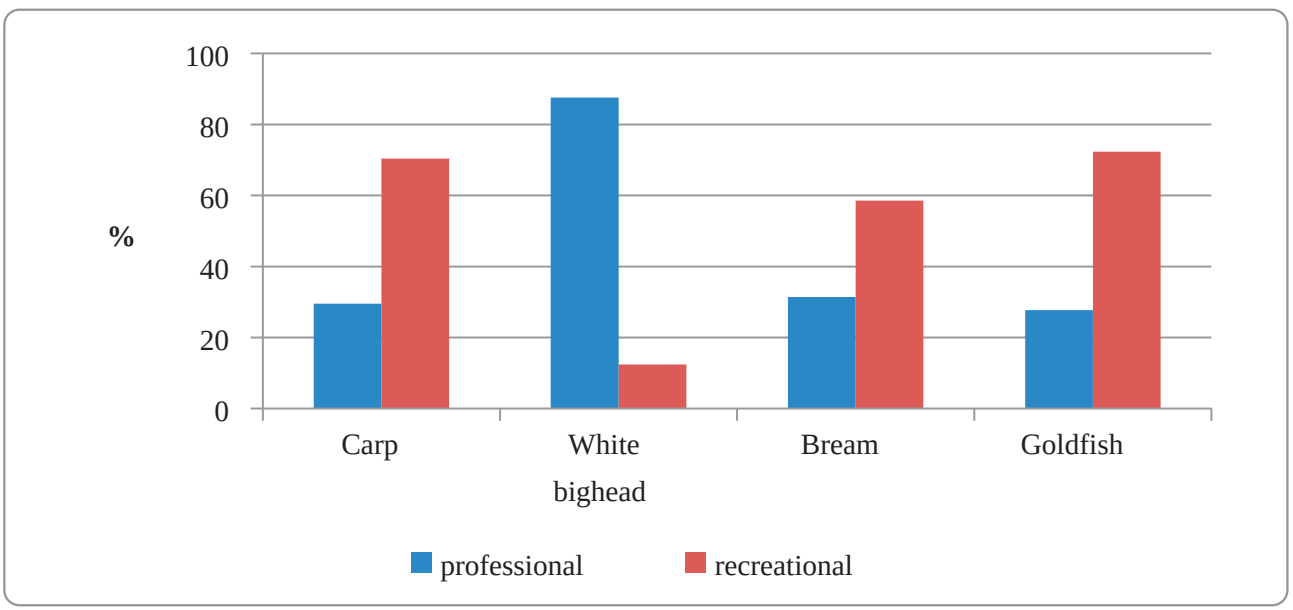


Figure 4. The percentage (%) contribution of carp, white bighead, bream, and goldfish catch from commercial and recreational fishing

duction of consumable fish in aquaculture, the proportion of carp over a ten-year period was 81.98%, while trout accounted for 9.02%. Based on the average areas of carp and trout ponds from 2012 to 2021, it was determined that the yield of carp per ha was 800 kg, and for trout per m^2 was 20 kg. The fish catch (commercial and recreational) was highest in 2013 (5,048 t) and lowest in 2000 (1,931 t). The data on fish catch refer only to the four most commonly caught fish species (carp, white bighead, bream, goldfish). The average ten-year total catch of these fish species was as follows: carp 257.6±133.30 t, white bighead 185.7±41.56 t, bream 229.0±126.00 t, and goldfish 511.7±186.50 t. The commercial catch of carp over the ten years was 76.1 t (29.54% of the total catch), while the recreational catch was 181.5 t (70.40%). In contrast to carp, the commercial catch of white bighead accounted for 87.56% (162.6 t), while the recreational catch was 12.44% (23.1 t). The recreational catch of bream was higher (58.56%; 134.1 t) compared to the commercial catch (31.44%; 94.9 t). This difference is even more pronounced for goldfish, since 72.31% (370.0 t) of the total catch of this fish was from recreational fishing, while 27.69% (141.7 t) was from commercial fishing. The data represent the average ten-year catch of these fish species. Figure 3. shows the trends in fish production, fish catch, and total fish supply in the Serbian market from 2012 to 2021.

The percentage (%) contribution of carp, white bighead, bream, and goldfish catch from commercial and recreational fishing is shown in Figure 4.

4. Discussion

The average annual production of consumable carp from 2006 to 2012 was 6,103±1,008 t, and trout production was 923.1±208.0 t (*Ivanović et al.,* 2015). Other fish species in carp aquaculture (white bighead, grass carp, occasionally catfish and pike) are produced in much smaller amounts than carp. Carp is the most commonly farmed fish species in aquaculture worldwide, especially in China and other Asian countries (Vietnam, India). In Serbia, as well as in most countries around the world, carp is farmed in a semi-intensive system. In this system, the majority of the feed comes from the natural ecosystem, with a smaller portion consisting of added nutrients. The semi-intensive nature of carp production in Serbia is evident from the data on the yield of harvested consumable carp per unit area (ha), which has been less than 1 t from 2012 to 2021. Carp production per ha can be increased by using grain (corn or pelleted feed) in the diet. By choosing high-quality feeds and implementing agrotechnical measures (such as water aeration), carp yields per hectare can exceed 3 t, and even reach 5 t or more in optimal conditions, such as cage culture systems (*Marković*, 2010). The lowest catch volume of fish from open waters (carp, grass carp, bream, and goldfish) in the observed period was in 2020, which can be attributed to it being the first year of the COVID-19 pandemic. Decreased fish catch and production in 2020 were observed globally in the fishing industry as a whole (*Boyd et al.,* 2022). Trout yield is highly dependent on feed, as well as other factors such as aeration and water temperature, and can range from 5 to 50 kg (*Aganović*, 1979). In addition to farmed fish in aquaculture, a portion of the fish in the Serbian market comes from commercial and recreational fishing. The contribution of fish from recreational fishing is greater than that from commercial fishing in the total fish catch. Within specific fish species, commercial fishing makes a higher contribution only in the case of common carp, while recreational fishing has a higher catch of carp, bream and goldfish.

In the Serbian market, in addition to fish farmed in aquaculture and fish from local catch, imported fish (mostly marine fish) also play a role. The volume of fish imports from 2012 to 2021 ranged from 30,000 to 40,000 tons, significantly impacting fish consumption in Serbia (*https://www.stat.gov.rs*). With fish from domestic sources alone, the average annual fish consumption per capita in Serbia would be 1.5 kg, while with imported fish, it reaches around 7 kg. The average global fish consumption in 2021 was 20.2 kg, which is double the amount in 1960 when it was 9.9 kg. Iceland has the highest consumption of fish per inhabitant per year (91 kg), followed by the Maldives, Portugal, and South Korea, and Afghanistan has the smallest (0.24 kg) (*Ali et al.*, 2022; *FAO*, 2022).

5. Conclusion

Fisheries in Serbia are sharing the fate of agricultural production, especially livestock farming, as fish production in aquaculture is decreasing. Serbia has the potential to increase fish production in existing fishponds, especially carp ponds, by revitalizing neglected fishponds and also by constructing new ones. The implementation of adequate agrotechnical measures and improved feeding practices would

contribute to higher fish production. Changes in the fish supply chain would also contribute to increased fish consumption. Currently, fish produced in Serbia is mostly sold live in the market, which is the least favorable method of fish supply, especially for urban populations.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143) and Contract number — Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Aganović, M. (1979). *Salmonidne vrste riba i njihov uzgoj.* Svjetlost, Sarajevo.

Ali, A., Wei, S., Ali, A., Khan, I., Sun, Q., Xia, Q.... & Liu, S. (2022). Research progress on nutritional value, preservation and processing of fish—A review. *Foods,* 11(22), 3669.

Baltić, M. Ž. & Teodorović, V. B. (1997). *Higijena mesa riba, rakova i školjki*. Veterinarski fakultet, Beograd.

Baltić, M. Ž., Bošković Cabrol, M., Dokmanović, M., Janjić, J., Glišić, M., Branković Lazić, I. & Dimitrijević, M. (2022). Meso in vitro-ante portas. *33. Savetovanje Veterinara Srbije, Zlatibor,* 08–11. Septembar 2022, 379–391.

Boyd, C. E., McNevin, A. A. & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food security,* 14(3), 805–827.

FAO, (2022). https://www.aquafeed.com/newsroom/reports/new-record-for-global-aquaculture-production-faos-2022-sofia-reports/#:~:text=Reports-,New%20record%20for%20global%20aquaculture%20production%2C%20FAO's%202022%20SOFIA%20reports,214%20million%20tonnes%20in%202020.

Ivanović, J., Baltić, Ž. M., Janjić, J., Marković, R., Bošković, M., Đorđević, V. & Dokmanović, M. (2015). Obim i struktura ulova i proizvodnje ribe u Srbiji od 2006. do 2012. godine. *Veterinarski glasnik,* 69(5–6), 453–465.

Marković, Z. (2010). *Šaran, Gajenje u ribnjacima i kaveznim sistemima*. Prof. dr Zoran Marković, 152, Beograd.

Pedro, S. & Nunes, M. L. (2019). Reducing salt levels in seafood products. In *Reducing Salt in Foods* (pp. 185–211). Woodhead Publishing.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.52.057(497.11)"2021/2023"

ID: 126498825

https://doi.org/10.18485/meattech.2023.64.2.63

Original scientific paper

The presence of nitrites and nitrates in various type of meat semi-products intended for grilling from the Serbian market

Vladimir Korićanac[a], Danijela Vranić[a], Dejana Trbović[a], Stefan Simunović[a] and Sara Simunović[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Nitrites
Nitrates
Food
Safety
Meat

ABSTRACT

The aim of this study was to examine the presence of nitrites and nitrates in different types of meat semi-products prepared for the grill, in accordance with the national Regulation that prohibits their use. A total of 724 meat semi-product samples, including dry-aged steaks, fresh sausages, marinated meat, and various types of minced meat, produced by the Serbian meat industry between January 2021 and January 2023, were tested. The findings revealed that none of the tested samples showed the presence of either nitrites or nitrates, aligning with the Regulation's prohibition on their use in meat semi-product production, including those intended for grilling.

1. Introduction

Meat consumption has been an integral part of human diets for centuries, providing essential nutrients and contributing to cultural and culinary traditions. As modern food processing techniques have evolved, so too has the production and preparation of meat products. One popular preparation method is grilling, which imparts a unique flavour profile and texture. However, concerns have arisen regarding the presence in meat semi-products intended for grilling of some hazards, like nitrites and nitrates, as these compounds have been associated with potential health risks. Nitrites and nitrates are chemical compounds commonly found in various food products, including meat. These compounds have attracted considerable attention due to their potential health effects and their role in food preservation. Understanding the presence of nitrites and nitrates in meat products, particularly those intended for grilling and available in the Serbian market, is crucial for evaluating their impact on human health and the development of various diseases in the Serbian population, but also, the responsibility of producers of meat and meat products in Serbia. This paper aims to review the presence of nitrites and nitrates in different types of meat products intended for grilling in the Serbian market, emphasizing the implications associated with their consumption. Nitrites (NO_{2-}) and nitrates (NO_{3-}) are naturally occurring compounds found in soil, water, and certain foods. They are also used as food additives, primarily in meat products, due to their antimicrobial properties and their ability to enhance flavour, colour and preservation (*Honikel*, 2008). Nitrites play a critical role in preventing bacterial growth, particularly the growth of *Clostridium botulinum*, which is responsible for botulism, a

***Corresponding author:** Vladimir Koricanac, *vladimir.koricanac@inmes.rs*

Paper received Jun 6th 2023. Paper accepted Jun 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

potentially fatal form of food poisoning (*Sebranek & Bacus*, 2007). However, concerns have been raised regarding the potential adverse effects of nitrites and nitrates on human health. Numerous studies have suggested a potential link between excessive consumption of nitrites and nitrates and adverse health consequences. Nitrites can react with certain compounds in the stomach, leading to the formation of nitrosamines, which are known to be carcinogenic (*Goulas et al.*, 2021; *World Health Organization [WHO]*, 2010). Nitrosamines have been associated with an increased risk of various types of cancer, including stomach, oesophageal, and colorectal cancer (*Cross et al.,* 2010; *Loh et al*., 2020). Moreover, high levels of nitrites can interfere with the normal functioning of haemoglobin, reducing its oxygen-carrying capacity and leading to a condition called methaemoglobinaemia (*Mashima & Nakazato*, 2020). In the Serbian market, meat products for grilling are widely available, including various types of sausages, kebabs, and marinated cuts of beef, pork, and poultry. These products may vary in terms of their composition, quality and processing techniques, which can influence the levels of nitrites and nitrates present (*Katsanidis et al.*, 2015). Previous studies have investigated the levels of nitrites and nitrates in meat products from different regions, providing valuable insights into their occurrence and potential health risks (*Chiavaro et al.*, 2015; *Stajić et al.*, 2020). Investigating the presence of nitrites and nitrates in different types of meat products for grilling available in the Serbian market is essential to assess the potential risks associated with their consumption. By conducting a comprehensive review of existing literature and available data, this paper aims to contribute to the understanding of nitrite and nitrate levels in Serbian meat products for grilling and their implications for human health. According to the Serbian Regulation on the quality of ground meat, meat preparations and meat products (*Republic of Serbia*, 2019, 2023), nitrites and nitrates are not allowed to be used in the production of meat semi-products, which includes meat products intended for grilling.

2. Materials and methods

2.1 Reagents

All chemicals used, for nitrites and nitrates testing in meat semi-products intended for grill, were of analytical grade and were used as received without any further purification unless otherwise stated.

2.2. Meat Semi-products and Sample Preparation

In this study, a total of 724 meat semi-product samples, produced by the Serbian meat industry or imported (6 dry-aged steaks, 234 fresh sausages, 64 marinated meat products, 420 various types of minced meat intended for grilling), were obtained from different regions from the Serbian retail market during period January 2021 to January 2023 and were analysed for nitrite and nitrate presence. In most of the meat semi-products, all parameters of quality defined by the legislation were examined, and in a smaller number, analyses were carried out as per the client's request. All meat products were kept at refrigeration temperature and analysed within 48 h. If the analyses were not conducted on the same day, the samples were stored in a refrigerator at 4°C until required for testing. The analysed samples were warmed to room temperature and blended in a commercial kitchen blender unit (Homogenizer Blixer 2, Robot Coupe, Vincennes, France (2.9 L) 700 w, 3000 rpm). For each sample, two composite samples were prepared. All samples were then analysed in duplicate.

2.3. Determination of Nitrite and Nitrate Content

The content of nitrites and nitrates in tested meat products was determined according to the respective standard ISO procedures (*ISO*, 1975a, b) with a limit of quantification (LOQ) of 0.03 mg kg^{-1} for both parameters. A representative sample amount was measured using an analytical balance (Mettler, AE 200, USA). Following the analytical procedure provided in ISO standards, absorbance was measured using a spectrophotometer (GENESYS 10S Series UV-Visible Spectrophotometers). A procedural blank was run with every batch of samples.

2.4. Analysis and graphical presentation of results

The results analysis and graphical presentation of their distribution was performed using Microsoft Office Excel 2016.

3. Results

The results of determination of nitrites and nitrates presence in the tested samples of meat semi-products intended for grill are shown in Table 1. Also, distributions of samples by type of the meat product, and by presence of nitrites and nitrates in all samples, are graphically presented in Figures 1 and 2.

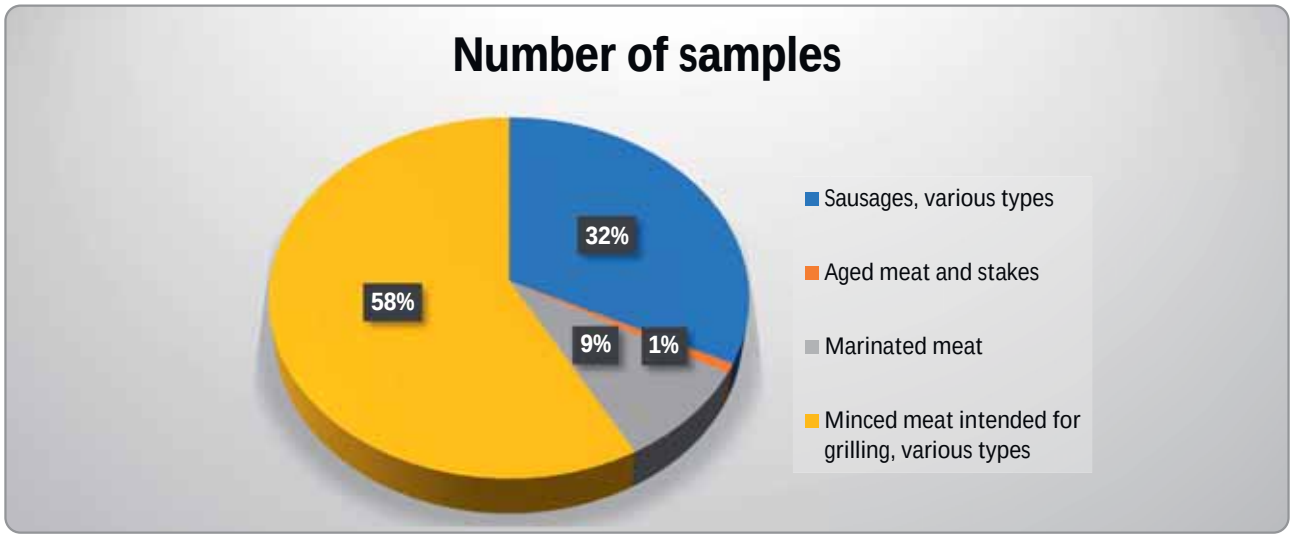


Figure 1. Distribution of samples by type of meat product

Table 1. Number of samples of meat semi-products intended for grill in which the presence of nitrites (as $NaNO_2$) and nitrates (as $NaNO_3$) was determined, in the period January 2021 to January 2023

Type of samples	Not detected ($NaNO_2$ <0.03 mg kg^{-1})	Not detected ($NaNO_3$ <0.03 mg kg^{-1})
Aged meat and steaks (6)	6	6
Marinated meat (64)	64	64
Sausages, various types (234)	234	234
Minced meat intended for grilling, various types (420)	420	420
All samples (724)	724	724

These results are graphically presented in Figures 2 and 3, respectively.

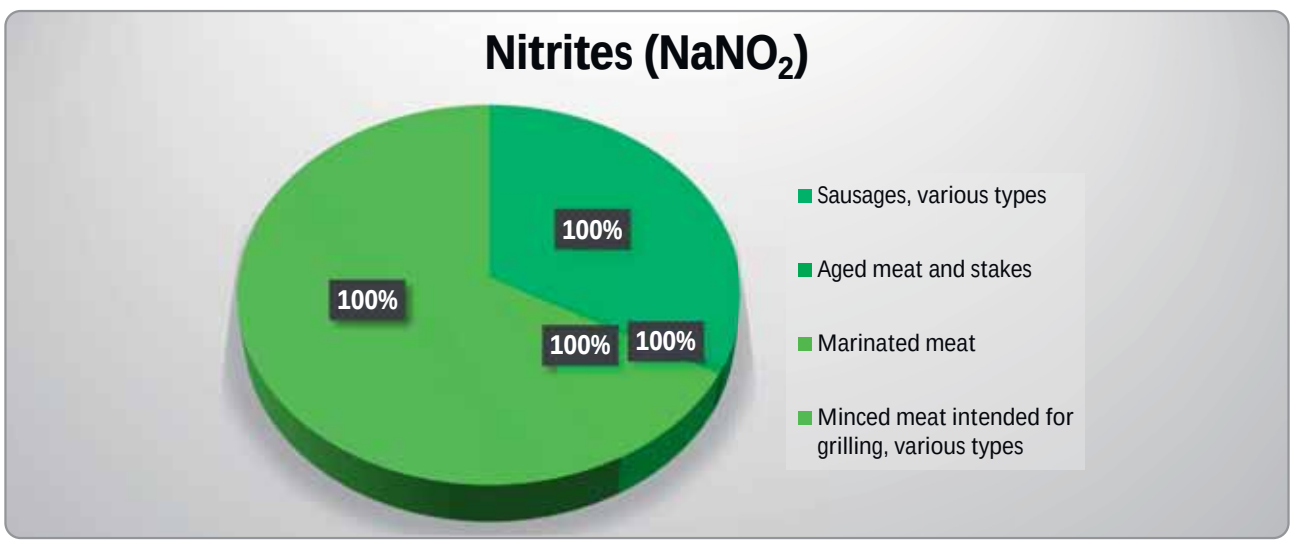


Figure 2. Percentage of samples by type of meat product in which nitrites were not detected

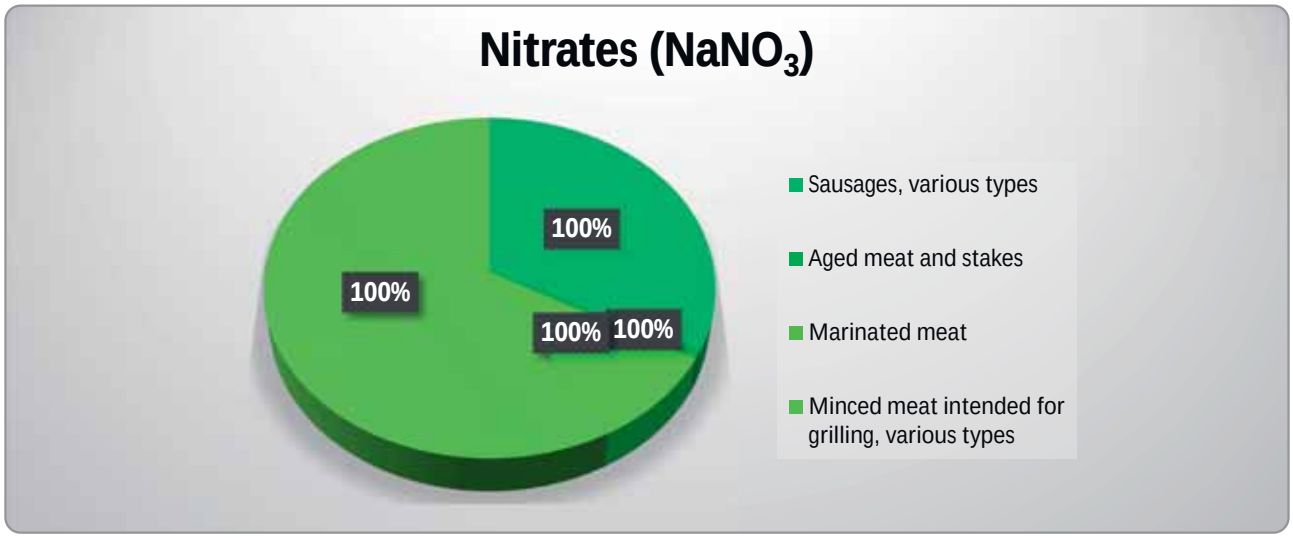


Figure 3. Percentage of samples by type of meat product in which nitrates were not detected

4. Discussion

The results obtained from the analysis of the various types of meat semi-products intended for grilling showed that none of the 724 samples contained nitrites or nitrates. This finding confirms that all the tested samples comply with the Serbian national regulation (*Republic of Serbia*, 2019, 2023), which strictly prohibits the addition of nitrites and nitrates in these types of meat semi-products. The absence of nitrites and nitrates in these samples suggests that the producers and manufacturers have complied with the regulations, ensuring that these meat semi-products are safe for consumption without the need for added nitrites or nitrates. This finding is significant from a food safety perspective, as excessive consumption of nitrites and nitrates has been associated with potential health risks, including the formation of potentially carcinogenic compounds.

5. Conclusion

The successful adherence to the regulation indicates the effectiveness of the monitoring and control measures implemented by the regulatory authorities and the food industry in Serbia. It showcases their commitment to ensuring the safety and quality of meat semi-products intended for grilling. It is important to note that the absence of nitrites and nitrates in these samples does not imply a lack of other potential contaminants or food safety concerns. It is crucial to continue monitoring and conducting regular quality assessments to ensure compliance with other safety parameters, such as microbial contamination, storage conditions, and labelling requirements.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M. T., Cerretani, L. & Zanardi, E. (2015). Nitrite, nitrate and other oxides of nitrogen in cured meat products and their effects on human health. In A. M. Holban, A. M. Grumezescu & M. V. Grumezescu (Eds.), Food Chemistry, Function and Analysis (pp. 311–336), Academic Press.

Cross, A. J., Sinha, R. & Ward, M. H. (2010). Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environmental and Molecular Mutagenesis*, 51(9), 947–963, https://doi.org/10.1002/em.20585

Goulas, A. E., Valero-Cases, E., Atalay, A. & Özogul, F. (2021). Nitrites, nitrates, and nitrosamines in processed meat products. In E. N. Voutsinas (Ed.), Processed Meats: Improving Safety, Nutrition, and Quality (pp. 65–89), Academic Press.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78(1–2), 68–76, https://doi.org/10.1016/j.meatsci.2007.05.030

International Standard Organization — ISO 2918:1975, (1975a). Meat and meat products — Determination of nitrite content (Reference method).

International Standard Organization — ISO 3091:1975, (1975b). Meat and meat products — Determination of nitrate content (Reference method).

Katsanidis, E., Goulas, A. E. & Kontominas, M. G. (2015). The effect of production process on the levels of nitrites, nitrates and residual hydrogen peroxide in traditional Greek cured meat products. *Food Chemistry*, 171, 361–367, https://doi.org/10.1016/j.foodchem.2014.08.075

Loh, Y. H., Jakszyn, P., Luben, R. N., Mulligan, A. A., Mitrou, P. N., Khaw, K. T. & Cross, A. J. (2020). N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *The American Journal of Clinical Nutrition*, 112(4), 934–943, https://doi.org/10.1093/ajcn/nqaa207

Mashima, K. & Nakazato, Y. (2020). Methemoglobinemia: a rare complication of nitrite poisoning. *Clinical Practice and Cases in Emergency Medicine*, 4(2), 204–207, https://doi.org/10.5811/cpcem.2020.2.45279

Official Gazette of the Republic of Serbia, (2019). Regulation on the quality of ground meat, meat preparations and meat products. *Official Gazette of the Republic of Serbia*, 50/2019.

Official Gazette of the Republic of Serbia, (2023). Regulation on the quality of ground meat, meat preparations and meat products. *Official Gazette of the Republic of Serbia*, 34/2023.

Sebranek, J. G. & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Science*, 77(1), 136–147, https://doi.org/10.1016/j.meatsci.2007.04.016

Stajić, S., Stanišić, N., Tasić, T., Pavlović, V., Petrović, L., Tomašević, I.... & Tomašević, M. (2020). Content of nitrates and nitrites in various types of meat products. *Quality Assurance and Safety of Crops & Foods*, 12(2), 135–142, https://doi.org/10.3920/QAS2020.1924

World Health Organization, (WHO). (2010). Nitrate and nitrite in drinking-water. World Health Organization. Retrieved from https://www.who.int/water_sanitation_health/publications/nitrite_drinking_water_fulltext.pdf

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.4.09:616.993

ID: 126500873

https://doi.org/10.18485/meattech.2023.64.2.64

Original scientific paper

Prevalence and main factors for Salmonella spreading in wild boars — a risk for food safety

Jelena Petrović[a], Jovan Mirčeta[b] and Jasna Prodanov-Radulović[a]

[a] *Scientific Veterinary Institute Novi Sad, Rumenacki put 20, 21000 Novi Sad, Serbia*
[b] *JP Vojvodinašume, Preradovićeva 2, 21132 Petrovaradin, Serbia*

ARTICLE INFO

Keywords:
Wild boars
Salmonella
Prevalence
Epidemiology
Anthropogenic influence

ABSTRACT

Salmonella is not a priority pathogen for wild boar health. However, it poses a hazard for meat safety. This paper presents the results of our multi-year research on the prevalence and epidemiology of *Salmonella* in hunting grounds in Vojvodina, Serbia. In total, 425 wild boars (25.3% of the total population) were studied. The overall *Salmonella* prevalence in Vojvodina boars was not high (3.1%) and was quite similar to findings from Spain, Germany and Japan. However, the prevalence in some hunting grounds was very high (13.3–33.3%). The anthropogenic impact is significant, as the prevalence is statistically significantly higher in open hunting grounds where animals have contact with domestic animals and access to animal waste. The pulsotype (PFGE) profiles confirmed a link between isolates from wild pigs and domestic animals. The category of wild boars in which *Salmonella* was most commonly found was sows older than 36 months and weighing more than 75 kg, which is a direct consequence of their increased need for protein during the lactation period when they exhibit scavenging and cannibalistic behaviour.

The measures taken against *Salmonella* in hunting grounds need to incorporate biosecurity measures that prevent anthropogenic influence. The hygienic and sanitary measures for the control of caught animals should also include enhanced measures when processing risk categories.

1. Introduction

Game meat is a highly biologically valuable foodstuff. It is not consumed often in Serbia, but it is present in the population's diet. Hunting tourism has the greatest commercial importance in our hunting grounds. However, with the opening of plants for the processing and packaging of game meat and increased interest in game farming, the availability of game meat to a wide range of consumers is increasing. Wild boar meat is associated with numerous hazards, such as microbial (*Trichinella*, *Toxoplasma*, *Alaria alata*, *Salmonella*, *Campylobacter*) (*Petrović et al.*, 2018; *Petrović et al.*, 2019; *Petersen et al.*, 2020; *Castillo-Contreras et al.*, 2022) and chemical hazards (pesticides, heavy metals) (*Petrović et al.*, 2021). Although it is considered that *Salmonella* is not a priority pathogen in wild pigs, it is certainly a relevant pathogen in the control of game meat safety. Biosecurity measures taken in the hunting grounds and proper hygiene procedures for hunted animals prevent meat contamination (*Mirčeta and Petrović*, 2020). Since the main source of contamination of wild boar carcasses is faeces, the study of the prevalence of *Salmonella* in hunting grounds directly indicates the risk for meat safety. Therefore, the aim of this paper was

***Corresponding author:** Jelena Petrović, *jelena@niv.ns.ac.rs*

Paper received Jun 9th 2023. Paper accepted Jun 20th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

to present the results of the research on the prevalence and risk factors that lead to the spread of *Salmonella* in wild boars in the hunting grounds of Vojvodina.

2. *Salmonella* prevalence in Vojvodina hunting grounds

We studied wild boar carcasses during two hunting seasons in Vojvodina region (*Petrović et al.*, 2022). The hunting grounds Vojvodina are specific in comparison with hunting grounds in mountainous regions. Vojvodina is flat, without major natural obstacles, but near the hunting grounds are human settlements having a population of domestic animals as well as industrial plants. The study included 12 hunting grounds: 10 fenced and two open hunting grounds. The total number of wild boars in all hunting estates during the two year study was estimated at 1,677, out of which 425 wild boars were examined, i.e. 25.3% of the total population. All animals were shot and sampled during official hunts

Salmonella was detected in eight hunting grounds (66.7% of the total number), with the prevalence ranging from 1.7% up to 33.3%. As can be seen in Table 1, where data from around the world are presented, the prevalence of *Salmonella* in wild pigs is highly variable and ranges from 0% (Denmark) to 43.9% (USA) (*Petersen et al.*, 2021; *Cummings et al.*, 2016). The low prevalence found in Serbia (3.1%) is similar to the prevalence found in Germany, 2.4% (*Plaza-Rodríguez et al.*, 2021), in some studies from Spain (2.9%–3.1%) (*Gil Molino et al.*, 2019; *Castillo-Contreras et al.*, 2022), and in Japan, 5.0% (*Sasaki et al.*, 2013).

3. Risk factors that affect the spread of *Salmonella* in hunting grounds

In the hunting grounds of Vojvodina (*Petrović et al*., 2022), differing prevalences of *Salmonella* were found (Table 2). In half of the hunting grounds a low prevalence was detected (1.5–3.1%). There was no *Salmonella* in four hunting grounds, while

Table 1. Prevalences and serovars of *Salmonella* in wild boar faeces (*Altissimi et al.*, 2023)

Prevalence	Frequency	Country	Serotype	Reference
3.1%	13/425	Serbia	*S*. Enteritidis, *S*. Infantis, *S*. Typhimurium	*Petrović et al.*, 2022
3.1%	4/130	Spain	*S*. Typhimurium, *S*. Bardo, *S*. Enteritidis	*Castillo-Contreras et al*., 2022
35.6%	32/90	Italy	*S*. Abony, *S*. Newport, *S*. Agona, *S*. Derby,	*Piras et al*., 2021
2.4%	13/562	Germany	*S*. Typhimurium, *S*. Enteritidis, *S*. Stanleyville	*Plaza-Rodríguez et al*., 2021
0%	0/115	Denmark	/	*Petersen et al.*, 2021
2.98%	25/838	Spain		*Gil Molino et al.*, 2019
43.9%	194/442	USA	*S*. Montevideo, *S*. Newport, *S*. Give	*Cummings et al.*, 2016
1.1%	1/88	Sweden	/	*Sannö et al.*, 2014
5%	2/40	Japan	*S*. Agona, *S*. Narashino, *S*. Enteritidis, *S*. Havana, *S*. Infantis, *S*. Thompson	*Sasaki et al.*, 2013
0.3%	1/333	Spain	*S*. Bardo, *S*. Montevideo, *S*. arizonae, *S*. Typhimurium	*Díaz-Sánchez et al.*, 2013
10.8%	54/499	Italy	*S*. Salamae, S. Diarizonae, *S*. Houtenae, *S*. Fischerhuette	*Zottola et al.*, 2013
24.82%	326/1313	Italy	*S. enterica* subsp. *enterica*	*Navarro-Gonzalez et al.*, 2012
15.4%	33/214	Spain	/	*Ranucci et al.*, 2021

Table 2. Prevalence of *Salmonella* in each hunting ground (HG) (*Petrović et al*., 2022)

Hunting ground	No of animals in HG per year	Examined animals (% in HG)	*Salmonella* positive animals (prevalence, %)
A	180	63 (35.0)	2 (3.2)
B	160	26 (16.3)	0
C	210	59 (28.1)	1 (1.7)
D	82	12 (14.6)	4 (33.3)
E	340	66 (19.4)	1 (1.5)
F	210	57 (27.1)	1 (1.8)
G	150	48 (32.0)	1 (2.1)
H	220	26 (11.8)	0
I	55	32 (58.2)	1 (3.1)
J	20	10 (50.0)	0
K*	35	15 (21.4)	2 (13.3)
L*	15	11 (73.3)	0
TOTAL	**1677**	**425 (25.3)**	**13 (3.1)**

* K and L are open hunting grounds and all others are fenced areas

in two hunting grounds, a very high prevalence was established (13.3–33.3%). Similar data were reported in Spain, where *Salmonella* was found in only 12% of hunting grounds and the prevalence ranged from 5% to 33% (*Díaz-Sánchez et al*., 2013). In our research, several epidemiologically significant characteristics of the hunting ground that had a high prevalence (hunting ground K) were observed: this hunting ground is open, i.e. the animals inside move freely to outside the area of the hunting ground; there is a human settlement (a village) in the immediate vicinity and; wild boars were found near the illegal dump.

Open hunting grounds typically have <5 animals/km^2 and are considered low-density hunting grounds. Statistical analysis of our data proved that the prevalence of *Salmonella* was significantly higher in the open hunting grounds compared to the fenced ones, although some authors believe that the prevalence of pathogens is higher in high-density hunting grounds (*Ortega et al*., 2020). However, the presence of high-risk factors, such as the illegal dump where the carcasses of domestic animals are dumped as well as the entrails after home slaughter, in this case poultry, has probably led to the spread of *Salmonella* in the low-density hunting grounds.

Salmonella is most commonly found in wild sows older than 36 months and weighing more than 75 kg, probably as the consequence of the sow behaviour that was already observed in previous studies from Vojvodina (*Prodanov-Radulović et al*., 2020). Due to offspring and lactation, sows have a greater need for proteins, they often roam searching for food and walk long distances, and often exhibit scavenging behaviour. They feed on carcasses of deer, boars, wild birds, rats and different domestic animals, which contributes to the transmission of pathogens. Other authors also believe that older animals carry pathogens more often, primarily due to longer exposure (*Closa-Sebastià et al.*, 2011).

4. *Salmonella* epidemiology

In open hunting areas on mountains, usually lacking a relevant domestic pig population, wild boars act as a host for *Salmonella* and some other food borne pathogens (*Mycobacterium*, *Leptospira*, *Erysipelothrix*), maintaining an active infection focus and pathogen circulation (*Cano-Manuel et al.*, 2014; *Prodanov-Radulović et al.*, 2020). However, in flat geographical areas — like Vojvodina — the situation is different. The identical and highly similar pulsed-field gel electrophoresis (PFGE) profiles found in wild boars and domestic pigs and poultry indicate the existence of molecular and potential epidemiological links. Generally, wild boars could

easily came into contact with domestic animals due to the lowland nature of the Vojvodina terrain. In our studies (*Petrović et al.*, 2019; *Petrović et al.*, 2014), it was concluded that one zoonotic pathogen (*Trichinella spiralis*) in Vojvodina circulates from domestic pigs to wild boars and *vice versa* in the opposite direction. Our conclusions are further supported by studies on wild boars living near human settlements in Spain, where the presence of anthropogenic *Salmonella* and *Campylobacter* spp. in wild boars has been confirmed (*Castillo-Contreras et al.*, 2022).

5. Importance for meat safety

Wild game meat hygiene is specific and differs from that of farmed animals — it primarily relies on the hunting ground management and the training and skills of hunt participants (*Mirčeta and Petrović*, 2020). Critical points in the hunting process and subsequent carcass processing are shooting, evisceration, skinning and cooling, whereas major sources of microbial contamination of carcasses are gut contents and skin/hair of game animals (*Mirceta et al.*, 2017).

6. Conclusion

The overall prevalence of *Salmonella* in Vojvodina hunting grounds is not high (3.1%). However, there are hunting grounds with a very high prevalence (up to 33.3%), which is a direct consequence of anthropogenic influence. Important risk factors include the following: proximity to human settlements, proximity to farms with terrestrial domestic animals (pigs, poultry, ruminants), and illegally disposed carcasses of domestic animals and waste after home slaughter. Research also points to a high-risk category of wild boar sows older than 36 months and weighing more than 75 kg. Therefore, measures taken to control the presence of *Salmonella* in Vojvodina's hunting grounds need to include suitable biosecurity measures that prevent potential anthropogenic influence, while hygienic and sanitary measures for control of hunted animals need to incorporate better surveillance in the processing of sows.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

References

Altissimi, C., Noé-Nordberg, C., Ranucci, D. & Paulsen, P. (2023). Presence of foodborne bacteria in wild boar and wild boar meat—a literature survey for the period 2012–2022. *Foods*, 12(8),1689.

Cano-Manuel, F. J., Lopez-Olvera, J., Fandos, P., Soriguer, R. C., Pérez J. M. & Granados, J. E. (2014). Long-term monitoring of 10 selected pathogens in wild boar (*Sus scrofa*) in Sierra Nevada national park, southern Spain. Veterinary Microbiology, 174(1–2), 148–154.

Castillo-Contreras, R., Marín, M., López-Olvera, J. R., Ayats, T., Fernandez Aguilar, X., Lavín, S., Mentaberre, G. & Cerdà-Cuéllar, M. (2022). Zoonotic *Campylobacter* spp. and *Salmonella* spp. carried by wild boars in a metropolitan area: Occurrence, antimicrobial susceptibility and public health relevance. *Science of the Total Environment*, 822, 153444. DOI: 10.1016/j.scitotenv.2022.153444

Closa-Sebastià, F., Casas-Díaz, E., Cuenca, R., Lavín, S., Mentaberre, G. & Marco, I. (2011). Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *European Journal of Wildlife Research*, 57(4), 977–981.

Cummings, K. J., Rodriguez-Rivera, L. D., Grigar, M. K., Rankin, S. C., Mesenbrink, B. T., Leland, B. R. & Bodenchuk, M. J. (2016). Prevalence and characterization of *Salmonella* isolated from feral pigs throughout Texas. *Zoonoses and Public Health*, 63, 436–441.

Díaz-Sánchez, S., Sánchez, S., Herrera-León, S., Porrero, C., Blanco, J., Dahbi, G., Blanco, J. E., Mora, A., Mateo, R. & Hanning, I. (2013). Prevalence of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacte*r spp. in large game animals intended for consumption: Relationship with management practices and livestock influence. *Veterinary Microbiology*, 163, 274–281.

Gil Molino, M., García Sánchez, A., Risco Pérez, D., Gonçalves Blanco, P., Quesada Molina, A., Rey Pérez, J., Martín Cano, F. E., Cerrato Horrillo, R. Hermoso-de-Mendoza Salcedo, J.; Fernández Llario, P. (2019). Prevalence of *Salmonella* spp. in tonsils, mandibular lymph nodes and faeces of wild boar from Spain and genetic relationship between isolates. *Transboundary and Emerging Diseases*, 66, 1218–1226.

Mirceta, J., Petrovic J., Malesevic M., Blagojevic B. & Antic, D. (2017). Assessment of microbial carcass contamination of hunted wild boars. *European Journal of Wildlife Research*, 63, Article 37, DOI 10.1007/s10344-017-1096-3

Mirčeta, J. & Petrović, J. (2020). The control methods for improvement of microbial contamination of wild boar meat in Serbia. *Balkan Journal of Wildlife Research,* 5(1), 21–25.

Navarro-Gonzalez, N., Mentaberre, G., Porrero, C. M., Serrano, E., Mateos, A., López-Martín, J. M., Lavín, S. & Domínguez, L. (2012). Effect of cattle on *Salmonella* carriage, diversity and antimicrobial resistance in free-ranging wild boar (*Sus scrofa*) in Northeastern Spain. *PLoS ONE*, 7, e51614.

Ortega, N., Fanelli, A., Serranoa, A., Martinez-Carrasco, C., Wscribano, F., Tizzani, P. & Candela, M. (2020). *Salmonella* seroprevalence in wild boar from Southeast Spain depends on host population density. *Research in Veterinary Science*, 132, 400–403.

Petersen, H. H., Takeuchi-Storm, N., Enemark, H. L., Nielsen, S.T., Larsen, G. & Chriél, M. (2020). Surveillance of Important Bacterial and Parasitic Infections in Danish Wild Boars (*Sus scrofa*). *Acta Veterinaria Scandinavica*, 62, 41.

Petrovic, J., Grgic, Z., Pusic, I. & Urosevic, M. (2014). Sylvatic trichinellosis in the Vojvodina region (Serbia), in: Paulsen, P., Bauer, A., Vodnansky, M., Winkelmayer, R., Smulders, F. J. M. (Ed.), Trends in game meat hygiene. Wageningen Academic Publishers, Wageningen, 175–181.

Petrović, J., Malešević, M., Mirčeta, J., Prodanov-Radulović, J., Ratajac, R., Stojanov, I. & Pušić I. (2018). Consumption of game meat: Risk of infection caused by *Alaria alata*. *Ecologica*, 25(91), 594–598.

Petrović, J., Grgić, Ž., Prodanov Radulović, J., Ratajac, R., Urošević, M., Pustahija, T. & Medić S. (2019). Epidemiology of human trichinellosis in Vojvodina Province, Serbia, from 2015 to 2016. *Acta Veterinaria Hungarica*, 67(1), 40–50.

Petrović J., B., Kartalović, J., Mirčeta, J., Prodanov-Radulović, R., Ratajac & Mastanijević, K. (2021). Organochlorine pesticides and NDL-PCBs in wild boars from flatland region with intensive agricultural activities. *Food Additives & Contaminants: part B*, https://doi.org/10.1080/19393210.2021.1976287

Petrović, J., Mirčeta, J., Babić, J., Malešević, M., Blagojević, B., Prodanov Radulović, J. & Antić D. (2022). S*almonella* in wild boars (*Sus scrofa*): Characterization and epidemiology. *Acta Veterinaria Belgrade*, 72(2), 184–194.

Piras, F., Spanu, V., Siddi, G., Gymoese, P., Spanu, C., Cibin, V., Schjørring, S., De Santis, E. P. L. & Scarano, C. (2021). Whole-genome sequencing analysis of highly prevalent *Salmonella* serovars in wild boars from a national park in Sardinia. *Food Control*, 130, 108247.

Plaza-Rodríguez, C., Alt, K., Grobbel, M., Hammerl, J. A., Irrgang, A., Szabo, I., Stingl, K., Schuh, E., Wiehle, L. & Pfefferkorn, B. (2021). Wildlife as sentinels of antimicrobial resistance in Germany? *Frontiers in Veterinary Science*, 7, 627821.

Prodanov-Radulović, J., Vučićević, I., Polaček, V. & Aleksić-Kovačević, S. (2020). Current swine respiratory diseases morphology in intensive swine production in Serbia. *Acta Veterinaria Belgrade*, 70(1), 1–36.

Ranucci, D., Roila, R., Onofri, A., Cambiotti, F., Primavilla, S., Miraglia, D., Andoni, E., Di Cerbo, A. & Branciari, R. (2021). Improving hunted wild boar carcass hygiene: Roles of different factors involved in the harvest phase. *Foods*, 10, 1548.

Sannö, A., Aspán, A., Hestvik, G. & Jacobson, M. (2014). Presence of *Salmonella s*pp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis* and *Escherichia coli* O157:H7 in wild boars. *Epidemiology and Infection*, 142, 2542–2547.

Sasaki, Y., Goshima, T., Mori, T., Murakami, M., Haruna, M., Ito, K. & Yamada, Y. (2013). Prevalence and antimicrobial susceptibility of foodborne bacteria in wild boars (*Sus scrofa*) and wild deer (*Cervus nippon*) in Japan. *Foodborne Pathogens and Diseases*, 10, 985–991.

Zottola, T., Montagnaro, S., Magnapera, C., Sasso, S., De Martino, L., Bragagnolo, A., D'Amici, L., Condoleo, R., Pisanelli, G. & Iovane, G. (2013). Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region—Italy. *Comparative Immunology, Microbiology and Infectious Diseases*, 36, 161–168.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 664.933:636.592

ID: 126547977

https://doi.org/10.18485/meattech.2023.64.2.65

Original scientific paper

Investigating the influence of rosehip tea marination on lipid oxidation in turkey breast meat

Meltem Serdaroğlu[a], Özlem Yüncü-Boyacı[a], Merih Karaman[a] and Hülya Serpil Kavuşan[a]*

[a] Ege University, Engineering Faculty, Food Engineering Department, 35100 Bornova, İzmir, Turkey

ARTICLE INFO

Keywords:
Turkey meat
Marination
Color
Rosehip
Antioxidant

ABSTRACT

The utilization of natural antioxidants has emerged as a potential strategy to inhibit or delay lipid oxidation in meat and meat products. Rosehip, the fruits of *Rosa canina*, has gained attention as a rich source of bioactive compounds with potent antioxidant properties. In this study, turkey breast slices were marinated in rosehip infusion prepared with varying amounts of rosehip powder (6.67% (R1), 10% (R2), and 13.33% (R3)). Total phenolic content and DPPH activity of the prepared marinades were found to be high. As a result of marinating turkey breast samples with rosehip infusion, a significant decrease in TBARS values was observed. For peroxide values, the marinating process was found to be effective starting from day 5 of storage. The pH of samples fluctuated during storage. Also, rosehip marination caused significant changes in the color parameters of the samples. While L* values of the turkey breast slices marinated with rosehip infusion decreased, a* and b* values increased. As a result, it was concluded that rosehip infusion can be used as a natural antioxidant in meat products.

1. Introduction

Turkey breast meat has gained popularity as a healthy alternative to other meats due to its high protein content (23.03–27.59%) and low fat content (0.42–3.08%). This nutritional profile makes it an attractive choice for health-conscious individuals (*Çelen et al.*, 2016; *Oblakova et al.*, 2016). Additionally, turkey breast meat is a good source of vitamins and minerals, including niacin, vitamin B6, phosphorus and selenium, which play important roles in supporting various body functions and promoting overall health. Despite the high nutritional value of turkey meat, its consumption remains limited, as the low fat content and tenderness of turkey breast meat have a negative impact on consumer preference.

Marination is a widely employed technique used to enhance the market share of meat products. Marination is a processing technique, traditionally used to improve organoleptic qualities and water retention of the meat while prolonging shelf-life (*Alvarado et al.*, 2007; *Kaewthong and Wattanachant*, 2018; *Çınar*, 2022). It offers various benefits such as improving aroma and flavour, enhancing tenderness and correcting color defects (*Barbanti and Pasquini*, 2005). Several studies have reported that marinating turkey breasts leads to improvements in textural and sensory parameters also retards oxidative changes (*Serdaroğlu et al.*, 2007; *Gök and Bor*, 2016; *Augustyńska-Prejsnar et al.*, 2021). Another description of marination in terms

***Corresponding author:** Meltem Serdaroğlu, *meltem.serdaroglu@ege.edu.tr*

Paper received Jun 9th 2023. Paper accepted Jun 21st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

of morphology is influencing the pH of the tissue to move away from its isoelectric point (around 5.2–5.3 for red meat), creating space between the myofilaments to retain sufficient water (*Önenç et al*., 2004). Wine, vinegar, lemon juice, fruit juices, milk, fermented milk products, oils and salt have been widely used for marinating different kinds of meat (*Goli et al.*, 2011). In addition to these main components, the utilization of fruit and vegetable juices and extracts in marinade solutions has also been investigated because of their antioxidant and antimicrobial properties *(Nile and Park*, 2014; *Afrin et al*., 2016; *Kalaycıoğlu and Erim*, 2017; *Saricaoglu et al.,* 2019; *Van de Velde et al.*, 2019; *Sengün et al.*, 2020).

Rosehip is the pseudo-fruit of the rose bush. Especially *Rosa canina* L. is abundantly rich and considered to be an excellent source of polyphenols and vitamin C (*Fan et al*., 2014). Rosehip contains 2–3 times as much ascorbic acid as kiwi, 3–5 times as much as peppers, and 5–6 times as much as citrus fruits (*Karhan et al.*, 2004). The high content of polyphenols, particularly flavonoids and phenolic acids, in rosehip tea contributes to its antioxidative potential (*Fan et al.*, 2014). These compounds have been reported to scavenge free radicals, chelate metal ions and inhibit lipid peroxidation. Studies investigating the effect of r osehip on lipid oxidation in various food matrices have shown promising results (*İlyasoğlu*, 2014; *Rivera Toapanta et al.*, 2022; *Vlaicu et al.,* 2022). However, there is limited research available regarding the use of rosehip infusion for marinating meat products, specifically turkey breast meat. Therefore, the objective of this study is to investigate the impact of marinating turkey breast meat with rosehip infusion on lipid oxidation, with the aim of assessing its potential as a natural antioxidant strategy to enhance meat quality and extend shelf life.

2. Materials and methods

2.1. Materials

The fresh skinless turkey breast meat was purchased from a local producer in İzmir. The breast samples were received free of visible blood spatter or bruises and had a pH of 5.91 to 5.93. Dried rosehips were obtained from a local herbalist in Izmir. All chemicals used were of analytical grade (Sigma-Aldrich Chemie GmbH, Germany).

2.1.1. Preparation of rosehip infusion

The dried rosehip fruits were ground using a Waring 8011 EB SET2 blender (Stamford, CT) at 2nd speed for 30 sec. The ground rosehips were wrapped in filter paper to obtain the determined concentrations. Rosehip powder was added to distilled water at 100°C at concentrations of 6.67% (10 g/150 mL) (R1), 10% (15 g/150 mL) (R2), and 13.33% (20g/150 mL) (R3) and infused for 30 min. The analysis of the obtained infusion was conducted once it had cooled to room temperature.

2.1.2. Marination and cooking process

The turkey breast meat underwent slicing into slices measuring 1 cm in thickness, 13 cm in length, with an average weight ranging from 100 g to 150 g. Meat slices were randomly submerged in marinade solutions containing various concentrations of rosehip infusion (C, R1, R2, and R3) at a ratio of 1:1 (meat:marinade) in plastic bags. The slices were then left in the marinade solution for 4 hours at 1°C to allow the ingredients to permeate the meat. The control treatment consisted of distilled water only. The samples were then vacuum-packed and sous-vide cooked (WiseBath, Germany) at 80°C until the core temperature reached 73°C. The samples were rapidly cooled to room temperature and then stored at 1°C for 7 days for further evaluation including pH measurement, color evaluation, peroxide analysis and thiobarbituric acid reactive substances (TBARS) analyses.

2.1.3. Analyses

The determination of the total phenolic content (TPC) in the rosehip infusion was carried out utilizing the Folin-Ciocalteu method (*Yılmaz et al*., 2015). DPPH analysis was performed by modification of the methods applied by *Grajeda-Iglesias et al.* (2016). Triplicate measurements of pH values were conducted using a WTW pH 3110 set 2 pH meter from Germany. Color parameters of the turkey slices were determined using a digital colorimeter (Chromameter CR 400, Minolta, Japan) to obtain the color parameters including lightness (L*), redness (a*), and yellowness (b*). The assessment of lipid oxidation was performed by analysing peroxide (*AOAC*, 2012) and TBARS (*Witte et al*., 1970). The impact of rosehip infusion and storage conditions on turkey breast slices was assessed using analysis of variance (ANOVA) followed by Duncan's post-hoc tests in the SPSS software.

3. Results and discussion

3.1. Analysis of the marinade solutions

Table 1 provides a summary of the phenolic content, DPPH values, and pH of the marinade solutions. The marinades exhibited a range of pH 3.58 to pH 3.71. As the concentration of rosehip in the infusion increased, the pH decreased due to the higher acidity. The total phenolic content (TPC) ranged from 138.47 to 241.13 mg GAE/g, while the DPPH values ranged from 33.58 to 52.67 µmolTE/g. Our TPC resth the findings reported in the literature (*Koczka et al*., 2018). Furthermore, both TPC and DPPH values showed an increasing trend with the rise in rosehip concentration in the infusion. This observation aligns with the findings of a previous study (*Orhan et al*., 2012).

3.2. Color

Color parameters of meat samples are given in Table 2. The marination process led to significant alterations in the color parameters ($P<0.05$). The L* values of marinated meat were lower than those of the control group ($P<0.05$), indicating a darker appearance of the samples. This finding is consistent with a previous study where camel meats marinated with ginger extract and citric acid also exhibited lower L* values (*Moeini et al.*, 2022). In contrast, the a* and b* values showed a significant increase ($P<0.05$) compared to the control group (C), indicating a shift towards more red and yellow colours. This color change can be attributed to the natural pigments present in the rosehip infusion, which imparts a red color to the marinade solution. During stor-

Table 1. The pH values, DPPH and total phenolic contents of marinades

Marinade	pH	Total phenolic content (mg GAE/g)	DPPH (µmolTE/g)
R1	3.71	138.47	33.58
R2	3.63	165.13	47.58
R3	3.58	241.13	52.67

R1: 6.67% rosehip tea, R2: 10% rosehip tea, R3: 13.33% rosehip tea

Table 2. Effect of marinade treatments on color parameters

Treatments*	Storage	C	R1	R2	R3
L*ults were consistent wi	0	$67.52\pm1.57^{a,Y}$	$60.63\pm1.25^{b,X}$	$61.21\pm1.19^{b,X}$	$52.93\pm0.69^{c,Y}$
	3	$70.98\pm1.74^{a,X}$	$51.53\pm1.15^{c,Y}$	$61.33\pm1.09^{b,X}$	$51.78\pm1.29^{c,Y}$
	5	$73.44\pm1.32^{a,X}$	$61.62\pm1.78^{b,X}$	$55.64\pm0.78^{d,Z}$	$58.10\pm0.61^{c,X}$
	7	$72.76\pm1.39^{a,X}$	$61.15\pm0.65^{b,X}$	$58.40\pm0.17^{c,Y}$	$57.47\pm0.35^{c,X}$
a*	0	$4.53\pm0.26^{c,X}$	$8.75\pm0.27^{b,Y}$	$8.63\pm0.26^{b,Y}$	$9.74\pm0.44^{a,Y}$
	3	$1.58\pm0.33^{c,Z}$	$10.86\pm0.46^{a,X}$	$7.31\pm0.21^{b,Z}$	$10.41\pm0.37^{a,X}$
	5	$0.52\pm0.23^{d,T}$	$6.47\pm0.43^{c,Z}$	$9.40\pm0.28^{a,X}$	$7.71\pm0.34^{b,Z}$
	7	$2.36\pm0.44^{b,Y}$	$6.98\pm0.57^{a,Z}$	$7.31\pm0.48^{a,Z}$	$7.56\pm0.13^{a,Z}$
b*	0	13.87 ± 0.34^{c}	$21.63\pm0.32^{a,X}$	$17.00\pm1.52^{b,Y}$	$21.06\pm0.49^{a,X}$
	3	14.06 ± 0.86^{d}	$21.79\pm0.15^{a,X}$	$16.31\pm0.40^{c,Y}$	$20.30\pm0.63^{b,X}$
	5	13.86 ± 0.55^{c}	$14.88\pm0.97^{c,Y}$	$19.10\pm0.60^{a,X}$	$17.55\pm0.09^{b,Y}$
	7	14.59 ± 0.35^{b}	$14.88\pm0.99^{b,Y}$	$15.55\pm0.48^{b,Y}$	$16.93\pm0.39^{a,Y}$

* C: distilled water. R1: 6.67% rosehip tea. R2: 10% rosehip tea. R3: 13.33% rosehip tea. $^{a-d}$ Different letters in the same row indicate a significant difference ($P < 0.05$). $^{X-T}$ Different letters in the same column indicate a significant difference ($P < 0.05$).

age, the lowest b* value was observed in the control (13.86), while the highest value was observed in the R1 (21.79) (P<0.05), this situation indicates a shift towards a more yellowish hue in groups containing rosehip infusion. Similarly, it has been reported by other researchers that b* values increase with marination (*Augustyńska-Prejsnar et al.*, 2021; *Moeini et al.*, 2022; *Unal et al.*, 2022). *Önenç et al.* (2004) reported that cattle meat samples marinated with citric acid had higher L* and a* values than control.

3.3 pH values

The pH of turkey meat plays a crucial role in determining its quality properties, such as tenderness, water-holding capacity, and microbial stability. Figure 1 presents the pH of the turkey breast slices; the initial pH of samples ranged between 5.75 (R3)–5.90 (C). The meat pH decreased with increasing rosehip concentration in the marinade. In studies using acidic marinade, it was reported that the pH value decreased (*Serdaroglu et al.*, 2007; *Gómez-Salazar et al.*, 2018; *Santos et al.*, 2020; *Cho et al.*, 2021). The R3 treatment had the lowest pH during storage (P<0.05). On day 5 of storage, the pH was increased in all treatment groups compared with at the beginning.

3.4. Lipid oxidation

Marinating with rosehip infusion was highly effective against lipid oxidation of samples. Peroxide values ranged between 1.03–4.10 meqO_2/kg (Table 3). On day 0 and 3, there were no significant differences in peroxide values among the groups (P>0.05). However, on day 5, the R3 group exhibited the highest peroxide value, and on the day 7, the R2 group had the highest value. The fact that the control group had lower peroxide value and higher TBARS value indicates that oxidation progressed faster in this group than in the marinated meat. TBARS values of samples ranged between 0.14–1.91 mg MA/kg. The marination process resulted in a significant reduction in TBARS compared to the control group (P<0.05). The highest value was observed in the control group during storage (P<0.05). The TBARS value, including the control group, was below the accepted limit value (<2 mg MA/kg, *Witte et al.*, 1970) in all samples. This finding indicates that rosehip infusion has antioxidant effects. Consistent with our results, several studies conducted on various meat products and acidic marinades have reported significant reductions in peroxide and/or TBARS values (*Blackhurst et al.*, 2011; *Arcanjo et al.,* 2019; *Rasuli et al.*, 2021). Lipid oxidation is a complex

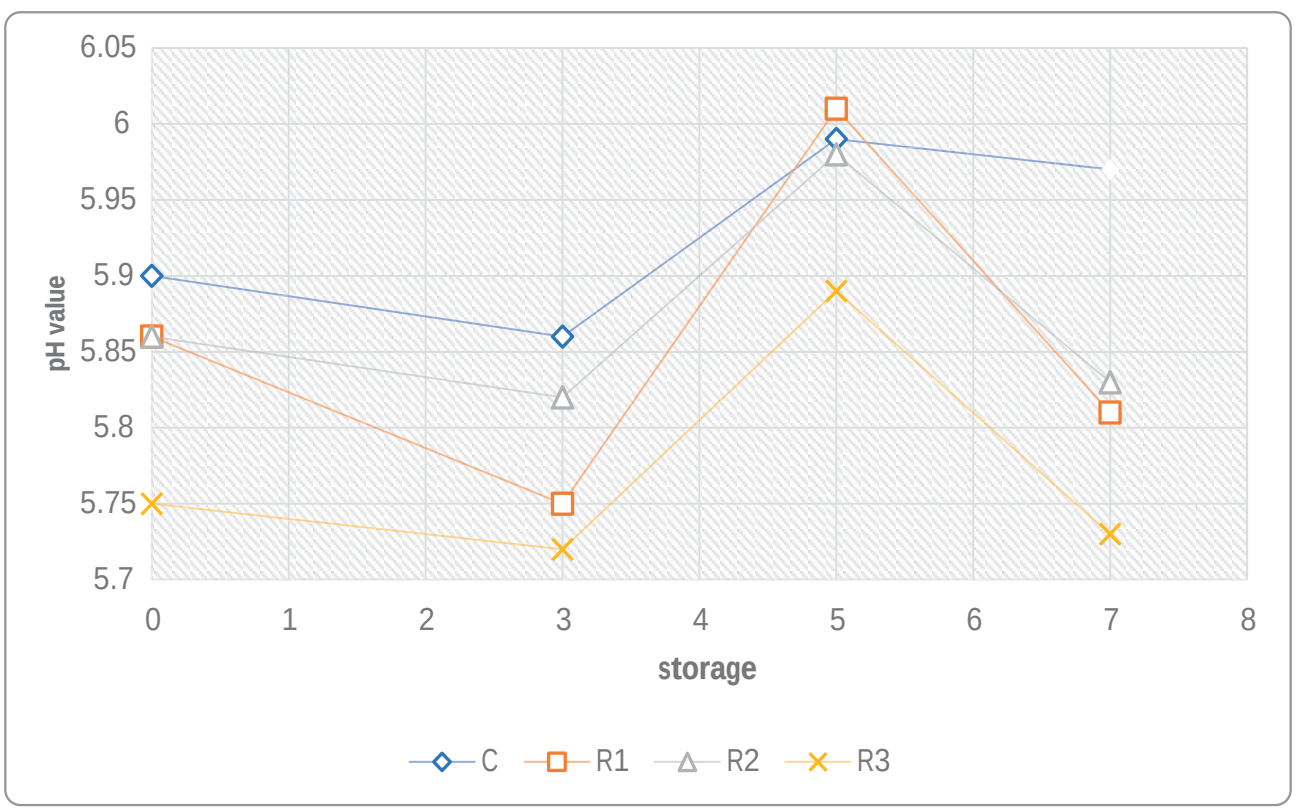


Figure 1. Effect of marinade treatments on meat pH during storage

Table 3. Effect of marinade treatments on lipid oxidation during storage

Lipid oxidation measurement*	Storage (days)	C	R1	R2	R3
Peroxide ($meqO_2$/kg)	0	3.45 ± 0.42^{X}	3.46 ± 0.12^{X}	4.10 ± 0.78^{X}	3.30 ± 0.46^{X}
	3	2.33 ± 0.30^{Y}	2.13 ± 0.81^{Y}	1.80 ± 0.40^{YZ}	2.13 ± 0.46^{Y}
	5	$1.03\pm0.06^{d,Z}$	$1.79\pm0.02^{b,Y}$	$1.57\pm0.06^{c,Z}$	$2.00\pm0.01^{a,Y}$
	7	$2.16\pm0.20^{b,Y}$	$1.33\pm0.10^{c,Y}$	$2.50\pm0.19^{a,Y}$	$1.33\pm0.11^{c,Y}$
TBARS (mg MA/kg)	0	$1.27\pm0.04^{a,Z}$	$0.26\pm0.11^{b,Z}$	$0.29\pm0.01^{b,Z}$	$0.14\pm0.01^{c,T}$
	3	$1.46\pm0.11^{a,Y}$	$0.43\pm0.06^{b,Y}$	$0.24\pm0.01^{c,T}$	$0.25\pm0.01^{c,Z}$
	5	$0.90\pm0.03^{a,T}$	$0.58\pm0.02^{b,X}$	$0.52\pm0.03^{c,Y}$	$0.58\pm0.16^{b,Y}$
	7	$1.91\pm0.02^{a,X}$	$0.63\pm0.01^{c,X}$	$0.64\pm0.01^{c,X}$	$0.67\pm0.01^{b,X}$

TBARS: thiobarbituric acid reactive substances. C: distilled water. R1: 6.67% rosehip tea. R2: 10% rosehip tea. R3: 13.33% rosehip tea. $^{a-d}$ Different letters in the same row indicate a significant difference ($P < 0.05$). $^{X-T}$ Different letters in the same column indicate a significant difference ($P < 0.05$).

chemical process that occurs in meat products, leading to the development of off-flavours, rancidity, and a decline in overall quality (*Gray et al*., 1996).

4. Conclusion

The findings of our study demonstrated that marinating turkey breast slices with rosehip infusion had antioxidative effects, attributed to the high phenolic content of rosehip. The marination process effectively reduced peroxide values after 5 days of storage. Additionally, TBARS values significantly decreased during storage. However, the incorporation of rosehip tea had a notable impact on the color parameters, while pH values decreased with increasing rosehip concentration. Further research should focus on investigating the effects of marinating with rosehip tea on the physicochemical, antimicrobial and technological properties of the product, as well as its application in various meat products.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Afrin, S., Gasparrini, M., Forbes-Hernandez, T. Y., Reboredo-Rodriguez, P., Mezzetti, B., Varela-López, A. & Battino, M. (2016). Promising health benefits of the strawberry: a focus on clinical studies. *Journal of Agricultural and Food Chemistry*, 64(22), 4435–4449.

Alvarado, C. & McKee, S. (2007). Marination to improve functional properties and safety of poultry meat. *Journal of Applied Poultry Research*, 16(1), 113–120.

AOAC, (2012). 19th ed. Association of Official Analytical Chemists. Gaithersburg. MD. USA.

Arcanjo, N. M., Ventanas, S., González-Mohíno, A., Madruga, M. S. & Estévez, M. (2019). Benefits of wine-based marination of strip steaks prior to roasting: inhibition of protein oxidation and impact on sensory properties. *Journal of the Science of Food and Agriculture*, 99(3), 1108–1116.

Augustyńska-Prejsnar, A., Hanus, P., Sokołowicz, Z. & Kačániová, M. (2021). Assessment of technological characteristics and microbiological quality of marinated turkey meat with the use of dairy products and lemon juice. *Animal Bioscience*, 34(12), 2003.

Barbanti, D. & Pasquini, M. (2005). Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat. *LWT-Food Science and Technology*, 38(8), 895–901.

Blackhurst, D. M., Pietersen, R. D., O'Neill, F. H. & Marais, A. D. (2011). Marinating beef with South African red wine may protect against lipid peroxidation during cooking. *African Journal of Food Science*, 5(12), 650–656.

Çelen, M. F., Söğüt, B., Zorba, Ö., Demirulus, H. & Tekeli, A. (2016). Comparison of normal and PSE turkey breast meat for chemical composition. pH, color, myoglobin and drip loss. *Revista Brasileira de Zootecnia*, 45, 441–444.

Cho, J., Kim, H. J., Kwon, J. S., Kim, H. J. & Jang, A. (2021). Effect of marination with black currant juice on the formation of biogenic amines in pork belly during refrigerated storage. *Food Science of Animal Resources*, 41(5), 763.

Çınar, S. (2022). Turunç Suyu (Citrus aurantium L.) ile marinasyonunun tavuk göğüs eti üzerine etkisi (Master's thesis, Fen Bilimleri Enstitüsü).

Fan, C., Pacier, C. & Martirosyan, D. M. (2014). Rose hip (Rosa canina L): A functional food perspective. *Functional Foods in Health and Disease*, 4(12), 493–509.

Gök, V. & Bor, Y. (2016). Effect of marination with fruit and vegetable juice on some quality characteristics of turkey breast meat. *Brazilian Journal of Poultry Science*, 18, 481–488.

Goli, T., Bohuon, P., Ricci, J., Trystram, G. & Collignan, A. (2011). Mass transfer dynamics during the acidic marination of turkey meat. *Journal of Food Engineering*, 104(1), 161–168.

Gómez-Salazar, J. A., Ochoa-Montes, D. A., Cerón-García, A., Ozuna, C. & Sosa-Morales, M. E. (2018). Effect of acid marination assisted by power ultrasound on the quality of rabbit meat. *Journal of Food Quality*, 1–6.

Grajeda-Iglesias, C., Salas, E., Barouh, N., Baréa, B., Panya, A. & Figueroa-Espinoza, M. C. (2016). Antioxidant activity of protocatechuates evaluated by DPPH, ORAC, and CAT methods. *Food Chemistry*, 194, 749–757.

Gray, J. I., Gomaa, E. A. & Buckley, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science*, 43, 111–123.

İlyasoğlu, H. (2014). Antioxidant effect of rosehip seed powder in raw and cooked meatballs during refrigerated storage. *Turkish Journal of Veterinary & Animal Sciences*, 38(1), 73–76.

Kaewthong, P. & Wattanachant, S. (2018). Optimizing the electrical conductivity of marinade solution for water-holding capacity of broiler breast meat. Poultry Science, 97(2), 701–708.

Kalaycıoğlu, Z. & Erim, F. B. (2017). Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide. *Food Chemistry*, 221, 496–507.

Karhan, M., Aksu, M., Tetik, N. & Turhan, I. (2004). Kinetic modeling of anaerobic thermal degradation of ascorbic acid in rose hip (Rosa Canina L) pulp. *Journal of Food quality*, 27(5), 311–319.

Koczka, N., Stefanovits-Bányai, É. & Ombódi, A. (2018). Total polyphenol content and antioxidant capacity of rosehips of some Rosa species. *Medicines*, 5(3), 84.

Moeini, R., Zamindar, N. & Aarabi Najvani, F. (2022). The effect of marination by using ginger extract and citric acid on physicochemical characteristics of camel meat. *Food Science and Technology International*, 10820132221136590.

Nile, S. H. & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. *Nutrition*, 30(2), 134–144.

Oblakova, M., Ribarski, S., Oblakov, N. & Hristakieva, P. (2016). Chemical composition and quality of turkey-broiler meat from crosses of layer light (LL) and meat heavy (MH) turkey. *Trakia Journal of Sciences,* 2, 142–147.

Önenç, A., Serdaroğlu, M. & Abdraimov, K. (2004). Effect of various additives to marinating baths on some properties of cattle meat. *European Food Research and Technology*, 218, 114 –117.

Orhan, D. D., Özlük, Ö. & Coskun, S. H. (2012). Antioxidant capacities. ascorbic acid and total phenol contents of the plants sold as rose hip in Turkey. *FABAD Journal of Pharmaceutical Sciences,* 37(3), 161.

Rasuli, N., Bintoro, V. P., Purnomoadi, A. & Nurwantoro, N. (2021). The shelf life of buffalo meat marinated with pomegranate (Punica granatum) peel extract. *Journal of Advanced Veterinary and Animal Research*, 8(4), 612.

Rivera Toapanta, E. A., Oruna, M. J., Terriente, C., Gonzalez, J., Guerrero, L., Claret Coma, A. & Oliver, A. (2022). Effects of rose hip (Rosa canina L.) extract as a natural ingredient on the nutritional composition, oxidative stability and sensory attributes of raw and cooked pork patties from Majorcan Black pig breed under retail conditions. *Acta Scientific Nutritional Health*.

Santos, D. I., Fraqueza, M. J., Pissarra, H., Saraiva, J. A., Vicente, A. A. & Moldão-Martins, M. (2020). Optimization of the effect of pineapple by-products enhanced in bromelain by hydrostatic pressure on the texture and overall quality of silverside beef cut. *Foods*, 9(12), 1752.

Saricaoglu, F. T., Atalar, I., Yilmaz, V. A., Odabas, H. I. & Gul, O. (2019). Application of multi pass high pressure homogenization to improve stability, physical and bioactive properties of rosehip (Rosa canina L.) nectar. *Food Chemistry*, 282, 67–75.

Serdaroğlu, M., Abdraimov, K. & Onenc, A. (2007). The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of turkey breast. *Journal of Muscle foods*, 18(2), 162–172.

Unal, K., Alagöz, E., Çelik, İ. & Sarıçoban, C. (2022). Marination with citric acid, lemon, and grapefruit affects the sensory, textural, and microstructure characteristics of poultry meat. *British Poultry Science*, 63(1), 31–38.

Van de Velde, F., Esposito, D., Grace, M. H., Pirovani, M. E. & Lila, M. A. (2019). Anti-inflammatory and wound healing properties of polyphenolic extracts from strawberry and blackberry fruits. *Food Research International*, 121, 453–462.

Vlaicu, P. A., Untea, A. E., Turcu, R. P., Panaite, T. D. & Saracila, M. (2022). Rosehip (Rosa canina L.) Meal as a Natural Antioxidant on Lipid and Protein Quality and Shelf-Life of Polyunsaturated Fatty Acids Enriched Eggs. *Antioxidants*, 11(10), 1948.

Witte, V. C., Krause, G. F. & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*, 35(5), 582–585.

Yılmaz, F. M., Karaaslan, M. & Vardin, H. (2015). Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (Prunus cerasus L.) pomace. *Journal of Food Science and Technology*, 52, 2851–2859.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5:639.21
621.798-98-021.146.4

ID: 126558473

https://doi.org/10.18485/meattech.2023.64.2.66

Original scientific paper

Changes of sensory attributes of carp steaks (*Cyprinus carpio*) packaged in vacuum and modified atmosphere

Jelena Babić Milijašević[a], Milan Milijašević[a], Slobodan Lilić[a], Branka Borović[a], Jasna Đinović-Stojanović[a], Jelena Jovanović[a] and Mirjana Lukić[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade, Serbia.*

ARTICLE INFO

Keywords:
Carp steaks
Sensory attributes
Shelf-life
Modified atmosphere packaging
Vacuum packaging

ABSTRACT

Consumers rate fish based on a number of parameters, the most important of which are the consumption safety, nutritional characteristics, taste, smell, color, texture, convenience for culinary processing and preservation. The shelf-life of fresh chilled fish can be extended by vacuum or modified atmosphere packaging. During the past decade, mixtures of gases with high concentrations of carbon dioxide and nitrogen have attracted the attention of researchers, who have been investigating packaging of fish. The aim of this research was to monitor changes of selected sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in modified atmosphere and vacuum during storage at 3±0.5°C and to determine the shelf-life of the products. Sensory evaluation was conducted on 1, 4, 7, 9, 12 and 15 days of storage. Different gas mixtures as well as vacuum packaging did not significantly affect the changes in color and meat texture of carp steaks, and they remained characteristic until the end of the experiment. The freshness and acceptability of fish was most influenced by the average ratings of odor. The shelf-life of carp steaks packaged in the gas mixture consisting of 60% CO_2 and 40% N_2 (MAP1) was 12 days, while samples packaged in a mixture of gases with 40% CO_2 and 60% N_2 (MAP2) were acceptable for 9 days. The shelf-life of carp steaks packaged in vacuum was 7 days. The gas mixture consisting of 60% CO_2 and 40% N_2 proved to be the most suitable for packaging fresh carp steaks regarding the selected sensory characteristics of odor, meat texture, meat color and overall acceptability.

1. Introduction

The fact that fresh fish is a very perishable food (pH> 6.0; a_w> 0.98) has influenced the producers to focus on finding the optimal method for fish preservation. In recent years, however, consumers worldwide increasingly demand that they have fresh fish at all times, since it is fresh fish that has the most acceptable sensory attributes. This trend has led to the development of the effective concept of modified atmosphere packaging (MAP), thus ensuring a longer shelf-life of fish and preserving the basic parameters of its freshness (*Gimenéz et al., 2002*).

Consumers rate fish based on a number of parameters the most important of which are the consumption safety, nutritional characteristics, taste, smell, color, texture, convenience for culinary processing and preservation.

Changes in fish meat begin at the moment fish dies, or already at the time of the catch, and are the result of the activities of their own enzymes, the metabolism of microorganisms and the oxidation

***Corresponding author:** Jelena Babić Milijasević, *jelena.babic@inmes.rs*

Paper received Jun 16th 2023. Paper accepted Jun 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

of lipids. Changes in the sensory characteristics of the fish result from microbial development. The decomposition of food ingredients and the growth of microorganisms cause an unpleasant smell and taste as well as the production of visible pigmented or unpigmented colonies. The synthesis of polysaccharide extracellular materials and diffuse pigments results in sensory changes in the form of mucus formation and discoloration (*Gram & Huss, 1996*). On the other hand, chemical changes such as auto oxidation or enzymatic hydrolysis of fats may cause the rise of unpleasant smell and taste or, in the latter case, the activity of tissue enzymes may lead to unacceptable softening of the fish meat.

The shelf-life of fresh chilled fish can be extended by vacuum or modified atmosphere packaging (MAP) (*Pastoriza et al., 1996*; *Siverstvik et al., 2002*; *Stamatis & Arkoudelos, 2007*; *Milijašević et al., 2010*). The most commonly used gases in modified atmosphere packaging technology are carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2) (*Martinez et al., 2006*). During the past decade, mixtures of gases with high concentrations of CO_2 and N_2 have attracted the attention of researchers, who have been investigating packaging of fish. The shelf-life of fresh chilled fish is relatively short and at temperatures of +2 ± 2 °C, it is about 2 to 3 days. It has been confirmed that packaging of fish in modified atmosphere significantly extends the shelf-life of product (*Masniyom et al., 2002*; *Stamatis & Arkoudelos, 2007*; *Provincial et al., 2010*).

The aims of this research were to monitor changes of selected sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in modified atmosphere and vacuum during storage at 3±0.5°C and to determine the shelf-life of the products.

2. Materials and methods

2.1. Sampling

Common carp (*Cyprinus carpio*) of average body weight 2.50±0.30 kg were obtained from a fishpond where a semi-intensive rearing system was used. Fish were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered and scaled, and carcasses were cut into steaks 2 cm thick and 220 g average weight. Three sample groups of carp steaks were formed. One group of steaks were vacuum packaged and were used as the control. The other two sample groups were packaged in modified atmospheres with different gas ratios: MAP1: 60% CO_2+40% N_2 and MAP2: 40% CO_2+60% N_2. The machine used for packaging was Variovac (Variovac Primus, Zarrentin, Germany), and the material used for packaging was foil OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene, Dynopack, Polimoon, Kristiansand, Norway) with low gas permeability (degree of permeability for O_2 – 3.2 cm^3/m^2/day at 23°C, for N_2 – 1 cm^3/m^2/day at 23°C, for CO_2 – 14 cm^3/m^2/day at 23°C and for steam 15 g/m^2/day at 38°C). The ratio of gas:fish steaks in the packages was 2:1. All fish steaks were stored in the same conditions at 3°C±0.5°C and on days 1, 4, 7, 9, 12 and 15 of storage, sensory testing was performed.

2.2. Sensory evaluation

The sensory evaluation was performed by six trained panelists. Prior the sensory evaluation, samples were taken out from the refrigerator and remained at room temperature for 15 min. For each day of examination, each assessor was provided with portion of carp steaks. The samples were evaluated for overall acceptability, with regard to odor, meat color, and meat texture using a 1–7 intensity score scale, with 7 corresponding to the most liked sample, and 1 corresponding to the least liked sample. The product was defined as unacceptable when a score of less than 3 points was recorded by at least of 50% of the panelists.

2.3. Statistical analysis

The mean values and standard deviations were calculated by using column statistics with the processing of 6 values for each analyzed group. Significant differences between groups were calculated using one-way ANOVA analysis by Tukey's comparative test in the program Microsoft Office Excel (2016). Differences were evaluated as significant at $p<0.05$.

3. Results and discussion

The results of the sensory evaluation of carp steaks are presented in Table 1.

At the beginning of the storage period, meat color, texture, odor and overall acceptability were evaluated with very high scores in all groups of carp steaks. Average scores of color acceptability decreased during the experiment and this was most pronounced in samples packaged in vacuum. Nevertheless, the color of fish meat was acceptable during the entire storage period in all groups of samples.

Table 1. Sensory evaluation of carp steaks packaged under different conditions during the storage period. MAP1: 60% CO_2+40% N_2 and MAP2: 40% CO_2+60% N_2

Sensory parameter	Packaging conditions	Storage time (days)					
		1	4	7	9	12	15
Odor	MAP1	7.0±0.0^a	7.0±0.0^a	6.8±0.4^a	5.1±0.2^a	3.6±0.4^a	1.3±0.4
	MAP2	7.0±0.0^a	7.0±0.0^a	6.6±0.5^a	3.7±0.5^b	1.2±0.3^b	ne
	Vacuum	7.0±0.0^a	6.5±0.5^b	4.7±0.6^b	1,0±0.0^c	ne	ne
Meat texture	MAP1	7.0±0.0^a	6.9±0.2^a	6.1±0.4^a	5.3±0.6^a	4.6±0.4^a	3.8±0.3
	MAP2	7.0±0.0^a	7.0±0.0^a	6.3±0.4^a	5.2±0.8^a	3.7±0.5^b	ne
	Vacuum	7.0±0.0^a	6.8±0.4^a	5.0±0.3^b	3.5±0.5^b	ne	ne
Meat color	MAP1	7.0±0.0^a	6.8±0.4^a	6.4±0.5^a	5.8±0.3^a	5.2±0.4^a	4.5±0.4
	MAP2	7.0±0.0^a	6.7±0.5^a	6.5±0.4^a	5.9±0.5^a	5.0±0.3^a	ne
	Vacuum	6.8±0.4^a	6.9±0.2^a	4.8±0.2^b	4.6±0.6^b	ne	ne
Overall acceptability	MAP1	7.0±0.0^a	7.0±0.0^a	6.5±0.5^a	4.8±0.4^a	4.1±0.2^a	1.2±0.3
	MAP2	7.0±0.0^a	7.0±0.0^a	6.2±0.4^a	4.6±0.5^a	2.2±0.4^b	ne
	Vacuum	7.0±0.0^a	6.2±0.4^b	4.5±0.3^b	1.5±0.4^b	ne	ne

Legend: Same lowercase letters in a column indicate no significant differences ($p>0.05$)
ne: not evaluated

Numerous studies in the European Union showed that the color of food products, specially fish, is a main parameter that influences consumer decisions to buy a particular type of food (*Espe et al., 2004*).

In our research, textural changes were detected in common carp muscle during the storage and was most evidenced in samples packaged in vacuum. Although the average texture scores were lower over time, at the end of the experiment, texture was rated as acceptable in all groups.

During the entire storage period, the average scores of the odor of carp steaks decreased in all experimental groups. Our results showed that odor changes were most pronounced in the vacuum packaged fish. The carp steaks packaged in a modified atmosphere consisting of 60% CO_2 and 40% N_2 received high odor scores, and they had an effect on high ratings of overall acceptability, considering that odor is the most significant sensory attribute in the evaluation of freshness and acceptability of fish as food. The odor of carp steaks packaged in MAP1 was evaluated as unacceptable on day 15 of the experiment, while in samples packaged in MAP2, odor was judged as unacceptable on day 12. The odor of vacuum packaged samples was evaluated as unacceptable on day 9.

The results of numerous studies indicate that samples of fish packaged in various gas mixtures have always shown higher sensory estimates of overall acceptability and, hence, a longer shelf-life in comparison with the fish stored primarily in the air, but also vacuum packaged fish. The results of the present study showed that the highest average rates of overall acceptability, which also proved to be significantly higher ($p< 0.05$), were established for carp steaks packaged in an atmosphere consisting of 60% CO_2 and 40% N_2. Carp steaks with somewhat smaller average ratings of overall acceptability were packaged in a mixture of gases with 40% CO_2 and 60% N_2; the lowest average ratings of overall acceptability were those of vacuum packaged samples. Statistically significantly higher sensory ratings during storage were established by *Masniyom et al., 2002* for samples of sea bass fillets packaged in various mixtures of gases in relation to fish stored in air. Similar results were obtained by *Goulas and Kontominas, 2007*, who examined modified atmosphere packaged and vacuum packaged mackerel. Such results can also be a confirmation of *Murcie et al., 2003*, who found that food packaged in a modified atmosphere retains a better appearance in comparison with vacuum packaged food.

4. Conclusion

Different gas mixtures as well as vacuum packaging did not significantly affect the changes in meat color and meat texture of carp steaks, and they remained characteristic until the end of the experiment. The freshness and acceptability of fish was most influenced by the average ratings of odor, so based on the results of the present study, it can be concluded that the shelf-life of carp steaks packaged in the gas mixture consisting of 60% CO_2 and 40% N_2 (MAP1) was 12 days, while samples packaged in a mixture of gases with 40% CO_2 and 60% N_2 (MAP2) were acceptable for 9 days. The shelf-life of carp steaks packaged in vacuum was 7 days.

The gas mixture consisting of 60% CO_2 and 40% N_2 proved to be the most suitable for packaging fresh carp steaks regarding the selected sensory characteristics of meat color, meat texture, odor and overall acceptability.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: The research results shown in this paper were financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, and based on the Agreement on the implementation and financing of scientific research work by the Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Espe M., Kiessling A., Lunestat B., Torrissen O. & Røra, A. B. (2004). Quality of cold smoked salmon collected in one French hypermarket during a period of 1 year. *Lebensmittel- Wissenschaft & Technologie*, 37, 627–638.

Gimenéz, B., Roncalés, P. & Beltrán, J.A. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture*, 82, 1154–1159.

Goulas, A. E. & Kontominas, M. G. (2007). Effect of modified atmosphere packaging and vacuum packaging on the shelf-life of refrigerated chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *European Food Research and Technology*, 224, 545–553.

Gram, L. & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33, 121–137.

Martinez, L., Djenane, D., Cilla, I., Beltran, J. A. & Roncales, P. (2006). Effect of varying oxygen concentrations on the shelf life of fresh pork sausages packaged in modified atmosphere. *Food Chemistry*, 94, 219–225.

Masniyom, P., Benjakul, S. & Visessanguan, W. (2002). Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging. *Journal of the Science of Food and Agriculture*, 82(8), 873–880.

Milijašević, M., Babić, J., Baltić, M., Spirić, A., Velebit, B., Borović, B. & Spirić, D. (2010). The influence of different gas mixtures on the changes of certain microbiological and chemical parameters in carp's (*Cyprinus carpio*) cuts packed in modified atmosphere. *Meat Technology*, 51 (1), 66–70.

Murcia M. A., Martinez-Tome M., Nicolas M.C. & Vera A. M. (2003). Extending the shelf-life and proximate composition stability of ready to eat foods in vacuum or modified atmosphere packaging. *Food Microbiology*, 20, 671–679.

Pastoriza L., Sampedroo G., Hherrera J. J. & Cabo M. L. (1996). Effect of carbon dioxide atmosphere on microbial growth and quality of Salmon slices. *Journal of the Science of Food and Agriculture*, 72, 348–352.

Provincial, L., Gil, M., Guille´n, E., Alonso, V., Roncales, P. & Beltran, J. A. (2010). Effect of modified atmosphere packaging using different CO_2 and N_2 combinations on physical, chemical, microbiological and sensory changes of fresh sea bass (*Dicentrarchus labrax*) fillets. *International Journal of Food Science & Technology*, 45, 1828–1836.

Stamatis, N. & Arkoudelos, J. S. (2007). Effect of modified atmosphere and vacuum packaging on microbial, chemical and sensory quality indicators of fresh, filleted *Sardina pilchardus* at 3°C. *Journal of Science of Food and Agriculture*, 87, 1164–1171.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 664.933:636.592

ID: 126582025

https://doi.org/10.18485/meattech.2023.64.2.67

Original scientific paper

Innovative coating approach: vacuum impregnation with chia mucilage and sage infusion for turkey fillets

Elnaz Sharefiabada[a], Hulya Serpil Kavusan[a] and Meltem Serdaroğlu[a]*

[a] Ege University, Engineering Faculty, Food Engineering Department, 35100 Bornova, İzmir, Turkey

ARTICLE INFO

Keywords:
Tturkey Meat
Coating
Vacuum impregnation
Chia mucilage
Sage infusion

ABSTRACT

This study aimed to investigate the application of chia mucilage and/or sage infusion as an antioxidant using the vacuum impregnation (VI) technique for coating turkey fillets. Fillets were divided into four groups; one was soaked in deionized water (C) and the following groups were fillets separately immersed in chia mucilage (CM), chia mucilage including sage infusion (CMS), and fresh turkey fillets without any treatment (B). Their impact on the physicochemical properties and sensory attributes was evaluated during 7 days of storage at 4°C. VI was effective in increasing moisture contents and coating solution uptake. It was observed that the application of VI increased the L* value and hardness while decreasing springiness and cohesiveness. The incorporation of CM or CMS using VI retarded lipid oxidation. Both CM and CMS influenced the sensorial properties of turkey breast. Taken together, the utilization of natural material coatings with VI revealed a suitable technique to improve meat quality and reduce waste in the meat industry.

1. Introduction

Turkey breast meat faces a considerable challenge in terms of its limited shelf life, even when stored under cold conditions, necessitating the employment of novel technologies to prolong its freshness (*Elmas et al*., 2020). Cold storage typically affords turkey meat a restricted shelf life of approximately two days. Thus, it becomes imperative to identify approaches that can preserve its quality for an extended duration. In order to extend the shelf life of fresh or minimally processed meats, edible films and coatings have gained recognition as viable solution. These films and coatings act as a protective barrier on the surface of foods, hindering deterioration meats (*Jooyandeh et al*., 2022). Vacuum impregnation (VI) is a widely employed method in the food industry for facilitating the infusion of various ingredients, such as salt, binding agents, coating materials, antioxidants, and antimicrobial agents, into different products. The VI technique plays a pivotal role in enhancing mass transfer across the pores of animal or vegetable tissues by utilizing varying levels of pressure. This technique aims to utilize the empty spaces within the food to impregnate substances. To prevent the deterioration of quality, VI has proven to be highly effective in integrating a coating solution into a porous solid matrix, resulting in a thicker and more uniform coating (*Kırmızıkaya and Çınar*, 2018; *Panayampadan et al.,* 2022).

Chia seeds (*Salvia hispanica* L.) and their mucilage offer great potential as a nutritional, functional, and pharmaceutical material in the food

***Corresponding author:** Meltem Serdaroğlu, *meltem.serdaroglu@ege.edu.tr*

Paper received Jun 19th 2023. Paper accepted July 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

industry. When chia seeds come into contact with water, they release a highly transparent and thick solution that adheres to the seed coat. Chia mucilage serves as an innovative source for developing edible coatings and films (*Brütsch et al*., 2019; *Akhavan et al.,* 2022). Sage (*Salvia officinalis* L.), an aromatic plant from the Lamiaceae family, is renowned for its medicinal properties. Sage has been documented to possess potent antibacterial, antifungal, and antioxidant characteristics (*Walch et al.*, 2011; *Yazgan,* 2020). Although several coating materials have been utilized in meat products (*Gagaoua et al*., 2021; *Li et al.*, 2023; *Smaoui et al.,* 2022), there is a notable gap in research regarding the application of chia mucilage and sage infusion as antioxidant agents and also the use of VI in turkey fillets. Consequently, the primary goal of this study was to enhance the shelf life of chilled fresh turkey breast by enhancing its technological and oxidative stability. The aim was to achieve this by employing chia mucilage either alone or in conjunction with sage infusion, which acts as an antioxidant agent.

2. Materials and methods

2.1. Materials

Chia mucilage was prepared according to the method of *Yüncü et al*. (2022). Subsequently, the mixture was transferred to centrifuge tubes and centrifuged at 4100 rpm for 10min (NF400, Nüve, Turkey). The resulting solution was then filtered to eliminate the seeds and obtain pure chia mucilage. Sage infusion was prepared using the method described by *Yıldırım et al*. (2000). The chia mucilage was mixed with the sage infusion to achieve a final concentration of 1.5% extract. Turkey breast was cut into similarly shaped fillets, 1 cm thick and weighing about 10±1 g. The fillets were randomly divided into four groups, one was soaked in deionized water (C), and the other groups were separately immersed in chia mucilage (CM), chia mucilage including sage infusion (CMS), with the ratio of fillets to solution being nearly 1:3, and the fourth group was fresh turkey breast without any treatment (B). VI was imposed according to the method applied by *Zhao et al.* (2021). The treated fillets were individually placed in ziploc bags and stored at 4°C for 7 days. Analysis of the samples was conducted on days 0, 3, 5, and 7 to assess their quality characteristics and changes over time.

2.2. Methods

The moisture and ash content were determined according to AOAC (2012) procedures. Protein content was assessed using the Dumas method with an automatic nitrogen analyzer (FP 528 LECO, USA). Fat content was evaluated following the methodology described by *Flynn and Bramblet* (1975). The pH of the samples was measured in triplicate using a pH meter (WTW pH 3110 set 2, Germany) equipped with a penetration probe. The uptake of the coating solution was determined by calculating the weight differences of the fillets before and after the VI process. Thiobarbituric Acid Reactive Substances (TBARS) value was measured using the method outlined by *Witte et al.* (1970). Surface color analysis was conducted using a portable colorimeter (CR400, Konica-Minolta, Japan). For sensory evaluation, warm fillets from each group were randomly served to a panel of five graduate students from the Food Engineering Department at Ege University. The panel assessed the samples for attributes such as appearance, color, texture, juiciness, flavor, and overall acceptability. All analyses were performed in triplicate. Statistical analysis was carried out using the IBM SPSS Statistics program (version 25.0), employing one-way and two-way analysis of variance (ANOVA). Differences among the means were examined using Duncan's multiple range test at a confidence level of 95%.software.

3. Results and discussion

3.1. Chemical composition, coating solution uptake and pH

The total moisture content ranged from 74.2% to 77.50%, protein content ranged from 21.27% to 23.87%, fat content ranged from 0.36% to 0.84%, and ash content ranged from 0.73% to 1.08% across the fillets (Table 1). The fillets treated with VI exhibited higher moisture content compared to the untreated fillets (B) ($P<0.05$). This could be attributed to the enhanced coating degree of the fillets when using the VI technique, as supported by the findings on coating solution uptake. Similar findings were reported by *Zhao et al.* (2021). The addition of chia mucilage and sage infusion resulted in increased coating uptake in the fillets when VI was applied. VI treatment of turkey fillet with deionized water (C) produced the lowest coating uptake followed by the chia mucilage treatment (CM). The presence of chia mucilage in the coating solution could enhance its

Table 1. Chemical composition, coating solution uptake, and pH of turkey fillets

Fillet group	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Coating solution uptake (%)	pH
B	23.87^{a}±0.19	74.2^{b}±0.36	0.84^{a}±0.08	1.08^{a}±0.13	0.00^{d}±0	5.80^{a}±0.01
C	22.23^{b}±0.98	76.44^{a}±0.91	0.60^{b}±0.05	0.73^{c}±0.06	1.30^{c}±0.22	5.76^{b}±0.01
CM	21.27^{b}±0.34	77.50^{a}±0.32	0.43^{c}±0.01	0.80^{bc}±0.04	4.37^{b}±0.24	5.71^{c}±0.01
CMS	21.36^{b}±0.34	77.38^{a}±0.36	0.36^{c}±0.05	0.89^{b}±0.03	4.96^{a}±0.40	5.78^{b}±0.03

All values are means ± SD, with the different letters showing significantly different (P<0.05). B, fresh turkey breast without any treatment; C, deionized water; CM, chia mucilage; CMS, chia mucilage including sage infusion.

ability to interact with the meat surface, leading to improved solution absorption efficiency. However, the combination of sage infusion with chia mucilage enhanced the viscosity of the solution and improved its adhesion to the surface of the fillets. Therefore, the highest solution uptake was observed in the CMS treatment. Similarly, the process yield of tilapia fillets was increased by coating them with fish gelatin and grape seed extract (*Zhao et al*., 2019). The pH of the turkey fillets ranged from 5.71 to 5.80, with the highest pH observed in the untreated fillets (B), followed by the fillets treated with deionized water (C) and chia mucilage including sage infusion (CMS). Therefore, the addition of sage infusion did not significantly increase the pH values beyond that of the untreated (B) fillets.

3.2. Color

The L* (lightness), a* (redness), and b* (yellowness) values of the turkey breast fillets ranged from 45.89 to 51.65, 3.61 to 5.26, and 3.24 to 4.25, respectively (Table 2). The application of VI resulted in an increase in the brightness (L*) of the turkey fillets. This can be attributed to the fact that the coating solution fills the gaps and voids within the meat, leading to a more uniform distribution of light and ultimately causing an overall increase in the lightness of the fillets. It should be acknowledged that variations in the composition of the coating solution, processing conditions, and the inherent characteristics of different meat species can contribute to differences in the observed color changes. The incorporation of sage infusion altered the a* and b* values. This suggests that sage infusion contributed to the development of a less pronounced red color and an enhanced yellow hue in the turkey fillets. CM or CMS treatment caused a decrement in a* value. These reductions in a* values could be associated with denser texture and thicker appearance of chia mucilage compared to the water, and to the natural color of the sage infusion, which could have influenced the overall redness of the fillets. B, C, and CM treatments had similar b* values. Additionally, the use of sage infusion increased the b* value, and similar results were reported by *Cegielka et al.* (2022) in chicken meatballs treated with sage.

3.3. Lipid oxidation

Fresh turkey meat is prone to oxidative changes due to the presence of lipids and endogenous enzymes. The application of coating solution and the use of antioxidants affected TBARS values (P<0.05). The initial TBARS values ranged

Table 2. Color parameters of turkey fillets

Fillet group	L*	a*	b*
B	45.89^{c}±1.02	3.61^{c}±0.29	3.30^{b}±0.11
C	49.28^{b}±0.42	5.26^{a}±0.41	3.24^{b}±0.68
CM	51.65^{a}±0.18	4.40^{b}±0.34	3.66^{b}±0.20
CMS	51.14^{a}±0.50	3.80^{c}±0.12	4.25^{a}±0.22

All values are means ± SD with the different letters showing significantly different color parameters (P<0.05). B, fresh turkey breast without any treatment; C, deionized water; CM, chia mucilage; CMS, chia mucilage including sage infusion.

Table 3. TBARS values of turkey fillets during storage at 4°C

Fillet group	0 days	3 days	5 days	7 days
B	$0.84^{bt}\pm0.02$	$0.99^{az}\pm0.00$	$1.54^{ax}\pm0.03$	$1.31^{ay}\pm0.01$
C	$1.02^{axy}\pm0.01$	$0.91^{bz}\pm0.08$	$0.96^{byz}\pm0.01$	$1.08^{bx}\pm0.02$
CM	$0.57^{cy}\pm0.01$	$0.43^{cz}\pm0.01$	$0.70^{cx}\pm0.05$	$0.77^{dx}\pm0.06$
CMS	$0.46^{dz}\pm0.01$	$0.32^{dt}\pm0.02$	$0.73^{cy}\pm0.03$	$0.98^{cx}\pm0.05$

All values are means ± SD of three replicates. The means within the same column with different superscripts (a-d) are different. The means within the same row with different superscripts (X-Z) are different. B, fresh turkey breast without any treatment; C, deionized water; CM, chia mucilage; CMS, chia mucilage including sage infusion.

from 0.46 to 1.02 mgMA/kg (Table 4). The highest TBARS value was observed in samples coated with water initially; however, the infusion of chia mucilage and sage reduced oxidation, and the lowest values were detected in the CMS treatment ($P<0.05$). While the untreated fillets (B) exhibited a continuous increase in oxidation values during storage, the other groups showed fluctuations ($P<0.05$). In the untreated (B) fillets, oxidation occurred at the highest level among the four groups, whereas the application of coating solution resulted in lower TBARS values due to the barrier properties of the coatings, which prevented contact between turkey meat and oxygen. Also, *Kanatt et al.* (2013) reported that chitosan coating was able to reduce oxidation in ready to cooked meat products. Furthermore, the presence of phenolic compounds in the chia mucilage and sage infusion led to the lowest values observed in the CM and CMS treatments throughout storage, with oxidation not exceeding 1 mgMA/kg in these groups. *Mariutti et al.* (2011) reported that the use of sage infusion was effective in controlling lipid oxidation. In line with our findings, sea bass fillets coated with chia mucilage and propolis extract resulted in lower TBARS values compared to the control (*Coban and Coban,* 2020).

3.4. Sensory evaluation

The sensory evaluation results of VI turkey fillets are presented in Figure 1. Coating with chia mucilage or chia mucilage and sage influenced the appearance and color attributes of the turkey fillets. The inclusion of sage infusion in the coating solution resulted in a decrease in the sensory scores for appearance and color. One potential explanation is the presence of certain chemical compounds in the sage infusion that interact with the pigments responsible for color in turkey meat. Chitosan coating consisting of grape seed extract and oregano essential oil also decreased the color and taste scores of turkey meat (*Mojaddar Langroodi et al.,* 2021). However, it can be inferred that the implemented procedure yielded acceptability levels comparable to those of the control group. A similar result was also reported in grass carp slices where fillets coated with chitosan containing plant extracts by VI (*Zhao et al.*, 2021).

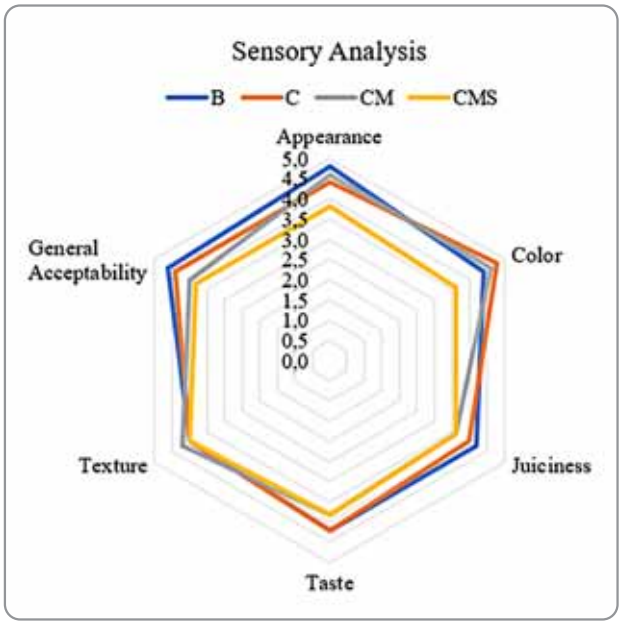


Figure 1. Sensory properties of turkey fillets. B, fresh turkey breast without any treatment; C, deionized water; CM, chia mucilage; CMS, chia mucilage including sage infusion.

4. Conclusion

Favorable impacts of VI on physicochemical properties, oxidation stability, and coating solution uptake were seen in turkey fillets. The results of the instrumental analysis (texture and color) showed

that VI had a significant effect on the turkey fillets compared to the fillets treated with deionized water. Sensory analysis showed that chia mucilage/sage infusion affected the evaluated parameters. In general, chia mucilage coating alone and a combination of chia mucilage with sage infusion had similar effects on the quality of fillets. However, sage infusion had a slightly adverse effect on sensorial properties.

Disclosure statement: No potential conflict of interest was reported by the authors.

Acknowledgments: The authors are thankful to Ege University Scientific Research Projects Coordination under project number 23867 for their financial support.

References

Akhavan, F., Sharifi Soltani, M., Zomorodi, S., Jafarian, S. & Khosrowshahi Asl, A. (2022). The effect of chia seed mucilage containing different concentrations of aqueous olive leaf extract on the shelf life of chicken fillets in the refrigerator. *Journal of Food Microbiology*, 9 (1), 55–67.

Association of Official Analytical Chemists, (2007). Official Methods of Analysis of AOAC International.

Brütsch, L., Stringer, F. J., Kuster, S., Windhab, E. J. & Fischer, P. (2019). Chia seed mucilage–a vegan thickener: isolation, tailoring viscoelasticity and rehydration. *Food & Function*, 10(8), 4854–4860, https://doi.org/10.1039/C8FO00173A

Cegiełka, A., Chmiel, M., Hać-Szymańczuk, E. & Pietrzak, D. (2022). Evaluation of the Effect of sage (*Salvia officinalis* L.) preparations on selected quality characteristics of vacuum-packed chicken meatballs containing mechanically separated meat. *Applied Sciences*, 12(24), 12890, https://doi.org/10.3390/app122412890

Coban, M. Z. & Coban, O. E. (2020). Potency and use of chia mucilage coating containing propolis liquid extract for improves shelf-life of sea bass fillets. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 19(3), 255–260, https://doi.org/10.17306/J.AFS.0843

Elmas, F., Bodruk, A., Köprüalan, Ö., Arıkaya, Ş., Koca, N., Serdaroğlu, F. M., Kaymak-Ertekin, F. & Koç, M. (2020). Drying kinetics behavior of turkey breast meat in different drying methods. *Journal of Food Process Engineering*, 43(10), e13487, https://doi.org/10.1111/jfpe.13487

Flynn, A. W. & Bramblett, V. D. (1975). Effects of frozen storage, cooking method and muscle quality on attributes of pork loins. *Journal of Food Science*, 40(3), 631–633, https://doi.org/10.1111/j.1365-2621.1975.tb12544.x

Gagaoua, M., Bhattacharya, T., Lamri, M., Oz, F., Dib, A. L., Oz, E., Uysal-Unalan, I. & Tomasevic, I. (2021). Green coating polymers in meat preservation. *Coatings*, 11(11), 1379, https://doi.org/10.3390/coatings11111379

Jooyandeh, H., Ebrahimi Hemmati Kaykha, M., Alizadeh Behbahani, B. & Noshad, M. (2022). Evaluating the quality of mutton meat coated with *Cordia myxa* fruit mucilage containing *Rosmarinus officinalis* essential oil during cold storage. *Journal of Food Measurement and Characterization*, 1–13, https://doi.org/10.1007/s11694-022-01788-2

Kanatt, S. R., Rao, M., Chawla, S. & Sharma, A. (2013). Effects of chitosan coating on shelf-life of ready-to-cook meat products during chilled storage. *LWT-Food Science and Technology*, 53(1), 321–326, https://doi.org/10.1016/j.lwt.2013.01.019

Kırmızıkaya, E. S. & Çınar, İ. (2018). Use Of Vacuum Impregnation in Food Enrichment Processes. 3rd International Energy & Engineering Congress Proceedings Book, Gaziantep University, Turkey.

Li, X.-L., Shen, Y., Hu, F., Zhang, X.-X., Thakur, K., Rengasamy, K. R., Khan, M. R., Busquets, R. & Wei, Z.-J. (2023). Fortification of polysaccharide-based packaging films and coatings with essential oils: A review of their preparation and use in meat preservation. *International Journal of Biological Macromolecules*, 124767, https://doi.org/10.1016/j.ijbiomac.2023.124767

Mariutti, L. R. B., Nogueira, G. C. & Bragagnolo, N. (2011). Lipid and cholesterol oxidation in chicken meat are inhibited by sage but not by garlic. *Journal of Food Science*, 76(6), C909–C915, https://doi.org/10.1111/j.1750-3841.2011.02274.x

Mojaddar Langroodi, A., Nematollahi, A. & Sayadi, M. (2021). Chitosan coating incorporated with grape seed extract and *Origanum vulgare* essential oil: an active packaging for turkey meat preservation. *Journal of Food Measurement and Characterization*, 15(3), 2790–2804, https://doi.org/10.1007/s11694-021-00867-0

Panayampadan, A. S., Alam, M. S., Aslam, R. & Kaur, J. (2022). Vacuum impregnation process and its potential in modifying sensory, physicochemical and nutritive characteristics of food products. *Food Engineering Reviews*, 1–28, https://doi.org/10.1007/s12393-022-09312-4

Smaoui, S., Hlima, H. B., Tavares, L., Braïek, O. B., Ennouri, K., Abdelkafi, S., Mellouli, L. & Khaneghah, A. M. (2022). Application of eco-friendly active films and coatings based on natural antioxidant in meat products: A review. *Progress in Organic Coatings*, 166, 106780, https://doi.org/10.1016/j.porgcoat.2022.106780

Walch, S. G., Tinzoh, L. N., Zimmermann, B. F., Stühlinger, W. & Lachenmeier, D. W. (2011). Antioxidant capacity and polyphenolic composition as quality indicators for aqueous infusions of *Salvia officinalis* L. (sage tea). *Frontiers in Pharmacology*, 2, 79, https://doi.org/10.3389/fphar.2011.00079

Witte, V. C., Krause, G. F. & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*, 35(5), 582–585, https://doi.org/10.1111/j.1365-2621.1970.tb04815.x

Yazgan, H. (2020). Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. *LWT*, 130, 109669, https://doi.org/10.1016/j.lwt.2020.109669

Yıldırım, A., Mavi, A., Oktay, M., Kara, A. A., Algur, Ö. F. & Bilaloğlu, V. (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf ex DC), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis*) extracts. *Journal of Agricultural and Food Chemistry*, 48(10), 5030–5034, https://doi.org/10.1021/jf000590k

Yüncü, Ö., Kavuşan, H. S. & Serdaroğlu, M. (2022). Chia (*Salvia hispanica* L.) mucilage as a novel fat replacer in beef patties cooked with different methods: physico-chemical, technological, and nutritional perspectives. *Journal of Culinary Science & Technology*, 1–29, https://doi.org/10.1080/15428052.2022.2115960

Zhao, W., Yu, D. & Xia, W. (2021). Vacuum impregnation of chitosan coating combined with water-soluble polyphenol extracts on sensory, physical state, microbiota composition and quality of refrigerated grass carp slices. *International Journal of Biological Macromolecules*, 193, 847 –855, https://doi.org/10.1016/j.ijbiomac.2021.10.190

Zhao, X., Zhou, Y., Zhao, L., Chen, L., He, Y. & Yang, H. (2019). Vacuum impregnation of fish gelatin combined with grape seed extract inhibits protein oxidation and degradation of chilled tilapia fillets. *Food Chemistry*, 294, 316–325, https://doi.org/10.1016/j.foodchem.2019.05.054

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.521.058
579.67

ID: 126588169

https://doi.org/10.18485/meattech.2023.64.2.68

Original scientific paper

Microbiological parameters and sensory characteristics of sliced meat products packaged in modified atmosphere throughout the shelf life

Jelena Vranešević[a], Suzana Vidaković Knežević[a], Anja Novaković[b], Anđela Pavlović[a] Snežana Škaljac[c], Jasna Kureljušić[d] and Dragan Vasilev[b]*

[a] *Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*
[c] *University of Novi Sad, Faculty of Technology, Boulevard Cara Lazara 1, 21101 Novi Sad, Serbia*
[d] *Scientific Veterinary Institute of Serbia, Janisa Janulisa 14, 11107 Belgrade, Serbia*

ARTICLE INFO

Keywords:
MAP
Pork
Ham
Sausage
Kulen
Pancetta

ABSTRACT

Demand for food, such as ready to eat food which is easy to consume with as long as possible shelf life, has continuously increased due to the modernization and growth of the human population. Sliced meat products that were normally packed and placed on the market in vacuum packaging, were packed in MAP with the aim of extending the shelf life. The research objective of this study was to determine the microbiological parameters and sensory characteristics of 6 sliced meat products packaged in modified atmosphere (smoked pork loin with added water, Budim sausage, Kamendin pancetta, Smoked pork neck with added water, Kulen, Ham for pizza with added water) during the expected shelf life. This study included sensory analysis and microbiological parameters (*Listeria monocytogenes*, *Enterobacteriaceae* and total aerobic mesophilic microorganisms). All samples of sliced meat products packaged in modified atmosphere had satisfactory microbiological and sensory characteristics during the expected shelf life which ranged from 30 to 90 days.

1. Introduction

Despite the growing number of vegans and vegetarians, i.e., people who endorse the benefits associated with adopting a meat-free diet (*Bolderdijk & Cornelissen*, 2022), meat and meat products are an important part of diet of a large number of people worldwide and represent an important source of protein in the human diet (*Font-i-Furnols & Guerrero*, 2014). The share of meat in the human diet has been constantly increasing since 1960s (*Halagarda & Wójciak*, 2022). The demand for food, such as ready to eat food that is easy to consume with a shelf life as long as possible, has continuously increased due to the modernization and growth of the human population.

Considering that consumers are the last step in the production chain, meeting their expectations is an important part of their satisfaction and purchasing behaviour. Multiple determinants shape consumer behaviour toward meat and meat products (*Font-i-Furnols & Guerrero*, 2014). In this regard, manufacturers make a great effort to meet all consumer expectations. When deciding what to buy, the consumer is guided by many factors such as motivation,

***Corresponding author:** Jelena Vranešević, *jelenababic@niv.ns.ac.rs*

Paper received Jun 19th 2023. Paper accepted July 1st 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

perception, attitudes and expectation. The consumer of the new age, in addition to a safe and high-quality product, also demands easy consumption, attractive packaging and a long shelf life. The advantages of food packaging in vacuum and modified atmosphere are reflected in extending the shelf life, increasing the efficiency of production and distribution, reducing costs and increasing the sale of products that meet the ever stricter demands of consumers for natural preservation of food quality, without additives and preservatives, market expansion, greater flexibility of packaging and distribution, availability of as much information as possible and better appearance. Because of that, sliced meat products that normally have been packed and placed on the market in vacuum packaging, were packed in MAP. The research objective of this study was to determine the microbiological parameters and sensory characteristics of six sliced meat products packaged in modified atmosphere (70% N and 30% CO_2) (smoked pork loin with added water, Budim sausage, Kamendin pancetta, smoked pork neck with added water, kulen, and ham for pizza with added water) during the expected shelf life.

2. Materials and methods

2.1. Meat product production

Smoked pork loin with added water was obtained from pork loin with injected brine, followed by mechanical processing of the loin in a tumbler during five hours. Heat treatment of the product was carried out in an ATMOS smoking chamber by warm and hot smoking, and then the loin was heated at pasteurization temperature until reaching 70ºC in the centre of product. After cooling to 4ºC, the product was sliced in a meat slicer and packed in MAP mixture for 45 days' storage. Budim sausage was obtained by cutting frozen pork meat (category I) into pieces on a frozen meat shredder. Fresh pork meat (category I) and solid fatty tissue were chopped in a meat grinder through a grid (diameter: 7.8 mm). Crushed pieces of frozen meat were chopped and combined with salt, additives and spices in a cutter. Ground fresh pork meat and solid fatty tissue were then added to the obtained mass, and the mass was then mixed in a cutter. The stuffing was filled using vacuum filler into artificial casings made of polyamide with a diameter of 60 mm, permeable to smoke and moisture. The product was then subjected to cold smoking treatment in a smoking chamber for 20 hours, followed by drying and ripening of the product under controlled conditions (humidity and temperature), until the appropriate quality was achieved. The finished product was sliced using a meat slicer and packed in MAP mixture for 90 days' storage. Kamendin pancetta was obtained from meat bacon, which was preserved by the process of dry salting and then aged for a defined number of days. After that, it was desalinated, dried, and smoked with beech wood in a smokehouse. Smoking was done in a classic smokehouse for 24 hours at up to 25ºC to produce cold smoking conditions in the chamber. The smoked bacon was then dried in a chamber with defined humidity and temperature. The finished product was sliced using a meat slicer and packed in MAP mixture for 90 days' storage. Smoked pork neck with added water was obtained from pork neck into which brine had been injected, followed by mechanical processing of the pork neck in a massaging device (tumbler), lasting five hours. Heat treatment of the product was carried out in the ATMOS smoking chamber by warm (65 °C for 10 min) and hot smoking (65 °C for 10 min), and then the product was heated at pasteurization temperature until reaching 70ºC in the thermal centre. After cooling to 4ºC, the product was sliced in a meat slicer and packed in MAP mixture for 45 days' storage. Kulen was obtained by chopping frozen pork meat (category I) into pieces on a device for crushing frozen meat. Fresh pork meat category I and solid fatty tissue were chopped on a meat grinder through a grid (diameter: 7.8 mm). Crushed pieces of frozen meat were chopped and combined with salt, additives and spices in a cutter. Ground fresh pork meat and solid fatty tissue were added to the obtained mass, and the mass was then mixed in a cutter. The stuffing was filled using vacuum filler into artificial casings made of polyamide with a diameter of 60 mm, permeable to smoke and moisture. The product was then subjected to cold smoking treatment in a smoking chamber for 20 hours, followed by drying and ripening of the product under controlled conditions (humidity and temperature), until the appropriate quality was achieved. The finished product is sliced in a meat slicer and packed in MAP mixture for 90 days' storage. Ham for pizza with added water was obtained by grinding pork shoulder meat on a grinding machine through a grid (diameter: 7.8 mm). Salt, additives and spices were dissolved in water and ice in a cutter, and then, minced meat was added to the resulting brine. After that, the mass was mixed for 40 min. The compact mass obtained was left to stand for 24 h at 0 to 4ºC. The mass was filled using a vacuum filler into polyamide casings and placed in an appropriate mould. Heat treatment was performed in the ATMOS smoking chamber by steaming until reaching 70ºC in the

thermal centre of the product. After cooling to 4ºC, the product was sliced using a meat slicer and packed in MAP mixture for 30 days' storage.

After production and packaging, sampling was performed (30 packages for each sample – 6 tests in 5 units). Samples were transported in a dedicated vehicle, with a refrigerator, at a temperature of 0°C to 4°C. All products were stored under estimated conditions in the fridge at 3ºC until examination.

2.2. Microbiological analysis and sensory evaluation

Determination of the presence of *Listeria monocytogenes* in the samples (on the first day of testing) was carried out by an ISO accredited method (ISO, 2017a). The number of *L. monocytogenes* in the samples was determined by an accredited method (ISO, 2017b). The number of *Enterobacteriaceae* in the samples was determined using an accredited method (ISO, 2017c). The total number of mesophilic aerobic bacteria in the samples was determined according to ISO (2014). Sensory evaluation was performed using an accredited method according to Sensory examination of foodstuffs — qualitative descriptive test.

3. Results

L. monocytogenes was not detected in any of the examined samples on the first day of the study. The numbers of *L. monocytogenes* and *Enterobacteriaceae* in all examined samples were below the limit of quantification (less than 10 cfu/g). The counts of total aerobic mesophilic bacteria are shown in Figures 1, 2 and 3.

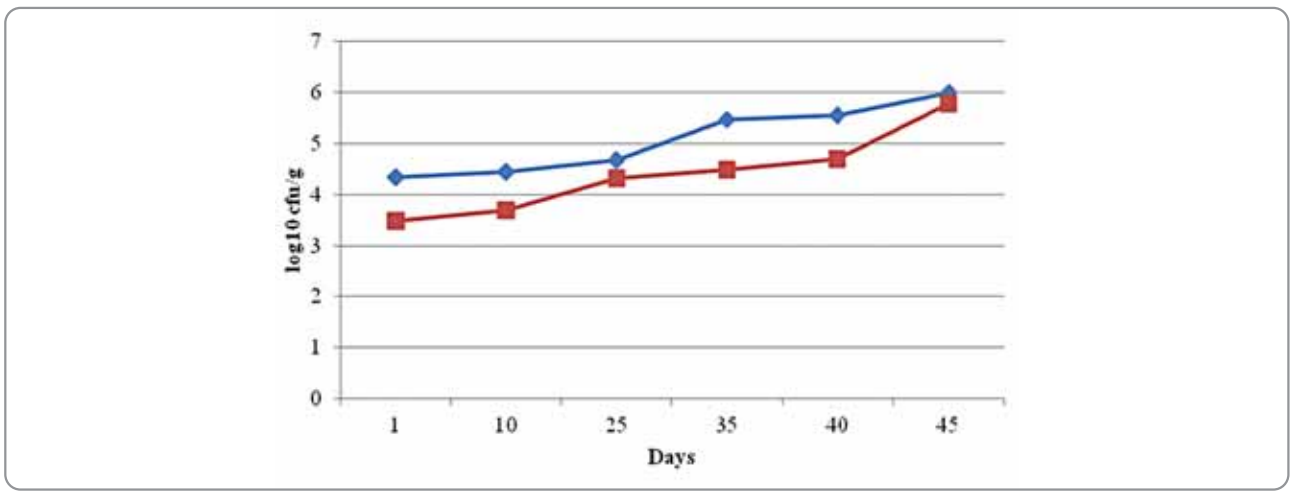


Figure 1. Total aerobic mesophilic bacteria during shelf life in smoked pork loin with added water (blue line) and smoked pork neck with added water (red line)

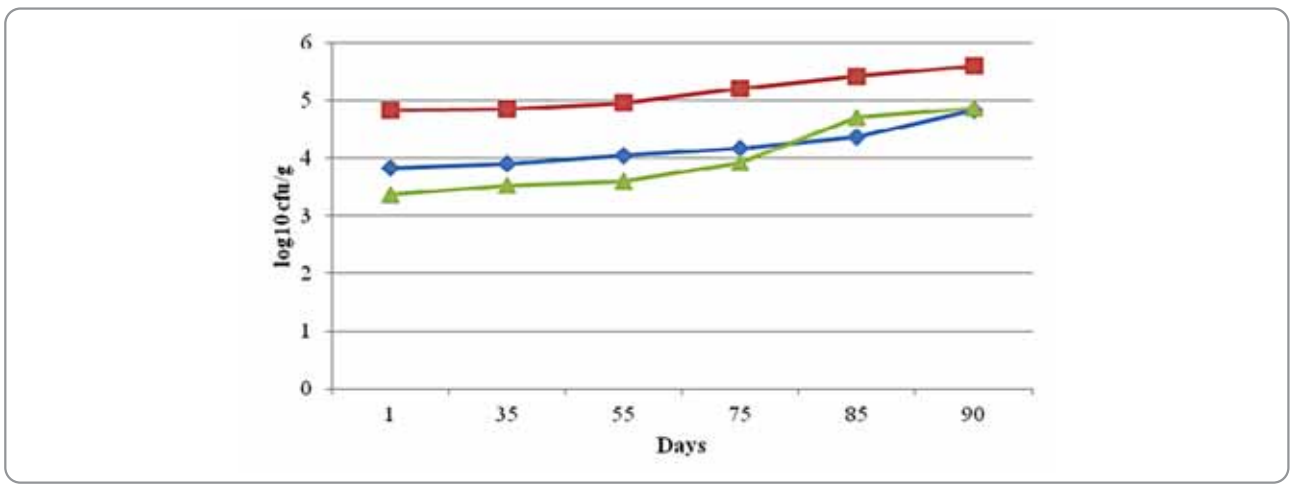


Figure 2. Total aerobic mesophilic bacteria during shelf life in Budim sausage (blue line), Kamendin pancetta (red line) and Kulen (green line)

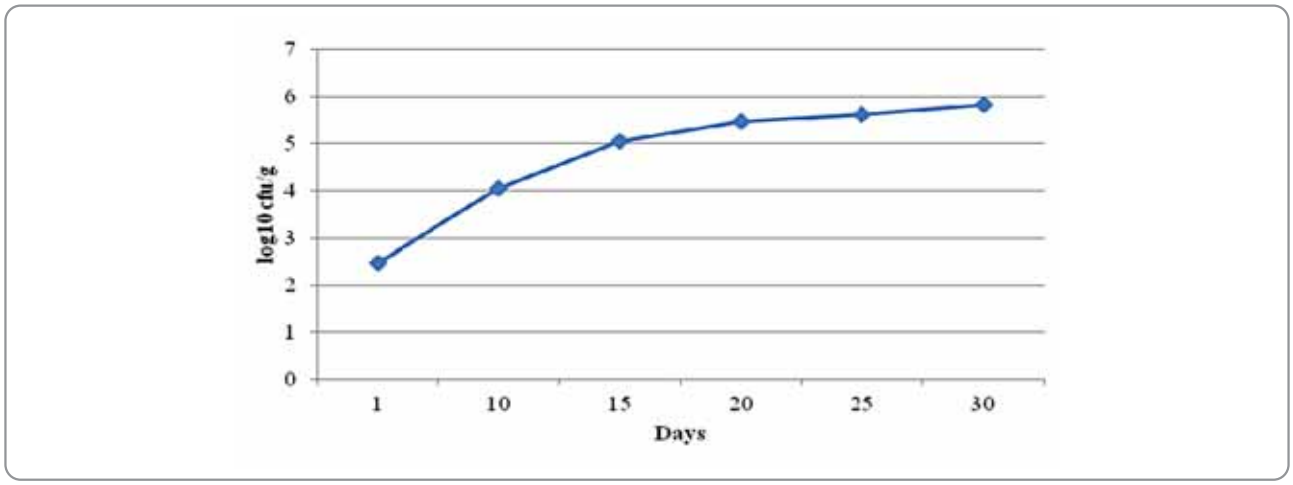


Figure 3. Total aerobic mesophilic bacteria during shelf life in Ham for pizza with added water

Sensory ratings of all tested smoked meat products during their expected shelf lives were in accordance with the criteria prescribed by legislation in Serbia (*Republic of Serbia*, 2019).

4. Discussion

The most common format of the sliced meat products for selling is vacuum packaging, but vacuum packaging has some negative consequences related to the plastic of the packaging. Plastic adheres closely to the product, so there is a problem of adhesion between the slices as well. The use of MAP is becoming more common on the market because this format avoids those problems (*Parra et al*, 2012). Regarding this packaging, MAP preserved better sensory characteristics (primarily color) of the *Iberian chorizo* slices than vacuum packaging for a long period (*García-Torres et al.*, 2021). MAP packaging is more in line with the current consumers trends and habits (*Ortiz et al.*, 2020).

Considering that *L.* monocytogenes is ubiquitous and has ability to grow at refrigeration temperature, *L. monocytogenes* is a significant threat to the safety of RTE meat products (*Zhu et al.*, 2005). Its absence primarily indicates good hygienic practice and good manufacturing practice during slicing and packaging after cooking.

A large number of studies have shown that ready to eat meat products sliced in retail shops often have a higher level of bacterial contamination than ready to eat meat products sliced and packed in meat factories (*Chaitiemwong et al.*, 2014; *Curpas et al*., 2018). The results of our study show that the number of total aerobic mesophilic microorganisms during the expected shelf life was satisfactory according to the recommendations by *Health Protection Agency* (2009), *Health Canada* (2010) and the *Centre for Food Safety* (2014).

When purchasing, consumers are guided by knowledge and tradition, which inclines them towards sliced meat products packed in vacuum packaging (*Ortiz et al*., 2020).

5. Conclusion

All samples of sliced meat products packaged in modified atmosphere had satisfactory microbiological and sensory characteristics during their expected shelf lives, which ranged from 30 to 90 days. This study enables this meat industry to offer more safe and trendy alternative packaging instead of the previously used vacuum packaging.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

References

Bolderdijk, J. W. & Cornelissen, G. (2022). "How do you know someone's vegan?" They won't always tell you. An empirical test of the do-gooder's dilemma. *Appetite*, 168, 105719.

Centre for Food Safety. (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Food and Environmental Hygiene Department, Hong Kong.

Chaitiemwong, N., Hazeleger, W.C., Beumer, R. R. & Zwietering, M. H. (2014). Quantification of transfer of *Listeria monocytogenes* between cooked ham and slicing machine surface. *Food Control*, 44, 177–184.

Font-i-Furnols, M. & Guerrero, L. (2014). Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science*, 98(3), 361–371.

García-Torres, S., Contador, R., Ortiz, A., Ramírez, R., López-Parra, M. M. & Tejerina, D. (2021). Physico-chemical and sensory characterization of sliced Iberian chorizo from raw material of three commercial categories and stability during refrigerated storage packaged under vacuum and modified atmospheres. *Food Chemistry*, 354, 129490.

Halagarda, M. & Wójciak, K. M. (2022). Health and safety aspects of traditional European meat products. A review. *Meat Science*, 184, 108623.

Health Canada. (2010). Microbial guidelines for ready-to-eat foods a guide for the conveyance industry and environment health officers (EHO).

Health Protection Agency. (2009). Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market.

ISO. (2017a). SRPS EN ISO 11290-1 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method.

ISO, (2017b). SRPS EN ISO 11290-2 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method.

ISO, (2017c). SRPS EN ISO 21528-2 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count technique.

ISO, (2014). SRPS EN ISO 4833-1 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique.

Ortiz, A., Tejerina, D., Díaz-Caro, C., Elghannam, A., García-Torres, S., Mesías, F. J.... & Crespo-Cebada, E. (2020). Is packaging affecting consumers' preferences for meat products? A study of modified atmosphere packaging and vacuum packaging in Iberian dry-cured ham. *Journal of Sensory Studies*, 35(4), e12575.

Parra, V., Viguera, J., Sánchez, J., Peinado, J., Espárrago, F., Gutierrez, J. I. & Andrés, A. I. (2012). Effect of exposure to light on physico-chemical quality attributes of sliced dry-cured Iberian ham under different packaging systems. *Meat Science*, 90(1), 236–243.

Republic of Serbia. (2019). Regulation on the quality of minced meat, semi-finished meat and meat products. *Official Gazette of the Republic of Serbia*, 50.

Zhu, M., Du, M., Cordray, J. & Ahn, D. U. (2005). Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Comprehensive Reviews in Food Science and Food Safety*, 4(2), 34–42.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 638.167:615.324

ID: 126592265

https://doi.org/10.18485/meattech.2023.64.2.69

Review paper

Bioactive compounds in honey: a literature overview

Tatjana Baltić[a], Jelena Ćirić[a], Sara Simunović[a], Ivana Branković Lazić[a], Vesna Đorđević[a], Nenad Parunović[a] and Nenad Katanić[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[b] *Ministry of Agriculture, Forestry and Water Management, Nemanjina 22–26, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Honey
Nutritional value
Bioactive compounds
Antioxidant
Antibacterial

ABSTRACT

Honey is a natural product used worldwide and has a multiple nutritional and health benefits. From ancient times, honey was used as a natural sweetener and healing agent. The specific composition of honey includes primarily carbohydrates, but organic acids, enzymes, vitamins, proteins, volatile compounds, phenols, flavonoids and minerals as well. These compounds are provenly related to honeys' antioxidant, anti-inflammatory and antimicrobial properties. This paper shows the latest scientific data about bioactive compounds and health benefits of honey.

1. Introduction

From ancient times, honey was not only used as a natural sweetener but also as a healing agent (*Helmi*, 2012). Increased rates of different cancers, autoimmune diseases and chronic non-infective and infective diseases have led to a search for new, reliable, non-synthetic, traditional and natural therapeutic products. Therefore, science is now returning to natural products with new approaches in an endeavour to understand older medicinal applications. In that respect, prevention or therapy of diseases by bee products is defined as a very ancient medical practice and is one of the areas in which bee products are used. Moreover, the use of natural products in the nutritional and/or therapeutic context has been growing.

According to the statistical data reported by the World Health Organisation, up to 80% of the population in some developed countries prefer natural products in primary health care (*WHO*, 2014). Nowadays, natural materials are more acceptable to consumers, and if these alternative approaches are effective, this could reduce the reliance on more synthetic substances (*Slover et al.*, 2009). Furthermore, since modern medicine is undergoing a crisis because of the adverse effects of synthetic drugs on human health and increased anti-microbial resistance, natural alternatives are more discussed. In that context, novel scientific data about health benefits of honey are presented in this paper.

2. Materials and methods

A literature search was conducted to identify recent scientific data about bioactive compounds and health benefits of honey. Scientific online databases including Web of Science, Science Direct and Pub Med were used. The following keywords were used individually and in combination as inclusion criteria for articles to be considered for this review: bee products, honey, health, antibacterial, antioxidant, phenolic compounds, and microorganisms.

***Corresponding author:** Dr Tatjana Baltić, *tatjana.baltic@inmes.rs*

Paper received Jun 21st 2023. Paper accepted July 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

3. Composition of honey

Codex Alimentarius (2001) define honey as natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature. Numerous studies from different countries have presented the high nutritional value and health benefits of bee products (honey, bee bread, bee pollen, beeswax, bee toxin, propolis, royal jelly and bee brood). The results of these studies have shown a positive impact on human health (antioxidant, antimicrobial, anti-fungal, anti-inflammatory, etc.), which correlated to the high contents of specific bioactive compounds. The popularity of using natural materials, such as honeys, is due to their potent activities and generally very low toxicity (*Albaridi*, 2019).

3.1. Nutritional value of honey

Different studies show that honeys have a high nutritional and biological value (*Tsavea et al*., 2022; *Ávila et al*., 2022; *Graikou et al*., 2022). Honey is an excellent source of energy, as 100 g of honey supplies about 306 kcal. Similarly, 20 g of honey is the usual quantity per serving or tablespoon that provides about 61.2 kcal, which represents more or less 3% of the energy necessary per day (*Bogdanov et al*., 2008). The main constituents of honey are the carbohydrates that are used for human body energy requirements after being rapidly absorbed into the blood without previous digestion (*Ajibola et al*., 2012). According to chemical composition, honey contains various sugars and other substances, such as organic acids, enzymes, vitamins, proteins, volatile compounds, several bioactive substances (phenols and flavonoids) and minerals.

The main sugars are carbohydrates (60–85%), predominantly fructose and glucose (*Samarghandian et al*. 2017; *Machado De-Melo et al*., 2018). The water content of honey is about 20% and it is related to different factors, such as the botanical and geographical origins of nectar, season of harvesting, intensity of nectar flux, degree of maturation, manipulation by beekeepers during the harvest period, as well as to the extraction, processing and storage conditions (*Ojeda de Rodrıguez et al*. 2004; *Pontara et al*., 2012; *Ciric et al*. 2019; *Ćirić et al*., 2021). Honey's protein content is very low and ranges up to 0.5%. The physicochemical characteristics and quality of honey are defined in different national and EU regulations.

3.2. Antioxidants in honey

The antioxidant activity of different honeys has been already measured using different in vitro methods (*Martinello and Mutinelli*, 2021). The characterization of the polyphenolic profile was carried out mainly to determine which specific compounds might have the strongest effect upon the antioxidant and antimicrobial properties. Twenty compounds were identified in honey samples, eleven of which were flavonoids (sakuranetin dimer, rutin, isorhamnetine 3-O-rutinoside, quercetin 3-O-glucuronide, orientin, vitexin, quercetin, epicatechin, kaempferol, pinobanksin and apigenin), eight were phenolic acids (gallic, neochlorogenic, chlorogenic, protocatechuic, caffeic, sinapic, 3,4-di-O-caffeoylquinic and protocatechuic acid-O-hexoside acids), and one compound belonged to ellagitannins (ellagic acid) (*Sawicki et al*., 2022). Gallic, ellagic, neochlorogenic, chlorogenic, protocatechuic and sinapic acids were detected in honeys in different studies (*Sawick et al*., 2022; *Yucel et al*., 2016; *Habryka et al*., 2021). Also, sakuranetin dimer, caffeic acid and quercetin were detected only in honey. Similar, *Habryka et al*. (2021) found six phenolic acids (ferulic, gallic, p-hydroxybenzoic, caffeic, p-coumaric and protocatechuic acids) and four flavonoids (kaempferol, chrysin, galangin and quercetin) in Polish multifloral honey. Differences in the polyphenolic profiles and contents can be related to the laboratory method of extraction and the sensitivity of the analytical technique. Moreover, the number of compounds detected could be related to the geographic region of honey origin.

In most of the studies, the polyphenolic compounds were measured by the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) assays and the photochemiluminescence (PCL) method. The order of average antioxidant activity for the honey was as follows: ABTS > ACL (lipophilic antioxidants) > ACW (hydrophilic antioxidants) > DPPH. The multifloral honeys were also tested by *Sawicki et al*. (2022) for their ability to scavenge superoxide anion radicals. The higher antioxidative ability of hydrophilic antioxidants was noticed in comparison to the honeys' lipophilic anti-

oxidants. Moreover, the results for honey are consistent with the findings from another study examining multifloral Polish honey (*Wesołowska and Dzugan*, 2017).

Different flavonoids in honey originate from pollen, nectar or propolis (*Khalil et al*., 2012). According to *Bogdanov et al*. (2008), the main flavonoids found in honey are pinocembrin, apigenin, campferol, quercetin, pinobanksin, luteolin, galangin, hesperetin and isorhamnetin. *Khalil et al*. (2011) found the total content of phenolic compounds in Tualang honeys ranged between 26.99 ± 0.13 μg/g and 42.23 ± 0.64 μg/g. *Lianda et al*. (2012) found that multifloral honeys have the highest content of phenolic compounds compared with other honey types. The highest flavonoid content was identified in heather honey (44.5 ±3.2 μg/g) followed by buckwheat honey (41.7 ±2.1 μg/g), lime honey (32.0 ±1.7 μg/g) and rape honey (13.5 ±1.3 μg/g) (*Kaškonienė et al*. 2009). *Cheung et al*. (2019) not detected flavonoids in wolfberry honey, acacia honey and loquat honey. The highest total content of phenolic compounds was found in Manuka honey (250.18 ± 14.39 μg/g). On the other hand, eucalyptus honey has the highest phenolic compounds (41.65 ± 10.35 μg/g) (*Cheung et al*., 2019).

3.3. Antimicrobial compounds in honey

Various components contribute to the antibacterial efficacy of honey: the sugar content, polyphenol compounds, hydrogen peroxide, 1,2-dicarbonyl compounds and bee defensin-1 (*Almasaudi*, 2021). Phenolic compounds are found at high levels in honey and may contribute to its antibacterial activity. *Estevinho et al*. (2008) found that dark honey has a high amount of flavonoids and this has been shown to have a good correlation with its higher antibacterial activity. However, the amount of phenolic acid in honey is influenced by the geographic location and the botanical source of the nectar. The sugar content, polyphenol compounds, hydrogen peroxide, 1,2-dicarbonyl compounds and bee defensin-1 in honey depend on the source of nectar, bee type and honey storage. These components in honey work synergistically, allowing honey to be potent against a variety of microorganisms, including multidrug resistant bacteria, and/or to modulate microorganisms' resistance to antimicrobial agents. The effectiveness and potency of honey against microorganisms depends on the type of honey produced, which is contingent on its botanical origin, the health of the bee, its geographical origin and the processing method.

The natural components of honey have various activities against different microorganisms. Honey has excellent antibacterial efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) and a variety of *Pseudomonas*, which are often associated with wound and burn infections (*Hazrati et al*. 2010). Interestingly, Manuka honey, which originates from New Zealand, differs from other types of honey in that it contains a high concentration of methylglyoxal, rather than hydrogen peroxide. This compound is considered the main antimicrobial agent in Manuka honey.

Also, honey has been used in the medical treatment of surface wounds, burns and inflammation, and has a synergistic effect when applied with antibiotics (*Samarghandian et al*., 2017). *Chein et al*. (2019) found that the application of antibiotics with honey yielded better antimicrobial potential, and synergistic effects were noted against biofilms.

4. Conclusion

Honey is a natural sweetener, rich in bioactive compounds that have proven health-promoting properties. It has antimicrobial potential, showing a broad spectrum of antibacterial activities against microorganisms. Many important factors contribute to its antimicrobial efficacy, including osmolarity, hydrogen peroxide content, low pH, phenolic acid levels, and flavonoids. Honey has been used in the treatment of surface wounds, burns and inflammation, and has a synergistic effect when applied with antibiotics. The antioxidant and antibacterial activity of honey could partly be due to the presence of enzymes, such as glucose oxidase and catalase, as well as to compounds, such as phenolic acids, flavonoids and organic acids. In the future, more research needs to be conducted to understand the full potential of honey use.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200050, from 03.02.2023) financially supported this paper.

References

Ajibola, A., Chamunorwa, J. & Erlwanger, K. H. (2012). Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition & Metabolism*, 9, 61–73, https://doi:10.1186/1743-7075-9-61

Albaridi, N. A. (2019). Antibacterial Potency of Honey. *International Journal of Microbiology*, Article ID 2464507 https://doi.org/10.1155/2019/2464507

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, 28, 2188–2196.

Ávila, S., Hornung, P. S., Teixeira, G. L., Malunga, L. N., Apea-Bah, F. B., Beux, M. R. & Ribani, R. H. (2019). Bioactive compounds and biological properties of Brazilian stingless bee honey have a strong relationship with the pollen floral origin. *Food Research International*, 123, 1–10.

Bogdanov, S., Jurendic, T., Sieber, R. & Gallmann, P. (2008). Honey for nutrition and health: A review. *Journal* of the *American College* of *Nutrition,* 27, 677–689. doi:10.1080/ 07315724.2008.10719745

Cheung, Y., Meenu, M., Yu, X. & Xu, B. (2019). Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources. *International Journal of Food Properties*, 22(1), 290 –308.

Ciric, J., Spiric, D., Baltic, T., Janjic, J., Petronijevic, R., Simunovic, S. & Djordjevic, V. (2019). Element concentration and fatty acid composition of Serbian bee bread. In IOP Conference Series: Earth and Environmental Science (Vol. 333, No. 1, p. 012050). IOP Publishing.

Ćirić, J., Spirić, D., Baltić, T., Lazić, I. B., Trbović, D., Parunović, N., Petronijević, R. & Đorđević, V. (2021). Honey bees and their products as indicators of environmental element deposition. *Biological Trace Elements Research*, 199(6), 2312–2319.

Codex, (1981). Codex Alimentarius standard for honey 12-1981. Revised Codex standard for honey. Standards and standard methods (Vol. 11). Retrieved December, 2014, from http:// www.codexalimentarius.net

Estevinho, L., Pereira, A. P., Moreira L., Dias, L. G. & Pereira E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, 46(12), 3774–3779.

Graikou, K., Andreou, A. & Chinou, I. (2022). Chemical profile of Greek *Arbutus unedo* honey: Biological properties. *Journal of Apicultural Research*, 61(1), 100–106.

Habryka, C., Socha, R. & Juszczak, L. (2021). Effect of bee pollen addition on the polyphenol content, antioxidant activity, and quality parameters of honey. *Antioxidants,* 10(5), 810.

Hazrati, M., Mehrabani D., Japoni, A., Montasery, H., Azarpira, N., Hamidian-shirazi, A. R. & Tanideh, N., (2010). Effect of honey on healing of *Pseudomonas aeruginosa* infected burn wounds in rat. *Journal of Applied Animal Research*, 37(2), 161–165.

Helmi, N. & El-Soud, A. (2012). Honey between traditional uses and recent medicine. *Macedonian Journal of Medical Sciences*, 5, 205–214.

Kaškonienė, V., Maruška, A., Kornyšova, O., Charczun, N., Ligor, M. & Buszewski, B. (2009). Quantitative and qualitative determination of phenolic compounds in honey. *Chemija ir Cheminė Technologija*, 52(3), 74–80.

Khalil, M. I., Alam, N., Moniruzzaman, M., Sulaiman, S. A. & Gan, S. H. (2011). Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science*, 76(6), 921–928.

Lianda, R. L., Sant'Ana, L. D. O., Echevarria, A. & Castro, R. N. (2012). Antioxidant activity and phenolic composition of Brazilian honeys and their extracts. *Journal of the Brazilian Chemical Society*, 23, 618–627.

Machado De-Melo, A. A., Almeida-Muradian, L. B. D., Sancho, M. T. & Pascual-Maté, A. (2018). Composition and properties of *Apis mellifera* honey: A review. *Journal of Apicultural Research*, 57(1), 5–37.

Martinello, M. & Mutinelli, F. (2021). Antioxidant activity in bee products: A review. *Antioxidants*, 10(1), 71.

Ojeda De Rodrıguez, G., Sulbaran De Ferrer, B., Ferrer, A. & Rodrıguez, B. (2004). Characterization of honey produced in Venezuela. *Food Chemistry,* 84, 499–502, doi:10.1016/ S0308-8146(02)00517-4

Pontara, L. P. M., Clemente, E., Oliveira D. M., Kwiatkowski, A., Rosa, C. I. L. F. & Saia, V. E. (2012). Physicochemical and microbiological characterization of cassava flower honey samples produced by Africanized honey bees. *Ciencia e Tecnologia de Alimento,* 32, 547–552, doi:10.1590/S0101- 20612012005000066

Samarghandian, S., Farkhondeh, T. & Samini F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy Research*, 9, 121–127.

Sawicki, T., Starowicz, M., Klębukowska, L. & Hanus, P. (2022). The profile of polyphenolic compounds, contents of total phenolics and flavonoids, and antioxidant and antimicrobial properties of bee products. *Molecules*, 27(4), 1301.

Slover, C. M., Danziger, L. H., Adeniyi, B. A. & Mahady, G. B. (2009). Use of natural products to combat multidrug-resistant bacteria. In I. Ahmad & F. Aqil (Eds.), New Strategies Combating Bacterial Infection (pp. 127–135). Wiley-VCH Verlag GmbH&Co. KGaA

Tsavea, E., Vardaka, F. P., Savvidaki, E., Kellil, A., Kanelis, D., Bucekova, M. & Mossialos, D. (2022). Physicochemical characterization and biological properties of pine honey produced across Greece. *Foods*, 11(7), 943.

Wesołowska, M. & Dżugan, M. (2017). The use of the Photochem device in evaluation of antioxidant activity of Polish honey. *Food Analytical Methods*, 10(5), 1568–1574.

World Health Organization (WHO), (2014). Traditional Medicine Strategy 2014–2023, World Health Organization (WHO), Geneva, Switzerland.

Yucel, B., Topal, E. & Kosoglu, M. (2017). Bee products as functional food. In Superfood and Functional Food—An Overview of Their Processing and Utilization; Waisundara, V., Shiomi, N., Eds.; IntechOpen: Rijeka, Croatia, 2017.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 613.291:637.521.4

ID: 126600713

https://doi.org/10.18485/meattech.2023.64.2.70

Original scientific paper

Sodium chloride replacement with other chloride salts in chicken burgers

Branka Borović[a], Slobodan Lilić[a], Danijela Vranić[a], Dragan Milićević[a], Slavica Vesković[a], Jelena Babić Milijašević[a] and Tamara Gerić[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia*

ARTICLE INFO

Keywords:
Chicken burgers
Sodium chloride reduction
Potassium chloride
Ammonium chloride

ABSTRACT

The aim of this paper was to investigate the influence of reducing the sodium chloride content in chicken burgers by partial replacement of the normal level of sodium chloride with potassium chloride or ammonium chloride. The experiment consisted of five groups. In the control group of chicken burgers, only the normal level of sodium chloride was added. One third of sodium chloride was replaced with potassium chloride in group 1; One half of the sodium chloride was replaced with potassium chloride in group 2; One third of the sodium chloride was replaced with ammonium chloride in group 3. In group 4, sodium chloride was reduced by one half and one quarter of ammonium chloride was added in relation to the control group. Burgers in all experimental groups had acceptable sensory attributes. A bitter taste was the most expressed in group 2, in which one half of the added sodium chloride was replaced with potassium chloride. The most expressed saltiness acceptability and taste acceptability were in control and group 1 burgers, without statistically significant differences ($p > 0.05$).

1. Introduction

Nowadays, dietary salt intake is very high, particularly in developed countries, mainly due to consumption of processed foods. Excessive dietary sodium intake is recognised as the main cause of essential hypertension (*Brown et al*., 2009). Studies show that as salt intake increases, body mass, total blood sodium content, extracellular volume, plasma and blood volume increase. At the same time, there is a decrease in the levels of renin, angiotensin and norepinephrine (*Haddy*, 2006).

The World Health Organisation (*WHO*, 2012) recommends a daily intake for adults of less than 5 grams of salt/2,000 mg of sodium, and less than 3,510 mg of potassium. The main source of sodium in food products is derived from sodium chloride, i.e., from table salt. One of the important sources of sodium in human diets are meat products. Products like burgers and kebabs are very popular and are mostly made from beef and pork. However, there is a need to produce these products from chicken meat. Total production and consumer interest in chicken meat are permanently increasing due to this meat's low cost and due to consumers' religious aspects.

One of the methods that can be used to limit the amount of sodium in human diets is reducing the salt content of meat products by partial replacement of sodium chloride with other chloride salts (potassium chloride, KCl; calcium chloride, $CaCl_2$; and magnesium chloride, $MgCl_2$) (*Sofos*, 1983; *Tarell*, 1983). The aim of this study was to investigate the possibility of reducing the sodium content in meat preparations, i.e.,

***Corresponding author:** Branka Borović, *branka.borovic@inmes.rs*

Paper received Jun 27th 2023. Paper accepted July 9th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Table 1. Composition of chicken burgers (g)

Group	Chicken minced meat	Sodium Chloride	Potassium chloride	Ammonium chloride
Control	589.8	10.2		-
1	589.8	6.79	3.41	-
2	589.8	5.1	5.1	-
3	589.8	6.79		3.41
4	592.35	5.1		

chicken burgers, by partially replacing sodium chloride (NaCl) with KCl or ammonium chloride (NH_4Cl).

2. Materials and methods

Five sample groups of chicken *burgers* were produced from chilled, category I chicken minced meat (drumstick and thigh without skin) with different salt mixtures. The trial design is shown in Table 1.

In the control group (C), only 10.2 g of sodium chloride was added, an amount that is common for this type of product. In group 1, one third of the sodium chloride was replaced with potassium chloride, while in group 2, one half of the sodium chloride was replaced with potassium chloride. In group 3, one third of the sodium chloride was replaced with ammonium chloride. In group 4, sodium chloride was reduced by half in the relation to the control group, and one quarter of ammonium chloride was added.

2.1. Sensory evaluation

After forming, the burgers were grilled and presented to ten trained assessors who evaluated sensory attributes using numeric scales. Colour acceptability, consistency, saltiness acceptability and taste acceptability were evaluated using a 1-5-point scale, where 1 was the least acceptable and 5 was the most acceptable attribute. Saltiness intensity and bitterness intensity were evaluated with a 1-5-point scale, whereby 5 was the most expressed attribute and 1 was the least expressed attribute. Preparation and presentation of the cooked burger samples to the assessors (number, coding and randomization) as well as the fitting out of the serving area (isolation of panellists, lighting conditions) were performed according to standards. The final ranking was done according to the sum of all sensory evaluation results, where the best scored chicken burgers was ranked first and the worst ranked in fifth place.

2.2 Statistical evaluation

The obtained results were statistically evaluated using Microsoft Excel 2010 and are presented as mean±SD. Statistical differences between means of the examined parameters were determined at the levels 0.05 and 0.01 by Student's t-test.

3. Results and discussion

In Table 2, the results of sensory evaluation of the chicken burgers are shown.

Burgers in all experimental groups had acceptable sensory characteristics. Saltiness as a sensory attribute is directly linked to amount of added

Table 2. Sensory evaluation of chicken burgers, Mean±SD, n = 10

	Colour acceptability	Consistency	Saltiness acceptability	Saltiness intensity	Taste acceptability	Bitter taste intensity	Overall acceptability
C	4.70±0.46	4.50±0.55	4.45±0.47 ^{a}	4.20±0.81 ^{a}	4.50±0.50^{ax}	1.65±1.14^{a,x}	4.60±0.49^{a,x}
1	4.60±0.66	4.15±0.78	4.10±0.86	3.70±0.75	4.05±1.01	2.65±1.10	3.95±0.65
2	4.65±0.55	3.70±0.71	3.60±1.18	2,95±0.79^{b,x}	3.40±0.49^{y}	3.30±1.19^{y}	3.00±1.20^{y}
3	4.55±0.57	3.90±0.66	3.15±1.10 ^{b}	4.30±0.75 ^{y}	3.15±0.87^{y}	2,70±1.03	3.30±0.75^{b}
4	4.50±0.81	4.05±0.88	3.40±1.09 ^{b}	3.15±1.16 ^{b}	3.30±1.33 ^{b}	3.20±1.35 ^{b}	3.05±1.29^{y}

$^{(a,b)}$ Values (m ean±SD) in columns with different superscript letters are significantly different (P≤0.05)

$^{(x,y)}$ Values (mean±SD) in columns with different superscript letters are significantly different (P≤0.01)

C = control

salt (sodium chloride). Only sodium chloride has a clearly salty taste, and adding any other salt instead of sodium chloride to food products degrades the products' saltiness.

The highest saltiness intensities were determined in control and group 3 burgers, which were significantly higher than the saltiness intensities of group 2 and 4 burgers ($p < 0.05$). However, a greater statistical difference in saltiness intensity was determined between group 2 and 3 burgers ($p < 0.01$).

Saltiness acceptability and taste acceptability were both evaluated as the best in the control and group 1 burgers, without any statistically significant difference between these two groups ($p > 0.05$). The lowest evaluations for saltiness acceptability and taste acceptability were given to burgers in groups 3 and 4. Saltiness acceptability scores in these two groups were significantly different from the control group burgers ($p < 0.05$), while group 3 burgers had significantly different taste acceptability from the control group burgers at the level of $p < 0.01$.

In accordance with the taste acceptability, the overall acceptability was evaluated similarly. The most acceptable burger products were the control group, while the group 1 burgers were a little less acceptable overall, but not different to the control. Group 3 burgers were significantly less acceptable than those of the control group ($p > 0.05$), as were burgers in groups 2 and 4 ($p > 0.01$).

Colour and consistency were acceptable in all groups and without statistical differences ($p > 0.05$). A bitter taste was the most expressed in the group 2 burgers in which one half of the normal amount of sodium chloride was replaced with potassium chloride. Adding potassium chloride had a negative influence on both the saltiness and taste acceptability due to the appearance of this bitter taste, particularly in the group 2 burgers. This result is in accordance with results of *De Almeida et al*. (2016) and *Inguglia et al*. (2017).

4. Conclusion

Chicken meat burgers in all experimental groups had acceptable sensory characteristics. Colour and consistency were not affected by the addition of the different salts. Saltiness acceptability and taste acceptability were rated the most highly in the control and group 1 burgers. However, for these two attributes, group 3 and 4 burgers received the lowest ratings, which were significantly lower than the ratings for control group burgers. A bitter taste was the most expressed in group 2 burgers due to this product formulation having the largest amount of added potassium chloride.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Brown, I. J, Tzoulaki, I., Candieas, V. & Elliot P. (2009). Salt intakes around the world: implications for public helth. *International Journal of Epidemiology*, 38, 791–813.

De Almeida, M. A., Montes-Villanueva, N. D., Pinto, J. S. S., Saldaña. E. & Contreras-Castillo, C. J. (2016). Sensory and physicochemical characteristics of low sodium salami. *Scientia Agropecuaria*, 73, 347–55.

Haddy F. J. (2006). Role of dietary salt in hypertension. *Life Sciences*, 79, 1585–1592.

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P. & Burgess, C. M. (2017). Salt reduction strategies in processed meat products — A review. *Trends in Food Science and Technology*, 59, 70–78.

Sofos, J. N. (1983). Effects of reduced salt (NaCl) levels on sensory and instrumental evaluation of frankfurters. *Journal of Food Science*, 48, 1691–1692.

Terell, R. N. (1983). Reducing the sodium content of processed meats. *Food Technology*, 37(77), 66–71.

WHO, (2012). Guideline: Sodium intake for adults and children 2012; 1. ISBN 978 92 4 150483 6 available: http://apps.who.int/iris/bitstream/10665/75146/1/9789241548441_eng.pdf

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.521.4.057
641.526.3:637.521.4

ID: 126608649

https://doi.org/10.18485/meattech.2023.64.2.71

Original scientific paper

The effect of sample temperature on sensory quality of caseless sausages — cevap

Čaba Silađi[a], Vesna Đorđević[a], Volker Heinz[b], Nino Terjung[b], Franziska Witte[b] and Igor Tomašević[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *DIL German Institute of Food Technologies, Prof.-v.-Klitzing-Str. 7, 49610 Quakenbrueck, Germany*

ARTICLE INFO

Keywords:
Sensory analysis
Pork meat
Beef meat
Algae

ABSTRACT

This study analyzes the impact of the addition of three different types of algae (White *Chlorella vulgaris*, *Himanthalia elongate* (sea spaghetti), and *Undaria pinnatifida* (wakame)) at two concentrations (1.5% and 3%) on the sensory characteristics of caseless sausages (cevap) served at two different temperatures. The aim of this study was to compare the color, smell, taste, texture, juiciness, and overall acceptability of the reheated samples with those served at room temperature. The results show that cevap with wakame had the lowest scores, while white *Chlorella* had no significant effect on the sensory parameters compared to the control sample. The overall acceptability of the three cevap types was significantly higher when they were served warm than when served cold, while the color, taste, texture, and juiciness parameters were different for one of the tested samples, proving that cevap needs to be served warm.

1. Introduction

The food industry and consumers are becoming more interested in food products supporting health and well-being. These foods are generally known as functional foods, as they provide health benefits above and beyond simple nutrition (*Sloan*, 1999). However, these types of healthy foods often have a negative impact on sensory attributes, so it is necessary to determine whether they are acceptable to potential consumers.

Over the past few decades, consumer evaluation has been widely used to assess the acceptability and quality of food products, including meat. One of the most established techniques in sensory characterization is asking consumers what they think of a product through liking or preference questions, using hedonic scales (*Torrico et al.,* 2018). The gold standard in sensory evaluation for liking is the *Peryam and Girardot* (1952) 9-point hedonic scale, although several modifications are used nowadays. This kind of estimation is common in the food and meat industries and offers accurate quantitative information on the acceptability of the product. Hedonic tests are used to determine how much a product is liked, using scales ranging from like extremely, through neither like nor dislike, to dislike extremely (*Torrico et al.,* 2018).

To avoid preparation effects, the samples for sensory comparison should all be prepared according to a uniform procedure. Before beginning sensory testing, preparation steps should be standardized throughout the preliminary testing and carefully recorded to ensure uniformity. Each sample should be offered at the same temperature as the usual serving temperature for the food type being evaluated. Some foods must be served warm or reheated to develop their particular flavor or aroma (*Watts et al.,* 1989).

***Corresponding author:** Čaba Silađi, *caba.siladji@inmes.rs*

Paper received Jun 23rd 2023. Paper accepted July 2nd 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

The evolution of consumer perceptions, expectations, and needs created additional quality and sensory criteria that meat producers must satisfy (*Bredahl,* 2004). For this reason, meat products are increasingly often improved and reformulated with bioactive components, while removing some of the fat and salt (*de Medeiros et al.,* 2021). Algae can be used to develop functional foods, as they are a significant source of biologically active substances, and their inclusion increases food quality, reduces the need for chemical preservatives, and provides other health benefits (*Scieszka & Klewicka,* 2019). Systematic information on the amounts of seaweed used to reformulate meat products cannot be provided, as these amounts depend on the desired technological, nutritional, functional, or sensory effects, as well as on the type of algae (*Gullón et al.,* 2020).

In this study, three types of algae (white *Chlorella vulgaris*, *Himanthalia elongata* (sea spaghetti), and *Undaria pinnatifida* (wakame)) were added to cevap in two concentrations (1.5% and 3%) to investigate the effects of these ingredients on sensory characteristics while served reheated on the first day and at room temperature on the second day. Also, this article aimed to examine the influence of the sample temperature on the same parameters.

2. Materials and methods

2.0.1. Ingredients

Commercial fresh post-rigor pork shoulder with fat, and beef neck and shoulder clod with fat were obtained from Landschlachterei G.H. Diekmann (Essen Oldenburg, Germany). White *Chlorella vulgaris* powder was purchased from Aliga microalgae (Hjørring, Denmark), while sea spaghetti and wakame powder were purchased from Alganex (Berlin, Germany).

2.0.2. Meat preparation

Cevap was prepared at the German Institute of Food Technologies (DIL e.V., Quakenbrück, Germany) according to an industrial processing protocol. The entire study was performed on two consecutive days. On each day, seven different cevap formulations (Table 1) were prepared according to a standard industrial recipe as follows: 89% meat mixture with fat (49% beef, 40% pork) and 11.0% ice water, while 1.4% salt, 0.6% dextrose and algae (1.5% and 3%) were added "on top". Pork and beef meats were standardized to S III (with 12% fat) and R II (with 8% fat), according to the GEHA meat classification system (*Hack et al.,* 1976). The meat was ground through a 7.8mm sieve, salted with NaCl, covered with foil, and stored overnight at 4°C. Dextrose, salt, and algae were then added to all treatments (except the control, which contained no algae) and mixed in a bowl chopper (5000 Express, 30 l, KILIA GmbH, Birmingham, UK). The mixtures were formed into cylindrical shapes of approximately 2 cm in diameter and 8 cm in length using a vacuum filler (VF 608 plus, Albert Handtmann Maschinenfabrik, Biberach der Riss, Germany). After shaping, the cevap was baked on an electric grill (GGM Gastro International, Gronau, Germany) until an internal temperature of 75°C was reached and cooled at room temperature.

Table 1. Formulation of cevap with different algae per batch

Ingredients (g)	Groups						
	K	**C 1.5%**	**C 3.0%**	**S 1.5%**	**S 3.0%**	**W 1.5%**	**W 3.0%**
Beef meat (R II)	2450	2450	2450	2450	2450	2450	2450
Pork meat (S III)	2000	2000	2000	2000	2000	2000	2000
Ice	550	550	550	550	550	550	550
Salt	80	78.5	75	78.5	75	78.5	75
Dextrose	27.5	27.5	27.5	27.5	27.5	27.5	27.5
White *C. vulgaris*	/	75	150	/	/	/	/
Sea spaghetti	/	/	/	75	150	/	/
Wakame	/	/	/	/	/	75	150

K – Control cevap, C – Cevap with C. vulgaris, S – Cevap with sea spaghetti, W – Cevap with wakame

2.0.3. Sensory evaluation

The sensory evaluation of the cevap (color, smell, taste, texture, juiciness, and overall acceptability) was performed with a panel of 14 trained people with experience in sensory tests of meat products and of good general health condition with BMI between 18 and 25 kg/height in m^2, as recommended by *Forde et al.* (2013), on two consecutive days. A 7-point hedonic scale test was used with the following attributes: 1 — I absolutely dislike it, 4 – I moderately like it, 7 – I absolutely like it. During the test, panelists received a cevap of each sample labeled with a randomized three-digit number. On the first day, the samples were reheated in a microwave oven at 800W for 30 seconds before the evaluation, while on the second day, the cevap was served at room temperature. Still mineral water was used to clean the palate between samples.

2.0.4. Statistical analysis

Statistical analysis was performed using SPSS (SPSS 23.0, Chicago, IL, USA) software. The difference between mean values were tested using one-way ANOVA, Tukey's post hoc test, and t-test of paired samples ($p < 0.05$).

3. Results and discussion

The results of the sensory analysis with reheated cevap (Table 2) indicated that the use of different algae had a significant influence ($p < 0.05$) on 4 out of 7 parameters.

In terms of color, the results showed that the cevap with Wakame was the least likable compared to the rest of the cevap. W3 received lower scores (3.14), compared to the control (4.57) and samples with other algae, while W1.5 (4.07) was less desirable only compared to S1.5, which received the highest scores (5.50). Regarding smell, W3 received significantly lower marks (3.42) compared to the control sample (4.92). In respect of taste, the control samples were the best (5.14), while cevaps with *Chlorella* were insignificantly lower rated. Both samples with wakame were significantly less tasty (3.21 and 2.64, respectively) relative to control and cevap with *Chlorella*, S3 had also weaker scores (3.71) than the control, and S1.5 showed significantly higher scores (4.21) compared to W3. Similarly, the overall acceptability was the highest in control cevap (5.42), while cevap containing wakame was the least preferable (3.35 and 2.64, respectively), since they had significantly poorer results compared to C1.5, C3, and S1.5. S3 also had significantly lower scores (3.64), compared to the control. No significant difference was found between the batches in terms of texture and juiciness.

The sensory evaluation of the cevap served at room temperature showed similar results to reheated samples in terms of the best and worst ranked samples, but generally with lower mean values for every parameter. Statistically significant differences between batches ($p < 0.05$) were found in color, smell, taste, and overall acceptability (Table 3).

W3 had the lowest score (2.00) for color that was significantly lower compared to all other samples, the color scores of which varied from 3.28 to 4.35. In terms of smell, only between control (4.28) and W3 (2.71) was a meaningful difference found. Cevap without algae earned the highest score for taste (4.64) and the panelists favored them com-

Table 2. Sensory properties of the reheated cevap containing algae

Parameters	K (N=14)	C1.5 (N=14)	C3 (N=14)	S1.5 (N=14)	S3 (N=14)	W1.5 (N=14)	W3 (N=14)
	Mean values						
Color	4.57^{ab}	4.64^{ab}	5.00^{ab}	5.50^{a}	5.00^{ab}	4.07^{bc}	3.14^{c}
Smell	4.92^{a}	4.50^{ab}	4.07^{ab}	4.50^{ab}	4.42^{ab}	3.64^{ab}	3.42^{b}
Taste	5.14^{a}	4.78^{ab}	4.50^{ab}	4.21^{abc}	3.71^{bcd}	3.21^{d}	2.64^{d}
Texture	4.64	4.42	4.85	4.85	4.28	4.28	3.92
Juiciness	4.92	4.28	5.14	5.07	3.85	4.50	4.00
Overall acceptability	5.42^{a}	4.57^{abc}	4.71^{ab}	4.50^{abc}	3.64^{bcd}	3.35^{cd}	2.64^{d}

^{a,b,c,d} Different superscripts within a row indicate a significant difference ($P < 0.05$)

K – Control cevap, C – Cevap with *C. vulgaris*, S – Cevap with sea spaghetti, W – Cevap with wakame

Table 3. Sensory properties of the cevap containing algae served at room temperature

Parameters	K (N=14)	C1.5 (N=14)	C3 (N=14)	S1.5 (N=14)	S3 (N=14)	W1.5 (N=14)	W3 (N=14)
	Mean values						
Color	4.21[a]	4.00[a]	4.21[a]	4.35[a]	3.85[a]	3.28[ab]	2.00[b]
Smell	4.28[a]	3.71[ab]	3.64[ab]	4.07[ab]	4.21[ab]	3.42[ab]	2.71[b]
Taste	4.64[a]	3.64[abc]	3.50[abc]	4.07[ab]	3.00[bc]	2.78[bc]	2.35[c]
Texture	4.35	3.78	4.28	3.64	3.71	3.71	3.42
Juiciness	4.42	4.21	4.14	4.71	3.92	4.42	4.21
Overall acceptability	4.50[a]	3.57[ab]	3.35[abc]	4.07[ab]	3.00[bc]	2.92[bc]	2.07[c]

[a,b,c,d] Different superscripts within a row indicate a significant difference ($P < 0.05$)

K – Control cevap, C – Cevap with *C. vulgaris*, S – Cevap with sea spaghetti, W – Cevap with wakame

Table 4. Differences in cevap sensory properties according to serving temperature of the same cevap

Parameters	K (N=14)	C1.5 (N=14)	C3 (N=14)	S1.5 (N=14)	S3 (N=14)	W1.5 (N=14)	W3 (N=14)
	Mean values						
				Color			
Reheated cevap	4.57	4.64	5	5.50[a]	5	4.07	3.14
Cevap at room temp.	4.21	4	4.21	4.35[b]	3.85	3.28	2
p	0.336	0.145	0.102	0.017	0.052	0.222	0.063
				Smell			
Reheated cevap	4.92	4.5	4.07	4.5	4.42	3.64	3.42
Cevap at room temp.	4.28	3.71	3.64	4.07	4.21	3.42	2.71
p	0.189	0.085	0.407	0.234	0.568	0.678	0.224
				Taste			
Reheated cevap	5.14	4.78[a]	4.5	4.21	3.71	3.21	2.64
Cevap at room temp.	4.64	3.64[b]	3.5	4.07	3	2.78	2.35
p	0.336	0.026	0.058	0.671	0.065	0.407	0.336
				Texture			
Reheated cevap	4.64	4.42	4.85	4.85[a]	4.28	4.28	3.92
Cevap at room temp.	4.35	3.78	4.28	3.64[b]	3.71	3.71	3.42
p	0.591	0.108	0.263	0.029	0.135	0.283	0.278
				Juiciness			
Reheated cevap	4.92	4.28	5.14[a]	5.07	3.85	4.5	4
Cevap at room temp.	4.42	4.21	4.14[b]	4.71	3.92	4.42	4.21
p	0.439	0.890	0.024	0.336	0.893	0.888	0.620
				Overall acceptability			
Reheated cevap	5.42	4.57[a]	4.71[a]	4.50	3.64	3.35	2.64[a]
Cevap at room temp.	4.50	3.57[b]	3.35[b]	4.07	3.00	2.92	2.07[b]
p	0.097	0.005	0.038	0.336	0.108	0.451	0.026

[a,b] Different superscripts within a column and within each sensory parameter indicate a significant difference ($P < 0.05$)

K – Control cevap, C – Cevap with *C. vulgaris*, S – Cevap with sea spaghetti, W – Cevap with wakame

pared to S3, W1.5, and W3. The only other cevap with a score above 4 was S1.5, which received also significantly higher grades compared to the least likable W3 (2.35). Similarly, in overall acceptability, the best cevap was the control (4.50), while only S1.5 received a similar score above 4.00. These two batches, followed by C1.5 (3.57) had a significantly higher grade compared to W3 (2.07). The control cevap was also considerably more acceptable than S3 (3.00) and W1.5 (2.92).

Within this research, the t-test of paired samples was used to examine whether there were statistically significant differences ($p < 0.05$) for the above-mentioned six parameters in respect of the serving temperature of the same cevap.

Based on the results shown in Table 4, it can be concluded that there was a statistically significant difference in the color of S1.5, the taste of C1.5, the texture of S1.5, the juiciness of C3, and the overall acceptability of C1.5, C3, and W3, where reheated cevap had a significantly higher degree of liking in all cases.

4. Conclusion

The addition of Wakame and Sea spaghetti had a significant impact on the sensory characteristics of cevap. Color, smell, taste, and overall acceptability scores were the most reduced with the addition of wakame, regardless of the cevap serving temperature. The incorporation of sea spaghetti at a higher concentration (3%) also decreased the results for taste and overall acceptability at both serving temperatures, while *Chlorella vulgaris* had no significant effect on the sensory properties of the cevap. On the other hand, the temperature of the cevap used for this study had a significant effect on the level of the reported sensory properties. The overall acceptability of the three cevap types was noticeably higher when they were served warm compared to colder ones, while the color, taste, texture, and juiciness parameters were different for one of the tested cevap. These results support the fact that cevap needs to be served fresh from the grill or reheated during sensory analysis to reach their full sensory potential.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023 and the German Federal Ministry of Education and Research (BMBF) (Bridge2ERA2021: 100579052).

Acknowledgements: The authors would like to thank the German Institute of Food Technologies (DIL e.V., Quakenbrück, Germany) for the excellent working conditions and the survey respondents for generously giving their time and sharing their experiences on meat consumption.

References

Bredahl, L. (2004). Cue utilisation and quality perception about branded beef. *Food Quality and Preference,* 15(1), 65–75, doi.org/10.1016/S0950-3293(03)00024-7

de Medeiros, V. P. B., Pimentel, T. C., Sant'Ana, A. S. & Magnani, M. (2021). Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredients. *Current Opinion in Food Science*, 37, 125–134, doi.org/10.1016/j.cofs.2020.10.014

Forde, C. G., van Kuijk, N., Thaler, T., de Graaf, C. & Martin, N. (2013). Oral processing characteristics of solid savoury meal components and relationship with food composition, sensory attributes, and expected satiation. *Appetite,* 60(1), 208–219, doi.org/10.1016/j.appet.2012.09.015

Gullón, B., Gagaoua, M., Barba, F. J., Gullón, P., Zhang, W. & Lorenzo, J. M. (2020). Seaweeds as a promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. *Trends in Food Science & Technology,* 100, 1–18, doi.org/10.1016/j.tifs.2020.03.039

Hack, K.-H., Gerhardt, U. & Staffe, E. (1976). Verarbeitungsmaterial-Atlas für die Fleisch- und Wurstwarenproduktion (3. Aufl ed.). Gewürzmüller.

Peryam, D. & Girardot, N. (1952). Advanced taste-test method. *Food Engineering*, 24, 194.

Scieszka, S. & Klewicka, E. (2019). Algae in food: a general review. *Critical Reviews in Food Science and Nutrition*, 59(21), 3538–3547, doi.org/10.1080/10408398.2018.1496319

Sloan, A. E. (1999). The new market: foods for the not-so-healthy. *Food Technology (Chicago),* 53(2), 54–60.

Torrico, D. D., Hutchings, S. C., Ha, M., Bittner, E. P., Fuentes, S., Warner, R. D. & Dunshea, F. R. (2018). Novel techniques to understand consumer responses towards food products: A review with a focus on meat. *Meat Science*, 144, 30–42, doi.org/10.1016/j.meatsci.2018.06.006

Watts, B. M., Ylimaki, G., Jeffery, L. & Elias, L. G. (1989). Basic Sensory Methods for Food Evaluation. *IDRC*, Ottawa, ON, CA.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 632.95.02

ID: 126617353

https://doi.org/10.18485/meattech.2023.64.2.72

Original scientific paper

Distribution of pyrethroids and piperonyl butoxide in foods and feeds analysed with GC-MS/MS in 2022–2023

Nikola Borjan[a], Stefan Simunović[a], Srđan Stefanović[a], Zoran Petrović[a], Jasna Đinović-Stojanović[a], Vedrana Jelušić[b] and Saša Janković[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *Veterinary Office of Bosnia and Herzegovina, Maršala Tita 9a/II, 71000 Sarajevo, Bosnia and Herzegovina*

ARTICLE INFO

Keywords:
Pesticides
Pyrethroids
Piperonyl butoxide
GC-MS/MS
Food

ABSTRACT

The production of pesticides in the world is increasing, newly synthesized ones are replacing old ones, so pyrethroids should replace organophosphorus pesticides. As the pests become resistant, other compounds are added to the formulations in addition to pesticides; the function of these added compounds is to enhance the effect of active substances by slowing down their degradation in the pests. A wide range of products, from vegetables, fruits, animal feed, fish feed, teas, spices and honey to cereals, was analysed in order to gain insight into the distribution of pesticides, with special reference to pyrethroids and piperonyl butoxide in the analysed samples. The technique of gas chromatography with tandem mass detection (GC-MS/MS) was used for these analyses.

1. Introduction

Pesticides are a very diverse group of toxic compounds that are used to reduce pest numbers. Pesticides include products that target viruses, bacteria, molluscs, birds and rodents. However, of major concern are products that target insects, plants and fungi, because they are so widely used across the expanse of agricultural lands, forests and residential areas (*Beasley, 2020; Poppenga et al., 2010*).

Pyrethrins are natural insecticides, which have been used for al least 2000 years, found in the flowers of the plant genus *Pyrethrum*. Pyrethrin, jasmolin and cinerin are representatives of active compounds in this group with insecticidal properties. Lack of stability of natural insecticides has led us to synthesize more stable compounds (less sensitive to hydrolysis and photodegradation), which are called pyrethroids (*Ensley, 2018*). Depending on whether they contain an α-cyano-3-phenoxybenzyl moiety, pyrethroids can be divided into two types, the first which does not contain this moiety: permethrin, allethrin, tetramethrin etc. and the second type which contains the α-cyano-3-phenoxybenzyl moiety: cyfluthrin, cypermethrin, deltamethrin etc. (*Lawrence et al., 1982*).

Mostly, research on pesticide impacts is based on the living world, and as the synergists are considered inactive components, their influence is marginalized. However, the influence of these components, which are found at levels several tens of times higher than the concentration of pesticides, is by no means negligible (*Tison et al., 2023*). Piperonyl butoxide (PBO) has been used as a pesticide synergist for more than 70 years. It was largely developed in the United States due to increased concern about the spread of insect-borne diseases (*Tozzi, 1999*).

***Corresponding author:** Nikola Borjan, nikola.borjan@inmes.rs*

Paper received Jun 27th 2023. Paper accepted July 8th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

PBO is currently found in about 1700 products, where sometimes it is indicated as an active ingredient, but sometimes it is considered an inert ingredient and is not on the declaration. Commercial names are pybuthrin and butacide (*US EPA, 2005*).

Pyrethroids achieve their insecticidal properties by affecting the action potential of insect nerves by modifying the kinetics of ion channels (primarily voltage-gated sodium channels, secondary γ-aminobutyric acid (GABA)-gated chloride channels and voltage-gated calcium channels), whereby they become more permeable to sodium ions, which can lead to depolarization of nerves and loss of nerve function (*Solderlund, 2010*). PBO works to enhance pesticide efficacy by inhibiting detoxification induced by cytochrome P450 enzymes (CYPs). CYPs are a very big family of enzymes that have a prosthetic haem group. They play major roles in xenobiotic metabolism (drugs, pesticides and other chemicals), and almost 75% of ingested drugs will be processed by this family of enzymes. Beside xenobiotic metabolism, the metabolism of a very big, endogenous group of compounds (steroids, fat-soluble vitamins, fatty acids etc.) is not possible without CYPs (*Zhao et al., 2021*). PBO inhibits CYPs in two phases, firstly by binding to the active site and secondly by forming an irreversible complex with CYP's haem group. PBO also inhibits esterase and glucuronosyltransferase enzymes (*Snoeck et al. 2017*).

2. Materials and methods

All food and feed samples (see Table 1) were divided into several groups according to origin, water, sugar and fat content according to *SRPS EN 15662* (2018). All samples were routinely analysed from May 2022 to May 2023. Pesticides (209 compounds) were extracted with acetonitrile (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and/or water (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and then purified using the QuEChERS technique. Identification and quantification was done using gas chromatography with a triple quadrupole mass spectrometer (GCMS-TQ8050 NX, Shimadzu Corporation, Japan).

Sample preparation was based on the QuEChERS method developed by *Anastassiades et al.* (2003) and specified in *SRPS EN 15662* (2018). Homogenized sample (5 g) was weighed into a 50 mL centrifuge tube, internal standard PCB 52 (Lab Instrument srl, Italy) and 10 mL of acetonitrile or/and water were added, and the tube content was shaken vigorously for 1 minute. Extraction was done using 10 mL of acetonitrile (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and QuEChERS Mix (4.0 g $MgSO_4$, 1 g NaCl, 1 g SCTD, 0.5 g SCDS; Phenomenex Inc., USA). Taking care not to form lumps, each sample was immediately shaken by hand for 1 minute followed by 15 min on a shaker (iRoll PR35, Neuation Technology Pvt. Ltd., India) and centrifuged (Sigma 2-16P, Sigma Laborzentrifugen GmbH, Germany) for 5 minutes at 3000 rpm. An aliquot of the extract was transferred to 15 mL centrifuge tube which contains dispersive SPE clean-up mixture (150 mg $MgSO_4$, 25 mg C18 sorbent, per mL of extract) (Phenomenex Inc., USA). The mixture was shaken by hand for 1 minute and centrifuged for 5 minutes at 3000 rpm. After centrifugation, the supernatant was transferred to a glass vial for gas-chromatography analysis. With each set of samples, a four-point matrix-matched series of calibration enriched samples (as well as a blank sample and a control enriched sample) were prepared so that final concentration were in range from 5 to 50 μg kg-1 (*Guidance SANTE, 2021*). All pesticide mixtures were bought from CPAchem Ltd, Bulgaria.

Chromatographic separation was done using a fused silica GC column (30 m × 0.25 μm ID and 0.25 df). All analytes were measured in MRM (multiple reaction monitoring) mode with one quantifier and at least one qualifier ion, with instrument setup according to the manufacturer (*Shimadzu application news*, 2022). Identification was done by comparing the retention times of the standards with the retention times of the peaks in the sample. The areas of the peaks in the standard and the control sample were determined and the yields calculated. Measured concentrations were corrected for the response factors of internal standards. Quantification was performed using Lab Solution data® processing software (Shimadzu Corporation, Japan).

Statistical analysis of experimental data was performed using software Minitab® 17.1.0 Statistical Software. Microsoft Office® Excel was used for graphic displays.

3. Results

The total number of analysed samples was 826, of which 145 were positive samples that contained at least one quantified pesticide (divided into the groups in Table 1). Detected pyrethroids were: bifenthrin (sum of all isomers), cypermethrin (sum of all isomers), deltamethrin (cis-deltamethrin), fenvalerate, λ-cyhalothrin (sum of all isomers), permethrin (sum

of all isomers), τ-fluvalinate, tefluthrin and tetramethrin. All positive samples were in compliance with Serbia's national legislation (*Serbia*, 2022). The results for positive samples in 2022 and 2023 are presented in Table 1. Among the groups, vegetables and fruits had the highest numbers of pyrethroid-positive samples, 11 and 9, respectively. The group with the highest number of samples containing PBO were, in order, animal feed, fish feed and cereals and cereal products. Figure 1 shows the ratios of pyrethroids, PBO and other pesticides in the total number of positive samples according to sample groups.

Altogether, 17.6% of all samples analysed contained pesticides. Of the total number of samples, other pesticides accounted for 8.5%, PBO accounted for 6.2% and pyrethroids accounted for 2.9% of positive samples.

Table 2 shows the range of pyrethroids and PBO levels according to the sample groups. The only group of samples where almost all pyrethroids and PBO were detected and were quantifiable, with the exception of fenvalerate, permethrin and τ-fluvalinate, was vegetables. The groups of samples with the lowest levels of pyrethroid pesticides were

Table 1. Occurrence of pyrethroids and piperonyl butoxide in analysed groups of foods and feeds

Sample group	Number of positive samples			
	Pyrethroids	PBO	Other pesticides	Total number of positive samples per group
Vegetables	11	1	33	45
Fruits	9	0	21	30
Animal feed	1	23	5	29
Fish feed	0	12	1	13
Cereals and cereal products	2	9	2	13
Tea, spices	1	5	7	13
Honey	0	1	1	2
TOTAL	24	51	70	145

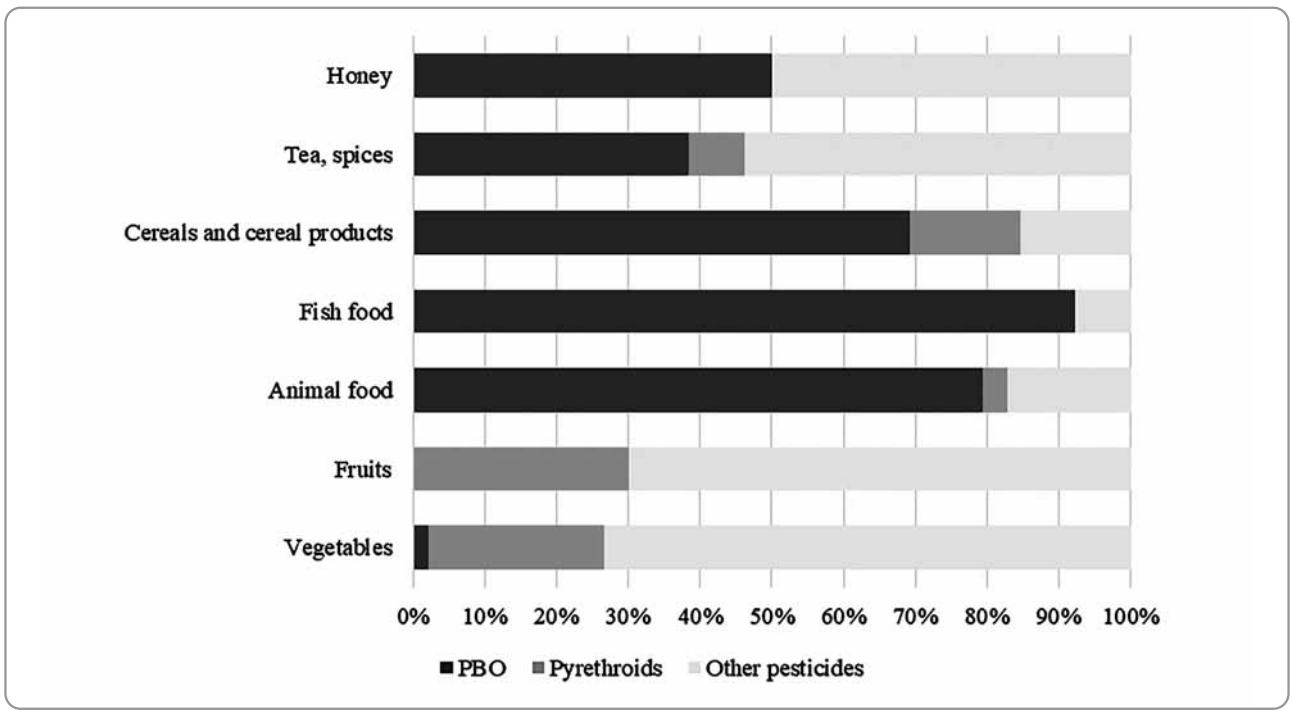


Figure 1. Ratios of piperonyl butoxide, pyrethroids and other pesticides among the pesticide-positive groups of foods and feeds

Table 2. Pyrethroid and piperonyl butoxide (PBO) levels (μg kg^{-1}) in grouped food and feed samples in 2022–2023

Sample group	Bif	Cyp	Del	Fen	Cyh	Per	Flu	Tef	Tet	PBO
Vegetables	9.2–30	6.1–1205	5.0–17	/	17 [a]	/	/	6.2–15	9.1–11	30 [a]
Fruits	/	5.3–190	5.0–19	/	18–23	/	464 [a]	/	/	/
Animal feed	/	9.4[a]	5.1–19	/	/	57–133	/	/	/	5.0–161
Fish feed	/	/	/	/	/	45–66	/	/	/	6.5–133
Cereals and cereal products	/	/	9.8–20	/	/	/	/	12 [a]	8.3–11	5.7–378
Tea, spices	/	21–7920	/	/	/	/	/	/	/	6.0–152
Honey	/	5.3 [a]	/	8.3 [a]	/	/	/	/	/	32 [a]

Bif – bifenthrin, Cyp – cypermethrin (sum of all isomers), Del – deltamethrin, Fen – fenvalerate, Cyh – λ-cyhalothrin, Per – permethrin, Flu – τ-fluvalinate, Tef – tefluthrin, Tet – tetramethrin,

[a] – one positive sample

fish feed and tea and spices. However, every sample of fish feed contained PBO. PBO was found in all sample groups except in fruits.

Cypermethrin with all of its isomers were quantified in all sample groups except in fish feed and cereals and cereal products. The highest quantified levels were for cypermethrin in spices and cypermethrin in vegetables, with levels of 7920 μg kg^{-1} and 1200 μg kg^{-1}, respectively. Some pyrethroids were found at low frequencies in the foods and feeds, e.g., fenvalerate in one honey (8.3 μg kg^{-1}) and τ-fluvalinate, which was quantified in one fruit at a level of 464 μg kg^{-1}.

4. Conclusion

In the human body, pyrethroids are degraded in up to 24 hours, but when they are combined with PBO and when their degradation is stopped, that time is significantly longer. The activity of CYPs and other enzymes is reduced in the presence of PBO, and therefore, enzyme function is weakened and the release of toxins is reduced (*Singh et al., 2022*). This happens during acute contact with the pyrethroid-PBO combination, but as to what actually happens with chronic exposure to this combination over a period of several years, we remain unsure. We have barely scratched the surface of this problem. Some studies have shown that pyrethroid-PBO causes enlargement of the liver (*Hoberman et al., 2022*), promotes cancer formation (*Okamiya et al., 1998*), reduces foetus size and is toxic to male reproductive system (*Bae et al., 2021*), and is linked to neurodevelopment problems (*Horton et al. 2011*). From the obtained results, there is a high prevalence of low or relatively low concentrations of pesticides in the food chain, and this can affect the health of people and animals. A study that will include the combination of pyrethroid-PBO is certainly needed.

Disclosure statement: No potential conflict of interest was reported by the authors.

Fundings: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, according to the provisions of the Contract on research financing 2023. No 451–03-47/2023-01/200050 dated from 03.02.2023.

References

Anastassiades, M., Lehotay S. J., Stajnbaher, D. & Schenck, F. J., (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International*, 86(2), 412–431.

Bae, J. & Kwon, W. (2021). Piperonyl butoxide, a synergist of pesticides can elicit male-mediated reproductive toxicity. *Reproductive Toxicology* 100, 120–125.

Beasley, V. R. (2020). Direct and Indirect Effects of Environmental Contaminants on Amphibians. *Reference Module in Earth Systems and Environmental Sciences,* https://doi.org/10.1016/B978-0-12-409548-9.11274-6.

Guidance SANTE 11312/2021, (2021). Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.

Gupta, R. C. (2018). Veterinary toxicology, Basic and Clinical Principles (3rd ed.) Ensley, S. M. (Chapter 39).

Hoberman, A. M. & Hauswirth, J. W. (2022). Developmental and reproduction toxicity of piperonyl butoxide part 1 developmental safety of piperonyl butoxide in the CD® (Sprague Dawley) rat. *Reproductive Toxicology* 112, 171–176.

Jones, D. G. (1999). Piperonyl Butoxide, The Insecticide Synergist. Tozzi. A. (Chapter 1)

Krieger, R. (2010). Hayes' Handbook of Pesticide Toxicology. Poppenga, R. H., Oehme, F. W., (Chapter 7).

Krieger, R. (2010). Hayes' Handbook of Pesticide Toxicology. Solderlund, D., M. (Chapter 77).

Lawrence, L. J., and Casida, J. E. (1982). Pyrethroid toxicology: mouse intracerebral structure-toxicity relationships. *Pesticide Biochemistry and Physiology*, 18, 9–14.

Okamiya, H., Mitsumori, K., Onodera, H., Ito, S., Imazawa, T., Yasuhara, K. & Takahashi, M. (1998). Mechanistic study on liver tumor promoting effects of piperonyl butoxide in rats. *Archives of Toxicology,* 72 (11), 744–750.

Serbia. (2022). *Official Gazette of the Republic of Serbia*, 91.

Shimadzu application news. (2022). Method for the determination of 431 Residual Pesticides in Honey using LCMS-8050 and GCMS-TQ8040 NX. Retrieved from https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/applications/application_note/16648/an_06-saip-f-03-en.pdf. Accessed May 3, 2022.

Singh, S., Mukherjee, A., Jaiswal, D. K., Prudêncio de Araujo Pereira, A., Prasad, R., Sharma, M., Kuhad, R. C., Shukla, A. C. & Verma, J. P. (2022). Advances and future prospects of pyrethroids: Toxicity and microbial degradation. *Science of the Total Environment* 829, 154561.

Snoeck, S., Greenhalgh, R., Tirry, L., Clark, R. M., Van Leeuwen, T. & Dermauw, W. (2017). The effect of insecticide synergist treatment on genome-wide gene expression in a polyphagous pest. *Scientific Reports* 7, 13440.

SRPS EN 15662:2018. (2018). Foods of plant origin — Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE — Modular QuEChERS-method.

Tison, L., Franc, C., Burkart, L., Jactel, H., Monceau, K., Revel, G. & Thiéry. D. (2023). Pesticide contamination in an intensive insect predator of honey bees. *Environment International*, 176, 107975.

US EPA, (2005). Overview of the Piperonyl Butoxide Risk Assessments. Retrieved from https://www.regulations.gov/document/EPA-HQ-OPP-2005-0042-0003. Accessed April 10, 2023.

Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., Huai, C., Shen, L., Zhang, N., He, L., Qin, S. (2021). Cytochrome P450 Enzymes and Drug Metabolism in Humans. *International Journal of Molecular Sciences,* 22, 12808.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.524.055
615.279:634.74

ID: 126626569

https://doi.org/10.18485/meattech.2023.64.2.73

Original scientific paper

Effect of chokeberry (*Aronia melanocarpa)* extract on the sensory properties of raw cooked meat products (frankfurters)

Andrea Mesárošová[a], Marek Bobko[a], Lukáš Jurčaga[a], Alica Bobková[a], Katarína Poláková[a], Alžbeta Demianová[a], Miroslav Kročko[a], Judita Lidiková[a], Ondřej Bučko[b] and Andrea Mendelová[a]*

[a] *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Science, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*
[b] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Animal Husbandry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

ARTICLE INFO

Keywords:
Chokeberry
Meat product
Sausages
Sensory properties
Frankfurters

ABSTRACT

The current research focuses on sensory and textural properties and color changes of soft meat products (frankfurters), which we observed on days 1 and 21 of storage. We incorporated chokeberry extract into frankfurters as natural antioxidants. We used two concentrations of extracts (3 mL kg^{-1} and 5 mL kg^{-1}), the control with the addition of vitamin C and negative control. A colorimeter and instrumental texture analyser were used to create the texture and color profiles. At the beginning of storage, the group with added vitamin C had the lightest L* parameter, and there were no statistically significant differences between the a* and b* parameters. After 21 days, the values of all color parameters decreased. When determining textural properties, no negative effect was observed after the addition of extracts. According to sensory evaluations, the groups supplemented with natural antioxidants, and the control supplemented with vitamin C improved consumer acceptability and preference.

1. Introduction

In the meat industry, there is a growing interest in the use of innovative processing methods, reformulated products and the replacement of synthetic ingredients with natural bioactive compounds. Innovative methods could minimize health problems and improve the overall organoleptic, nutritional and health properties of processed meat (*Jiang & Xiong*, 2016). These strategies are in line with customer expectations, who increasingly prefer the addition of natural antioxidants and colorants derived from plants over synthetic varieties (*Kowalczyk et al.*, 2023). The main components of plant materials in this context are phenolic acids, which contribute to their antioxidant capacity (*Munekata et al.*, 2016; *Şahin et al*., 2017). The concentration of antioxidant activity differs in different plant materials and therefore the dosage in meat products differs. Berries and their extracts are rich in polyphenols and are suitable for use in meat products (*Lorenzo et al.,* 2018).

2. Materials and methods

The fruit of *Aronia melanocarpa* was provided by the Botanic Garden of Slovak University of Agriculture in Nitra. The meat (loin and shoulder) for the meat product manufacturing were bought at a local butcher shop.

***Corresponding author:** Andrea Mesárošová, *xmesarosova@uniag.sk*

Paper received Jun 29th 2023. Paper accepted July 9th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2.1 Extract preparation

According to *Jurčaga et al.* (2021), *Aronia melanocarpa* was extracted. Fruits that had been dried and homogenized (20 g) were combined with 100 mL of 80% ethanol in a shaker and allow to rest for 24 hours at room temperature and in the dark. Ethanol was added to the filtrate to a maximum volume of 100 mL. In a vacuum rotary evaporator, the liquid portion was then evaporated until dry at 65°C. Resolving the weighted dry residue in 50 mL of water. The finished extract was stored in the dark at 4°C.

2.2 Frankfurters preparation

The following materials were used for preparing the meat product: pork meat, water, a salting mixture with 0.3% sodium nitrite concentration, black pepper, sweet and sour paprika, and nutmeg. The antioxidants were then incorporated after mixing all of the ingredients together. The control group (Con) was prepared with no antioxidant additive at all. The second group (Con-C) contained 0.7 g kg^{-1} of citric acid. The third (AM-1) and the fourth group (AM-2) contained 3 mL kg^{-1} and 5 mL kg^{-1} of extracts of *Aronia melanocarpa*, respectively. The finished pork frankfurters were heat-cured by wet smoking to obtain a temperature of 70°C in the core for at least 10 minutes, cooled down, and packaged, vacuum sealed, and stored at 4°C for 21 days.

2.3 Color determination

The color of each sample was measured using a spectrophotometer (Konica Minolta CM-2600d, Osaka, Japan) set to Specular Component Included (SCI) after each sample had been homogenized. We used a 10° observer with an 8 mm-diameter port and the D65 light source. According to the manual, the white plate calibration was performed at a temperature of 23°C. The results of the experiment were represented as coordinates in the CIELab color interface, where L* indicates lightness, a* for redness-greenness, and b* for yellowness-blueness. Color measurements were made on days 1 and 21 of storage.

2.4 Texture analysis

The TA.XTplus Texture Analyzer (Godalming UK) was used as the texture analyzer machine to determine the textural characteristics. Before the analysis, the frankfurters were cooked to a core temperature of 70°C. The materials were cut into 1 × 1 cm blocks before analysis. With a Warner-Bratzler probe (V-blade) chosen from the analyzer library, we used the default settings for the hot-dog analysis. We observed the firmness and toughness measurements. Texture analysis measurements were made on days 1 and 21.

2.5 Sensory evaluation

A professional panel performed the sensory evaluation on days 1 and 21 after preparation. Before the evaluation, all of the samples were heated. Five sensory parameters were observed: appearance (surface and on a cut), color, odor, consistency, and taste. Every parameter was rated on a scale of 1 to 5. A score of 5 represented the parameter's best score and 1 the worst score for each selected parameter. We calculated the arithmetic mean from the assigned values. The sensory panel was made up of 10 trained evaluators, both male and female, ranging in age from 25 to 50. The Department of Technology and Quality of Animal Products provided all of the evaluators, all of whom have experience rating the quality of animal products.

2.6 Statistical analysis

ANOVA analysis with a Duncan test was performed to compare the findings of the various analysis groups. The level of significance was set to 0.05 for each test. The statistical and data analysis solution XLSTAT (Addinsoft, 2021, New York, NY, USA), was used for the analysis.

3. Results and discussion

3.1 Color analysis

Color determination of all the samples was carried out on the days 1 and 21 of storage. The main objective of our research was to observe changes between the negative control (Con. — without antioxidants) and experimental groups (Con-C, AM-1, AM-2). For the lightness parameter, on day 1, Con-C showed the highest value compared to the other groups. On day 1, there were no significant differences between the negative control and experimental groups in yellowness and redness. At the end of the storage period, on the day 21, no statistically significant differences were found in the lightness of the groups of frankfurters. For the yellowness parameter, the control with added vitamin C (Con-C) clearly showed the highest value compared to the other groups. A statistically significant difference was found in redness between the negative control (Con.) and the experimental group AM-2. *Muzolf-Panek et al.* (2015) reported that the addition

Table 1. Color analysis results of frankfurter sausages

Group	Day 1			Day 21		
	L*(D65)	a*(D65)	b*(D65)	L*(D65)	a*(D65)	b*(D65)
Con.	65.80± 1.224^{b}	12.38± 0.706^{a}	19.34± 0.896^{a}	66.11± 1.378^{a}	11.86± 0.320^{b}	18.78± 0.512^{b}
Con-C	67.69± 0.907^{a}	12.96± 0.367^{a}	19.44± 0.325^{a}	66.28± 0.923^{a}	12.72± 0.651^{a}	19.16± 0.677^{ab}
AM-1	65.70± 0.900^{b}	12.91± 0.793^{a}	19.20± 0.838^{a}	66.77± 1.062^{a}	11.65± 0.664^{b}	19.18± 0.694^{ab}
AM-2	66.77± 1.381^{ab}	12.87± 0.813^{a}	19.40± 0.690^{a}	66.62± 0.867^{a}	12.17± 0.564^{b}	19.82± 0.576^{a}
p-value	0.002	0.277	0.900	0.544	0.002	0.011

Note: Con.= negative control, Con-C= control with 0.7 g kg^{-1} vit. C, AM-1= 3 mL chokeberry extract, AM-2= 5mL chokeberry extract, results are expressed as value ± S.D.; L* = measured lightness, a* = measured redness, b* = measured yellowness, D65-standard illumination used for measurement; a, ab, b as upper index represent statistically significant differences between groups in columns.

of blueberry extract caused a significant decrease in the values of the L* parameter, which results from the dark blue color of blueberries. Similar findings were reported by *Garrido et al.* (2011), *Selani et al.* (2011) and *Jia et al.* (2012) after the addition of grape and blackcurrant extracts. The blueberry extract also caused a significant reduction in the redness of the pork loin compared to the control sample. The b* parameter was also affected by the addition of blueberry extract, which significantly reduced the yellowness of pork meatloaf. Similar to our study, *Muzolf-Panek et al.* (2015) reported that the parameter b* was affected after the addition of blueberry extract, which significantly reduced the yellowness of the pork cutlet.

3.2 Textural analysis

The textural analysis of all the samples was carried out on days 1 and 21 of storage. All samples were cut into 1 × 1 cm squares. The objective of our study was to find out whether the addition of the extract would have an impact on the final meat

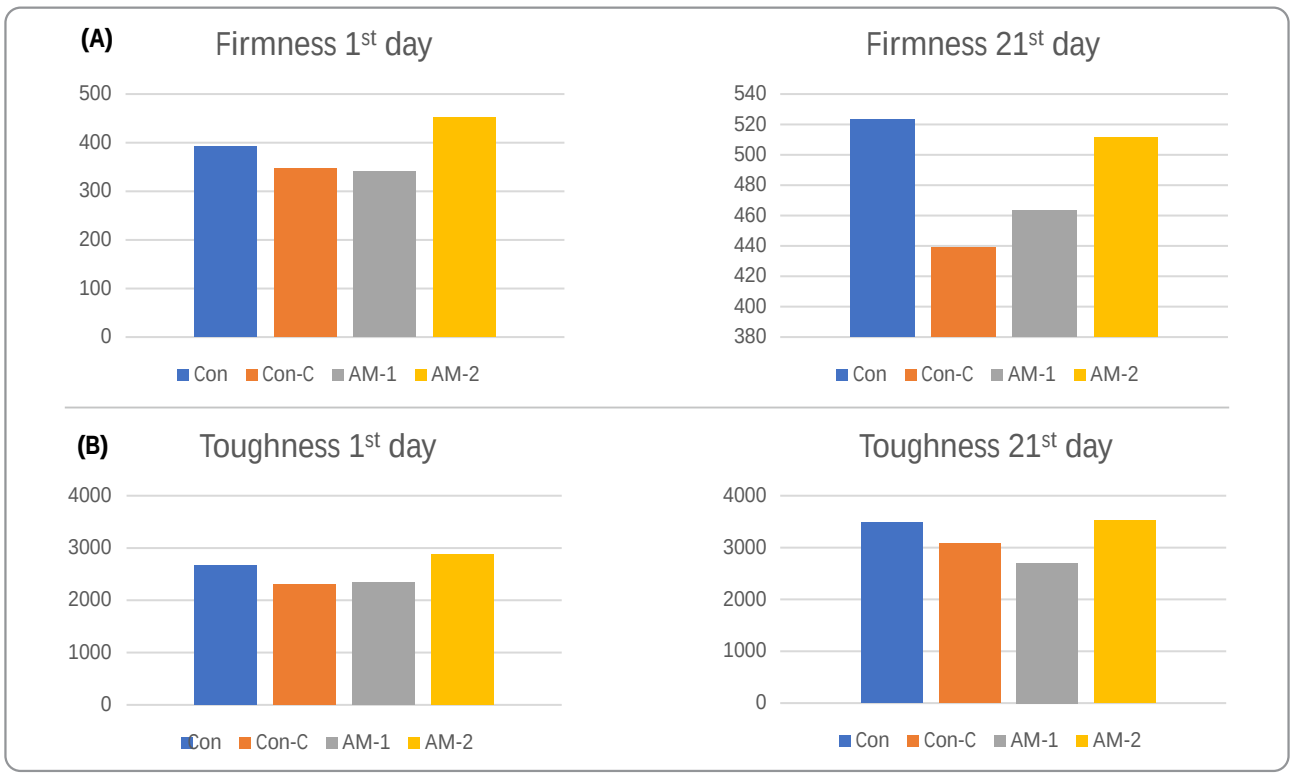


Figure 1. (A) Results of textural analysis of frankfurter sausages — Firmness (g); **(B)** Results of textural analysis of frankfurter sausages — Toughness (g.s)

Note: Con.= negative control, Con-C= control with 0.7 g kg^{-1} vitamin C, AM-1= 3 m L chokeberry extract, AM-2= 5 mL chokeberry extract, a, ab, b as upper index represent statistically significant differences between groups on each day of measurement.

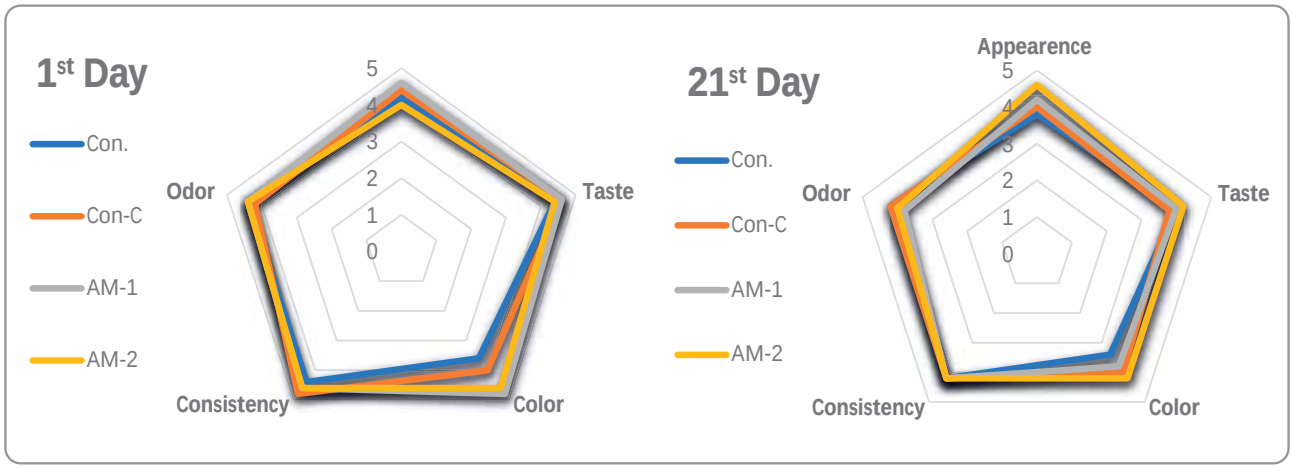


Figure 2. Results of sensory evaluation for frankfurter sausages

product's firmness and toughness over the course of storage. At the beginning of the storage period, the highest values of firmness and toughness were measured in the experimental group AM-2 compared to the other groups. After 21 days of storage, no statistically significant differences between the groups were demonstrated in firmness or toughness. The observed differences between the analyzed groups could be due to the technological procedure of dough filling rather than the addition of antioxidants. *Jurčaga et al.* (2021) in their analysis noted no significant differences in strength between all groups during the storage period. They noted a significant difference in the toughness parameter between the negative control and the groups with the addition of antioxidants.

3.3 Sensory evaluation

Possibly the most important factor in determining customer satisfaction is the product's sensory quality. Any experimental addition cannot affect quality indicators like taste or odor. Our objective was to monitor changes in the chosen parameters over the period of storage. After the first day of storage, the most favorably evaluated sample was experimental group AM-1, which achieved the highest average scores for appearance, taste, color and smell. This sample also achieved the best overall score. The group with the addition of vitamin C (Con-C) achieved the best scores for taste and consistency. After 21 days of storage, we observed a decrease in scores for all groups. The best rated was the experimental group AM-2, which achieved the best rating in all monitored parameters. After 1 and 21 days of storage, both experimental groups (AM-1 and AM-2) achieved satisfactory scores, indicating that chokeberry extracts can improve the sensory quality of meat products. Barberry extract was added to chicken frankfurters by *Jaberi et al.* in concentrations of 0.75, 1.5, and 3%. Additionally, in their research, the group that received 3% barberry extract had the highest color value. In an experiment with blackcurrant extract, *Jurčaga et al.* (2021) reported similar results. After adding extracts from black chokeberry, blackberry, blueberry and red currant pomace, *Babaoglu et al.* (2022) found that extracts from the pomace of various berries preserved the sensory properties of beef patties despite advances in refrigerated storage. Similar results were reported by *Turan and Şimşek* (2021), who added 0.1% and 0.2% black mulberry water extract and found that the extracts did not affect the color, texture, aroma or taste scores of cooked beef patties.

4. Conclusion

In our study, we observed the effect of chokeberry extracts on the sensory and textural properties and color changes of frankfurters compared to a negative control and a group with vitamin C supplementation. The frankfurters were stored for 21 days in vacuum packaging and were kept in a refrigerator. During the storage of the products, we did not observe any negative effects of the added extracts on the textural properties and color changes. During the sensory evaluation at the end of the storage period, the experimental groups with a higher amount of extract showed the best point evaluation in appearance, taste, color and consistency. From the findings, chokeberry extracts seem to be suitable as natural antioxidants in meat products, but further experiments would be necessary.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Slovak Research and Development Agency under grant KEGA 001 SPU — 4/2023 and project Visegrad+ Grant 22230075 — Food Quality in Digital Age.

References

Babaoğlu, A. S., Unal, K., Dilek, N. M., Poçan, H. B. & Karakaya, M. (2022). Antioxidant and antimicrobial effects of blackberry, black chokeberry, blueberry and redcurrant pomace extracts on beef patties subject to refrigerated storage. *Meat Science*, 187, https://doi.org/10.1016/j.meatsci.2022.108765

Garrido, M. D., Auqui, M., Martí, N. & Linares, M. B. (2011). Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers. *LWT — Food Science Technology,* 44, 2238–2243, https://doi.org/10.1016/j.lwt.2011.07.003

Jaberi, R., Kaban, G. & Kaya, M. (2020). The effect of barberry (*Berberis vulgaris* L.) extract on the physicochemical properties, sensory characteristics and volatile compounds of chicken frankfurters. *Journal of Food Processing and Preservation*, 44, https://doi.org/10.1111/jfpp.14501

Jia, N., Kong, B., Liu, Q., Diao, X. & Xia, X. (2012). Antioxidant activity of black currant (*Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Science,* 91, 533–539, https://doi.org/10.1016/j.meatsci.2012.03.010

Jiang, J. & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, 120, 107–117, https://doi.org/10.1016/j.meatsci.2016.04.005

Jurčaga, L., Bobko, M., Kolesárová, A., Bobková, A., Demianová, A., Belej, Ľ., Mendelová, A., Bučko, O., Kročko, M. & Čech, M. (2021). Blackcurrant (*Ribes nigrum* L.) and Kamchatka Honeysuckle (*Lonicera caerulea* var. Kamtschtica) extract effects on technological properties, sensory quality and lipid oxidation of raw-cooked meat product (frankfurters). *Foods,* 10, 2957, https://doi.org/10.3390/foods10122957

Kowalczyk, M., Domaradzki, P., Materska, M., Florek, M., Kaliniak-Dziura, A., Skałecki, P., Żółkiewski, P., Grenda, T. & Pabich, M. (2023). Effect od the addition of chokeberry leaf extract on the physicochemical and sensory properties of burgers from dark cutting veal. Food *Chemistry*, 399, https://doi.org/10.1016/j.foodchem.2022.133978

Muzolf-Panek, M., Waśkiewicz, A., Kowalski, R. & Konieczny, P. (2015). The effect of Blueberries on the oxidative stability of pork meatloaf during chilled storage. *Journal of Food Processing and Preservation,* 40, 899–909, https://doi.org/10.1111/jfpp.12668

Selani, M. M., Contreras-Castillo, C. J., Shirahigue, L. D., Gallo, C. R., Plata-Oviedo, M. & Montes-Villanueva, N. D. (2011). Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. *Meat Science*, 88, 397–403, https://doi.org/10.1016/j.meatsci.2011.01.017

Turan, E. & Şimşek, A. (2021). Effects of lyophilized black mulberry water extract on lipid oxidation, metmyoglobin formation, color stability, microbial quality and sensory properties of beef patties stored under aerobic and vacuum packaging conditions. *Meat Science*, 178, https://doi.org/10.1016/j.meatsci.2021.108522

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.52.083.6

ID: 126630921

https://doi.org/10.18485/meattech.2023.64.2.74

Original scientific paper

Nutritional strategies to reduce ammonia and carbon dioxide production in intensive livestock production

Miljana Krstić[a], Vesna Đorđević[a], Jelena Ćirić[a], Tatjana Baltić[a], Aleksandar Bajčić[a], Sara Simunović[a] and Dejan Perić[b]

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11 000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Poultry production
Ammonia
Carbon dioxide
Animal nutrition

ABSTRACT

Poultry production is an example of mass livestock production, so intensive production of fattening broilers involves raising broilers on farms with a capacity of 5,000 to 50,000 units or more at a density of 0.06 m^2 per bird. Modern poultry farms are constructed with the task of reducing heat loss, i.e. improving energy efficiency, which very often in combination with reduced ventilation can lead to increased levels of ammonia (NH_3), carbon dioxide (CO_2) and other air pollutants, and thus adversely affect animal health and productivity. The speed of gas emissions is influenced by many factors, such as the composition of feed and the efficiency of feed use (conversion), the quality of the litter and the microclimatic conditions on the farm. The litter on intensive poultry farms usually contains 4 to 6% of nitrogen, most of which is in NH_3 or NH_4^+ form. The mixture of litter and manure is a storage of nitrogen which is released in the form of ammonia under appropriate conditions. On the other hand, the main source of carbon dioxide in livestock is the product of animal respiration, so there is a connection between the levels of animal metabolism and CO_2 production on farms. The production of carbon dioxide in birds is proportional to their metabolic heat production, and thus to the metabolic body mass of the bird, which is affected by temperature and activity. The aim of the study was to examine the effect of a nutritional supplement, Eubiotic, added to broiler feed on the NH_3 and CO_2 emissions in a broiler farm. The values of NH_3 and CO_2 emissions in the facility for breeding fattening broilers that received Eubiotic in feed were numerically lower, which can be explained by better digestibility of basic nutrients, primarily proteins, present in feed.

1. Introduction

Poultry production is an example of mass livestock production, so the intensive production of fattening broilers implies raising broilers on farms with a capacity of 5,000 to 50,000 individuals or more at a density of 0.06 m^2 per bird. The way broilers are raised has a direct impact on pollution with harmful compounds, dust emissions and microbiological pollution on farms, and among them chemical pollutants are hazards that are equally dangerous for human and animal health. Modern poultry farms are constructed with the task of reducing heat loss, i.e., improving energy efficiency, which very often in combination with a reduced level of ven-

***Corresponding author:** Krstić Miljana, *miljana.krstic@inmes.rs*

Paper received Jun 30th 2023. Paper accepted July 12th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

tilation can lead to increased levels of ammonia (NH_3), carbon dioxide (CO_2) and other air pollutants, and thus negatively affect the health and productivity of animals (*Brouček and Čermák*, 2015). The production and emission of gases in poultry or any animal housing facility involves complex biological, physical and chemical processes. The rate of gas emission is influenced by many factors, such as feed composition and feed utilization efficiency (conversion), litter quality and microclimatic conditions on the farm.

2. Ammonia production on broiler farms

Litter on intensive poultry farms typically contains 4 to 6% nitrogen, most of which is in the NH_3 or NH_4^+ form. The mixture of litter and manure is a storehouse of nitrogen (N) which, under suitable conditions, is released in the form of ammonia. Many factors, such as season, facility temperature, relative humidity and broiler health, can affect the level of ammonia release in broiler farms. Ammonia is formed by the decomposition of nitrogenous waste products in manure (undigested protein from food and uric acid) under the action of exogenous enzymes produced by microorganisms. Factors that exert direct control over these processes are the pH of the manure, and the temperature and humidity of the facility, and they are strongly influenced by the age of the flock, that is, the age of the birds (*Knížatová et al.*, 2010b). It has been proven that the rate of ammonia emission increases with the age of the flock from an almost zero value at the beginning of the production cycle, to a maximum value at the end. Lower ammonia concentrations and higher ventilation rates were noted during warm summer months, while ammonia concentrations were higher during cold weather when low ventilation rates provided less fresh air for ammonia dilution (*Gates et al.*, 2005). Elevated concentrations of ammonia on broiler breeding farms, in addition to having a negative impact on the smell component in the facility, reduce food intake and slow down the growth rate of animals, and have a negative effect on the respiratory tract, increasing susceptibility to Newcastle virus and *Mycoplasma gallisepticum*, as well as the incidence of airborne sacculitis and keratoconjunctivitis (*Liu et al*., 2009). The main source of ammonia is the urine of animals, while 70% of nitrogenous substances in excrement come from urine and 30% from faeces.

3. Production of carbon dioxide on broiler farms

CO_2 is one of the main products of burning fossil fuels and makes a major contribution to the greenhouse effect, which is why it is directly linked to climate change. The main source of CO_2 in animal husbandry is animal respiration, so there is a connection between the level of animal metabolism and the production of CO_2 on farms. CO_2 production in birds is proportional to their metabolic heat production, and thus to the bird's metabolic body mass, which is affected by temperature and activity (*Knížatová et al.*, 2010a). On farms with inadequate ventilation, oxygen becomes a limiting factor in broiler health, production performance and welfare. On the other hand, animal health conditions including factors such as CO_2 and oxygen levels are known to influence the occurrence of ascites in broilers. It is interesting that the research showed no difference in CO_2 emissions in relation to the age of the animals, i.e. the period of fattening.

4. Objective of the study

The aim of the conducted research was to examine the influence of the nutritional supplement Eubiotic added to feed for broilers on emissions of NH_3 and CO_2 in a facility for breeding broilers.

5. Materials and methods

The experiment was carried out on a farm for growing fattening broilers in Žablje, in two buildings with a capacity of 8500 broilers each, with an area of 530 m^2. Broilers of Cobb 500 provenance were used, which were fed complete mixtures of standard raw materials and chemical composition that fully corresponded to the nutritional needs depending on the age of the animals. The experimental group, unlike the control group, received Eubiotic in the feed during the entire fattening period, at the level of 1 kg/ton of feed. Measurements of gas emissions (NH_3 and CO_2) in the buildings were carried out on days 28 and 35 in the morning hours with a multigas detector manufactured by Dräger — Germany.

6. Results

Determined values of NH_3 and CO_2 emissions in the broiler breeding facility after 28 days of the production cycle are reported in Tables 1–6.

Table 1. NH_3 emissions at the entrance to the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	5.36	0.086	0.035	5.25	5.45	1.61%
Experimental	5.07	0.211	0.086	4.87	5.31	4.17%

Table 2. NH_3 emissions in the central part of the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	10.74	0.250	0.102	10.25	10.98	2.33%
Experimental	10.12	0.203	0.083	9.88	10.42	2.01%

Table 3. NH_3 emissions at the end of the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	5.38	0.110	0.045	5.19	5.49	2.04%
Experimental	5.09	0.166	0.068	4.88	5.31	3.27%

Table 4. CO_2 emissions at the entrance to the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1371.00	74.080	30.240	1240.00	1448.00	5.40%
Experimental	1334.00	36.660	14.970	1293.00	1382.00	2.75%

Table 5. CO_2 emissions in the central part of the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1890.00	51.500	21.030	1828.00	1984.00	2.72%
Experimental	1834.00	132.900	54.240	1655.00	1952.00	7.24%

Table 6. CO_2 emissions at the end of the facility on day 28 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1546.00	20.750	8.470	1519.00	1574.00	1.34%
Experimental	1502.00	53.830	21.980	1441.00	1602.00	3.58%

Determined values of NH_3 and CO_2 emissions in the broiler breeding facility after 35 days of the production cycle are shown (Tables 7–12).

Table 7. NH_3 emissions at the entrance to the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	12.36	0.836	0.341	11.36	13.50	6.76%
Experimental	11.67	1.199	0.490	10.52	13.29	10.28%

Table 8. NH_3 emissions in the central part of the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	17.72	2.283	0.932	14.63	20.26	12.89%
Experimental	16.67	1.543	0.630	14.99	18.75	9.26%

Table 9. NH_3 emissions at the end of the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	15.53	0.978	0.399	14.63	17.22	6.29%
Experimental	14.68	0.651	0.266	14.01	15.80	4.43%

Table 10. CO_2 emissions at the entrance to the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1343.00	43.590	17.800	1281.00	1384.00	3.25%
Experimental	1305.00	42.770	17.460	1245.00	1361.00	3.28%

Table 11. CO_2 emissions in the central part of the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1790.00	56.790	23.180	1737.00	1883.00	3.17%
Experimental	1734.00	135.500	55.330	1502.00	1892.00	7.82%

Table 12. CO_2 emissions at the end of the facility on day 35 (ppm)

Group	$\overline{X}$	Measurements				
		Sd	Se	X_{min}	X_{max}	C_v (%)
Control	1511.00	84.440	34.470	1400.00	1622.00	5.59%
Experimental	1467.00	76.960	31.420	1390.00	1585.00	5.25%

7. Conclusion

From the results shown, we can conclude that the emission values of NH_3 and CO_2 in the facility for growing fattening broilers that received Eubiotic in their feed were numerically lower, which can be explained by the better digestibility of basic nutrients, primarily proteins present in the feed. The above results indicate that the use of Eubiotic has a positive effect on the zoohygienic conditions of the environment and thus consequently leads to an increased resistance of broilers to diseases caused by emissions of harmful gases and all accompanying effects.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The Ministry of Science, Technological Development, and Innovation, Republic of Serbia (Grant No. 451-03-47/2023-01/200050, from 03.02.2023) financially supported this paper.

References

Brouček, J. & Čermák B. (2015). Emission of harmful gases from poultry farms and possibilities of their reduction. *Ekológia (Bratislava)*, 34(1), 89–100.

Gates, R. S., Xin, H., Casey, K. D., Liang Y. & Wheeler E. F. (2005). Method for Measuring Ammonia Emissions from Poultry Houses. *Journal of Applied Poultry Research*, 14, 622–634.

Knížatová, M., Mihina, Š., Brouček, J., Karandušovská, I. & J. Mačuhová J. (2010b). The influence of litter age, litter temperature and ventilation rate on ammonia emissions from a broiler rearing facility. *Czech Journal of Animal Science*, 55(8), 337–345.

Knížatová, M., Mihina, Š., Brouček, J., Karandušovská, I., Sauter, G. J. & Mačuhová J. (2010a). Effect of the age and season of fattening period on carbon dioxide emissions from broiler housing. *Czech Journal of Animal Science*, 55(10), 436–444.

Liu, Z., Wang, L., Beasley, D. B. & Shah S. B. (2009). Modeling ammonia emissions from broiler litter at laboratory scale. *American Society of Agricultural and Biological Engineers*, 52(5), 1683–1694.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523.043

ID: 126632969

https://doi.org/10.18485/meattech.2023.64.2.75

Original scientific paper

Fermented dry Sremska sausages made of pork meat from various breeds — chemical content and sensory properties

Nenad Parunović[a], Jelena Ćirić[a], Tatjana Baltić[a], Dejana Trbović[a], Nikola Betić[a], Čedomir Radović[c] and Radomir Savić[b]*

[a] *Institute of Meat Hygiene and Technology, 11000 Belgrade, Serbia*
[b] *Institute of Animal Sciences, Faculty of Agriculture, University of Belgrade, 11040 Belgrade, Zemun, Serbia*
[c] *Institute for Animal Husbandry, 11040 Belgrade, Zemun, Serbia*

ARTICLE INFO

Keywords:
Mangalitsa
Autochthonous pork meat products
Chemical composition
Sensory qualities

ABSTRACT

In this study, the fatty acid composition, sensory qualities, and cholesterol levels of fermented dry Sremska sausages were examined. Pork meat and fat from three different pig breeds — autochthonous Mangalitsa, Moravka, and commercial Swedish Landrace — were used to create Sremska sausages. The sausage with the most cholesterol was created from Swedish Landrace meat and fat. On the other hand, compared with other sausage types, the sausage made from Mangalitsa pork included more monounsaturated fatty acids (MUFA) and unsaturated fatty acids (USFA) and less saturated fatty acids (SFA). Sausages made from Swedish Landrace pig meat had much greater PUFA content than other kinds. These differences were mostly due to higher overall n-6 PUFA contents. The health lipid markers for atherogenicity (IA) and thrombogenesis (IT) were lower in Mangalitsa pork sausage than in the two other sausage types. The Mangalitsa pork used for manufacturing Sremska sausage was exceptional in terms of appearance, flavor, aftertaste, and overall approval. The chemical and sensory qualities of dry-fermented Sremska sausages are modified by pig breed, according to this study.

1. Introduction

Autochthonous pork products made from regional pig breeds that passed through intensive, sustainable breeding programs are becoming increasingly popular in Europe, particularly in Mediterranean nations. Traditional breed meat and meat products usually have positive public and media perceptions and are frequently seen to be superior than meat and meat products from newer pig breeds and crossbreeds in terms of taste and quality. The carcass sides of Mangalitsa pigs are usually composed of between 65–70% fat and 30–35% meat (*Egerszegi et al*., 2003**)**. The Mangalitsa pig's meat was darker in color, its fat was whiter, and it included significantly more intramuscular fat and back fat than meat from other pig breeds. From the perspective of human nutrition, the meat from these fat pig breeds is preferable because it contains less saturated fatty acid (SFA) and a larger proportion of unsaturated fatty acid (USFA) than meat from other fat pig breeds (*Holló et al.*, 2003; *Parunović et al.,* 2012).

However, native meat products manufactured from regional breeds are becoming more popular in keeping with contemporary trends aimed at recovering

***Corresponding author:** Nenad Parunović, *nenad.parunovic@inmes.rs*

Paper received Jun 30th 2023. Paper accepted July 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

and developing traditional food production procedures. Additionally, a lot of research has been performed to identify the features of regionally traditional and naturally fermented sausages (*Di cagno* et al., 2008; *El Malti & Amarouch*, 2009; *Parunović et al.*, 2014).

The aim of this study was to analyze the chemical composition and sensory quality, and to detect potential differences in traditionally fermented dry Sremska sausage manufactured from the meat and fat of three pig breeds: Mangalitsa, Moravka and Swedish Landrace. Mangalitsa and Moravka breeds were selected as autochthonous Serbian pig breeds, while Swedish Landrace was chosen as the most common commercial meat/fattening pig breed in Serbia.

2. Materials and methods

All of the animals were reared on the Institute for Animal Husbandry's experimental farm in Belgrade, Serbia. With the addition of a feed concentrate made of maize and wheat, the pigs had unlimited access to green forages including pasture and clover. At a nearby slaughterhouse, animals were stunned before being slaughtered and exsanguinated. Twenty-four hours after slaughter and chilling, the meat was processed.

The Sremska sausage varieties that were under investigation (Table 1) were produced in the Animal Husbandry Institute's processing department. For all studies, three sausages of each variety were used, and each assay was carried out twice. The Sremska sausage varieties under examination were made on the same day and in the same way. A cutter (Seydelman K60, Germany) was used to grind the meat and fat (85:15) to an 8 mm size. Variations of the sausage were made using the same proportions of the following ingredients: 2.3% salt, 0.011% $NaNO_2$, 0.3% dextrose, 0.20% garlic, and 0.5% sweet red paprika. Pork small intestines with a diameter of around 32 mm were filled with the mixture. Following filling, the sausages were hung on poles and allowed to mature in a controlled environment (Maurer, Germany).

Total lipids were extracted using the accelerated solvent extraction technique on the Dionex ASE 200 to ascertain the content of fatty acids. By using a flame ionization detector and capillary gas chromatography, fatty acids were identified as methyl esters. According to *Maraschiello et al.* (1996), cholesterol content was assessed using an HPLC/PDA on a Waters 2695 Separations Module with a Waters 2996 Photo Diode Array Detector.

The following calculations were made using the information on the fatty acid composition:

1) Index of atherogenicity (IA): This statistic compares the total of the main groups of saturated and unsaturated fatty acids [8, 9].

The following equation was applied:

$$IA = [(4 \times C14{:}0) + C16{:}0 + C18{:}0] / [\Sigma MUFA + \Sigma PUFA\text{-}n6 + \Sigma PUFA\text{-}n3]$$

2) Index of thrombogenicity (IT): This measurement illustrates the propensity for blood clots to develop. This is referred to as the interaction between the pro-thrombogenic (saturated) and the anti-thrombogenic (MUFA, PUFA-n6 and PUFA-n3) fatty acids (*Ulbritch & Southgate*, 1991; *Senso et al.*, 2007).

The following equation was applied:

$$IT = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times MUFA + 0.5 \times PUFA\text{-}n6 + 3 \times PUFA\text{-}n3 + PUFA\text{-}n3/PUFA\text{-}n6}$$

Two samples of each type of dry fermented sausage were utilized for the analysis. Each parameter was determined using six repeats for each sample. The taste of Sremska sausages was evaluated using quantitative descriptive analysis. The sensory properties of Sremska were examined by ten experienced assessors who were carefully chosen and competent. Assessors were asked to rate the Sremska sausage's nine distinct characteristics, including appearance, cross-section, color intensity, odor intensity, taste, consistency, acidity, aftertaste, and overall accepta-

Table 1. The percentage of meat originating from specified pig breeds in different types of fermented dry Sremska sausages

Pig meat	Fermented dry Sremska sausage types		
%	SM	SMM	SL
Mangalitsa	100	50	-
Moravka	-	50	-
Swedish Landrace	-	-	100

bility, on a numeric-descriptive scale ranging from 1 (extremely unacceptable) to 7 (extremely acceptable). Each Sremska sausage sample was offered to the testers one at a time.

Means and standard error for descriptive statistics were determined. ANOVA with a single factor was used to process the findings. Tukey's approach was used to compare the differences between the various sausage varieties. Statistica 7.0 (Statsoft Inc.) was used to do the calculations.

3. Results

Sremska sausages manufactured from Swedish Landrace pork meat had considerably higher PUFA contents (P<0.001) than other varieties (Table 1). Higher overall n-6 PUFA content was the primary factor causing these changes (P=0.001). In sausages of type SM (Mangalitsa pigs), lower n-6/n-3 ratios were established. Despite this, the SM sausage type's n-6/n-3 ratio of unsaturated fatty acids was 17 and

Table 2. Different fermented dry Sremska sausages were studied for their fatty acid composition (%), cholesterol content (mg/100g), index of atherogenicity (IA), and index of thrombogenicity (IT) (LSM standard error).

Traits	Fermented dry sausage types			
	Swedish Landrace	Mangalitsa	Mangalitsa and Moravka	P[1]
C14:0	1.04±0.02 [b]	1.20±0.04 [a]	1.08±0.03 [ab]	**
C16:0	23.93±0.06[c]	25.86±0.06[a]	25.23±0.07[b]	***
C16:1	1.72±0.07 [c]	3.83±0.07[a]	2.11±0.07[b]	***
C17:0	0.31±0.03	0.28±0.03	0.32±0.02	NS
C18:0	14.17±0.07[a]	10.85±0.06[b]	14.07±0.06[a]	***
C18:1c9	37.76±0.07[c]	43.44±0.06[a]	38.73±0.05[b]	***
C18:1c11	2.93±0.06[a]	4.56±0.06[b]	3.19±0.06[a]	***
C18:2n6	14.42±0.08[a]	6.57±0.07[c]	11.93±0.07[b]	***
C18:3n6	ND	ND	ND	
C18:3n3	0.45±0.04	0.46±0.04	0.36±0.05	NS
C20:0	0.23±0.03	0.18±0.02	0.18±0.02	NS
C20:1	0.73±0.04	0.86±0.04	0.72±0.04	NS
C20:2	0.92±0.04[a]	0.55±0.04[b]	0.84±0.04[a]	***
C20:3n6	1.05±0.05[ab]	1.14±0.04[a]	0.92±0.05[b]	*
C20:3n3	ND[b]	0.14±0.03[a]	0.08±0.05[ab]	*
C22:1/C20:4	0.36±0.02[a]	0.15±0.02[c]	0.25±0.03[b]	***
SFA	39.68±0.12[b]	38.43±0.10[c]	40.95±0.13[a]	***
MUFA	43.53±0.14[c]	52.76±0.15[a]	45.07±0.145[b]	***
PUFA	16.77±0.14[a]	8.73±0.14[c]	14.02±0.13[b]	***
USFA	60.26±0.21[b]	61.46±0.22[a]	59.06±0.20[c]	**
MU/PU	2.56±0.05[c]	6.07±0.04[a]	3.23±0.05[b]	***
MU/SF	1.12±0.00[b]	1.36±0.00[a]	1.10±0.00[b]	***
PU/SF	0.43±0.00[a]	0.22±0.00[c]	0.34±0.00[b]	***
n-3	0.45±0.04	0.46±0.05	0.36±0.05	NS
n-6	15.44±0.08[a]	7.67±0.08[c]	12.83±0.08[b]	***
n-6/n-3	38.93±3.88[b]	17.34±3.86[a]	37.37±3.86[b]	**
Cholesterol	64.94±0.16[a]	59.66±0.17[b]	53.46±0.16[c]	***
IA	0.72±0.01[b]	0.67±0.00[c]	0.75±0.00[a]	***
IT	1.26±0.01[b]	1.21±0.01[c]	1.36±0.01[a]	***

[1]NS-not significant ($P>0.05$); [*]:Statistical significance at the level of $P<0.05$;[**]:Statistical significance at the level of $P<0.01$;[***]:Statistical significance at the level of $P<0.001$;

[a-c]Means in the same row with different letters are significantly different (P<0.05).

higher, above the advised range of 1:1–5:1 (*Simopoulos*, 2004). In independent studies, *Hoz* (2004) and Valencia (2006) both discovered lower ratios of n-6/n-3 fatty acids (12.05 and 13.86, respectively) in their control groups of fermented dry sausages than our findings. Linoleic acid, an important PUFA, was present in sausage types SM and SL in differing amounts (6.58% to 14.40%; P<0.001). Mangalitsa pork meat sausages had greater MUFA levels than other varieties (P<0.001) of sausages. Higher quantities of oleic acid, cis-vaccenic acid (C18:1 cis-11), and palmitic acid (C16:1) in these sausages were the key contributors to these differences. Significant differences were found for specific fatty acids in respect to the SFA percentage, leading to comparable levels for the overall fraction. Sausage type SM had the lowest overall SFA level, whereas sausage type SMM (mixed Mangalitsa and Moravka) had the highest. Stearic acid (C18:0), one of the main SFAs, was present in considerably different amounts in the various sausage varieties (P<0.001).

In our investigation, fermented sausages manufactured from Mangalitsa pork meat were found to have the lowest PUFA/SFA ratio (0.23). With notable variations amongst the samples (P< 0.001), the mean cholesterol levels in fermented sausages ranged from 53.47 mg/100g (SMM) to 64.92 mg/100g (SL). For an Italian-style salami, *Baggio and Bragagnolo* (2006) discovered that the cholesterol level ranged from 48 to 57 mg/100g. According to *Pleadin et al.,* (2010), in researching fermented sausages in Croatia, the average cholesterol amount in commercially produced fermented sausages ranged from 58.48 to 105.24 mg/100g, whereas home-made fermented sausages had a maximum cholesterol content of 75.07 mg/100g. From a nutritional perspective, the fatty acid composition of lipids is significant, particularly the ratio between PUFA and SFA, the ratio between "bad" and "good" fatty acids (IA and IT), and the n-6/n-3 ratio. Foods with lower IA and IT have reduced atherogenic and thrombogenic potential. In stark contrast to other sausages produced, the IA and IT were lower in Sremska sausages produced from Mangalitsa pork meat. Pork has an IA of 0.60, poultry 0.50, and beef 0.72 (*Žlender & Gapšerlin*, 2005).

Table 3 presents the findings of professional trained assessors' sensory evaluations. The Sremska sausage SMM (mixed Mangalitsa and Moravka) received the lowest cross-sectional scores and consequently had the least acceptable appearance. The fermented dry sausage type SL was considered to be the best. The most reliable sausage was of type SL, whereas SMM sausage received the lowest consistency rating. The color of the meat used in the manufacturing of the product had a connection to this. Relationships between physical meat quality traits and sensory traits, such as muscle fiber and overall softness, as well as between the amount and composition of intramuscular fat and taste, have been documented (*Hoffman et al.*, 2007; *Muchenje* et al., 2008; *Calkins & Hodgen,* 2007). The sensory indication most impacted by pig breed was odor. The Mangalitsa breed's meat was used to make the most traditional and finest sausage. Sremska sausage SM received the top ratings from expert judges for both flavor and aftertaste. The Sremska sausage of type SM had the most stable quality, according to the total sensory acceptance scores of the goods under examination.

Table 3. Professional assessors' evaluations of the sensory qualities of three types of fermented dry Sremska sausages (scale test rating)

	Fermented dry sausage types		
Sensory properties	**SL***	**SM**	**SMM**
Appearance	6.06	5.52	4.68
Cross-section	5.24	5.35	3.74
Consistency	5.43	5.18	4.85
Color	5.51	5.76	5.07
Odor	5.65	6.34	6.35
Taste	5.26	6.07	5.06
Acidity	4.57	4.93	4.02
Aftertaste	5.26	5.32	5.26
Overall acceptability	5.26	5.53	4.84

*SL, Swedish Landrace; SM, Mangalitsa; SMM, Mangalitsa and Moravka

4. Conclusion

The findings of this study showed that the chemical and sensory properties of dry-fermented Sremska sausages are influenced by pig breed. According to the findings of the current study, Sremska sausages can be produced with the right proportion of meat

and fat from native pig breeds alone. These sausages will have a respectable chemical content, a good and reasonably healthy fatty acid composition, and sensory qualities that will appeal to discerning consumers. These outcomes ought to help encourage the farming and conservation of endangered Mangalitsa pigs, assuming there are markets for Sremska sausage.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Egerszegi, I., Ratky, J., Solti, L. & Brussow, K. P. (2003). Mangalica — an indigenous swine breed from Hungary (Review). *Archiv fur Tierzucht*, 46, 245–256.

Holló, G., Seregi, J., Ender, K., Nürnberg, K., Wegner, J., Seenger, J. & et al. (2003). Examination of meat quality and fatty acid composition of Mangalitsa. *Acta Agraria Kaposvariensis*, 7(2), 19–32.

Parunović, N., Petrović, M., Matekalo-Sverak, V., Trbović, D., Mijatović, M. & Radović, Č. (2012). Fatty acid profile and cholesterol content of *m. longissimus* of free-range and conventionally reared Mangalitsa pigs. *South African Journal of Animal Science*, 42, 101–113.

Di cagno, R., Lopez, C. C., Tofalo, R., Gallo, G., De Angelis, M., Paparella, A., Hammes, W. P. & Gobbetti, M. (2008). Comparison of the compositional, microbiological, biochemical and volatile profile characteristics of three Italian PDO fermented sausages. *Meat Science*, 79, 224–235.

El Malti, J. & Amarouch, H. (2009). Microbiological and physicochemical characterization of the natural fermented camel meat sausage. *African Journal of Biotechnology*, 8, 4199–4206.

Parunović, N., Petrović, M., Matekalo-Sverak, V., Radojković, D. & Radović, Č. (2014). Fatty acid profiles, chemical content and sensory properties of traditional fermented dry kulen sausages. *Journal of Food Processing and Preservation*, 38, 2061–2068.

Maraschiello, C., Diaz, I. & Regueiro, J. A. G. (1996). Determination of cholesterol in fat and muscle of pig by HPLC and capillary gas chromatography with solvent venting injection. *Journal of High Resolution Chromatography*, 19, 165–168.

Ulbritch, T. L. V. & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, 338, 985–992.

Senso, L., Suarez, M. D., Ruiz-Cara, T. & Garcia- Gallego, M. (2007). On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). *Food Chemistry*, 101(1), 298–307.

Simopoulos, A. P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International*, 20, 77–90.

Hoz, L. (2004). Development of an n-3 fatty acid and tocopherol enriched dry fermented sausage. *Meat Science*, 67, 485–495.

Valencia, I., Ansorena, D. & Astiasaran, I. (2006). Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. *Meat Science*, 72, 727–733.

Baggio, S. R. & Bragagnolo, N. (2006). Cholesterol oxide, cholesterol, total lipid and fatty acid contents in processed meat products during storage. *Food Science and Technology (LWT)*, 39(5), 513–520.

Pleadin, J., Vahčić, N., Perši, N., Vulić, A., Volarić, M. & Vraneš, I. (2010). Sadržaj kolesterola u domaćim i industrijskim kobasicama. *Meso*, 12(3), 156–161.

Žlender, B. & Gapšerlin, L. (2005). Značaj i uloga lipida mesa u bezbednoj i balansiranoj ishrani. *Tehnologija Mesa*, 46(1–2), 11–21.

Hoffman, L. C., Kroucamp, M. & Manley, M. (2007). Meat quality characteristics of springbok *(Antidorcas marsupialis*). Sensory meat evaluation as influenced by age, gender and production region. *Meat Science*, 76, 774–778.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A. & Raats, J. G. (2008). Sensory evaluation and its relationship with physical meat characteristics of beef from Nguni and Bonsmara steers raised on natural pasture. *Animal*, 2, 1700–1706.

Calkins, C. R. & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, 77, 63–80.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.412.065

ID: 126656777

https://doi.org/10.18485/meattech.2023.64.2.76

Original scientific paper

Reduction of eggshell microbial load of table eggs by ultra-violet treatment

Suzana Vidaković Knežević [a], Slobodan Knežević[a], Jelena Vranešević[a], Marko Pajić[a], Zoran Ružić[b], Sava Spiridonović[b] and Mirjana Đukić Stojčić[b]*

[a] *Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia*
[b] *University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Table eggs
Ultraviolet irradiation
Decontamination
Bacteria
Molds and yeast

ABSTRACT

Table eggs *are an excellent animal-based food with an important role in human diet. However, eggs are often associated with* foodborne illnesses. The aim of this study was to evaluate the commercially available ultraviolet irradiation system incorporated in an egg grading and packing machine, and to compare the number of naturally present microorganisms on eggshells and the number of microorganisms on eggshells after ultra-violet treatment. The study showed reductions of the total number of aerobic microorganisms, *Enterobacteriaceae*, *Escherichia coli*, and the number of yeasts and molds on the eggshells, while the pathogenic bacteria, including *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp., as well as the number of coagulase-positive staphylococci, were under the limit of quantification on the shells of both not ultra-violet treated and ultra-violet treated table eggs. In conclusion, the 7 s ultra-violet treatment was effective in reducing the bacteria, molds and yeast on the eggshells, which could contribute to better health of consumers.

1. Introduction

The table eggs are considered as a complete food in the human diet providing a well-balanced source of nutrients. They consist of a protective eggshell, egg white, and egg yolk contained within thin membranes. The presence of microorganisms on the eggshell can affect the safety of table eggs. Two sources of contamination of the contents of intact table eggs can be distinguished. In the case of horizontal transmission, microorganisms penetrate through the eggshell, while vertical transmission refers to the transovarial route and the presence of microorganisms in the reproductive organs (*Gautron et al*., 2022). Penetration of microorganisms through the pores of the eggshell can cause the spoilage of table eggs, while the presence of foodborne pathogens can cause severe food poisoning (*Eke et al*., 2013). Therefore, disinfection of the eggshell surface is an important tool to prevent egg spoilage and egg-related illnesses. However, the washing and cleaning of Grade A eggs in Serbia, before and after sorting, is prohibited (*Official Gazette of the Republic of Serbia*, 2019), because such practices can cause damage to the eggshell. Such damage may cause increase chances of bacterial cross-contamination (*Musgrove*, 2011). Contaminated table eggs are a significant public human health hazard worldwide (*Abebe et al*., 2020).

***Corresponding author:** Suzana Vidaković Knežević, *suzana@niv.ns.ac.rs*

Paper received Jun 30th 2023. Paper accepted July 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Ultraviolet radiation (UV) could be a more favorable alternative for eggshell decontamination. Previous studies show that UV radiation is effective in reducing the bacterial load on the surface of visibly clean eggs (*De Reu et al*., 2006;).

The aim of this study was to evaluate UV treatment of a commercially available egg grading and packing machine, comparing the number of naturally present microorganisms on eggshells and the number of microorganisms on eggshells after UV treatment.

2. Materials and methods

2.1. Egg samples

The table eggs originated from a local farm and were produced by 32-week-old Isa Brown laying hens reared in enriched cages. All eggs were collected the day of lay. Average weight of eggs was 58.20 ± 1.68 g, which falls within the classification of "medium" eggs (*Official Gazette of the Republic of Serbia*, 2019). A total of 60 visibly clean table eggs were collected in 3 successive trials. For each trial, 20 table eggs were tested: 10 samples of table eggs collected before the UV treatment and 10 samples of table eggs collected after the UV treatment. Each table egg was sampled in a sterile Whirl-Pak bag, and transported to the laboratory in cold storage conditions within 2 h.

2.2. Ultra-violet treatment

The UV treatment was conducted by a commercial UV-C disinfection system, incorporated in an egg grading and packing machine (MOBA Omnia 125, Barneveld, the Netherlands), having a wavelength of 253.7 nm with an intensity of 10 mW cm^2. The speed of the conveyor belt was 0.142 m/s. As the UV-C disinfection system had a length of 100 cm, the exposure time for each egg was 7 s.

2.3. Microbial analysis of table eggs

At the laboratory, 18 mL of saline peptone solution was added to each bag with the table egg. The surface of each table egg was gently hand massaged through the bag for 5 minutes to detach the bacteria. The rinse solution was diluted, and volumes of 1.0 mL of suitable dilutions were further used. To quantify the total number of aerobic microorganisms, *Enterobacteriaceae,* and *Escherichia coli*, pour plate methods were used. Volumes of 1.0 mL of suitable dilutions were plated in plate count agar (PCA), violet red bile glucose (VRBG) agar and tryptone bile agar (TBX). The PCA plates were incubated aerobically at 30°C for 72 h (*ISO*, 2013), VRBG plates were incubated at 37°C for 24 h (ISO, 2017a), and TBX plates were incubated at 44°C for 24 h (*ISO*, 2008). Enumeration of coagulase-positive staphylococci (*ISO*, 2021), *Listeria monocytogenes* (*ISO*, 2017b), and *Campylobacter* spp. (*ISO*, 2017c) were carried out using the spread plate technique. Briefly, 1.0 mL of initial dilution was spread on three plates of Baird-Parker agar, agar Listeria according to Ottaviani and Agosti (ALOA), and modified charcoal cefoperazone deoxycholate (mCCD) agar, respectively. Both Baird-Parker and ALOA agar plates were incubated at 37 °C for 24–48 h, while mCCD agar plates were incubated in a microaerobic atmosphere at 41.5 °C for 48 h. *Salmonella* was determined by direct isolation on selective agar, xylose lysine deoxycholate agar (XLD), incubated at 37°C for 24 h. Enumeration of yeasts and molds was performed on Dichloran 18% glycerol agar (DG-18), incubated at 25°C for 5 days. All microbial media, except for mCCD agar which was purchased from Oxoid, used in the study were purchased from Biokar Diagnostics (Beauvais, France). To estimate the microbial load recovered from egg surfaces, the colony counts were calculated and transformed to $\log_{10}$ colony-forming units per egg ($\log_{10}$ cfu/eggshell). The lowest detection level for this procedure was 1.26 $\log_{10}$ cfu/eggshell.

2.4. Statistical analysis

The experiments were replicated three times. Descriptive statistics and t-test at a 0.05 level of significance were used to analyze the data.

3. Results and discussion

The visibly clean eggshell surfaces of table eggs were initially contaminated with microorganisms up to 5.57 $\log_{10}$ cfu/eggshell (Table 1). The total numbers of aerobic microorganisms, *Enterobacteriaceae*, *Escherichia coli*, and yeasts and molds on the eggshells were significantly ($p<0.05$) reduced after exposure to UV disinfection for 7 s. A decrease of 0.85 $\log_{10}$ cfu/eggshell of the total number of aerobic microorganisms was registered in our study. Similar results were obtained by *De Reu et al*. (2006) and *Pasquali et al.* (2014), when the natural bacterial load on eggshell was reduced by 0.9 $\log_{10}$

Table 1. Microbial reduction on the eggshells of table eggs after UV treatment.

Microorganism	Before UV treatment	After UV treatment
Total number of aerobic microorganisms	5.57±0.83[a]	4.72±0.74[b]
Enterobacteriaceae	1.44±0.49[a]	<1.26±0.00[b]
Escherichia coli	1.43±0.41[a]	<1.26±0.00[b]
Coagulase-positive staphylococci	<1.26±0.00	<1.26±0.00
Listeria monocytogenes	<1.26±0.00	<1.26±0.00
Campylobacter spp.	<1.26±0.00	<1.26±0.00
Salmonella spp.	<1.26±0.00	<1.26±0.00
Yeasts and molds	2.34±0.53[a]	1.57±0.44[b]

Results are presented as mean (log10 cfu/eggshell) ± standard deviation. Values within the same row marked with different letters (a, b) in superscript indicate statistically significant differences ($P < 0.05$).

cfu/eggshell after 4.7 s of UV treatment, and by 1.04 $\log_{10}$ cfu/eggshell after exposure to UV disinfection for 7 s, respectively.

Bacteria from the *Enterobacteriaceae* family were similar in numbers to *Escherichia coli*. This may be attributed to the fact that this bacterium is a normal inhabitant of intestinal tracts of hens. Number of *Enterobacteriaceae* and *Escherichia coli* were reduced by >0.18 $\log_{10}$ cfu/eggshell and >0.17 $\log_{10}$ cfu/eggshell, respectively. The number of yeasts and molds was reduced by 0.77 $\log_{10}$ cfu/eggshell. The UV treatment was effective in this study. Other researchers also found the UV treatment as significantly effective in eggshell decontamination (*Chavez et al.*, 2002; *Turtoi and Borda*, 2014; *Clímaco et al*., 2018).

The number of pathogenic bacteria, *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp., as well as the number of coagulase-positive staphylococci, were under the limit of quantification (<1.26 $\log_{10}$ cfu/eggshell) on the eggshells of both not UV treated and UV treated table eggs. A similar finding was reported in Iran (*Safaei et al*., 2011). However, these pathogenic bacteria have been isolated from eggshell previously (*De Reu et al*., 2005).

Generally, the population of microorganisms on eggs can vary (*Musgrove*, 2011), and usually represents the farm hygiene. Moreover, a variety of methods have been developed for the recovery of microorganisms from eggshells. Different methods, including surface rinsing, shaking crushed shells with glass beads, blending eggshells and membranes, and surface swabbing, aggravate comparison of results gained in different studies. In this study, we massaged the egg by hand for 5 min in saline peptone solution, while other studies used different times for massaging. This could also have impact in the number of detached microorganisms.

Although table eggs are estimated to be microbiologically sterile at oviposition, cross-contamination occurs at the moment of lay. Sources of contamination are feces, dust, caging and nesting materials, hands, transport belting and other matrices (*Musgrove*, 2011). In a previous study, among the microorganisms isolated from egg samples were *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Shigella* spp., *Proteus*, *Klebsiella*, *Citrobacter*, *Corynebacterium*, *Bacillus* spp., *Escherichia coli*, and fungi of the genus *Aspergillus* (*Salihu et al*., 2015). The microorganisms on eggshell could cause severe health problems like, diarrhea, nausea and abdominal pain.

The UV-C treatment involves short UV waves ranging from 200 to 280 nm. The UV light is lethal to most microorganisms, causing damage of nucleic acids (DNA and RNA). The damage does not kill the microorganisms, but it prevents cell replication. Therefore, an adequate UV treatment must provide a sufficiently high level of disruption to ensure that the nucleic acid is damaged beyond the repairable stage (*Turtoi and Borda*, 2014).

4. Conclusion

The results of this study showed that the commercially available UV disinfection system was effective in reducing different microorganisms on the shells of table eggs. Data show that UV light can significantly reduce microbial populations on eggshells,

allowing fewer bacteria to penetrate and contaminate the interior of the egg. This decontamination would result in fewer bacteria that could cause spoilage of table eggs and foodborne illnesses. Future studies remain to investigate the ideal length of UV treatment against the natural eggshell microbiota.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

References

Abebe, E., Gugsa, G. & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of Tropical Medicine*, doi.org/10.1155/2020/4674235

Chavez, C., Knape, K. D., Coufal, C. D. & Carey, J. B. (2002). Reduction of eggshell aerobic plate counts by ultraviolet irradiation. *Poultry Science*, 81(8), 1132–1135, doi.org/10.1093/ps/81.8.1132

Clímaco, W. L. D. S., Melo, É. D. F., Vaz, D. P., Saldanha, M. M., Pinto, M. F. V. D. S., Fernandes, L. C. C., Baião, N. C., de Oliveira, L. G., de Sant'Anna, F. M., de Souza., M. R. & Lara, L. J. C. (2018). Eggshell microbiology and quality of hatching eggs subjected to different sanitizing procedures. *Pesquisa Agropecuária Brasileira*, 53, 1177–1183.

De Reu, K., Grijspeerdt, K., Herman, L., Heyndrickx, M., Uyttendaele, M., Debevere, J., Putirulan, F. F. & Bolder, N. M. (2006). The effect of a commercial UV disinfection system on the bacterial load of shell eggs. Letters in applied microbiology, 42(2), 144–148, doi.org/10.1111/j.1472-765X.2005.01825.x

De Reu, K., Grijspeerdt, K., Heyndrickx, M., Zoons, J., De Baere, K., Uyttendaele, M., Debevere, J. & Herman, L. (2005). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. *British Poultry Science*, 46(2), 149 –155, doi.org/10.1080/00071660500065359

Eke, M. O., Olaitan, N. I. & Ochefu, J. H. (2013). Effect of storage conditions on the quality attributes of shell (table) eggs. *Nigerian Food Journal*, 31(2), 18–24, doi.org/10.1016/S0189-7241(15)30072-2

Gautron, J., Dombre, C., Nau, F., Feidt, C. & Guillier, L. (2022). Production factors affecting the quality of chicken table eggs and egg products in Europe. *Animal*, 16, 100425, doi.org/10.1016/j.animal.2021.100425.

ISO, (2008). Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide (SRPS ISO 16649-2:2008)

ISO, (2013). Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013).

ISO, (2017a). Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* — Part 2: Colony-count technique (ISO 21528-2:2017)

ISO, (2017b). Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method (ISO 11290-2:2017)

ISO. (2017c). Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 2: Colony-count technique (ISO 10272-2:2017)

ISO, (2021). Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1: Method using Baird-Parker agar medium (ISO 6888-1:2021)

Musgrove, M. T. (2011). Microbiology and safety of table eggs. In F. Van Immerseel, Y. Nys & M. Bain (Eds.), Improving the safety and quality of eggs and egg products (pp. 3–33). Woodhead Publishing.

Official Gazette of the Republic of Serbia, (2019). Regulation on egg quality, No. 7/2019, 35/2019 and 78/2019.

Pasquali, F., Rocculi, P., De Cesare, A., Bovo, F., Olivi, P., Lucchi, A. & Meluzzi, A. (2014). New technologies to enhance quality and safety of table eggs: ultra-violet treatment and modified atmosphere packaging. *Italian Journal of Food Safety*, 3(4), 192–195, doi.org/10.4081/ijfs.2014.4462

Safaei, H. G., Jalali, M., Hosseini, A., Narimani, T., Sharifzadeh, A. & Raheimi, E. (2011). The prevalence of bacterial contamination of table eggs from retails markets by Salmonella spp., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. *Jundishapur Journal of Microbiology*, 4(4), 249–253.

Salihu, M. D., Garba, B. & Isah, Y. (2015). Evaluation of microbial contents of table eggs at retail outlets in Sokoto metropolis, Nigeria. *Sokoto Journal of Veterinary Sciences*, 13(1), 22–28. doi: 10.4314/sokjvs.v13i1.4

Turtoi, M. & Borda, D. (2014). Decontamination of egg shells using ultraviolet light treatment. *World's Poultry Science Journal*, 70(2), 265–278, doi:10.1017/S0043933914000282

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.057:636.1

ID: 126661385

https://doi.org/10.18485/meattech.2023.64.2.77

Review paper

Proteomics as an emerging tool in equine meat research: an overview

Antonella della Malva[a], Mohammed Gagaoua[b*], Agostino Sevi[a] and Marzia Albenzio[a]*

[a] *University of Foggia, Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), Via Napoli 25, 71121 Foggia, Italy*
[b] *PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France*

ARTICLE INFO

Keywords:
Horse
Donkey
Meat quality
Biochemical pathways
Biomarkers
Muscle Proteome

ABSTRACT

Proteomics tools in the field of equine meat research have been very recently applied to explore the changes in the post-mortem muscle proteome and to discover biomarkers to monitor the variations in its different meat quality traits. The current advances achieved by proteomics in equine meat research are reviewed. Different proteomics techniques (sodium dodecyl sulphate-polyacrylamide gel electrophoresis; two-dimensional polyacrylamide gel electrophoresis; fluorescent two-dimensional difference gel electrophoresis; targeted proteomics; tandem-mass tag labeled proteomics; data-independent analysis proteomics) have been applied in the study of the equine muscle/meat. The studies revealed the biochemical pathways involved in the development of several donkey and horse (foal) meat quality variation. The current knowledge would be useful to develop high-quality products.

1. Introduction

In the last years, the increase in consumer health consciousness led to increased demand and consumption of alternative meat sources worldwide, with consumers choosing products with high nutritional value. Equine meat (from donkeys and horses) is widely recognized as a health-beneficial food due to its greater content of vitamins, minerals, conjugated fatty acids, and low fat and cholesterol content (*Marino et al.*, 2022). Despite its excellent nutritional value, preferences and perceptions of equine meat differ significantly due to the social, historical, ethical, and psychological characteristics of consumers (*Lopez-Pedrouso et al.*, 2023). In fact, recent trends show an increase in production and consumer demand for equine meat (*FAOSTAT*, 2021). Improving consumer confidence in equine meat consumption including its nutritional features could offer an opportunity for farming systems to enlarge the livestock species, to differentiate the market, and to promote environmental sustainability. In terms of sensory properties, equine meat is considered a tough and dark red meat, which drives its consumer acceptability, and consequently, the purchasing decision at the point of sale. Proteomics-based techniques have been employed to decipher the proteome changes and the underlying molecular mechanisms related to different meat organoleptic traits in different species (*Di Luca et al.*, 2011; *della Malva et al.*, 2017; *Lopez-Pedrouso et al.*, 2020; *Gagaoua et al.*, 2020; *Gagaoua et al.,* 2021*; Lamri et al.*, 2023). In the particular case of equids, proteomics has been very recently applied, and to the best of our knowledge, in less than 10 studies (Table 1). Such studies have as their ultimate goal the development of high-qual-

***Corresponding authors:** Antonella della Malva, *antonella.dellamalva@unifg.it*; Mohammed Gagaoua, *mohammed.gagaoua@inrae.fr*

Paper received July 3rd 2023. Paper accepted July 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ity equine products and, more specifically, the goal of better understanding the biochemical pathways behind the variability of equine meat quality. Therefore, this paper aims to briefly introduce the basis of proteomic approaches in the assessment of equine meat quality variation by highlighting the applications of this powerful tool in both donkey and horse meat research with a focus on i) post-mortem changes and underlying mechanisms and ii) meat quality traits.

2. Brief overview of proteomics in meat research

In the field of meat research, proteomics is an adapted tool for in-depth characterization and to explore the biochemical processes taking place during the conversion of muscle into meat or during the post-mortem time (*D'Alessandro & Zolla*, 2013; *Purslow et al.*, 2021; *Gagaoua et al.*, 2022). Several strategies and methodologies can be used. Gel-based approaches (sodium dodecyl sulphate-polyacrylamide gel electrophoresis, SDS-PAGE; two-dimensional polyacrylamide gel electrophoresis, 2D-PAGE; fluorescent two-dimensional difference gel electrophoresis, 2D-DIGE) coupled with mass spectrometry (MS) are widely applied and have, so far, been the main methods used to investigate muscle proteome changes (For review: *Gagaoua et al.*, 2022). However, in recent years, gel-free quantitative techniques (label-free or label-based approaches) have gradually taken over, leading to high accuracy and sensitivity in the quantification of proteins, including intact proteoforms and post-translational protein modifications (*Li et al.*, 2021; *Lamri et al.*, 2023). Furthermore, the tremendous progress in powerful bioinformatics tools and software have recently enabled the expansion and identification of new features related to several meat quality traits (*Kiyimba et al.*, 2022). Generally, the muscle undergoes several dynamic modifications during the animal's life due to intrinsic and extrinsic factors. Several studies on beef evidenced that those factors are responsible for huge variations in the muscle proteome, which consequently impact the final quality traits of muscle foods (*Sierra et al.,* 2021; *Di Luca et al.*, 2022; *Gagaoua et al.*, 2022). Proteomics technologies were successfully applied to decipher several of these changes and mechanisms, including in the field of biomarkers discovery to monitor meat tenderness, color, and water holding capacity (*Gagaoua et al.,* 2022; *Gagaoua and Picard*, 2022).

3. Proteomics applications in horse meat research

Proteomics tools in horsemeat research have been applied with the objectives of monitoring and exploring the tenderization rate and the quality traits of different muscles during aging and also for achieving a better understanding of the muscle proteome differences among different breeds and diets (Table 1). A 2D-PAGE approach combined with liquid chromatography-tandem mass spectrometry (LC/MS-MS) was used by *della Malva et al*. (2019) to investigate the post-mortem changes in the myofibrillar muscle proteome and the tenderization rate during the aging time of different muscles (*Longissimus lumborum*, *Semimembranosus* and *Semitendinosus*) from Italian Heavy Draft Horses. The authors identified 22 myofibrillar and sarcoplasmic protein biomarkers to follow up on the influence of aging time on proteolysis. The 22 proteins belong, based on Gene Ontology analysis, to six biological cluster pathways, these being muscle contraction (GO: 0006936), NADH regeneration (GO: 0006735), regulation of ATP-dependent activity (GO: 0043462), muscle structure development (GO: 0061061), response to estradiol (GO: 0032355), and organophosphate biosynthetic process (GO: 0090407). In addition, *della Malva et al*. (2019) revealed differences in the rates of tenderization among muscles during aging, showing a greater accumulation of MYL1 and MYL2 fragments in *Semitendinosus* muscle with an extending aging time (14 days). When investigating the changes in the sarcoplasmic proteome in comparison with the meat organoleptic characteristics, another study by *della Malva et al.* (2022) found 24 muscle-specific protein patterns during aging, from which TPM1 and TPM2 cytoskeletal proteins were potential biomarkers of intense proteolysis for *Longissimus* muscle. The authors also indicated mitochondrial and glycolytic proteins (SOD, PGM1, and MB) as putative biomarkers to monitor the meat quality characteristics of horse *Semitendinosus* muscle.

Beldarrain et al. (2022) applied the OFFGEL proteomics-based approach on the Hispano-Breton *Longissimus thoracis et lumborum* (LTL) horse muscle to unveil changes in the myofibrillar proteome during three weeks of aging time. The authors indicated that aging-induced significant abundance changes of muscle structure proteins (MYL1, MYBPC1, TNNT3, and TNNI2) as key players of horse meat tenderization. To better understand the changes

found in the myofibrillar proteome of horse meat during aging, a 2D-DIGE approach was further applied by the same authors (*Beldarrain et al.*, 2023) to gain insights into the biochemistry of horse muscles and identify candidate protein biomarkers to monitor meat tenderness. Five putative protein biomarkers (TNNT3, MYBPC1, MYBPC2, ACTA1, and GAPDH) evidenced/expressed a great potential to monitor the changes in horse meat tenderization.

Targeted proteomics (SWATH-MS: Sequential Window Acquisition of all Theoretical Mass Spectra) has been recently applied by *Lopez-Pedrouso et al.* (2023) on two horse breeds (Burguete *vs* Jaca Navarra) finished with conventional concentrate and straw *or* silage and organic feed diets to identify biomarkers of multiple horsemeat quality traits (tenderness, color, and intramuscular fat). The authors built a database of 294 proteins, from which 23 proteins were candidate biomarkers of intramuscular fat content, while eight proteins, from the energy metabolism (ALDOA, CKM, TPI1, and PGMA2) and the muscle structure (ACTA1, MYBPH, MYL1, and TNNC1) pathways were identified as biomarkers to monitor the tenderization process. Regarding horse meat color determination, seven potential protein biomarkers related to energy metabolism (ALDOA, PKM, PFKM, and CKM), and oxidative stress (HSPA1A, SOD2, and PRDX2) were identified.

4. Proteomics applications in donkey meat research

Proteomics investigations in donkey meat has been applied to decipher the biological mechanisms governing the meat tenderness and intramuscular fat variation and, consequently, to reveal the underlying pathways including those for the identification of protein biomarkers of the desirable meat quality (Table 1). In the frame of understanding the tenderization rate and the proteins changes occurring during the aging time of Martina Franca donkey meat, a gel-based 2DE proteomics approach coupled with LC/MS-MS was applied by *della Malva et al.* (2022). The authors proposed the first repertoire of 15 meat tenderness biomarkers for the donkey meat species. Using bioinformatics, the candidate biomarkers were allocated to three interconnected pathways: nine proteins were from muscle contraction, structure pathway (MYH1, MYH2, ACTA1, MYLPF, MYL6B, MYL1, TNNC2, TPM1, and TPM2); five from energy metabolism (ATP-5PD, UQCRC1, COX5A, GAPDH, and CKM), and one protein from the response to stress pathway (HSPB1), thus evidencing a key insight into the pathways and processes involved in the tenderness development of donkey meat.

Intramuscular fat content plays a pivotal role in the quality of muscle foods, thus affecting the flavor, juiciness, and tenderness of the end product. A tandem-mass tag (TMT) labeled proteomics study conducted by *Tan et al.* (2022) identified 30 differentially abundant proteins strictly related to the intramuscular fat deposition of the donkey *Longissimus thoracis* muscle. The functional enrichment analysis confirmed that the main biological pathways are involved in lipid metabolism and adipogenesis, thus confirming their role in the biological mechanisms that regulate meat quality variation.

Chai et al. (2022) used a data-independent analysis (DIA) proteomics approach to investigate the proteome differences of donkey muscles (*Semitendinosus* (ST), *Longissimus thoracis* (LT), and *Gluteus maximus* (GM)) related to meat quality parameters. The pairwise comparisons of the ST/LT and GM/LT allowed identification of, respectively, 111 and 127 differentially abundant proteins involved mainly in the MARK signaling pathway, fat digestion and absorption, and regulation of actin cytoskeleton. The focus given by these studies on the strong role of different biological pathways in the post-mortem processes linked with the donkey meat quality variation emphasizes the need for future research to explain the molecular basis of variations in donkey meat quality for developing high-quality products from this sustainable species.

5. Future perspectives/Conclusion

The potential of proteomic tools to decipher and understand biological mechanisms and pathways underlying the equine meat quality variations has barely/recently been explored. The current few proteomics studies, developed on equids, allowed us to gain more insights about the biological mechanisms responsible for the variations in meat quality, in terms of tenderness, color, and intramuscular fat content. Several biological pathways have been discovered including proteins from the energy metabolism, muscle structure, oxidative stress, lipid metabolism, and adipogenesis, although further post-mortem muscle proteome studies and multi-omics approaches will be necessary to validate the biochemical pathways and the proposed candidate biomarkers, with the aim of monitoring equine meat quality

Table 1. Proteomics approaches in the selection of biomarkers related to meat quality in equids.

Proteomic approach	Muscle/cut (breed)	Considered variable(s)/ effects	Identified proteins/candidate biomarkers[1]	References
		Horse		
SDS-PAGE, 2DE, HPLC/ Q-TOF mass spectrometry, and Western Blotting	*Longissimus lumborum* (LL), *Semitendinosus* (ST) and *Semimembranosus* (SM) (Italian Heavy Draft Horse)	Aging – Muscle	MYL1, TNNT3, MYLPF, MYL3, TPM2, CKM, ENO2, AK1, PGK1, TPI1, GPX1	*della Malva et al.*, 2019
SDS-PAGE, 2DE, and HPLC/Q-TOF mass spectrometry	*Longissimus lumborum* (LL), *Semitendinosus* (ST) and *Semimembranosus* (SM) (Italian Heavy Draft Horse)	Aging – Muscle	PGM1, CKM, TPM1, TPM2, ENO3, ALDOB, GPD1, GAPDH, TPI1, AK1, MB, SOD1	*della Malva et al.*, 2022
OFFGEL, SDS-PAGE, LC-MS/MS	*Longissimus thoracis et lumborum* (LTL) (Hispano-Breton horse)	Aging	MYL1, TUBB4A, TNNT3, CRYAB, CKM, ENO3, ALDOA, GAPDH, LDHA, TNNI2, MYBC1, TPM2, ALDOA, CAPZA2, LDHA, MDH2, VDAC3, ATP5F1C, CA3, PGAM2, MYL3, ATP5PO, MYL1, MYLPF	*Beldarrain et al.*, 2022
2-D DIGE, LC-MS/MS and Immunoblotting	*Longissimus thoracis et lumborum* (LTL) (Hispano-Breton horse)	Aging	ACTA1, MYBPC2, MYBPC1, PYGM, HSPA1A, DLAT, ALB, MYBPC2, SDHA, DES, TNNT3, ALDOA, CKM, LDHA, MYOZ1, ENO3, PHB, NDUFS3, HSPB1, ATP5PD, GAPDH,	*Beldarrain et al.*, 2023
Shotgun data-dependent acquisition proteomic approach by micro-LC-MS/MS, Data-independent acquisition (DIA), SWATH-MS	*Longissimus thoracis and lumborum* (LTL) (Jaca Navarra and Burguete horses)	Breed – Feeding – Meat quality	**WBSF** *Burguete:* OBSCN, MYBPC1, MYL1, AHNAK, FLNC, NEB, SMTNL1, PDLIM5, CKM, MYOM1, LDB3, ACTN3, MSN, PAICS, ALDOA, ACTA1, HBA2, PGAM2, ARHGDIA, NME2, MYBPH, WARS1, TTN, GSTO1, MYBPC2, MYOM2, PFN1, TPI1, MACROD1 *Jaca Navarra:* TNNC2, CKM, EEF1G, NDUFV2, ADSS1, FABP4); **Lightness (L*)** *Burguete:* IGL, PGK1, ALDOA, CKM, MACROD1, ORM1, ACYP2, GSTP1, PCMT1, SDHB, PKM, ALB, A1BG, ATP5F1B, UAB1, GSTO1, PDLIM5, CES1 *Jaca Navarra:* HIBADH, BIN1, CKM, HSPA1A, PCMT1, SOD2, EEF1A2, GAPDH, GOT2, MYBPH, GOT1); **Redness (a*)** *Burguete:* ECH1, VDAC2, PFKM, EEF1G, DES, SOD2, TRIM72, PGP, *Jaca Navarra:* HBA2, PPIA, GOT1, GLNA1, PRDX2, MYL1, CS, PHGDH), **Yellowness (b*)** *Burguete:* A1BG, TRIM72, VCL, ALDOA, ANXA7, ST13, NPEPPS, PSMA4, ORM1, CSRP3, MACROD1, DES *Jaca Navarra:* PCMT1, MYBPC1, ARHGDIA, AHCY); **IMF** *Burguete:* ACTA1, MSN, HSPD1, MYH1, PDLIM3, NME2, ART3, ALDH2 *Jaca Navarra:* HSPA5, FBP1, TF, LDHB, ANXA1, STIP1, EEF1G, A1BG, GPD1, TNNC2	*Lopez-Pedrouso et al.*, 2023

Proteomic approach	Muscle/cut (breed)	Considered variable(s)/ effects	Identified proteins/candidate biomarkers[1]	References
		Donkey		
SDS-PAGE, 2DE, LC-MS/MS, and Western Blotting	*Longissimus thoracis et lumborum* (LTL) (Martina Franca donkey)	Aging	MYLPF, TPM2, TPM1, MYL1, ACTA1, GAPDH, TNNC2, MYH1, MYH2, UQCRC1, HSPB1, MYL6B, COX5A, ATP5PD, CKM	*della Malva et al.*, 2022
Tandem-mass-tag, LC-MS/MS	*Longissimus dorsi* (LD) (Liaoxi donkey)	Meat quality	MAP4K4, PDLIM3, ADGRV1, ACTB, H2AJ, FLOT1, TPM1, UBR4, TRMT61A, PRMT3, C9, RPL32, MPC2, MBP, FHL3, LHPP, TAPT1, F11R, RPL27A, GLDN, PYCARD, COG3, CFAP251, SPAG8, GALNS, ROCK2, PRKCQ, K9KFH7, LUC7L2, ATP5MJ	*Tan et al.*, 2022
Data-independent acquisition (DIA) and nano-LC–MS/MS	*Longissimus thoracis* (LT), *Gluteus maximus* (GM), and *Semitendinosus* (ST) (Dezhou donkey)	Muscle	111 proteins in the ST/LT and 127 proteins in the GM/LT muscles comparison were differentially expressed	*Chai et al.*, 2022

[1]The full names of the proteins (gene names) are retrieved from Uniprot database (https://www.uniprot.org).

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Beldarrain, L. R., Sentandreu, E., Aldai, N. & Sentandreu, M. A. (2022). Horse meat tenderization in relation to post-mortem evolution of the myofibrillar sub-proteome. *Meat Science*, 188, 108804.

Beldarrain, L. R., Sentandreu, E., Aldai, N., Sentandreu, M. Á. & Miller, I. (2023). Application of 2-D DIGE to study the effect of ageing on horse meat myofibrillar sub-proteome. *Journal of Proteomics*, 272, 104770.

Chai, W., Xu, J., Qu, H., Ma, Q., Zhu, M., Li, M.... & Wang, C. (2022). Differential proteomic analysis to identify potential biomarkers associated with quality traits of Dezhou donkey meat using a data-independent acquisition (DIA) strategy. *LWT*, 166, 113792.

D'Alessandro, A. & Zolla, L. (2013). Meat science: From proteomics to integrated omics towards system biology. *Journal of Proteomics*, 78, 558–577.

della Malva, A., Marino, R., Santillo, A., Annicchiarico, G., Caroprese, M., Sevi, A. & Albenzio, M. (2017). Proteomic approach to investigate the impact of different dietary supplementation on lamb meat tenderness. *Meat Science*, 13, 74–81.

della Malva, A., De Palo, P., Lorenzo, J. M., Maggiolino, A., Albenzio, M. & Marino, R. (2019). Application of proteomic to investigate the post-mortem tenderization rate of different horse muscles. *Meat Science*, 157, 107885.

della Malva, A., Maggiolino, A., De Palo, P., Albenzio, M., Lorenzo, J. M., Sevi, A. & Marino, R. (2022a). Proteomic analysis to understand the relationship between the sarcoplasmic protein patterns and meat organoleptic characteristics in different horse muscles during aging. *Meat Science*, 184, 108686.

della Malva, A., Gagaoua, M., Santillo, A., De Palo, P., Sevi, A. & Albenzio, M. (2022b). First insights about the underlying mechanisms of Martina Franca donkey meat tenderization during aging: A proteomic approach. *Meat Science*, 193, 108925.

Di Luca, A., Mullen, A. M., Elia, G., Davey, G. & Hamill, R. M. (2011). Centrifugal drip is an accessible source for protein indicators of pork ageing and water-holding capacity. *Meat Science*, 88(2), 261–270.

Di Luca, A., Ianni, A., Bennato, F., Henry, M., Meleady, P. & Martino, G. (2022). A label- free quantitative analysis for the search of proteomic differences between goat breeds. *Animals*, 12(23), 3336.

FAOSTAT, (2021). Production quantities of Meat, horse. Retrieved October 14, 2021, from https://www.fao.org/faostat/en/#data/QCL/visualize.

Gagaoua, M., Hughes, J., Terlouw, E. M. C., Warner, R. D., Purslow, P. P., Lorenzo, J. M. & Picard, B. (2020). Proteomic biomarkers of beef colour. *Trends in Food Science & Technology*, 101, 234–252.

Gagaoua, M., Terlouw, E. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M. ... & Picard, B. (2021). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science*, 172, 108311.

Gagaoua, M., Schilling, W. M., Zhang, X. & Suman, S. P. (2022). Applications of proteomics in meat research. Reference module in food science. Elsevier.

Gagaoua, M. & Picard, B., 2022. Proteomics to explain and predict meat quality. Chapter 14. In: Purslow, P. (Ed.), New Aspects of Meat Quality, second ed. Woodhead Publishing, pp. 393–431.

Kiyimba, F., Gagaoua, M., Suman, S., Mafi, G. & Ramanathan, R. (2022). Bioinformatics: in-depth analyses of omics data in the field of muscle biology and meat biochemistry. Reference module in food science. Elsevier.

Lamri, M., della Malva, A., Djenane, D., Lopez-Pedrouso, M., Franco, D., Albenzio, M., ... Gagaoua, M. (2023). Towards the discovery of goat meat quality biomarkers using label-free proteomics. *Journal of Proteomics*, 278, 104868.

Li, X., Zhang, D., Ren, C., Bai, Y., Ijaz, M., Hou, C. & Chen, L. (2021). Effects of protein posttranslational modifications on meat quality: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 289–331.

Lopez-Pedrouso, M., Lorenzo, J. M., Gagaoua, M. & Franco, D. (2020). Application of proteomic technologies to assess the quality of raw pork and pork products: An overview from farm-to-fork. *Biology*, 9(11), 393.

López-Pedrouso, M., Lorenzo, J. M., Cittadini, A., Sarries, M. V., Gagaoua, M. & Franco, D. (2023). A proteomic approach to identify biomarkers of foal meat quality: A focus on tenderness, color and intramuscular fat traits. *Food Chemistry*, 405, 134805.

Marino R., della Malva A., Maggiolino A., De Palo P., d'Angelo F., Lorenzo J. M., Sevi A. & Albenzio M. (2022). Nutritional profile of donkey and horse meat: Effect of muscle and aging time. *Animals,* 12(6), 746.

Purslow, P. P., Gagaoua, M. & Warner, R. D. (2021). Insights on meat quality from combining traditional studies and proteomics. *Meat Science*, 174, 108423.

Sierra, V., Gonzalez-Blanco, L., Dineiro, Y., Díaz, F., García-Espina, M. J., CotoMontes, A., Gagaoua, M. & Olivan, M. (2021). New insights on the impact of cattle handling on post-mortem myofibrillar muscle proteome and meat tenderization. *Foods*, 10(12), 3115.

Tan, X., He, Y., Qin, Y., Yan, Z., Chen, J., Zhao, R., Zhou, S., Irwin, D.M., Li, B. & Zhang, S. (2023). Comparative analysis of differentially abundant proteins between high and low intramuscular fat content groups in donkeys. *Frontiers in Veterinary Science*, 9, 951168

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.521.056

ID: 126664201

https://doi.org/10.18485/meattech.2023.64.2.78

Original scientific paper

The effect of Swiss chard powder and starter cultures on color development and stability in dry cured pork loin

Aleksandra Silovska Nikolova[a], Zlatko Pejkovski[a], Daniela Belichovska[b] and Katerina Belichovska[a]*

[a] *Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Blvd. 16-ta Makedonska brigada 3, 1000 Skopje, North Macedonia*
[b] *Ss. Cyril and Methodius University in Skopje, Institute of Animal Science and Fishery, Blvd. Ilinden 92a, 1000 Skopje, North Macedonia*

ARTICLE INFO

Keywords:
Dry cured loin
Color quality
Starter culture
Nitrite
Swiss chard powder

ABSTRACT

Dry meat products are highly demanded and valued in the market. When choosing them, the consumer, initially, notices their color. Nitrite is responsible for red-pinkish color development. Modern consumers are looking for processed meat with low contents of additives. The paper focuses on the influence of starter culture and Swiss chard powder added to dry cured pork loin on instrumentally measured L^*, a^*, b^* values, as well as on product's color stability at room temperature (20 ± 2°C), when the meat products were kept in normal daylight for a duration of up to 120 minutes. Five groups of cured pork loins were produced: I – Control (negative), using table salt and dextrose; II – Control (positive), using nitrite curing salt and dextrose; III – nitrite curing salt, dextrose and starter culture; IV – Swiss chard powder (first producer), dextrose and starter culture and V – Swiss chard powder (second producer), dextrose and starter culture. L^*-values ranged between 28.42 (group III) and 34.23 (group I). The highest share of red color (10.86) was measured in group III. The share of yellow color ranges between 2.11 (group I) and 2.84 (group IV). Starter culture had a statistically significant ($p \leq 0.05$) impact on color development and stability of cured pork loin produced with and without nitrite curing salt.

1. Introduction

Dry cured meat products have a long history of production in Europe. It is assumed that the tradition for their production originated from the Mediterranean countries due to the specific climatic conditions that allow natural drying and ripening of dry cured meat products. These products occupy a position on the consumers' shopping list due to their high nutritional value and long shelf life. As pointed out by *Iaccarino et al.*, (2006) they have a significant place in the global gastronomic heritage. Pork and beef are, most often, used as a raw material for their production.

Based on a wide variety of studies, color of meat and meat products is an important factor for consumers in deciding their choice (*Lawrie and Ledward*, 2006; *Møller and Skibsted*, 2007; *Kolev et. al.*, 2022). At the beginning of the last century, it was determined that nitrites are the main factor in development of characteristic, stable, red-pinkish color in meat products. Added nitrites in meat products, as stated by *Gøtterup et. al.* (2008), are a source of nitric oxide (NO) which reacts with myoglobin (Mb) to form nitrosomyoglobin (NOMb). Nitrates are reservoirs of nitrites. When nitrates are used in meat products, it is necessary to reduce them

***Corresponding author:** Aleksandra Silovska Nikolova, *silovska@fznh.ukim.edu.mk*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

to nitrites with the bacterial enzyme nitrate reductase (*Arnau et. al.*, 2007). Based on their technological function, nitrites and nitrates are food additives that are included in the functional class of preservatives. From a technological point of view *Alahakoon et al*. (2015) state that they are important for color and aroma development, and they prevent oxidation. In recent years, consumers have been searching the market for meat products with lower amounts of additives, mainly focusing on nitrites. (*Wolk,* 2017; *Alirezalu et al*., 2020).

In the production of meat products, Swiss chard powder can be used as a substitute for synthetic nitrite due to its high nitrate content that could be converted into nitrite by the action of starter cultures containing nitrate reducing bacteria.

Numerous authors point out the positive effects of using the starter cultures in meat industry. Their application increases the safety of the product, helps the development of color, texture, taste, aroma, generally affects the development of characteristic properties of the product, increases the durability of the product, reduces the variation in the quality of the product, and makes production more profitable due to acceleration of fermentation, etc. (*Kovačević*, 2001; *Incze*, 2002; *Martinović and Vesković Moračanin*, 2006).

This paper aims to examine whether the usage of starter cultures influences the development and stability of color in cured pork loin produced with and without nitrite salt.

2. Materials and methods

2.1. Materials

Dry pork loin, dry-salted, produced in industrial conditions was used in the analysis. As an alternative to nitrite curing salt, in dry pork loin production, Swiss chard powder from two different producers, was used in combination with starter culture BactoFerm Rosa, produced by Chr. Hansen, Denmark.

The experiment was conducted in a commercial meat processing factory (Rimes MS Group). Their usual technological method for producing dry pork loin was taken as a basis for our study. Five groups of dry cured pork loins were produced, as follows: group I: control (negative), using table salt and dextrose; group II: control (positive), using nitrite curing salt and dextrose; group III: nitrite curing salt, dextrose and starter culture (BactoFerm Rosa); group IV: Swiss chard powder (first producer), dextrose and starter culture (BactoFerm Rosa) and group V: Swiss chard powder (second producer), dextrose and starter culture (BactoFerm Rosa).

The frozen outer part of pork loin (*m. longissimus dorsi*) was used. Bones, connective and adipose tissue were previously removed from it. The raw material was thawed by dry defrosting. Technology for production of dry cured pork loin includes salting the pork loin. Salted pieces were left in carts for 21 days in a dark room, with a constant mode of calm cooling, at a temperature of 0–40°C and a relative humidity of 85 to 90%. This was followed by cold smoking for 40 minutes. During the remaining 19 hours and 20 minutes, it was smoked twice more for 40 minutes each time, the chamber temperature was 22 °C, and the relative humidity 82%. The carts were then transferred into the air conditioning chamber where fermentation took place. At the beginning, the temperature was 22 °C and the relative humidity 82%. Then the temperature was gradually lowered to 12 °C and relative humidity was, also, lowered to 72%.

2.2. Methods

Instrumental color analysis was performed after ripening, on the fresh cross section and every 30 minutes for a 120 minute period of air exposure. The color measurements were performed at a room temperature (20 ± 2°C) and samples were kept at normal daylight. All measurements were performed five times in non-overlapping zones. A color difference meter (Dr. Lange) was used to determine these parameters. Before the start of the measurement it was calibrated with a black and white calibration plate, according to the standard procedure of the manufacturer. The color characteristics are expressed in three coordinates L* a* b*, (*CIE*, 1976).

The data collected in the experiment were processed and edited using the program Excel XP. The normality of the distribution of the values was checked by analyzing the homogeneity of the variances. If the homogeneity was confirmed, the analysis was continued with the multivariate general linear model (GLM) or the ANOVA test (comparison of three or more groups), and the associations between the parameters with the multivariate linear descriptive analysis (LDA) (IBM SPSS Statistics 23, release 23.0.0.0).

3. Results and discussion

3.1. Dynamics of L-value*

Table 1 shows that L*-values measured after production (0 minutes) on fresh cross-section ranged between 28.42 and 34.23. The L*values were lower than L*-values measured in similar dry cured meat products. When it comes to Spanish dry cured-hams, *Pérez-Alvarez et al.* (1998) determined that L*-values ranged between 34.8 and 38.8, while *Laureati et al.* (2014) stated that the value for Italian prosciutto ranged between 37.9 and 38.0. According to some authors, the lower L*-value in our study is due to the decrease in water content during drying and ripening. The lower water content contributes to a higher density in the piece of meat, which in turn causes a more intense absorption of light and the color is perceived as darker (*Hunt*, 1980; *De Maere et al.*, 2016).

In groups where starter culture was added (III, IV and V), even after 120 min of light exposure of the fresh cross-section at room temperature, no statistically significant ($p \leq 0.05$) changes were observed in terms of the L*-values, compared to the groups in which no starter cultures were added (I and II) (Table 1).

3.2. Dynamics of a*-value

It is understandable (Table 2) that group I cured pork loin, where only common table salt was added, has the lowest a*-value (3.09). The highest a*-value, i.e. the largest share of red color, was observed in group III (10.86), in which nitrite curing salt and starter culture were added. Starter culture had a statistically significant ($p \leq 0.05$) impact on the share of red color compared to group II positive control, where nitrite curing salt was added. In groups IV and V, where Swiss

Table 1. Dynamics of L*-values in cured pork loin exposed at room temperature (20 ± 2°C) to normal day light for periods of up to 120 minutes

Duration of exposure to light	Groups of dry pork loin				
	I	II	III	IV	V
	$\bar{X} \pm SD$				
0 minutes	34.23 ± 1.24^{aB}	31.60 ± 1.17^{bB}	28.42 ± 0.97^{cA}	28.98 ± 1.47^{cBA}	31.19 ± 1.47^{bA}
30 minutes	33.88 ± 1.24^{aB}	31.28 ± 1.16^{bB}	28.15 ± 0.96^{cBA}	28.69 ± 1.45^{cBA}	30.88 ± 1.45^{bBA}
60 minutes	33.53 ± 1.23^{aB}	30.97 ± 1.15^{bB}	27.87 ± 0.95^{cB}	28.26 ± 1.43^{cB}	30.36 ± 1.43^{bB}
90 minutes	34.22 ± 1.25^{aB}	31.61 ± 1.17^{bB}	28.29 ± 0.97^{cBA}	28.80 ± 1.46^{cBA}	31.00 ± 1.46^{bBA}
120 minutes	37.87 ± 1.44^{aA}	32.54 ± 1.20^{bA}	28.49 ± 0.97^{eA}	29.21 ± 1.45^{dA}	31.52 ± 1.48^{cA}

$\bar{X}$ – mean value, SD standard deviation, statistically insignificant effect; means with a different letter ($^{a-d}$) within a row are statistically significantly different ($p \leq 0.05$; significance of group differences), means with a different letter ($^{A-C}$) within a column and parameter are statistically significantly different ($p \leq 0.05$; significance of sampling differences).

Table 2. Dynamics of a*-values in cured por k loin exposed at room temperature (20 ± 2°C**)** to normal day light for periods of up to 120 minutes

Duration of exposure to light	Groups of dry pork loin				
	I	II	III	IV	V
	$\bar{X} \pm SD$				
0 minutes	3.09 ± 0.97^{eA}	9.62 ± 1.08^{bA}	10.86 ± 0.84^{aA}	7.34 ± 0.69^{cA}	6.73 ± 0.68^{dA}
30 minutes	3.06 ± 0.96^{eA}	9.50 ± 1.07^{bA}	10.75 ± 0.84^{aA}	7.23 ± 0.68^{cBA}	6.62 ± 0.67^{dBA}
60 minutes	3.03 ± 0.95^{eA}	9.41 ± 1.06^{bA}	10.64 ± 0.83^{aA}	7.10 ± 0.67^{cBA}	6.50 ± 0.65^{dBA}
90 minutes	2.92 ± 0.91^{eA}	9.12 ± 1.02^{bA}	10.48 ± 0.81^{aBA}	6.95 ± 0.65^{cCB}	6.32 ± 0.64^{dCB}
120 minutes	2.30 ± 0.86^{eB}	8.53 ± 1.01^{bB}	10.10 ± 0.73^{aB}	6.63 ± 0.63^{cC}	5.99 ± 0.60^{dC}

$\bar{X}$ – mean value, SD standard deviation, statistically insignificant effect; means with a different letter ($^{a-d}$) within a row are statistically significantly different ($p \leq 0.05$; significance of group differences), means with a different letter ($^{A-C}$) within a column and parameter are statistically significantly different ($p \leq 0.05$; significance of sampling differences).

chard powder and starter culture were added, the a*-values ranged between 6.73 (group V) and 7.34 (group IV). The difference seen between these two groups, although small, was statistically significant ($p \leq 0.05$). This difference may be caused by the different content of nitrates contained in the Swiss chard powder, since products from two different producers were used.

It is clear (Table 2) that a*-value declined in all groups at the end of light exposure, after 120 minutes, which is consistent with the information in the relevant professional literature, where it is stated that exposure to light and air causes a decrease in the a*-value, i.e., a decrease in the share of red color. (*Hunt et al*., 2012; *Kolev at al*., 2022). A smaller decrease in the a*-value was observed in the groups of cured pork loin in which starter culture was added (III, IV and V).

3.3. Dynamics of b*-value

The share of yellow color (Table 3) on a fresh cross-section after production ranged between 2.11 (group I) and 2.84 (group IV). The b*-values obtained were lower than the results obtained for similar dry cured meat products. *Marušić et. alt*. (2014 found that b*-values ranged between 7.3 and 10.4 in Dalmatian smoked prosciutto.

It was observed that no statistically significant difference in the change of b*-value during exposure to light and heat existed in any group of cured pork loin.

4. Conclusion

In view of the results obtained, BactoFerm Rosa starter culture was shown to have a positive effect on the parameters related to color measurement in cured pork loin. This starter culture had a positive impact on development and durability of a beautiful red color, even after 120 minutes of exposing the pork loin to air, normal day light and room temperature (20 ± 2°C). Usage of starter cultures in meat industry has a positive effect on color development and stability. The starter culture could affect the reduction of nitrites in the finished product.

Table 3. Dynamics of b*-values in cured pork loin exposed at room temperature (20 ± 2°C**)** to normal day light for periods of up to 120 minutes

Duration of exposure to light	Groups of dry pork loin				
	I	II	III	IV	V
	$\bar{X} \pm SD$				
0 minutes	2.11 ± 0.75^{cA}	2.68 ± 1.12^{baA}	2.70 ± 0.96^{baA}	2.84 ± 1.09^{aA}	2.26 ± 0.85^{cbA}
30 minutes	2.09 ± 0.74^{cA}	2.66 ± 1.11^{baA}	2.68 ± 0.95^{baA}	2.81 ± 1.08^{aA}	2.24 ± 0.84^{cbA}
60 minutes	2.07 ± 0.74^{cA}	2.63 ± 1.10^{baA}	2.65 ± 0.94^{baA}	2.77 ± 1.06^{aA}	2.20 ± 0.83^{cbA}
90 minutes	2.05 ± 0.73^{cA}	2.60 ± 1.09^{baA}	2.61 ± 0.93^{baA}	2.71 ± 1.04^{aA}	2.15 ± 0.81^{cbA}
120 minutes	2.03 ± 0.72^{cA}	2.51 ± 1.09^{cbaA}	2.59 ± 0.92^{baA}	2.67 ± 1.02^{aA}	2.11 ± 0.80^{cbA}

$\bar{X}$ – mean value, SD standard deviation, statistically insignificant effect; means with a different letter ($^{a-d}$) within a row are statistically significantly different ($p \leq 0.05$; significance of group differences), means with a different letter ($^{A-C}$) within a column and parameter are statistically significantly different ($p \leq 0.05$; significance of sampling differences).

Disclosure statement: No potential conflict of interests was reported by authors.

References

Alahakoon, A. U., Jayasena D. D., Ramachandra S. & Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. *Trends in Food Science and Technology*, 45(1), 37–49, https://doi.org/10.1016/j.tifs.2015.05.008

Alirezalu, K., Pateiro, M., Yaghoubi, M., Alirezalu, A., Peighambardoust, S. H. & Lorenzo, J. M. (2020). Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive review. *Trends in Food Science and Technology*, 100, 292–306, https://doi.org/10.1016/j.tifs.2020.04.010

Arnau, J., Serra, X., Comaposada, J., Gou, P. & Garriga, M. (2007). Technologies to shorten the drying period of dry-cured meat products. *Meat Science*, 77, 81–89, https://doi.org/10.1016/j.meatsci.2007.03.015

CIE, (1976). International Commission on Illumination, Colorimetry: Official Recommendation of the International Commission on Illumination Publication CIE No. (E-1.31). Bureau Central de la CIE, Paris, France.

De Maere, H., Fraeye, I., De Mey, E., Dewulf, L., Michiels, C., Paelinck, H. & Chollet, S. (2016). Formation of naturally occurring pigments during the production of nitrite-free dry fermented sausages. *Meat Science*, 114, 1–‒7, https://doi.org/10.1016/j.meatsci.2015.11.024

Gøtterup, J., Olsen, K., Knochel, S., Tjener, K., Stahnke, L. H. & Møller, J. K. S. (2008). Colour formation in fermented sausages by meat-associated staphylococci with different nitrite-and nitrate-reductase activities. *Meat Science*, 78(4), 492–501, https://doi.org/10.1016/j.meatsci.2007.07.023

Hunt, M. C. (1980). Meat color measurements. Proceedings of 33rd annual reciprocal meat conference Chicago.

Hunt, M. C., King, A., Barbut, S., Clause, J., Cornforth, D., Hanson, D. & Weber, M. (2012). AMSA meat color measurement guidelines. American Meat Science Association, Champaign, Illinois USA, 61820*,* 1–135.

Iaccarino, T., Di Monaco, R., Mincione, A., Cavella, S. & Masi, P. (2006). Influence of information on origin and technology on the consumer response: the case of soppressata salami. *Food Quality Preference*, 17 (1–2), 76–84, https://doi.org/10.1016/j.foodqual.2005.08.005

Incze, K. (2002). Fermented meat products — A review of current research topics. *Fleischwirtschaft*, 82(4), 112–118.

Kolev, N. D., Vlahova-Vangelova, D. B., Balev, D. K. & Dragoev, S. G. (2022). Color dynamics of cooked sausages after nitrite reduction and incorporation of biologically active substances. *The Journal of Almaty Technological University*, 3, 130–137, https://doi.org/10.48184/2304-568X-2022-3-130-137

Kovačević, D. (2001). Kemija i tehnologija mesa i ribe. Sveučilište J.J. Strossmayera, Prehrambeno tehnološki fakultet, Osijek, Hrvatska.

Laureati, M., Buratti, S., Giovanelli, G., Corazzin, M., Lo Fiego, P. D. & Pagliarini, E. (2014). Characterization and differentiation of Italian Parma, San Daniele and Toscano dry-cured hams: A multi-disciplinary approach. *Meat Science*, 96, 288–294, https://doi.org/10.1016/j.meatsci.2013.07.014

Lawrie, R. A. & Ledward, D. A. (2006). Lawrie's meat science, seventh edition. Abington, Cambridge: Woodhead Publishing Limited.

Martinović, A. & Vesković Moračanin, S. (2006). Primena starter kultura u industriji mesa. *Tehnologija Mesa*, 47(5–6), 216–230.

Marušić, N., Vidaček, S., Janči, T., Petrak, T. & Medić, H. (2014). Determination of volatile compounds and quality parameters of traditional Istrian dry-cured ham. *Meat Science*, 96 (4), 1409–1416, https://doi.org/10.1016/j.meatsci.2013.12.003

Møller, J. K. S. & Skibsted, L. H. (2007). Color. In: Handbook of fermented meat and poultry, edited by F. Toldrá, Y.H. Hui, I. Astiasarán, W.K. Nip, J.G. Sebranek, E.T.F. Silveira, L.H. Stahnke, R. Talon (pp. 203–216). Ames, Iowa, USA: Blackwell Publishing.

Pérez-Alvarez, J. A., Sayas-Barberá, M. E., Fernández-López, J., Gago-Gago, M. A., Pagán-Moreno, M. J. & Aranda-Catalá, V. (1998). Chemical and colour characteristics of Spanish dry-cured ham at the end of the aging process. *Journal of Muscle Foods*, 10(2), 195–201, https://doi.org/10.1111/j.1745–4573.1999.tb00395.x

Wolk, A. (2017). Potential health hazards of eating red meat. *Journal of Internal Medicine*, 281(2), 106–122, https://doi.org/10.1111/joim.12543

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.524.056

ID: 126667017

https://doi.org/10.18485/meattech.2023.64.2.79

Original scientific paper

Sensory and chemical characteristics of dry fermented sausages

Jelena Jovanović[a], Ivana Branković Lazić[a], Aleksandra Nikolić[a], Mladen Rašeta[a], Boris Mrdović[a], Tatjana Baltić[a] and Jelena Babić Milijašević[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Fermented sausages
Sensory analysis
Chemical analysis

ABSTRACT

The aim of this study was to investigate the sensory and chemical characteristics of dry fermented sausages produced according to two recipes (group I and group II), which differed in the amount of pork meat and solid fat tissue, while the amount of other ingredients, and the technological process were the same. The obtained results showed higher scores in taste, consistency, salinity, and seasoning for group I dry fermented sausages. Chemical results show higher protein content in group I (35.57%) than in group II, which had 30.70%. The collagen content in meat proteins was higher in group II compared to group I (5.53% and 4.52%, respectively). The average moisture content in both sausage groups was less than 35%. The chloride content in sausages was 5.15% (group I) and 4.42% (group II). The fat content in group II was higher than in group I (33.70% and 21.50%, respectively). The results of this study indicate that different quantities of pork meat and solid fat tissue can influence the sensory and chemical properties of dry fermented sausages.

1. Introduction

Fermentation and drying can be considered to be the oldest way to preserve raw food materials. The first documented sausage production was in ancient Greece, and the Romans inherited this tradition, from where the production of fermented sausages spread through Europe (*Vignolo*, 2010). Fermented sausages are considered high-quality products. They are made from minced meat and fatty tissue, with added spices, sugars, additives, starter cultures, and other ingredients, stuffed into casings (natural or artificial) and then preserved by drying, with or without smoking, during which the sausages mature and acquire characteristic quality properties (*Vuković et al.*, 2009). The drying process is carried out at a low temperature, and only then do sausages get their characteristic aroma, solid consistency, and extended product shelf life during the ripening process (*Vuković*, 2012). The shelf life of fermented sausages is based on low values of pH and water activity (a_w), on the basis of which they can be stored at higher temperatures than are usual for meat (*Teodorović et al.*, 2015). According to the degree of drying and consistency, fermented sausages are divided into dry fermented sausages and semi-dry fermented sausages. Dry fermented sausages contain less than 35% moisture, and their a_w is less than 0.90. In Serbia, this type of sausage is mostly industrially produced, which means the quality of this product is not standardized, but it is acceptable for the majority of the population because it is charac-

***Corresponding author:** Jelena Jovanović, *jelena.jovanovic@inmes.rs*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

terized by an attractive appearance, good grinding ability, and pleasant aroma (*Lazic et al*., 2019a).

The aim of this study was to investigate the sensory and chemical characteristics of dry fermented sausages produced according to two recipes, which differed in the amount of pork meat and solid fat tissue, while the amount of other ingredients, and the technological process were the same.

2. Materials and methods

Fermented dry sausages examined in this study were produced according to two recipes, which differed in the amount of pork meat and solid fat tissue, while the technological process was the same for both sausage groups. Table 1 shows the percentage distribution of the used ingredients.

The technological processing of the sausages took place in industrial conditions. The raw materials, pork meat (−3.6ºC), and solid fat tissue (−4ºC) were minced in the cutter. After that, the other ingredients were added, while the starter culture was added at the end of the process. The homogenization was carried out until a 5 mm granulation mosaic was attained. The sausage filling was stuffed into collagen casings, diameter of ⌀55 mm. After that sausages were hung on horizontal bars of drying racks and left in the anteroom of the automatic air conditioning chamber for about 3 h. This procedure was used to optimize the process of fermentation/ripening because it is necessary to raise the temperature of the filling as close as possible to the optimal temperature (recommendation: to achieve at least 18–19ºC, and ideally, 22–24ºC) before the fermentation process starts to ensure optimal conditions for the metabolism of starter cultures (*Lazic et al*., 2019b). The production process (fermentation/drying and smoking, ripening) was a combination of automatic air conditioning and a traditional smoke chamber. This process lasted for 26 days.

2.1. Laboratory analyses

Sausages were analysed after production in sensory and chemical laboratories accredited according to SRPS ISO/IEC17025:2017.

2.1.1. Sensory analyses

Sensory properties of sausages (appearance, surface colour, cross-section colour, cross-section appearance, odour, taste, consistency, salinity, seasoning, and overall acceptability) were assessed using a quantitive descriptive test (*SRPS ISO*, 2018), with a grading scale from one to five (1 – unacceptable, 5 – extremely acceptable) (Table 2). A panel consisting of five trained members of different ages performed the sensory evaluation. Panellists were previously tested for detecting and recognizing various tastes (*SRPS ISO*, 2016) and odours (*SRPS ISO*, 2014). Sensory property results were the mean value given by the five panellists.

Table 1. The ingredients used to produce two groups of fermented dry sausages

Raw material	Fermented dry sausage — group I (%)	Fermented dry sausage — group II (%)
Pork meat category 1	70	60
Pork meat category 2	15.9	15.9
Solid fat tissue	10	20
Nitrite salt	2.5	2.5
RADA*ferm*	0.05	0.05
Dextrose	0.2	0.2
Ascorbic acid	0.05	0.05
Sweet pepper (oleoresin)	0.8	0.8
Cayenne pepper (oleoresin)	0.1	0.1
Garlic	0.2	0.2
Onion	0.2	0.2

Table 2. Numerical descriptive scale for the assessment of sensory properties

Number rating	Descriptive rating
5	extremely acceptable
4	very acceptable
3	acceptable
2	at the margin of acceptability
1	unacceptable

The sausages were prepared as follows: after removing the casing, sausages were cut into slices thick 5 mm and served at room temperature on white, plastic plates. Three slices of each product were served to each panellist.

2.1.2. Chemical analyses

After sensory analyses, samples from both sausage groups were taken for chemical composition analysis. Hydroxyproline content (*SRPS ISO*, 2002), moisture content (*SRPS ISO*, 1998a), total fat content (*SRPS ISO*, 1998b), NaCl (*SRPS ISO*, 1999), and pH (*SRPS ISO*, 2004) were determined using standard reference methods. Nitrogen content was determined by an in-house method, the Kjeldahl method, and protein content was estimated by multiplying the nitrogen content by 6.25 (Kjeltec Auto 1030 Analyzer, Tecator, Sweden).

3. Results and discussion

The results of sensory analyses performed by professionally trained assessors are presented in Figure 1.

The sensory results showed the average scores for all tested sensory properties were similar between the two groups of dry fermented sausages. A higher rating was given to group I, which contained a slightly higher amount of pork meat category 1 and a slightly lower amount of solid fat tissue. These higher scores of group I sausages were noticed in taste, consistency, salinity, and seasoning, while group II had better ratings in appearance and cross-section colour. These results confirm that the quality of the raw material has a great influence on the sensory characteristics of fermented sausages (*Vuković et al*., 2009; **Živković** *et al*., 2011). However, in the study conducted by *Mendoza et al*. (2001), sausages with a smaller fat content were less juicy, have more solid consistency, and uneven and wrinkled surfaces.

The chemical composition of the two groups of dry fermented sausages is given in Table 3. The obtained results showed that group I sausages had a higher protein content (35.57%) than group II which had 30.70%, while collagen content in meat proteins was higher in group II compared to group I (5.53%

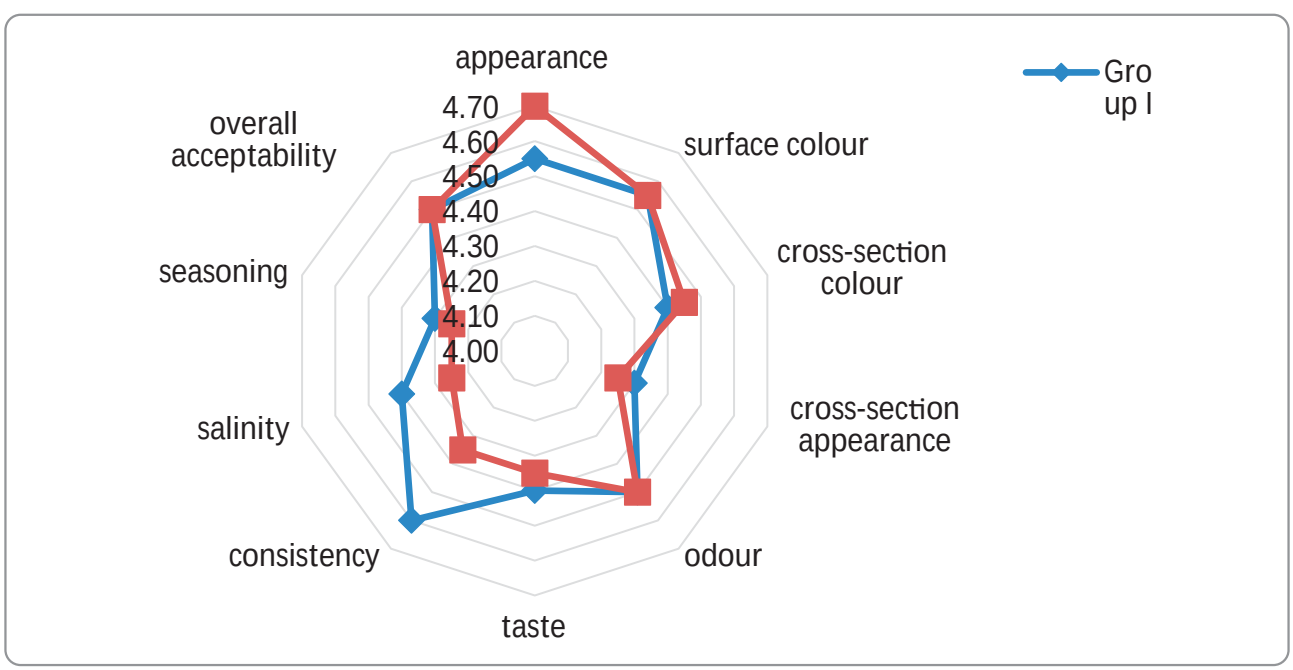


Figure 1. Sensory properties of dry fermented sausages (group I and group II)

Table 3. Chemical composition of fermented dry sausages

Attribute	Group I	Group II
Protein (%)	35.57	30.70
Collagen (%)	4.52	5.53
Water (%)	34.24	33.00
Fat (%)	21.50	33.70
NaCl (%)	5.15	4.42
pH	5.30	5.32
a_w	0.855	0.846

respectively 4.52%). The average moisture content (e.g. water) in both sausage groups was less than 35%, and the pH was at least 5.0, which meets the requirements of Serbian Regulations (*Anon*, 2019). The chloride content in sausages was 5.15% (group I) and 4.42% (group II). The fat content in group II was much higher than in group I (33.70% respectively 21.50%).

4. Conclusion

The results of this study indicate that different quantities of pork meat and solid fat tissue, can affect some sensory properties of dry fermented sausages (taste, consistency, salinity, seasoning, appearance, and cross-section colour). Also, this difference in the amount of raw materials can result in different protein and fat contents in final products. For that reason, the raw materials used in the production of dry fermented sausages can affect the quality of the final products.

Disclosure statement: No potential conflict of interest was reported by the authors.

Fundings: This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia, according to the provisions of the Contract on research financing in 2023. year (No. 451-03-47/2023-01/200050 dated 03. 02. 2023).

References

Anon, (2019). Regulation of quality of minced meat, meat preparations and meat products (*Official Gazette of the Republic of Serbia*, No. 50/2019).

Lazic, I. B., Jovanovic, J., Simunovic, S., Raseta, M., Trbovic, D., Baltic, T. & Ciric, J. (2019a). Evaluation of sensory and chemical parameters of fermented sausages. *Meat Technology*, 60(2), 84–90.

Lazic, I. B., Lilic, S., Jovanovic, J., Raseta, M., Mrdovic, B., Trbovic, D. & Nastasijevic, I. (2019b). Assessment of sensory and chemical parameters of tea sausage. In *IOP Conference Series: Earth and Environmental Science* **(Vol. 333, No. 1, p. 012048). IOP Publishing.**

Mendoza, E., Garcıa, M. L., Casas, C. & Selgas, M. D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science*, 57(4), 387–393.

SRPS ISO, (1998a). SRPS ISO 1442:1998 — Meat and meat products — Determination of moisture content.

SRPS ISO, (1998b). SRPS ISO 1444:1998 — Meat and meat products — Determination of free fat content.

SRPS ISO, (1999). SRPS ISO 1841-1:1999 — Meat and meat products — Determination of chloride content — Part 1: Volhard method.

SRPS ISO, (2002). SRPS ISO 3496:2002 — Meat and meat products — Determination of hydroxyproline content.

SRPS ISO, (2004). SRPS ISO 2917:2004 — Meat and meat products — Measurement of pH.

SRPS ISO, (2014). SRPS ISO 5496:2014 — Sensory analysis — Methodology — Initiation and training of assessors in the detection and recognition of odours.

SRPS ISO, (2016). SRPS ISO 3972:2016 — Sensory analysis — Methodology — Method of investigating sensitivity of taste.

SRPS ISO, (2018). SRPS ISO 6658:2018 — Sensory analysis — Methodology — General guidance.

SRPS ISO/IEC 17025:2017, (2017). General requirements for the competence of testing and calibration laboratories.

Teodorović, V., Karabasil, N., Dimitrijević, M. & Vasilev, D. (2015). Higijena i tehnologija mesa [Meat hygiene and technology].

Vignolo, G., Fontana, C. & Fadda, S. (2010). Semidry and dry fermented sausages. *Handbook of Meat Processing*, 561, 379–398.

Vuković I. (2012). Osnove tehnologije mesa

Vuković, I., Saičić, S., Vasilev, D., Tubić, M., Vasiljević, N. & Milanović-Stevanović, M. (2009). Neki parametri kvaliteta i nutritivna vrednost funkcionalnih fermentisanih kobasica. *Meat Technology*, 50(1–2), 68–74.

Živković, D., Tomović, V., Perunović, M., Stajić, S., Stanišić, N. & Bogićević, N. (2011). Senzorna prihvatljivost sremske kobasice izrađene od mesa svinja različite starosti. *Meat Technology*, 52(2), 52–61.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.528.056

ID: 126676233

https://doi.org/10.18485/meattech.2023.64.2.80

Original scientific paper

Physicochemical and sensory properties of pork liver pâté formulated with sunflower oleogel as fat substituent

Miloš Županjac[a], Predrag Ikonić[a], Branislav Šojić[b] and Branislava Đermanović[a]*

[a] *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[b] *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Liver pâté
Oleogel
Sunflower oil
Fat substituent

ABSTRACT

This study investigated the effects of back fat substitution with sunflower oleogel on the lipid, moisture and protein content, as well as texture, and color characteristics of liver pâté. Formulations with oleogel had higher lipid content ($p<0.05$) in comparison to control pâté made just with backfat. On the contrary, moisture and protein content were found to be significantly lower ($p<0.05$) in pâté formulated with oleogel compared to the control, regardless the level (%) of substitution. Color analysis revealed that oleogel-added pâté exhibited darker, redder, and more yellow ($p<0.05$) tones compared to the control. Texture analysis showed significant differences ($p<0.05$) in firmness, work of shear, and spreadability. Further research on sensory attributes and consumer acceptance will be performed.

1. Introduction

Pâté, also known as Braunschweiger, is a paste-based meat product with a pleasant flavor and a soft, oily texture that is widely consumed around the world (*Morales-Irigoyen et al.*, 2012). The main ingredients used in the manufacture of pork liver pâté are pork backfat, liver, spices, and, on rare occasions, low-quality meat (*Barbut et al.*, 2021; *Martins*, 2020). Pâtés are commonly known to contain a significant proportion of animal fat, typically ranging from 35% to 50% (*Pan*, 2021). The relatively high fat content in these emulsified meat products affects their sensory, physicochemical, and nutritional properties, as well as their texture (*Rezler et al.*, 2020; *Tobin et al.*, 2013; *Perta-Crisan et al.,* 2023). Saturated fat is the main type of fat found in meat products. However, there is an increasing trend toward healthier meat products with less saturated fat due to the well-documented link between saturated fat consumption and an increased risk of cardiovascular disease (CVD) and other physiological disorders, such as type 2 diabetes, high blood lipid levels, inflammation, and oxidative stress (*Schwingshackl et al.*, 2022; *Maki et al.*, 2021; *López-Pedrouso et al.*, 2021; *Astrup et al.*, 2020; *Silva et al.*, 2023; *Barbut et al.*, 2021; *Mensink et al.*, 2016). Although trans and saturated fats have been shown to positively impact the structure and texture of food products (*WHO,* 2013), the World Health Organization suggests limiting dietary fat to 15–30% of total daily energy intake, with saturated fats accounting for no more than 10% of the total (*Badar et al.*, 2021; *Lima et al.*, 2022; *Khan et al.*, 2015). The reduction of fat in this type of meat product or its replacement with a more unsaturated fat might affect pâté's technological or sensory characteristics. Plant-based oils, which contain a low share of saturated fats and high percentage of unsat-

***Corresponding author:** Miloš Županjac, *milos.zupanjac@fins.uns.ac.rs*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

urated fatty acid (UFA) (monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA)), have enormous potential for use in the production of healthier meat products with improved nutritional quality (*Romanić*, 2020; *Ramadan*, 2020). Sunflower oil is particularly rich in linoleic acid (*Singh et al*., 2017), an essential n-6 polyunsaturated fatty acid, that has been shown to reduce total cholesterol by acting on low-density lipoprotein (LDL), while the level of protective high-density lipoprotein (HDL) remains unchanged (*Guo et al*., 2023).

Cold pressing is a method that provides a secure and non-toxic approach for extracting and processing edible oils. This technique ensures the preservation of bioactive components by avoiding the use of heat, chemicals, and refining procedures (*Demirkesen and Mert*, 2020). Edible oleogels have been developed in response to the trend of substituting animal fat with vegetable oils structured by organogelators (*Patel and Hartel*, 2015; *Perta-Crisan* et al., 2023; *Aranda-Ledesma et al.*, 2022). Oleogels can be described as semi-solid systems with viscoelastic properties and hydrophobic nature, formed by organogelation. Organogelation involves the entrapment of a significant amount of liquid oil, especially vegetable oils, within a three-dimensional network using one or more organogelators (*ISO*, 1997; *Aranda-Ledesma et al.*, 2022). Organogelators can be categorized into different groups, including crystalline particles, low molecular weight compounds, polymers, and inorganic particles (*ISO*, 1978). Candelilla wax (CW) is a plant-derived natural wax obtained from *Euphorbia antisyphilitica* Zucc., a plant species found in the arid regions of northern Mexico. CW has gained attention as a potential organogelator for the creation of food formulations (*ISO*, 1973).

The aim of this study was to investigate how the replacement of pork backfat (lard) with different levels of oleogel affected the physicochemical properties of pork liver pâté.

2. Materials and methods

2.1.1. Raw materials and oleogels production

Pâté formulation comprised the formation of an emulsion constituted from pork backfat, sodium caseinate, hot broth, lean pork meat, chopped liver, salt, spice mix (comprising pepper, dried onion, and marjoram). For the production of Candelilla-based oleogel, a commercial cold-pressed sunflower oil (BISER, Velika Plana, Serbia) was used as the oil phase.

Oleogel with 5% (w/w) of gelator was produced for all the fat replacement experiments. Candelilla wax (Alekpharm, Beograd, Serbia) was dispersed in sunflower oil under stirring at 80°C (above wax melting point) for at least 30 min. After that period of time, the gels were left cooling at room temperature until full gel formation, for at least 24 h.

2.1.2. Pâté manufacturing

Pâtés were prepared using a combination of pork meat, liver, and fat, along with a spice mix (Table 1). The pork meat and fat were boiled in water until they reached a tender state. Subsequently, the meat was mixed with the hot broth obtained from cooking of the meat, and spice mix. Further, the mixture was processed in a Thermomix® TM5 mincer (Vorwerk Elektrowerke GmbH & Co. KG,

Table 1. Formulation of elaborated pâtés.

Raw Materials (%, w/w)	P-CO	P-20	P-40
Pork backfat	40	32	24
Sunflower oleogel	/	8	16
Sodium caseinate	2	2	2
Hot broth	22	22	22
Laean meat	15	15	15
Liver	18	18	18
Nitrite curing salt	2	2	2
Spice mix	1	1	1

P-CO: control **pâté**;
P-20 and P-40: **pâté** with 20% and 40% of pork fat replacement, respectively.

Wuppertal, Germany) at a temperature of 55°C, velocity set at position 6, for 10 minutes. At this stage, homogenized raw pork liver was also added. Oleogel was added to the minced meat and the grinding operation was continued until homogeneous consistency was obtained. Finally, the resulting mass was stuffed into 200 ml jars and cooked until a core temperature exceeded 80 ºC for a period of 3 minutes. The prepared pâtés were subsequently cooled using cold water and stored in a refrigerator prior to further analyses.

2.1.3. Chemical composition of elaborated pâtés

The moisture, protein, and fat content were analyzed using the methods outlined by the International Organization for Standardization (ISO) in ISO 1442 (*ISO*, 1997), ISO 937 (*ISO*, 1978), and ISO 1443 (*ISO*, 1973) procedures, respectively.

2.14. Textural and color analysis

The textural characteristics of the pâtés were assessed using a Texture Analyzer TA.XT Plus texturometer (Stable Micro System, England), following the Margarine Spreadability method — MAR4_SR.PRJ. This method involves employing the Spreadability Rig HDP/SR accessory, comprising an upper conical measuring component affixed to a metal platform. The accessory is calibrated to maintain a height of 30 mm above the static component, which consists of a conical cup. Throughout the measurement, the distance between the upper and lower parts of the accessory remained constant at 23 mm. The analysis employed the following parameters: a 5 kg load cell, a temperature of 22°C, a pre-analysis test speed of 1.0 mm/s, an analysis test speed of 3.0 mm/s, and a post-analysis test speed of 10.0 mm/s. The measurement involved monitoring the force exerted during penetration until it reached its maximum depth. The force values at specific penetration depths corresponded to the hardness of the spread, while the area under the force-time curve represented the work of shearing.

Color measurements were conducted on the pâtés using a portable colorimeter (CR-400, Konica, Minolta, Tokyo, Japan) equipped with a light protection tube (CR-A33b). The colorimeter employed a 0 degrees viewing angle geometry and an 8 mm aperture size. The measurements were performed after the canning procedure to assess the pâté color in the CIELAB color space, specifically the lightness (L^*), redness (a^*), and yellowness (b^*) values. Three distinct points on each sample were selected from homogeneous and representative areas.

2.1.5. Statistical analysis

Statistical analysis was done using Factorial ANOVA (Statistica 14.0.0.15 — TIBCO Software Inc., USA). Duncan's post hoc test was performed for comparison of mean values. Differences were considered significant at $p<0.05$.

3. Results and discussion

The total lipid content primarily consisted of the fat used, with smaller contributions from the liver and muscle. In the oleogel formulations, the lipid content (P-20: 39.21±0.22^a and P-40: 39.69±0.20^a) was higher compared to the backfat formulation, which had a significantly lower ($p<0.05$) fat percentage (34.55±0.56^b). These observations can be explained by the compositions of both the sunflower oil, used for the oleogel preparation, and the backfat. Sunflower oil contains 99.90% lipids, while backfat, besides fat, also contains smaller proportions of proteins and moisture.

Table 2. Chemical composition of pasteurized pâté samples.

	Chemical component (%)		
Pâté	**Moisture**	**Protein**	**Fat**
P-CO	52.77±0.16^b	11.17±0.03^c	34.55±0.56^b
P-20	47.47±0.16^a	10.88±0.03^b	39.21±0.22^a
P40	47.35±0.16^a	10.55±0.10^a	39.69±0.19^a

P-CO: control **pâté**; P-20 and P-40: **pâtés** with 20% and 40% of pork fat replacement

P-20 and P-40: samples with 20% and 40% of pork fat replacement

^{a,b,c} Different letters in the column mean that **pâtés** are statistically different

Table 3. Color parameters (L^*, a^*, b^*) of pâté samples.

Pâté	L^*	a^*	b^*
P-CO	69.54±0.50[a]	10.65±0.11[a]	15.17±0.17[a]
P-20	67.85±0.39[b]	11.66±0.17[b]	15.58±0.25[b]
P40	65.87±0.50[c]	12.63±0.18[c]	16.41±0.15[c]

P-CO: control **pâté**; P-20 and P-40: **pâtés** with 20% and 40% of pork fat replacement
P-20 and P-40: samples with 20% and 40% of pork fat replacement
[a,b,c] Different letters in the column mean that **pâtés** are statistically different

Moreover, the substitution of animal fat with oleogel led to a decrease in moisture content. The lower moisture content (Table 2) observed in P-20 (47.47±0.16[a]) and P-40 (47.35±0.16[a]) compared to the control pâté P-CO (52.77±0.16[b]) could be attributed to the backfat moisture content. The pâtés with oleogels did not differ significantly ($p>0.05$) in terms of fat and moisture content.

The control pâté exhibited a significantly higher ($p<0.05$) protein content (11.17±0.02[c]) compared to the P-20 (10.88±0.03[b]) and P-40 (10.55±0.10[a]) pâtés. This difference can be attributed to the presence of approximately 9% protein in pork backfat (*Vargas-Ramella et al.*, 2020). Consequently, the partial replacement of backfat with oleogel, which lacks protein, led to a significant decrease in the overall protein content. Our findings align with previous studies conducted on pâté (*Barbut*, 2015; *Martins et al*., 2020), further corroborating the observed trend.

According to the instrumental measurement of color, liver pâtés with the addition of oleogel were significantly ($p<0.05$) darker, redder, and more yellow compared to the control pâté (Table 3). These color changes can be attributed to the properties of the added oleogel, because most vegetable oils, obtained by cold pressing, are yellow or yellowish-brown, with red or green tone. The most common natural pigments in the oils are carotenoids (yellow) and chlorophylls, green color pigments (*Romanić et al*., 2021). Also, yellow tonality is a contribution from the presence of (opaque characteristic) CW.

Analyzing the texture characteristics of the pâtés, the statistical findings allow comparison of firmness and work of shear, which in turn determine the products' spreadability (*Shakerardekani et al.*, 2013). Spreadability (work of shear) is an extremely important attribute of spreadable products, as it is related to how easy the product is uniformly distributed over a surface (*Daubert et al*., 1998). The control pâté (P-CO), which did not contain oleogel, exhibited the highest firmness (357.88±14.37[a]) and work of shear (374.85±25.07[a]), indicating the lowest spreadability. On the other hand, the pâté with 40% substitution demonstrated significantly lower ($p<0.05$) firmness (213.10±16.14[c]) and work of share (201.32±32.76[b]), and thus, higher spreadability. Texture was improved since oleogel addition resulted in softer and more spreadable pâté (*Morales-Irigoyen*, 2012). According to (*Shakerardekani et al.*, 2013) the oleogel implementation resulted in a soft texture of the meat product which was preferred by consumers compared to other treatments. Consumer sensory evaluation revealed significantly higher overall liking scores ($p<0.05$) of meat products containing oleogel, thus indicating that the oleogel treatment enhanced the sensory quality and acceptability of

Table 4. Texture (firmness and work of shear) for P-CO, P-20 and P-40 pâté samples, as a result of texture profile analysis (TPA)

Pâté	Firmness (g)	Work of shear (g sec)
P-CO	357.88±14.37[a]	374.85±25.07[a]
P-20	300.85±10.18[b]	312.43±17.58[a]
P40	213.10±16.14[c]	201.32±32.76[b]

P-CO: control pâté; P-20 and P-40: pâtés with 20% and 40% of pork fat replacement
P-20 and P-40: samples with 20% and 40% of pork fat replacement
[a,b,c] Different letters in the column mean that pâtés are statistically different

the pâté. These findings highlight the importance of texture in consumer satisfaction and suggest that the use of oleogel incorporation can contribute to creating more desirable meat products that meet consumer preferences (*Issara et al*., 2022).

4. Conclusion

Overall, the utilization of oleogel as a substitute for animal fat in pâté formulations resulted in changes in lipid, moisture, and protein content. The incorporation of oleogel in liver pâté formulation influenced its color characteristics, making it darker, redder, and more yellow compared to the control pâté. These color changes can be attributed to the properties of the added oleogel and offer potential for tailoring the visual appearance of liver pâté products. The incorporation of oleogels in pâtés can modify their textural properties, potentially providing options to alter the spreadability and overall consumer acceptance of these products. These findings highlight the potential for modifying the composition of pâté and offer insights into the effects of incorporating oleogel in such meat-based products.

Disclosure statement: No potential conflict of interest was reporting by authors.

References

Aranda-Ledesma, N. E., Bautista-Hernandez, I., Rojas, R., Aguilar-Zarate, P., Medina-Herrera, N. P., Castro-Lopez, C. & Martínez-Avila, G. C. G. (2022). Candelilla wax: Prospective suitable applications within the food field. *LWT*, 159, 113170.

Astrup, A., Magkos, F., Bier, D. M., Brenna, J. T., de Oliveira Otto, M. C., Hill, J. O., King, J. C., Mente, A., Ordovas, J. M., Volek, J. S., Yusuf, S. & Krauss, R. M. (2020). Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations: JACC State-of-the-Art Review. *Journal of the American College of Cardiology*, 76, 844–857.

Badar, I. H., Liu, H., Chen, Q., Xia, X. & Kong, B. (2021). Future trends of processed meat products concerning perceived healthiness: a review. *Comprehensive Reviews in Food Science and Food Safety*, 20(5), 4739–4778, http://dx.doi.org/10.1111/1541-4337.12813. PMid:34378319

Barbut, S. (2015). Processing categories of meat products. The Science of Poultry and Meat Processing. Retrieved from http://www.poultryandmeatprocessing.com.

Barbut, S., Tiensa, B. E. & Marangoni, A. G. (2021). Partial fat replacement in liver pate using canola oil organogel. *LWT*, 139, 110428.

Daubert, C. R., Tkachuk, J. A. & Truong, V. D. (1998). Quantitative measurement of food spreadability using the vane method. *Journal of Texture Studies*, 29(4), 427–435.

Demirkesen, I. & Mert, B. (2020). Recent developments of oleogel utilizations in bakery products. *Critical Reviews in Food Science and Nutrition*, 60(14), 2460–2479, https:// doi.org/10.1080/10408398.2019.1649243

Guo, J., Cui, L. & Meng, Z. (2023). Oleogels/emulsion gels as novel saturated fat replacers in meat products: A review. *Food Hydrocolloids*, 137, 108313.

ISO, (1973). Meat and Meat Products—Determination of Total Fat Content; ISO 1443:1973; International Organization for Standardization: Geneva, Switzerland, 1973.

ISO, (1978). Meat and Meat Products—Determination of Nitrogen Content; ISO 937:1978; International Organization for Standardization: Geneva, Switzerland, 1978.

ISO, (1997). Meat and Meat Products—Determination of Moisture Content (Reference Method); ISO 1442:1997; International Organization for Standardization: Geneva, Switzerland, 1997.

Issara, U., Suwannakam, M. & Park, S. (2022). Effect of traditional fat replacement by oleogel made of beeswax and canola oil on processed meat (steak type) quality. *Food Research*, 6(5), 289–299.

Khan, S., Choudhary, S., Pandey, A., Khan, M. K. & Thomas, G. (2015). Sunflower Oil: Efficient Oil Source for Human Consumption.

Lima, T. L. S., Costa, G. F., Alves, R. N., Araújo, C. D. L., Silva, G. F. G., Ribeiro, N. L., Figueiredo, C. F. V. & Andrade, R. O. (2022). Vegetable oils in emulsified meat products: a new strategy to replace animal fat. *LWT,* 139, 110428.

López-Pedrouso, M., Lorenzo, J. M., Gullón, B., Campagnol, P. C. B. & Franco, D. (2021). Novel strategy for developing healthy meat products replacing saturated fat with oleogels. *Current Opinion in Food Science*, 40, 40–45.

Maki, K. C., Dicklin, M. R. & Kirkpatrick, C. F. (2021). Saturated fats and cardiovascular health: Current evidence and controversies. *Journal of Clinical Lipidology*, 15, 765–772.

Martins, A. J., Lorenzo, J. M., Franco, D., Pateiro, M., Domínguez, R., Munekata, P. E. S., Pastrana, L. M., Vicente, A. A., Cunha, R. L. & Cerqueira, M. A. (2020). Characterization of enriched meat-based pâté manufactured with oleogels as fat substitutes. **Gels**, 6, 17.

Mensink, R. P. (2016). Effects of saturated fatty acids on serum lipids and lipoproteins: A systematic review and regression analysis. Geneva, CH: World Health Organization. URL , Retrieved from http://www.who.int/nutrition/publications/nutrientrequirements/sfa_systematic_review

Morales-Irigoyen, E. E., Severiano-Pérez, P., Rodriguez-Huezo, M. E. & Totosaus, A. (2012). Textural, physicochemical and sensory properties compensation of fat replacing in pork liver pâté incorporating emulsified canola oil. *Food Science and Technology International*, 18(4), 413–421.

Pan, N., Dong, C., Du, X., Kong, B., Sun, J. & Xia, X. (2021). Effect of freeze-thaw cycles on the quality of quick-frozen pork patty with different fat content by consumer assessment and instrument-based detection. *Meat Science*, 172, https://doi.org/ 10.1016/j.meatsci.2020.108313

Patel, A. R. & Hartel, R. W. (2015). Alternative routes to oil structuring. Springer briefs in food, health, and nutrition, https://doi.org/10.1007/978-3-319-19138-6

Perta-Crisan, S., Ursachi, C. S., Chereji B. D. & Munteanu, F. D. (2023). Oleogels—Innovative Technological Solution for the Nutritional Improvement of Meat Products. *Foods*, 12, 131, https://doi.org/10.3390/foods12010131

Ramadan, M. F. (2020). Cold Pressed Oils. Green Technology, Bioactive Compounds, Functionality, and Applications. Academic Press, 1–5.

Rezler, R., Krzywdzinska-Bartkowiak, M. & Piątek, M. (2020). The influence of the substitution of fat with modified starch on the quality of pork liver pâtés. *LWT*, 135, 110264.

Romanić, R. (2020). Cold Pressed Oils. Cold pressed sunflower (Helianthus annuus L.) oil. Academic Press, 17.

Romanić, R., Lužaić, T., Grahovac, N., Hladni, N., Kravić, S. & Stojanović, Z. (2021). Color characteristic of non-refined oils obtained by cold pressing of the seeds oils obtained from confectionary sunflower hybrids. XII International Scientific Agriculture Symposium "AGROSYM 2021". Book of abstracts. Jahorina, October 07–10.

Schwingshackl, L., Heseker, H., Kiesswetter, E. & Koletzko, B. (2022). Reprint of: Dietary fat and fatty foods in the prevention of non-communicable diseases: A review of the evidence. *Trends in Food Science and Technology*, 130, 20–31.

Shakerardekani, A., Karim, R., Ghazali, H. M. & Chin, N. L. (2013). The effect of monoglyceride addition on the rheological properties of pistachio spread. *Journal of American Oil Chemists' Society*, 90, 1517–1521.

Silva, R. C. D., Ferdaus, M. J., Foguel, A. & da Silva, T. L. T. (2023). Oleogels as a fat substitute in food: A current review. *Gels*, 9(3), 180.

Singh, A., Auzanneau, F. I. & Rogers, M. A. (2017). Advances in edible oleogel technologies: a decade in review. *Food Research International*, 97, 307–317, http://dx.doi.org/10.1016/j.foodres.2017.04.022. PMid:28578056.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M. & Kerry, J. P. (2013). The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Science*, 93(2), 145–152.

Vargas-Ramella, M., Pateiro, M., Barba, F. J., Franco, D., Campagnol, P. C. B., Munekata, P. E. S., Tomasevic, I., Domínguez, R. & Lorenzo, J. M. (2020). Microencapsulation of healthier oils to enhance the physicochemical and nutritional properties of deer pâté. *LWT*, 125, 109223.

WHO, (2013). Global initiative on diet, physical activity and health. Geneva, Switzerland: World Health Organization. Retrieved from http://www.who.int/gho/ncd/risk_fact ors/unhealthy_diet_text/en/.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.1.043:636.1
636.1.085

ID: 126702345

https://doi.org/10.18485/meattech.2023.64.2.81

Original scientific paper

Influence of feed for horse nutrition on the chemical parameters and fatty acid composition of mare's milk

Aleksandra Nikolić[a], Jelena Jovanović[a], Vesna Djordjević[a], Dejana Trbović[a], Milijana Sinđić[b], Jelena Babić Milijašević[a] and Dragan Milićević[a]

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Mare's milk
Chemical composition
Horse feed
Fatty acid milk

ABSTRACT

The aim of this research was to determine the influence of horse feed on selected nutritionally important components of mare's milk with a focus on fat content: total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), stearic fatty acid, n-3 PUFA, n-6 PUFA, linoleic (LA) and α-linolenic (ALA) fatty acids. Also, the chemical parameters of the fatty acid composition of feed for horses (briquettes and meadow hay) were examined. Research results showed that complete feed contains a higher crude protein and fat content than hay. In addition, there was a difference in the composition of fatty acids in the milk of mares fed meadow hay compared to briquettes. Accordingly, the diet of mares has an influence on the chemical and fatty acid composition of milk, but it is not the only factor that has an influence.

1. Introduction

Mare's milk is highly appreciated, due to its unique nutritional profile. The chemical composition of mare's milk is similar to human milk, allowing its use in infant feeding as a substitute for human breast milk. Researchers from other countries have conducted a significant number of studies in the last few years, in order to examine the nutritional composition, the presence of bioactive components and certain therapeutic and preventive properties, showing a strong similarity between human breast milk and mare's milk. Mare's milk is attracting increasing interest from consumers due to its high content of vitamins and minerals, better digestibility and lower content of fat in comparison with cow's milk (*Sheng and Fang*, 2009).

Mare's milk is characterized by a high lactose content, while the fat and protein content is low (*Jastrzębska et al.,* 2017). The composition of fatty acids in milk fat is very diverse, due to the presence of over 400 fatty acids (*Trbovic et al*., 2018). The main nutritional components that are important for human nutrition and have an impact on human health are the fatty acids present in mare's milk. Although the fat content of mare's milk is low, the content of polyunsaturated fatty acids (PUFA) is high (*Shaikh et al.*, 2022). The composition of mare's milk is influenced by genetic, physiological and nutritional factors as well as environmental conditions. The aim of this the research was to determine the influence of horse feed on selected nutritionally important components of mare's milk with a focus on fat content: total fat, saturated fatty acids (SFA), monounsaturat-

***Corresponding author:** Aleksandra Nikolić, *aleksandra.nikolic@inmes.rs*

Paper received July 10th 2023. Paper accepted July 19th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ed fatty acids (MUFA), polyunsaturated fatty acids (PUFA), stearic fatty acid, n-3 PUFA, n-6 PUFA, linoleic (LA) and α-linolenic (ALA) fatty acids.

2. Materials and methods

The feed for the horses included two rations, briquettes (oats, corn and bran) and a ration of meadow hay. After mares consumed the feed, milk samples were taken to examined the chemical parameters and composition of fatty acids in the milk. Samples of the rations were also tested (granules and meadow hay) for the same parameters. In this study, samples of mare's milk collected during a period of 6 months of lactation were examined. Milk was from mares of the Friesian horse breed.

ISO standard methods were used to determine chemical parameters (moisture, fat, crude protein, ash, crude fibre, calcium and phosphorus) in horse feed (briquettes and meadow hay). Moisture was determined by drying the sample to a constant mass (ISO 6496:1999), fat content was determined after acid hydrolysis in a Sohlet apparatus (ISO 6492:1999), crude protein, were determined using the Kjeldahl method on the apparatus (Kjeldahl 8400, Foss, Denmark), ash was determined by burning the sample in a muffle furnace (ISO 5984:2022), crude fibre were determined by the intermediate filtration method (ISO 6865:2000), calcium by volumetric and phosphorus by spectrophotometric method (ISO 6490-1:1985, ISO 6491:1998, respectively).

The following chemical parameters were tested in mare's milk samples: lactose content by high-performance liquid chromatography, protein content according to Kjeldahl and fat content by the Gerber method (ISO 22662:2013; ISO 8968-1:2014; ISO 19662:2018, respectively).

2.1. Fatty acid analysis by capillary gas chromatography

In this analysis, fatty acid derivatives, fatty acid methyl esters (FAMEs) (Christie et al. 2001) were detected by gas-liquid chromatography (GLC, Shimadzu 2010, Japan) combined with a flame ionization detector and a capillary HP-88 column (length 100m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperatures were maintained at 250°C and 280°C, respectively. Nitrogen was used as the carrier gas at flow rate of 1.87 mL min^{-1}. The injector split ratio was set to 1:50 and injection volume was 1 μL. In order to achieve complete separation of the examined compounds, a programmed column oven temperature starting at 50°C and ending at 230°C was applied. The total analysis time was 63.12 min. The chromatographic peaks in the samples were identified by comparing FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, PA) and to which a mixture of 5 mg mL^{-1} conjugated linoleic acid (CLA) was added (O5632, Sigma Aldrich).

Nitrogen-free extractives as a measure of the soluble carbohydrates in the feed, such as percentage of starch and sugar, were calculated according to the equation:

$$NFE = 100 - (\text{moisture} + \text{protein} + \text{total fat} + \text{ash} + \text{fibre})$$

3. Results and discussion

Complete feed (briquettes) had a higher crude protein content than meadow hay, while the moisture content was similar, as shown in Table 1. Briquettes contained more fat, less cellulose and more nitrogen-free extractives (carbohydrates) compared to meadow hay. Also, the composition of fatty acids of hay and complete food differed. Table 1 shows that meadow hay contained more MUFA, while briquettes contained more SFA and PUFA. Of PUFA, linoleic acid (C18:2n-6) was more abundant in briquettes than in meadow hay, while linolenic acid (C18:3n-3) content was higher in meadow hay, although it was present in a significant amount in briquettes.

The nutritional content of mare's milk is shown in Tables 2 and 3, where it can be seen that the protein and fat content was slightly higher in the milk of mares fed with meadow hay compared to concentrated food (briquettes), while the lactose content was lower. Also, some authors (*Doreau et al.,* 1992) reported that mare's milk contains more protein and fat and less lactose when mares had more hay in their diet compared to concentrated feed. Additionally, observed by (*Barłowska et al.*, 2023), significantly increased content of dry matter, fat, lactose and ash occur in the milk of mares that do not have access to pasture, while the protein content of milk is increased in mares that have access to pasture.

The content of palmitic acid (C16:0) and palmitoleic acid (C16:1) was higher in mare's milk than in complete feed and meadow hay, shown in Tables 4 and 5. The reason for this is the fact that these fatty acids are synthesis products in mare's milk (*Djordjevic et al.*, 2019). Linoleic acid (C18:2n-6) was twice

Table 1. Chemical composition and composition of fatty acids in meadow hay and briquettes

Parameters	Meadow hay, g/100 g	Briquettes, g/100 g
Crude proteins, g	4.52	11.94
Moisture, g	10.30	11.02
Crude total fat, g	0.85	4.40
Crude ash, g	7.99	3.57
Crude fibre, %	30.57	10.24
Ca, g	0.68	0.25
P, g	0.21	0.50
NFE, g	53.76	62.40
Fatty acid composition		
C16:0	23.94	15.02
C18:0	3.52	1.49
C18:1n-9	10.05	30.70
C18:2n-6	23.59	49.09
C18:3n-3	5.63	2.12
SFA	5.90	17.40
MUFA	36.55	30.87
PUFA	35.82	51.92

NFE – nitrogen-free extractives; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

as abundant in complete feed and meadow hay than in mare's milk, which indicates that linoleic acid is incorporated unchanged into mare's milk. The content of linoleic acid (C18:2n-6) was higher in the milk of mares fed with bulky and concentrated feed, while the content of α-linolenic acid (C18:n3-3) was lower, established by *Naert et al.,* (2013), which coincides with our research.

In our study, the content of SFA in mare's milk was increased, and the content of PUFA was decreased in relation to the diet (meadow hay and briquettes). The content of MUFA was lower in mare's milk compared to meadow hay, and higher compared to in complete feed. Also, some authors (*Barłowska et al.*, 2023) reported higher levels of SFA in milk from mares with access to pasture, and lower levels of MUFA and PUFA. These differences in results can be attributed to the influence of other factors such as lactation of horse breeds and others.

The content of linolenic acid was higher in the milk of mares that had meadow hay in their diet, shown in Table 4. Also, greater amounts of α-linolenic (C18:3 n-3) acid were in the milk of mares that had access to pasture (*Barłowska et al.*, 2023). Linoleic acid (C18.2n-6) was higher in the milk of mares fed complete feed (briquettes), shown in Table 5, as well as in milk from mares without access to pasture (*Barłowska et al.,* 2023).

Table 2. Chemical composition of mare's milk (fed with meadow hay)

Nutrient	100 g
protein, g	2.67
fat, g	1.10
lactose, g	5.78
energy value, kcal	39.79
energy value, kJ	169.14

Table 3. Chemical composition of mare's milk (fed with Briquettes)

Nutrient	100 g
protein, g	2.15
fat, g	0.63
lactose, g	6.90
energy value, kcal	36.56
energy value, kJ	155.33

Table 4. Fatty acid composition of mare's milk (fed with meadow hay)

Fatty acids	Mare's milk
C4:0	No data
C6:0	No data
C8:0	1.02
C10:0	3.42
C12:0	4.22
C14:0	6.27
C15:0	0.49
C16:0	25.23
C16:1	4.89
C17:0	0.47
C18:0	3.55
C18:1TRANS-11	NO DATA
C18:1CIS-9	19.81
C18:2N-6	8.10
C20:0+C18:3N-6	NO DATA
C18:3N-3	19.50
C9T11CLA	NO DATA
C20:2N-6	0.15
C20:4N-6	NO DATA
C20:5N-3	NO DATA
C22:6N-3	NO DATA
SFA	45.69
MUFA	26.06
PUFA	28.57

Table 5. Fatty acid composition of mare's milk (fed with briquettes)

Fatty acids	Mare's milk
C4:0	0.08
C6:0	0.22
C8:0	2.07
C10:0	4.09
C12:0	3.28
C14:0	4.49
C15:0	0.22
C16:0	22.50
C16:1	7.37
C17:0	0.16
C18:0	1.23
C18:1TRANS-11	0.20
C18:1CIS-9	34.36
C18:2N-6	13.36
C20:0+C18:3N-6	0.06
C18:3N-3	3.12
C9T11CLA	0.50
C20:2N-6	0.35
C20:4N-6	0.10
C20:5N-3	0.06
C22:6N-3	0.07
SFA	38.34
MUFA	47.23
PUFA	14.44

Values represent mean ± SEM, n – number of samples; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

4. Conclusion

Our results showed that mare's milk is rich in linoleic and linolenic acids, which are necessary for the growth and development of the nervous system in humans. This indicates the importance of mare's milk in human diets. Variations in the composition of milk should be minimal in order to talk about the impact of mare's milk on human health. In our study, it can be seen that horse feed has an effect on the chemical composition and fatty acid composition of mare's milk. In addition to nutrition, many other factors affect the composition of milk, such as genetics, lactation, breed and environmental conditions. Accordingly, it is necessary to examine the influence of other factors on the composition of mare's milk.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, and based on the Contract on the implementation and financing of scientific research work in 2023 (No 451-03-47/2023-01/200050 dated 03.02.2023).

References

Barłowska, J., Polak, G., Janczarek, I. & Tkaczyk, E. (2023). The influence of selected factors on the nutritional value of the milk of cold-blooded mares: The example of the Sokólski breed. *Animals,* 13(7), 1152.

Christie, W. W., Sébédio, J. L. & Juanéda, P. (2001). A practical guide to the analysis of Conjugated linoleic acid (CLA). *Inform*, 12, 147–152.

Djordjevic, J., Ledina, T., Baltić, Z. M., Trbović, D., Babić, M. & Bulajić, S. (2019). Fatty acid profile of milk. *IOP Conference Series: Earth and Environmental Science*, 333(1).

Doreau, M., Boult, S., Bauchart, D., Barlet, J. P. & Martin-Rosset, W. (1992). Voluntary intake, milk production and plasma metabolites in nursing mares fed two different diets. The *Journal of Nutrition*, 122(4), 992–999.

ISO 19662:2018, (2018). Milk — Determination of fat content — Acido-butyrometric (Gerber method), International Organization for Standardization, Geneva, Switzerland.

ISO 22662:2007, (2007). Milk and milk products — Determination of lactose content by high-performance liquid chromatography (Reference method), International Organization for Standardization, Geneva, Switzerland.

ISO 5984:2022, (2022). Animal feeding stuffs — Determination of crude ash, International Organization for Standardization, Geneva, Switzerland.

ISO 6490-1:1985, (1985). Animal feeding stuffs — Determination of calcium content — Part 1: Titrimetric method, International Organization for Standardization, Geneva, Switzerland.

ISO 6491:1998, (1998). Animal feeding stuffs — Determination of phosphorus content — Spectrometric method, International Organization for Standardization, Geneva, Switzerland.

ISO 6492:1999, (1999). Animal feeding stuffs — Determination of fat content, International Organization for Standardization, Geneva, Switzerland, International Organization for Standardization, Geneva, Switzerland.

ISO 6496:2002, (2002). Animal feeding stuffs — Determination of moisture and other volatile matter content, International Organization for Standardization, Geneva, Switzerland.

ISO 6865:2000, (2000). Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration, International Organization for Standardization, Geneva, Switzerland.

ISO 8968-1:2014, (2014). Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation, International Organization for Standardization, Geneva, Switzerland.

Jastrzębska, E., Wadas, E., Daszkiewicz, T. & Fiećko, R. P. (2017). Nutritional value and health-promoting properties of mare's milk− a review. *Czech Journal of Animal Science*, 62(12), 511–518.

Naert, L., Vande, G., Verhoeven, L., Duchateau, S. & De Smet, F. (2013). Assessing heterogeneity of the composition of mare's milk in Flanders. *Vlaams Diergeneeskundig Tijdschrift*, 82(1), 23–30.

Shaikh, A., Mehta, B. M. & Atanu, H. J. (2022). Chemistry, nutritional properties and application of mare's milk: A review. *Agricultural Reviews*, 43(3), 355–361.

Sheng, Q. & Xinping, F. (2009). Bioactive components in mare milk. *Bioactive Components in Milk and Dairy Products*, 195.

Trbovic, D., Spiric, D., Lukic, M., Petrovic, Z., Parunovic, N., Djordjevic, V. & Milicevic, D. (2018). Fatty acid composition of cow's milk: opportunities and challenges for Serbian dairy producers. *Meat Technology*, 58(2), 118–124

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.52:577.112:542.943
615.279

ID: 126695945

https://doi.org/10.18485/meattech.2023.64.2.82

Review paper

Protein oxidation in meat products: exploring the role of natural antioxidants in preservation and quality enhancement

Meltem Serdaroğlu[a*]

[a] *Ege University Food Engineering Department 35100 Bornova Izmir, Turkey*

ARTICLE INFO

Keywords:
Protein oxidation
Meat products
Natural antioxidants
Sausages

ABSTRACT

Protein oxidation is a complex process involving the oxidative damage of proteins by reactive oxygen species and other oxidizing agents. It can lead to structural and functional alterations in proteins, impacting their biological activity. The primary cause of protein oxidation in meat products is the presence of endogenous enzymes, such as myoglobin and hemoglobin, which contain iron and catalyze oxidation reactions. Additionally, the presence of heme pigments, unsaturated fatty acids, and transition metal ions in meat can further promote protein oxidation. Protein oxidation in meat products can lead to several undesirable changes. One of the most noticeable effects is the development of off-flavors and off-odors, commonly described as rancidity. Natural antioxidants are compounds found in various plant-based sources, such as fruits, vegetables, herbs, and spices. These antioxidants possess the ability to scavenge free radicals and inhibit oxidative reactions, including the oxidation of proteins in meat. By exploring the effects of natural antioxidants on protein oxidation in meat products, it is possible to gain insights into their potential as functional additives for enhancing product stability and maintaining desired sensory characteristics. This paper aims to delve into the impact of natural antioxidants on protein oxidation in meat products and shed light on their role in preserving product quality and extending shelf life.

1. Introduction

Meat and meat products are highly nutritious and contain significant amounts of moisture with a neutral pH, making them prone to spoilage. To maintain their quality, and safety, and prevent potential health risks, proper preservation methods are crucial (*Aminzare et al*., 2016). Oxidation and microbial degradation are the primary factors that limit the quality and acceptability of meat products. These processes negatively impact the taste, color, texture, and nutritional composition (*Kavuşan and Serdaroğlu*, 2021; *Das et al*., 2020). While oxidative damage leading to a quality loss in meat products has been primarily attributed to lipids, it has been recognized that proteins also serve as substrates in oxidation reactions (*Zungur-Bastıoğlu et al*., 2016). Protein oxidation is a complex chemical process that occurs in meat and meat products, leading to detrimental effects on technological and sensory quality and nutritional value (*Serdaroğlu et al*., 2017). Protein oxidation, in a general sense, refers to the covalent modification of proteins induced either directly by reactive oxygen species (ROS) such as OH• and H_2O_2, or indirectly by

***Corresponding author:** Meltem Serdaroğlu, *meltem.serdaroglu@ege.edu.tr*

Paper received July 11th 2023. Paper accepted July 19th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

the secondary products of oxidative stress (*Estevez*, 2011; *Zhang et al.*, 2013; *Zungur-Bastıoğlu et al.*, 2016). The protein oxidation formation mechanism resembles the lipid oxidation reaction. In the initial stage, reactive oxygen and nitrogen species, such as hydroperoxides, aldehydes, OH, O_2, and ROO, metals like iron and copper, and hydrogen atom separation from amino acids cause protein radicals to form. These reactions result in the binding of protein radicals with OH ions, leading to the formation of water (H_2O) (*Zhang et al*., 2013). The generated protein radical reacts with oxygen, giving rise to protein peroxyl radicals (POO•), which then acquire hydrogen atoms to form protein hydroperoxides (POOH) and new protein radicals (P•) (*Bao et al*., 2018). Due to their instability, hydroperoxides rapidly decompose into alkoxy radicals (PO•) and hydroxyl radicals (HO•). This results in the conversion of certain amino acid residues into carbonyl compounds (*Ergezer et al*., 2016). Changes in particular amino acid residues, such as proline, arginine, lysine, and threonine, occur as a result of protein oxidation, leading to an increase in carbonyl content (*Dominguez et al*., 2021; *Hellwig*, 2020). Decreases in sulfhydryl and tyrosine content are observed due to the formation of disulfide bonds and dityrosine bridges through oxidation (*Ergezer et al*., 2016).

In the meat industry, there is growing interest in identifying effective strategies to mitigate protein oxidation and preserve the overall quality of meat products. To mitigate the negative effects of protein oxidation on meat product quality, various strategies can be employed (*Paterio et al.*, 2018; *Nawaz et al*., 2022). These include the addition of antioxidants, such as natural extracts or synthetic antioxidants, to inhibit oxidation reactions (*Munekata et al*., 2020). Proper packaging techniques, such as vacuum packaging or modified atmosphere packaging (MAP), can minimize exposure to oxygen and delay oxidative processes (*Zhang et al*., 2013). Additionally, appropriate storage conditions, including refrigeration or freezing, can help slow down oxidation reactions and extend the shelf life of meat products. Herbs, spices, fruits, plant essential oils, and plant extracts are valuable plant materials that provide abundant sources of bioactive phenolic compounds (*Paterio et al*., 2018; *Hadidi et al*., 2022). They have emerged as effective alternatives to synthetic antioxidants in various applications. Natural extracts obtained from plant material can be added directly to meat products or incorporated into marinades, coatings, or casings to provide antioxidant protection. They can scavenge free radicals, chelate metal ions that promote oxidation, and inhibit enzymatic reactions associated with protein oxidation. By incorporating natural extracts into meat formulations, manufacturers can potentially extend the shelf life, maintain product quality, and enhance the nutritional value of meat products while meeting consumer demands for clean label ingredients (*Paterio et al.*, 2018 ; *Lorenzo et al*., 2019). This review provides an overview of recent advancements in the application of natural antioxidant compounds in meat and meat products, aiming to enhance their quality and extend their shelf life.

2. Protein oxidation in meat and meat products and the effects on the quality

Susceptibility to protein oxidation in muscle foods is influenced by a combination of intrinsic and extrinsic factors. Intrinsic factors encompass the animal species, animal origin, muscle type, and composition of the product. Extrinsic factors comprise processing conditions, packaging conditions, and preparation techniques. Together, these factors play a role in determining the extent of protein oxidation in muscle foods (*Dominguez et al.*, 2021).

Protein oxidation has been demonstrated to impact the quality of muscle foods in various aspects (*Zungur-Bastıoğlu et al*. 2015; *Zhang et al*., 2013). Protein oxidation can result in the development of off-flavors and off-odors in meat products. Oxidation of amino acids in proteins can generate volatile compounds, such as aldehydes and ketones, which contribute to rancid flavor and undesirable aromas. These flavor changes can negatively affect the sensory characteristics and consumer acceptance of meat products. Oxidized proteins in meat products can undergo structural changes, including cross-linking and aggregation (*Bao et al*., 2018). Consequently, cross-linking reinforces the protein structure, leading to a reduction in water-holding capacity. Moreover, the inhibition of proteolytic reactions, as mentioned earlier, due to protein oxidation can also have a detrimental impact on water-holding capacity. These modifications can lead to alterations in the meat's texture, making it tougher and less tender. The formation of disulfide cross-links in myofibrillar proteins due to oxidation strengthens the structure of actin and myosin, resulting in a decrease in important quality characteristics such as tenderness and water-holding capacity (*Zakrys-Waliwander* et al., 2012; *Bao and Ertbjerg*, 2019).

Protein oxidation can also impact the color of meat products. Oxidized proteins can lead to the formation of pigments, such as metmyoglobin, which can result in color changes, such as a brown or gray discoloration (*Tao et al*., 2021). This can negatively affect the visual appeal and perceived freshness of meat products. The oxidative changes of proteins in meat products can lead to an undesirable effect, namely a loss of nutritional value. Nevertheless, it is crucial to acknowledge that the impact of protein oxidation on digestibility is contingent upon the degree or severity of oxidation (*Dominguez et al*., 2021). Oxidized proteins can become less digestible and can result in a reduced bioavailability of essential amino acids. This can impact the protein quality and nutritional value of the meat, which is a crucial consideration for consumers seeking adequate protein intake from meat products (*Öztürk-Kerimoğlu et al.*, 2019). Protein oxidation is associated with the deterioration of meat product shelf life. Oxidation reactions can promote the development of lipid oxidation, leading to the generation of off-flavors, rancidity, and texture changes (*Kavuşan and Serdaroğlu*, 2021; *Serdaroglu et al.,* 2017). The combined effects of protein and lipid oxidation can accelerate the spoilage process and reduce the overall storage stability of meat products. Furthermore, various compounds resulting from protein oxidation, such as heterocyclic aromatic amines, advanced glycation end products, α-aminoadipic semialdehyde, kynurenines, and others, are deemed harmful. Consequently, protein oxidation has been demonstrated to produce potentially toxic compounds, leading to a decrease in the nutritional quality of muscle foods (*Hu et al*., 2017).

3. Plant extracts can play a role in preventing protein oxidation

The use of synthetic antioxidants in mitigating oxidative damage of meat products raises concerns regarding consumer safety. Therefore, there has been a shift towards replacing synthetic antioxidants with natural bioactive compounds due to increased consumer awareness of the potential hazards associated with synthetic alternatives (*Aminzare et al*., 2019; *Munekata et al*., 2020). Plant materials, such as herbs, spices, fruits, plant essential oils, and extracts, offer rich sources of bioactive phenolic compounds and serve as effective alternatives to synthetic anti-

Table 1. Effect of different plant extracts on protein oxidation in meat products

Product	Type of treatment	Effects on product	References
Beef patties	Rosemary extract	Samples with rosemary extract had lower carbonyl content	*Lund et al*., 2007).
Cold stored porcine liver pâté.	Sage extract significantly inhibited the increase of protein carbonyls.	Protein oxidation has been prevented.	*Estevez et al.*, 2006
Cooked burger patties	Strawberry tree, common hawthorn, dog rose and elm-leaf blackberry extracts.	Fruits tested displayed intense antioxidant activity against protein carbonylation.	*Ganhão et al.,* 2010
Beef patties	Artichoke by-product extract	The addition of AE significantly inhibited the formation of protein carbonyls in beef patties during cold storage.	*Ergezer and Serdaroğlu,* 2018
Dry minced pork slice	Mulberry extract	Concentrated mulberry juice was found to be effective antioxidant in dried-minced pork slice, inhibiting both lipid and protein oxidation.	*Cheng et al*., 2018
Beef burgers	Elderberry encapsulated extract	Elderberry extract delayed protein oxidation	*Rocchetti et al*., 2022
Dry uncured sausages	Pomegranate peel extract	Pomegranate peel extract added at 1 or 2% (v/w) improved oxidative stability lowering the formation of carbonyls from protein carbonylation during drying.	*Cava and Ladero*, 2023
Heat treated fermented sausages	Arugula and barberry extract	Reduced carbonylation	*Serdaroğlu et al.,* 2023

oxidants. It is important to note that the effectiveness of natural extracts as antioxidants may vary depending on factors such as extract concentration, extraction method, and the specific meat product formulation (*Lorenzo et al*., 2019). Fruits and vegetables are widely recognized as rich sources of antioxidants. Various fruits, including pomegranates, strawberries, kinnows, acerola, white grapes, plums, blackcurrants, annatto, bearberries, bananas, and sapodilla, contain notable concentrations of antioxidants. These fruits have been extensively studied for their antioxidant activity and find applications in various industries. Polyphenols, acting as natural antioxidants, exhibit remarkable capacity for absorbing radicals and possess potent hydrogen atom-donating activity (*Falowo et al.*, 2014). Among the main antioxidative polyphenols are phenolic acids, flavonoids, and essential oils. Certain polyphenols regulate the formation and spread of free radicals and ROS, while others directly eliminate free radicals and bind to transition metals (*Hadidi et al*., 2022). Table 1 presents the impact of different plant extracts on protein oxidation in meat products.

4. Conclusion

In conclusion, protein oxidation poses significant challenges to the quality and shelf life of meat products. Natural plant extracts with antioxidant properties offer promising solutions to mitigate protein oxidation. These plant extracts contain bioactive compounds that can effectively scavenge free radicals and inhibit oxidative reactions, thus protecting proteins from oxidation. Additionally, the use of natural plant extracts as antioxidants offers a more desirable and consumer-frien dly alternative to synthetic antioxidants, aligning with the increasing demand for clean label and natural food ingredients. It is worth highlighting that the efficacy of natural extracts as antioxidants can vary based on factors including extract concentration, extraction method, and the specific formulation of the meat product. Therefore, it is essential to conduct appropriate studies and optimize the application of natural extracts to achieve the desired antioxidant effects while ensuring food safety and regulatory compliance.

References

Aminzare, M., Hashemi, M., Hassanzad Azar, H. & Hejazi, J. (2016). The use of herbal extracts and essential oils as a potential antimicrobial in meat and meat products; a review. *Journal of Environmental and Public Health,* 1, 63–74.

Aminzare, M., Hashemi, M., Ansarian, E., Bimkar, M., Azar, H. H., Mehrasbi, M. R., Daneshamooz, S., Raeisi, M., Jannat, B. & Afshari, A. (2019). Using natural antioxidants in meat and meat products as preservatives: A review. *Advances in Animal and Veterinary Sciences*, 7, 417– 426.

Bao, Y. & Ertbjerg, P. (2019). Effects of protein oxidation on the texture and water-holding of meat: a review. *Critical Reviews in Food Science and Nutrition*, 59, 3564–3578.

Bao Y., Boeren S. & Ertbjerg P. (2018). Myofibrillar protein oxidation affects filament charges, aggregation and water-holding. *Meat Science*, 135, 102–108.

Cava, R. & Ladero, L. (2023). Pomegranate peel as a source of antioxidants for the control of lipid and protein oxidation during the ripening of Iberian dry uncured sausages. *Meat Science*, 109198.

Cheng, J-R., Liu, X-M., Zhang,W., Chen, Z-Y. & Wang, X-P. (2018). Stability of phenolic compounds and antioxidant capacity of concentrated mulberry juice-enriched dried-minced pork slices during preparation and storage. *Food Control*, 89, 187–195.

Das, A. K., Nanda, P. K., Bandyopadhyay, S., Banerjee, R. & Biswas, S. (2020). Application of nanoemulsion based approaches for improving the quality and safety of muscle foods: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 19, 2677–2700.

Domínguez, R., Pateiro, M., Munekata, P. E. S., Zhang, W., Garcia-Oliveira, P., Carpena, M., Prieto, M. A., Bohrer, B. & Lorenzo, J. M. (2021). Protein Oxidation in Muscle Foods: A Comprehensive Review. *Antioxidants*, c 11, 60.

Ergezer, H., Gökçe, R., Hozer, Ş. & Akcan, T. (2016). Et ve ürünlerinde protein oksidasyonu: etki mekanizması, tespit yöntemleri ve etkileri. *Akademik Gıda*, 14(1), 54–60.

Ergezer, H. & Serdaroglu, M. (2018). Antioxidant potential of artichoke (Cynara scolymus L.) by-products extracted in raw beef patties during refrigerated storage. *Journal of Food Measurement and Characterization*, 12, 982–991.

Estévez, M., Ventanas, S. & Cava, R. (2006). Effect of natural and synthetic antioxidants on protein oxidation and colour and texture changes in refrigerated stored porcine liver pâté. *Meat Science*, 74, 369–403.

Estévez, M. (2011). Protein carbonyls in meat systems: a review. *Meat Science*, 89, 259–279.

Falowo, A. B., Fayemi, O. P. & Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International*, 64, 171–181.

Ganhão, R., Morcuende, M. & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science*, 85, 402–409.

Hadidi, M., Orellana-Palacios, J. C., Aghababaei, F., Gonzalez-Serrano, D. J., Moreno, A. & Lorenzo, J. M. (2022). Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. *LWT Food Science and Technology,* 69, 114003.

Hellwig, M. (2020). Analysis of protein oxidation in foods and feed products. *Journal of Agricultural and Food Chemistry*, 68, 12870–12885.

Hu, L., Shen, Q. & Ling, J. (2017). Protein oxidation and proteolysis during roasting and in vitro digestion of fish (*Acipenser gueldenstaedtii*). *Journal of the Science of Food and Agriculture,* 98, 27496–27505.

Kavuşan, H. S. & Serdaroğlu, M. (2021). Oxidative changes in fermented meat products and their effects on product quality. *The Journal of Food*, 46, 443– 462.

Lorenzo, J. M., Lorenzo, M. A., Trindade, D. U. & Ahn, F. J. (2019). Natural antioxidants to reduce the oxidation process of meat and meat products. *Food Research International,* 115, 377–378.

Lund, M. N., Hviid, M. S. & Skibsted, L. H. (2007). The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science*, 76, 226–233.

Nawaz, A., Irshad, S., Ali Khan, I., Khalifa, I., Walayat, N., Muhammad Aadil, R., Kumar, M., Wang, M., Chen, F., Cheng, K.W. & Lorenzo, J.M. (2022). Protein oxidation in muscle-based products: Effects on physicochemical properties, quality concerns, and challenges to food industry. *Food Research International*, 157, 111322.

Munekata, P. E. S., Rocchetti, G., Pateiro, M., Lucini, L., Domínguez, R. & Lorenzo, J. M. (2020). Addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes: An overview. *Current Opinion in Food Science,* 31, 81–87.

Öztürk-Kerimoğlu, B., Nacak, B., Özyurt, V. H. & Serdaroğlu, M. (2019). Protein oxidation and in vitro digestibility of heat-treated fermented sausages: How do they change with the effect of lipid formulation during processing? *Journal of Food Biochemistry*, 43, e13007.

Paterio, M., Barba, F. J., Domingez, R., Sant'Ana, A. S., Khaneghah, A. M., Gavahian, M. & Lorenzo, J. M. (2018). Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food Research International,* 113, 156–166.

Rocchetti, G., Becchi, P. P., Lucini, L., Cittadini, A., Munekata, E. S., Pateiro, M., Domínguez, R & Lorenzo, J. M. (2022). Elderberry (*Sambucus nigra* L.) Encapsulated extracts as meat extenders against lipid and protein oxidation during the shelf-life of beef burgers. *Antioxidants*, 11, 21301.

Serdaroğlu, M., Özyurt, H., Bastıoğlu, A. Z., Öztürk, B. & Ötleş, S. (2017). The effects of replacing beef fat with olive oil on protein oxidation products (α-aminoadipic semialdehydes-AAS and γ-glutamic semialdehydes-GGS) in Turkish dried fermented sausage. 63rd International Congress of Meat Science and Technology, 13–18 August, Cork, Ireland, 735 p.

Serdaroğlu, M., Can, H., Sarı, B., Kavuşan, H. S. & Yılmaz, F. M (2023). Effects of natural nitrite sources from arugula and barberry extract on quality characteristic of heat-treated fermented sausages. *Meat Science,* 198, 109090.

Tao, Y., Ma, L., Li, D., Tian, Y., Liu, J. & Liu, D. (2021). Proteomics analysis to investigate the effect of oxidized protein on meat color and water holding capacity in Tan mutton under low temperature storage. *LWT-Food Science and Technology*, 146, 111429.

Zhang W., Xiao S. & Ahn, D. U. (2013). Protein Oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition*, 53, 1191–1201.

Zakrys-Waliwander, I., O'Sullivan, M. G., O'Neill, E. E. & Kerry, J. P. (2012). The effects of high oxygen modified atmosphere packaging on protein oxidation of bovine M. longissimus dorsi muscle during chilled storage. *Food Chemistry*, 131, 527–532.

Zungur-Bastıoğlu, A., Serdaroğlu, M., Nacak, B. & Öztürk, B. (2015). Effects of olive oil as partial replacer of animal fat in sucuk on oxidation and some quality properties during production. 61st International Congress of Meat Science and Technology, 23–28th August 2015, Clermont-Ferrand, France.

Zungur-Bastıoğlu, A., Serdaroğlu, M. & Nacak, B. (2016). Protein Oxidation in Meat and Meat Products. *Journal of Food and Health Science,* 2, 171–183.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.513.1/.2(494)

ID: 126692361

https://doi.org/10.18485/meattech.2023.64.2.83

Review paper

On-farm killing as a method to minimize pre-slaughter stress: a qualitative analysis from Switzerland

Lisa Märcz[a], Anna Francesca Corradini[b], Eugenio Demartini[b] and Michael Gibbert[a]*

[a] *Faculty of Communication, Culture, and Society, University of Lugano,Via Buffi 13, 6900 Lugano, TI, Switzerland*
[b] *Department of Veterinary Science for Health, Animal Production and Food Safety, University of Milano, Via dell'Università 6, 26900 Lodi, LO, Italy*

ARTICLE INFO

Keywords:
On-Farm Killing
Pre-slaughter stress
Live transportation
Gunshot method
Cattle
Switzerland

ABSTRACT

Pre-slaughter stress is often caused during the different stages of animal handling, live transportation, stunning and dry bleeding. Such condition has a negative impact on animals and operators, meat quality and consumer satisfaction. A slaughter practice that intends to minimize pre-slaughter stress applicable to the small-scale commercial sector is on-farm killing (OFK) using the captive bolt pistol or gunshot method. A trust-based training of operators and cattle facilitates the killing process at the farm and thereby represents a viable substitute for live animal transportation. Our paper presents the results of qualitative research on the use of OFK methods after its legalization in Switzerland in 2020. Eight farms participated in this study, and results suggest that OFK methods are technically and economically viable in Switzerland. In fact, after the six-year-long pilot phase, farmers declare that OFK mitigates the stress for cattle, provides for less hazardous work and improves consumers' preference for their meat. In this sense, OFK may serve as a contribution to alternative slaughter methods in industrial countries. Nonetheless, the dimension of the farm still represents a major constraint for the application of these methods. In fact, OFK as a replacement for live transportation is usually viable in small-scale contexts with fewer animals, shorter distances to slaughterhouses and minimal logistical challenges.

1. Introduction

Pre-slaughter stress and how to minimize it in beef production is widely discussed (*Harris*, 2001; *Speer et al.,* 2001; *Ferguson & Warner*, 2008; *Muchenje et al.*, 2009; *Probst et al.* 2012; *Schwartzkopf-Genswein et al.*, 2012; *Wigham et al.*, 2018; *Hultgren et al.*, 2020; *Terlouw*, 2020). It involves animal handling, live transportation, stunning and dry bleeding. If mismanaged, these stages can trigger strong adaptive responses in cattle which may have a negative impact on their welfare and also on meat quality (*Muchenje et al.,* 2009; *Wigham et al.,* 2018; *Jorquera-Chavez* 2019; *Reiche et al.*, 2019; *Terlouw,* 2020), causing considerable economic losses (*Ferguson & Wagner*, 2008; *Muchenje et al.*, 2009; *Probst et al.* 2012; *Wigham et al.*, 2018; *Terlouw*, 2020) and concerns among consumers (*Vanhonacker & Verbeke*, 2014; *Buddle et al.*, 2018). The main hazards for animals and operators can occur during live transportation due to loading density, travel duration and distance, feed and water withdrawal, weather and driving conditions, handling of animals and animal juvenility (*Schwartzkopf-Gens-*

***Corresponding author:** Lisa Märcz, *lisa.maercz@usi.ch*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

wein et al., 2016). Other hazards relate to the stunning phase due to poor skills of staff as well as inadequate facilities and equipment (*EFSA*, 2020). Although the issue of live cattle transport is widely studied and reported in the literature (*Hultgren et al.*, 2020, *Schwartzkopf-Genswein et al.*, 2012), it can be considered an indispensable prerogative for the large-scale beef production process (*Harris,* 2001; *Speer et al.,* 2001).

On the other hand, looking at small-scale beef production, some solutions have been recently proposed. On-farm killing (OFK) minimizes pre-slaughter stress for animals by eliminating the element of live transportation, and facilitating a handling and stunning process that is grounded in specialized and skilled training of all involved entities. Two of these methods described in the literature involve either a mobile slaughter unit (MSU), also known as mobile abattoir, in which animals are stunned with a captive bolt pistol; and the so-called gunshot method by which animals are shot without restraint at close range (*Schiffer*, 2015; *Hultgren et al.*, 2022). Several experiences in the use of MSUs for red meat production from private small-scale meat producers in industrialized countries have been reported in literature since the early 2000s. The earliest use of licensed MSUs were reported in 2002 in the USA (*USDA*, 2010) and in 2006 in Canada (*Pinkney,* 2014), as a way for farmers in marginal areas to reduce the costs of animal transportation. Today, MSUs are known to be used across all continents except for Antarctica (*Hultgren et al.*, 2022). The use of the gunshot method, however, seems less common. It has been so far documented in. Australia, Canada, Germany, New Zealand, Switzerland and the USA (*Hultgren et al*., 2022). To our knowledge, Switzerland is currently the only country that legalized a third variant of OFK for which the acts of stunning and dry bleeding are performed with a CBP but without an MSU.

The pros and cons of OFK methods were recently evaluated (*Berger Richardson*, 2022). While the potential of OFK to replace large scale slaughter as an integral part of the contemporary meat production process seems unlikely (*Harris*, 2001; *Speer et al.*, 2001), it nonetheless is a viable option for small-scale businesses to reduce pre-slaughter stress and the related hazards for animals and operators, and consequently improve meat quality (*Ferguson*, 2008; *Jorquera-Chavez*, 2019) and consumer satisfaction (*Lagerkvist & Hess*, 2011; *Marescotti et al.*, 2020). Little is known in the literature about the particular application and implementations of OFK in farmer practices, especially in the commercial sector. When Switzerland legalized OFK in July 2020, we took this as the starting point for the present study which pursues the questions: how do farmers implement OFK methods? What advantages and disadvantages might this practice entail? What significance does OFK have for the mitigation of pre-stress slaughter? To address these questions, we provide context on the Swiss legislation and present original empirical data of a qualitative study on eight different farms that delivers the first complete insight into the implementations of OFK in the European area. Results of the present research contribute to the literature on alternative slaughter methods, as this seminal study could be highly valuable for scholars and different stakeholders within the meat industry.

2. Materials and methods

2.1 Data collection

Given the scarcity of farms that adopted OFK in Switzerland, and in view of the novelty of the topic, we opted for a data collection using qualitative methods, which was carried out in two stages.

During the first stage, material was collected to study the legal framework of OFK methods and reconstruct their introduction in the Swiss pilot phase (2014–2020) as well as the newly extended Swiss legislation concerning slaughter in 2020 (VSFK, SR 817.190, §2a., Art. 9a). Between April 2020 and February 2021, we studied the context of Swiss OFK via governmental guidelines and press reports; via the Research Institute of Organic Agriculture, FiBL, which was assigned to the OFK project in 2014; by studying cantonal veterinary documents; and via the websites of several farms who adopted OFK. This first stage enabled us to generate codes for the interview guidelines (*Mattimoe*, 2021), which were applied in the second stage of the research. The initial codes involved “farm development”, “farm transition to OFK”, “human-cattle relations”, “producer-consumer relations” and “marketing”. We used the analysis of these sources to create a semi-structured interview protocol, which included open-ended and generative questions.

The second stage of the study was carried out through the application of qualitative research methods, which allowed empirical *in situ* data collection. Empirical data were collected from March

2021 to April 2023, using ethnographic methods (*O'Reilly*, 2011; *Breidenstein et al.*, 2013). Specifically, we conducted semi-structured interviews with eight Swiss farmers and engaged in overt observations and participant observation, a method that describes a research process of conscious involvement of the researcher, alternating with a structured detachment to ensure objectivity of the findings (*Tedlock*, 1991). In a reiterative cycle, involvement allowed us to personally engage with the specific OFK context of each farm individually (*Collins & Gallinat*, 2010), while detachment facilitated the reflection, analysis, and interpretation of data and experiences on a regular basis (*Miles & Huberman*, 1994; *O'Reilly*, 2011; *DeMello,* 2012, *Fassin*, 2013; *Atkinson*, 2008; *Ejimabo*, 2015). Thus, the present research profits from unique first-hand insights which involved extended *in situ* stays at the studied farms. The choice of methods were applied over different periods of time (Table 1) to understand the different implementations of OFK and the required cattle handling.

2.2 Sampling and data analysis

Given the small number of farms that adopted OFK in Switzerland, a non-probabilistic method was implemented for the selection of the eight farms included in the final sample. Some farms were selected according to relevance and diversity of OFK methods (i.e., farms 1, 3, 4 and 8 were pioneers during the pilot phase of Swiss OFK from 2014–2020), while the remaining farms were contacted by snowball sampling (farms 2, 5, 6 and 7). The characteristics of the farms are described in Table 1. Farms 1, 6, 7 and 8 use the captive bolt pistol method for stunning, combined with a lifting arm attached to a tractor or the stable ceiling for dry bleeding while hanging, and a T-Trailer for carcass transportation (https://www.innovative-schlachtsysteme.de/t-trailer). Farms 2, 4 and 5 use the captive bolt pistol method for stunning, combined with a mobile slaughter unit (MSE-200) for dry bleeding and carcass transportation. Farm 3 practices the gunshot method from a high porch and uses a lifting arm attached to a tractor and a T-Trailer.

Table 1. Characteristics of the interviewed Swiss farms

On-farm killing solution / performance option	Swiss Canton	OFK since	Farm*	Herd size	Cattle breed	Participant	Periods of conducted research
Captive Bolt Pistol / Mobile Slaughter Unit MS-200	Lucerne	2021	Farm 2	35	Limousin	Female (f1)**	Aug. 2021
		2015	Farm 4	20	Miniature Zebu, Aubrac	Male (m3)	Sept. 2021
	Berne	2021	Farm 5	100	Montbélliarde, Norwegian Red, Holstein	Male (m4)	Sept. 2021
Captive Bolt Pistol / Lifting arm and T-Trailer	Grisons	2015	Farm 1	30	Grauvieh	Male (m1)	March / Sept. 2021
	Lucerne	2020	Farm 6	17	Grauvieh	Female (f2)	Oct. 2021 – April 2023
	Zurich	2021	Farm 7	20	Piedmontese, Highland, Black Angus, Grauvieh	Male (m6)	Oct. 2021
	Solothurn	2015	Farm 8	80	Red Angus	Male (m7)	Feb. 2022
Gunshot / Lifting arm and T-Trailer	Zurich	2014	Farm 3	25	Red and Black Angus	Male (m2)	Sept. 2021

*Numbers indicate the chronology of research visits, **Number in brackets indicate the data source code

Between March 2021 and April 2023, we conducted interviews with the participants, made observations about the farm infrastructure, and participated in four successful (Farms 2 and 6) and one failed (Farm 5) OFK. The authors triangulated (*Basit*, 2003; *Ritchie*, 2003) all material gathered from the first and second stage to present a holistic representation of OFK, how the methods are carried out in practice and why they work or fail in individual cases. Interviews were written in real time or recorded electronically and later transcribed and coded manually to select themes and assign categories (*Basit*, 2003; *Mattimoe*, 2021), based on the relevance of OFK as alternative slaughter method in theory and in practice. Results were not quantified but rather qualitatively explored and presented as individual business cases (*Basit*, 2003).

3. Preliminary results

3.1 Captive bolt pistol in combination with a mobile slaughter unit (MSU)

According to the data collected at Farms 2, 4 and 5, the success of the use of captive bolt pistol in combination with a mobile slaughter unit as OFK method depends on trust-based training between farmer and cattle as well as a skilled routine of both farmer and MSU operator. Cattle are required to willingly enter the ramp outside the MSU and be fixated in the attached head gate where they are stunned with a captive bolt pistol. This has to be practiced in advance by positive conditioning, enticing the animal with their favourite food. The ramp is then reeled into the MSU, and the lying carcass is stabbed in the chest for dry bleeding inside the shut trailer (*FiBL*, 2020).

The main advantages and disadvantages of this method seem to be the following:

- Advantages: the described method is the most hygienic as it collects the blood in a closed system. According to the MSU operator, with the mobile abattoir it was also much quicker than with other OFK methods, "because you didn't have to hang the cow for dry bleeding" (m3, Table 1). He explained that it was also safer, referring to his experiences of cattle falling off the crane to which they were not properly hooked.
- Disadvantages: some Swiss farmers reported an increasing amount of cancelled or failed OFKs in Switzerland and are under the impression that this is due to the cattle's unfamiliarity of the MSU trailer: farmers rent the platform for the training in advance but not always the whole unit. This way, only at the day of slaughter the cattle are facing the unfamiliar open trailer head-on and sometimes refuse to approach. We witnessed a failed OFK on farm 5 due to insufficient familiarization of the cattle with the stunning area.

3.2 Captive bolt pistol in combination with a T-Trailer

The use of captive bolt pistol in combination with a T-Trailer is similar to the one described in 3.1 but involves a different stunning and dry bleeding facility. The head gate here is part of a stable annex which is ideally a frequented, accessible area for the cattle. After a successful stunning, the head gate is opened, and the carcass released before it is attached to the chain of a lifting arm by one leg and hung up just above the blood container for dry bleeding. The hanging carcass can be stabbed either in the chest or the throat (*FiBL*, 2020). After the main gush is collected, the carcass is lifted onto the T-Trailer, which is then sealed for transportation.

The main advantages and disadvantages of this method seem to be the following:

- Advantages: the costs of the required infrastructure (head gate, area barrier and blood collection equipment) and vehicle (tractor with lifting arm) can be lowered by material shared with other farmers, which is practiced by some in Switzerland.
- Disadvantages: to chain and lift the carcass requires a well-rehearsed teamwork and timing (the maximum allowed duration between stunning and dry bleeding is 60 seconds). Further, the trust-based human-cattle training requires individual attention of single cattle characters (*Ferguson*, 2008) and a dedicated trial phase before the OFK appointment.

3.3. Gunshot method in combination with a T-Trailer

The use of the gunshot method in combination with a T-Trailer needs human-cattle shooting practice on a regular basis to keep the shooter in trainings and to familiarize the herd with the

sound. Our research participant from farm 3 (m2, Table 1) shoots his cattle from a high perch at close range with a red dot visor .22 magnum and lead soft point bullets, to have no exiting of the bullet from the head and, therefore, no energy waste of the slug expansion. Several potential slaughter cattle are gathered below in a small paddock and as soon as one looks up to him, he shoots. After the successful shot, the remainder of this OFK method is the same as in 3.2, with the exception that the maximum allowed duration between stunning and dry bleeding is 90 seconds.

The main advantages and disadvantages of this method seem to be the following:

- Advantages: direct contact with cattle is possible (as practiced at farm 3) but not required for the training. Handling can be done with the method of low-stress stockpersonship (LSS) (*Stookey & Watts*, 2014; *Barnes & Hibbard*, 2016). Furthermore, the infrastructure is not complex, and therefore, is less costly than for other OFK methods.
- Disadvantages: this method requires a rifle license and precision shooting.

4. Discussion and conclusion

The present study provides the first preliminary qualitative insights into the main methods of commercial OFK (*Collins & Gallinat*, 2010) and carves out their advantages and disadvantages to support a well-informed and individually suited implementation for the adoption of either a yard or pasture infrastructure (*Pinkney*, 2014). OFK definitely does not provide a "one size fits all" approach, being most suitable in small farms, where, however, it generates a premium product by minimizing the level of stress emanating from handling and reducing to zero the stressors of live transportation and hazardous stunning (*Ferguson & Warner*, 2008; *Jorquera-Chavez*, 2019). As such, OFK provides a window of opportunity to rethink the value chain of meat production and uses its very last stage as a means to provide consumers with a high animal-welfare product (*Probst et al.* 2012; *Schwartzkopf-Genswein et al.*, 2016; *FiBL,* 2020). This study contributes to the stream of literature on alternative slaughter techniques and OFK methods in particular (*Schiffer*, 2015; *Hultgren et al*., 2022), which seem to present a viable repertoire of ways to solve the long-lasting problem of pre-slaughter stress, at least in the small-scale context.

Disclosure statement: No potential conflict of interest was reported by the authors.

Acknowledgements: The authors would like to thank all research participants for generously giving their time, for welcoming us into their homes, and for sharing their experiences on on-farm killing.

References

Atkinson, P. (2006). Why do fieldwork? *Sociologisk Forskning*, 43(2), 128–134, https://doi.org/10.37062/sf.43.19303

Barnes, M. & Hibbard, W. (2016). Strategic Grazing Management Using Low-Stress Herding and Night Penning For Animal Impact. *Stockmanship Journal,* 5(2), 57–71. Retrieved from https://www.academia.edu/27838803/Strategic_Grazing_Management_Using_Low_Stress_Herding_and_Night_Penning_For_Animal_Impact

Basit, T. N. (2003). Manual or electronic? The role of coding in qualitative data analysis. *Educational Research*, 45(2), 143–154, https://doi.org/10.1080/0013188032000133548

Berger Richardson, S. (2022). Responding to Regulatory Barriers to "Ethical Meat": Are On-Farm Slaughter Exemptions the Solution? *Canadian Journal of Law and Society,* 37(2), 295–316, https://doi:10.1017/cls.2022.10

Breidenstein, G., Hirschauer, S., Kalthoff, H. & Nieswand, B. (2013). Ethnografie: Die Praxis der Feldforschung. [Ethnography: the practice of fieldwork.] UVK Verlagsgesellschaft, https://DOI:10.36198/9783838539799

Buddle, E. A., Bray, H. J. & Ankeny, R. A. (2018). "I feel sorry for them": Australian meat consumers' perceptions about sheep and beef cattle transportation. *Animals*, 8(10), 171, https://doi.org/10.3390/ani8100171

Collins, P. & Gallinat, A. (2010). The ethnographic self as resource. Writing memory and experience into ethnography. Berghahn Books, https://doi.org/10.3167/9781845456566

DeMello, M. (2012). Animals and Society. An Introduction to Human-Animal Studies. Columbia University Press, https://doi.org/10.7312/deme19484

EFSA (European Food Safety Authority), (2020). Welfare of cattle during killing for purposes other than slaughter. *EFSA Journal,*18 (11), 1–61, https://doi.org/10.2903/j.efsa.2020.6312

Ejimabo, N. O. (2015). The effective research process: Unlocking the advantages of ethnographic strategies in the qualitative research methods. *European Scientific Journal,* 11(23), 356–383, Retrieved from https://core.ac.uk/download/pdf/236404786.pdf

Fassin, D. 2013. Why ethnography matters: on anthropology and its publics. *Cultural Anthropology,* 28(4), 621–646, https://doi.org/10.1111/cuan.12030

Ferguson, D. M. & Warner, R. D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Science*, 80(1), 12–19, https://doi.org/10.1016/j.meatsci.2008.05.004

FiBL, (2020). Hof- und Weidetötung zur Fleischgewinnung. Stressarmes Töten von Rindern auf dem Landwirtschaftsbetrieb. [Yard- and pasture killing for meat production. Low stress killing of cattle in agricultural businesses.] *Merkblatt,* 1094*,* 1–28, Retrieved from https://www.fibl.org/fileadmin/documents/shop/1094-hof-weidetoetung.pdf

Harris, T. (2001). The history and development of European and North American transport regulations and international trade issues. *Journal of Animal Science*, 79(E-2001), 73–85, https://doi.org/10.2527/jas2001.79e-supple73x

Hultgren, J. (2022). Chapter 10: Avoiding live-animal transport to slaughter: mobile abattoirs. In Faucitano, L. (Ed.) *Preslaughter handling and slaughter of meat* (391–434). Wageningen Academic Publishers, https://doi.org/10.3920/978-90-8686-924-4_10

Hultgren, J., Arvidsson Segerkvist, K., Berg, C., Karlsson, A. H. & Algers, B. (2020). Animal handling and stress-related behaviour at mobile slaughter of cattle. *Preventive Veterinary Medicine,* 177*,* 1–11, https://doi.org/10.1016/j.prevetmed.2020.104959

Jorquera-Chavez, M., Fuentes, S., Dunshea, F. R., Jongman, E. C. & Warner, R. D. (2019). Computer vision and remote sensing to assess physiological responses of cattle to pre-slaughter stress, and its impact on beef quality: A review. *Meat Science*, 156, 11–22, https://doi.org/10.1016/j.meatsci.2019.05.007

Lagerkvist, C. J. & Hess, S. (2011). A meta-analysis of consumer willingness to pay for farm animal welfare. *European Review of Agricultural Economics*, 38(1), 55–78, https://doi.org/10.1093/erae/jbq043

Marescotti, M. E., Caputo, V., Demartini, E. & Gaviglio, A. (2020). Consumer preferences for wild game cured meat label: do attitudes towards animal welfare matter? *International Food and Agribusiness Management Review*, 23(4), 599–618, https://doi.org/10.22434/ifamr2019.0203

Mattimoe, R., Hayden, M. T., Murphy, B. & Ballantine, J. (2021). Approaches to analysis of qualitative research data: A reflection on the manual and technological approaches. *Accounting, Finance & Governance Review,* 27(1), 1–15, https://doras.dcu.ie/25848/1/22026-approaches-to-analysis-of-qualitative-research-data-a-reflection-on-the-manual-and-technological-approaches.pdf

Miles, M. B. & Huberman, A. M. (1994). *Qualitative data analysis: An expanded sourcebook*. SAGE, https://doi.org/10.1016/S0272-4944(05)80231-2

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E. & Raats, J. G. (2009). Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. *Meat Science*, 81(4), 653–657, https://doi.org/10.1016/j.meatsci.2008.11.004

O'Reilly, K. 2011. Ethnographic methods. Routledge, https://doi.org/10.4324/9780203864722

Probst, J. K., Spengler Neff, A., Leiber, F., Kreuzer, M. & Hillmann, E. (2012). Gentle touching in early life reduces avoidance distance and slaughter stress in beef cattle. *Applied Animal Behaviour Science*, 139(1–2), 42–49, https://doi.org/10.1016/j.applanim.2012.03.002

Pinkney, D. (2014). Mobile Abattoirs in the Context of Canadian Local Food System Development: Niches and the Potential for Local Food System Resiliency. Master thesis at The University of Guelph. Retrieved from https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/8292/Pinkney_Desiree_201408_MSc.pdf?sequence=1&isAllowed=y

Reiche, A.-M., Oberson, J.-L., Silacci, P., Messadène-Chelali, J., Hess, H. D., Dohme-Meier, F., Dufey, P.-A. & Terlouw, E. M. C. (2019). Pre-slaughter stress and horn status influence physiology and meat quality of young bulls. *Meat Science*, 158, 107892, https://doi.org/10.1016/j.meatsci.2019.107892

Ritchie, J. & Jane, L. (2003). Qualitative Research Practice. A Guide for Social Science Students and Researchers. SAGE, https://doi.org/10.5860/choice.41–1319

Schiffer, K. J. (2015). On-farm slaughter of cattle via gunshot method. Shaker Verlag. Retrieved from https://www.shaker.de/de/content/catalogue/index.asp?ID=8&ISBN=978-3-8440-3951-1

Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A. & Crowe, T. G. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass, and meat quality: A review. *Meat Science*, 92(3), 227–243, https://doi.org/10.1016/j.meatsci.2012.04.010

Schwartzkopf-Genswein, K., Ahola, J., Edwards-Callaway, L., Hale, D. & Paterson, J. (2016). Symposium Paper: Transportation issues affecting cattle well-being and considerations for the future. *The Professional Animal Scientist,* 32*,* 707–716, https://doi.org/10.15232/pas.2016–01517

Speer, N. C., Slack, G. & Troyer, E. (2001). Economic factors associated with livestock transportation. *Journal of Animal Science*, 79, https://doi.org/10.2527/jas2001.79E-SupplE166x

Stookey, J. M. Watts & J.M. (2014). Low-stress restraint, handling and sorting of cattle. In Grandin, T. (Ed.), *Livestock handling and transport, 4* (pp. 65–76). CABI Books, https://doi.org/10.1079/9781780643212.0065

Tedlock, B. (1991). From Participant Observation to the Observation of Participation: The Emergence of Narrative Ethnography. *Journal of Anthropological Research*, 47(1), 69–94, https://doi.org/10.1086/jar.47.1.3630581

Terlouw, C. (2020). Stress reactivity, stress at slaughter and meat quality. *Livestock Production Science,* 94(1–2), 125–135, https://doi.org/10.1016/j.livprodsci.2004.11.032

USDA, (2010). An Introduction to Mobile Slaughter Units. Retrieved from https://www.usda.gov/media/blog/2010/08/30/introduction-mobile-slaughter-units

Vanhonacker, F. & Verbeke, W. (2014). Public and consumer policies for higher welfare food products: Challenges and opportunities. *Journal of Agricultural and Environmental Ethics*, 27, 153–171, https://doi.org/10.1007/s10806-013-9479-2

Wigham, E. E., Butterworth, A. & Wotton, S. (2018). Assessing cattle welfare at slaughter — Why is it important and what challenges are faced? *Meat Science,* 145*,* 171–177, https://doi.org/10.1016/j.meatsci.2018.06.010

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.514.9.05:636.5
637.54:636.5]:664.932

ID: 126687497

https://doi.org/10.18485/meattech.2023.64.2.84

Original scientific paper

Effects of replacing chicken meat with chicken liver on some quality characteristics of model system chicken meat emulsions

Berker Nacak[a*]

[a] *Uşak University, Engineering Faculty, Food Engineering Department, 64000, Uşak, Türkiye*

ARTICLE INFO

Keywords:
Chicken liver
Chicken meat replacer
Model system chicken meat emulsion
Technological properties
Quality characteristics

ABSTRACT

The present study was set the purpose of investigating the effects of chicken liver as a chicken meat replacer on some quality characteristics of model system chicken meat emulsions. For this purpose, one control (100% chicken meat) and three different chicken liver treatments were formulated as follows: L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver). Chicken liver replacement ratios significantly affected moisture and protein contents and pH ($p<0.05$). The lowest emulsion stability and water holding capacity was observed in L75 ($p<0.05$). L75 also had the highest cooking loss among the emulsions ($p<0.05$). Chicken liver replacement ratio increments resulted in lower L* and b* values and higher a* values ($p<0.05$). Textural properties of emulsions were significantly affected by the presence of chicken liver in formulations, and softer emulsions were achieved with increasing chicken liver addition ($p<0.05$). The present study showed that chicken liver could be a good chicken meat replacer at up to 50%; however, this ratio could be increased by the addition of binders/fillers in meat products.

1. Introduction

Meat products are consumed world-wide in a variety of forms. They are among the foods that offer the best opportunity to deliver high amounts of protein, essential amino acids, minerals such as iron and selenium, vitamins and other nutrients (*Bolger et al.*, 2017). Different strategies have been used to change ingredients to improve the quality and presence of bioactive compounds in meat products (*Jiménez-Colmenero et al.*, 2001).

Chicken production and consumption have steadily increased globally. This implies that chicken edible by-products are also increasing day by day. Chicken liver, which is around 1.6–2.3% of a chicken's weight, is one of the main chicken by-products (*Ockerman & Basu,* 2004). According to *Ockerman and Basu* (2004) and *Seong et al.* (2015), chicken liver could be a good source of vitamins A, B_{12}, and some minerals, such as iron (Fe). Thus, using chicken liver in meat formulations could be a good strategy to provide novel products with high nutritional quality. However, using chicken liver in meat formulations could affect some quality characteristics, such as emulsion stability, water holding capacity (WHC), cooking loss, texture and color. For this reason, the replacement ratio becomes an important challenge for producing novel formulations.

***Corresponding author:** Berker Nacak, *berker.nacak@usak.edu.tr*

Paper received July 13th 2023. Paper accepted July 27th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

With the aforementioned facts, it is clearly understandable that using chicken liver in chicken meat formulations could increase the functionality of the products. Hence, the present study was set the purpose of investigating the effects of chicken liver as a chicken meat replacer on some quality characteristics of model system chicken meat emulsions.

2. Materials and methods

2.1. Materials

Chicken meat, chicken liver and beef fat were purchased from a local butcher. Sodium tripolyphosphate and sodium chloride were purchased from Kimbiotek (İstanbul, Türkiye).

2.2. Production of emulsions

In each treatment, the total proportion of chicken meat and chicken liver was 68%, and four different treatments were produced by replacing chicken meat with chicken liver as follows: C (100% chicken meat+0% chicken liver), L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver). Chicken meat, chicken liver and beef fat were passed through a grinder with a 3mm plate (Arnica Meatchef, Türkiye). After homogenization of chicken meat, chicken liver and half of the ice (5%) for 1 minute in kitchen type blender (Fakir Mr Chef Quadro, Türkiye), beef fat (20%), salt (1.5%), sodium tripolyphosphate (0.5%), and rest of the ice (5%) were added to the blender and mixed to provide a uniform blend. After obtaining a uniform blend, portions of each emulsion (approximately 25 g) were placed in Falcon tubes (50 mL) and were hermetically sealed. The tubes were heated for 30 min in a 70°C water bath. Emulsions were cooled to room temperature and analyzed.

2.3. Methods

2.3.1. Chemical composition

Moisture, fat, protein and ash contents of the samples were determined according to *AOAC* (2005).

2.3.2. Emulsion stability, water holding capacity and cooking loss

Emulsion characteristics of treatments in terms of total expressible fluid (TEF) and WHC were determined according to *Hughes et al*. (1997). Cooking loss (CL) was calculated according to sample weight difference before and after cooking. Total expressible fat (TFAT) was calculated with a modified procedure of *Hughes et al*. (1997) as follows:

2.3.3. pH

The pH of raw and cooked emulsions was measured using a pH meter (Hanna Instruments Inc., USA) on a homogenate of 10 g sample in 90 ml of distilled water.

2.3.4. Color

Lightness (L*), redness (a*) and yellowness (b*) parameters of treatments were determined by using a portable colorimeter (Chromameter CR400, Minolta, Japan). Emulsions were cut in half and color was determined on the inside cut surfaces.

2.3.5. Textural properties

Texture profile analysis (TPA) of cooked emulsions was performed using a texture analyzer (CT3-4500; Brookfield Engineering Laboratories, USA) with TA4/1000 probe. Samples (10 mm length, 20 mm diameter cylinder) were taken and compressed to 50% of their original height with a crosshead speed of 1 mm/s and 4500 g load cell. Texture Expert version 1.0 software (Stable Micro Systems, England) was used to collect and process the data.

2.3.6. Statistical analysis

All analyses were carried out in triplicate and one-way analysis of variance (ANOVA) was applied in order to observe the effect of using chicken liver as chicken meat replacer. Significant differences that had an effect were further analyzed by Duncan's multiple range test at 95% confidence level using SPSS for Windows statistical package program (version 23, IBM, USA).

3. Results and discussion

The chemical composition of emulsions is shown in Table 1. Moisture, fat, protein and ash contents of samples ranged between 58.97–61.15%, 19.82–20.80%, 16.48–17.29%, and 2.61–2.80%, respectively. According to the results, fat and ash contents of the emulsions were similar ($p>0.05$). However, significantly lower protein content and higher moisture content was found L75 than in the

Table 1. Chemical composition and pH of emulsions

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	pH	
					Raw	Cooked
C	59.41±0.64[b]	20.16±0.37	17.07±0.31[a]	2.77±0.05	6.16±0.01[d]	6.24±0.01[d]
L25	59.14±0.18[b]	20.06±0.17	17.29±0.25[a]	2.61±0.14	6.29±0.01[c]	6.41±0.01[c]
L50	58.97±0.94[b]	19.82±0.86	17.01±0.63[a]	2.78±0.32	6.46±0.01[b]	6.52±0.01[b]
L75	61.15±1.00[a]	20.80±0.43	16.48±0.13[b]	2.80±0.33	6.57±0.01[a]	6.61±0.01[a]

[a–b] Means in a same column with different letters are significantly different ($p<0.05$). C (100% chicken meat+0% chicken liver), L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver).

other emulsions ($p<0.05$). Similar to our results, *Wijayanti et al.* (2013) stated that chicken liver addition ratio could decrease protein contents of broiler nuggets.

The pH of raw and cooked emulsions was between 6.16–6.57 and 6.24–6.61, respectively. Similar to previous reports (*Dourou et al*., 2021), in the present study, the pH of fresh chicken liver was 6.60±0.02. Thus, with respect to chicken liver ratio, the pH of raw and cooked emulsions was increased ($p<0.05$).

WHC, emulsion stability (total expressible fluid and total expressible fat), and cooking loss are presented in Table 2. WHC, total expressible fluid, total expressible fat and cooking loss were between 74.43–83.19%, 1.13–6.41%, 0.23–5.22%, and 3.89–7.03%, respectively. Using chicken liver as chicken meat replacer at up to 50% did not affect the emulsion stability ($p>0.05$). However, using chicken liver at more than 50% significantly degraded the emulsion stability and WHC, and increased the cooking loss of the emulsion ($p<0.05$). Even though the pH of L75 was higher than in other emulsions, L75's emulsion stability was the lowest ($p<0.05$). Protein provides good emulsifying ability in emulsions (*Tamnak et al.*, 2016). For this reason, lower protein content and higher moisture content could be the result of lower emulsion stability in L75 ($p<0.05$). Cooking loss is associated with fat and water retention of products. *Afshari et al. (2017)* stated that high fat and moisture losses resulted in higher cooking losses in meat products. Thus, lower total expressible fluid and total expressible fat caused higher cooking loss in L75 ($p<0.05$).

Color is one of the main factors affects the consumer preference. The addition of non-meat ingredients could result in undesirable color changes (*Serdaroğlu et al.,* 2018)P2 (2% PM. Interior color parameters of the emulsions are presented in Table 3. L*, a*, and b* values ranged between 56.78–73.79, 3.64–14.83, and 10.95–13.84, respectively. Chicken liver replacement ratio increments resulted in darker, redder and less yellow products due to the characteristic color differences between chicken meat and chicken liver ($p<0.05$). These color differences might be the result of minerals, such as iron and zinc (*Permatasari et al.*, 2020). *Seong et al*. (2015) stated that chicken liver has higher levels of trace elements than most edible by-products and muscle tissues.

Table 2. Water holding capacity (WHC), total expressible fluid (TEF), total expressible fat (TFAT), and cooking loss (CL) of samples

	WHC (%)	TEF (%)	TFAT (%)	CL (%)
C	83.19±1.81[a]	1.22±0.03[b]	0.30±0.02[b]	3.89±.041[b]
L25	82.93±2.06[a]	1.22±0.06[b]	0.23±0.13[b]	4.24±1.02[b]
L50	82.45±1.98[a]	1.13±0.06[b]	0.64±0.31[b]	3.98±0.45[b]
L75	74.43±1.97[b]	6.41±0.29[a]	5.22±0.33[a]	7.03±1.29[a]

[a–b] Means in a same column with different letters are significantly different ($p<0.05$). C (100% chicken meat+0% chicken liver), L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver).

Table 3. Color of emulsions

	L*	a*	b*
C	73.79±0.85[a]	3.64±0.19[d]	13.45±0.23[a]
L25	65.64±0.36[b]	10.34±0.54[c]	13.84±0.29[a]
L50	61.52±0.46[c]	13.04±0.40[b]	12.82±0.13[b]
L75	56.78±1.58[d]	14.83±0.40[a]	10.95±0.42[c]

[a–b] Means in a same column with different letters are significantly different ($p<0.05$). C (100% chicken meat+0% chicken liver), L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver).

Table 4. Textural properties of samples

	Hardness (g)	Springiness (mm)	Cohesiveness (mJ)	Gumminess (g)	Chewiness (g)
C	4605.00±718.00[a]	3.23±0.24[a]	0.70±0.01[a]	3217.50±467.50[a]	102.84±22.19[a]
L25	2420.55±258.54[b]	3.11±0.32[b]	0.66±0.01[ab]	1594.92±173.65[ab]	49.07±10.29[b]
L50	1286.00±146.00[c]	2.74±0.28[c]	0.58±0.07[bc]	725.20±30.00[bc]	19.49±2.00[c]
L75	557.77±59.94[d]	2.60±0.08[c]	0.50±0.09[c]	272.29±52.18[c]	6.97±2.38[c]

[a–b] Means in a same column with different letters are significantly different ($p<0.05$). C (100% chicken meat+0% chicken liver), L25 (75% chicken meat+25% chicken liver), L50 (50% chicken meat+50% chicken liver), and L75 (25% chicken meat+75% chicken liver).

Textural properties of the emulsions are shown in Table 4. Chicken liver addition affected the texture of samples significantly ($p<0.05$). Due to the softer texture of chicken liver, incremental addition of chicken liver resulted in softer samples ($p<0.05$). Similar results were observed by *Amertaningtyas et al.* (2023) and *Wijayanti et al.* (2013). *Amertaningtyas et al.* (2023) stated that increasing the chicken liver substitution ratio could result in softer products, since chicken liver does not have muscle fibers. All textural properties of L75 were similar to those of L50 ($p>0.05$), except the hardness. However, these two emulsions had significantly lower textural properties than the control ($p<0.05$).

4. Conclusion

The main aim of this study was to investigate effects of using chicken liver as a chicken meat replacer on some quality characteristics of model system chicken meat emulsions. The chicken liver replacement ratio significantly affected moisture and protein contents of the emulsions as well as the pH values ($p<0.05$). The lowest emulsion stability, WHC, and the highest cooking loss was observed in L75 ($p<0.05$). However, using chicken liver as a chicken meat replacer at up to 50% did not affected the emulsion stability ($p>0.05$). With respect to chicken liver replacement ratio increments, lower L* and b* values and higher a* values were observed ($p<0.05$). Textural properties of emulsions were significantly affected by the presence of chicken liver in the formulations ($p<0.05$). The present study showed that chicken liver could be a good chicken meat replacer at up to 50%; however, this ratio could be increased by the addition of binders/fillers. Thus, further studies should be conducted to determine the effects of using chicken liver as a chicken meat replacer in meat products such as sausages, nuggets etc.

Disclosure statement: No potential conflict of interest was reported by authors.

References

Afshari, R., Hosseini, H., Mousavi Khaneghah, A. & Khaksar, R. (2017). Physico-chemical properties of functional low-fat beef burgers: Fatty acid profile modification. *LWT — Food Science and Technology*, 78, 325–331, https://doi.org/10.1016/j.lwt.2016.12.054

Amertaningtyas, D., Evanuarini, H., Mutia, S. H. & Zahra, L. L. (2023). The effect of the addition of skim milk powder to broiler liver nuggets on physicochemical and organoleptic quality. *Jurnal Ilmu dan Teknologi Hasil Ternak*, 18(1), 50–63, https://doi.org/10.21776/ub.jitek.2023.018.01.6

AOAC, (2005). Official Method of Analysis. 18th Edition. Association of Official Analysis Chemists International Press, Maryland, USA.

Bolger, Z., Brunton, N. P. & Monahan, F. J. (2017). Effect of mode of addition of flaxseed oil on the quality characteristics of chicken sausage containing Vitamin E and omega 3 fatty acids at levels to support a health claim. *Food and Function*, 8(10), 3563–3575, https://doi.org/10.1039/c7fo00929a

Choe, J., Kim, G. W. & Kim, H. Y. (2019). Effects of green tea leaf, lotus leaf, and kimchi powders on quality characteristics of chicken liver sausages. *Journal of Animal Science and Technology*, 61(1), 28–34, https://doi.org/10.5187/jast.2019.61.1.28

Dourou, D., Grounta, A., Argyri, A. A., Froutis, G., Tsakanikas, P., Nychas, G.-J. E., Doulgeraki, A. I., Chorianopoulos, N. G. & Tassou, C. C. (2021). Rapid Microbial Quality Assessment of Chicken Liver Inoculated or Not With Salmonella Using FTIR Spectroscopy and Machine Learning. *Frontiers in Microbiology*, 11, https://doi.org/10.3389/fmicb.2020.623788

Hughes, E., Cofrades, S. & Troy, D. J. (1997). Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 30% fat. *Meat Science*, 45(3), 273–281, https://doi.org/10.1016/S0309-1740(96)00109-X

Jiménez-Colmenero, F., Carballo, J. & Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. *Meat Science*, 59(1), 5–13, https://doi.org/10.1016/S0309-1740(01)00053-5

Ockerman, H. W. & Basu, L. (2004). By-products| Edible, for Human Consumption. In *Encyclopedia of Meat Sciences,* (pp. 104–112), Elsevier, https://doi.org/10.1016/B0-12-464970-X/00046-5

Permatasari, N., Angkasa, D., Dhyani, P., Melani, V. & Purwara, L. (2020). Pengembangan Biskuit MPASI Tinggi Besi dan Seng dari Tepung Kacang Tunggak (Vignia unguiculata L.) dan Hati Ayam. *Jurnal Pangan Dan Gizi*, 10(02), 33–48.

Seong, P. N., Cho, S. H., Park, K. M., Kang, G. H., Park, B. Y., Moon, S. S. & Ba, H. Van. (2015). Characterization of chicken by-products by mean of proximate and nutritional compositions. *Korean Journal for Food Science of Animal Resources*, 35(2), 179–188, https://doi.org/10.5851/kosfa.2015.35.2.179

Serdaroğlu, M., Kavuşan, H. S., İpek, G. & Öztürk, B. (2018). Evaluation of the quality of beef patties formulated with dried pumpkin pulp and seed. *Korean Journal for Food Science of Animal Resources*, 38(1), 3–13, https://doi.org/10.5851/kosfa.2018.38.1.001

Tamnak, S., Mirhosseini, H., Tan, C. P., Ghazali, H. M. & Muhammad, K. (2016). Physicochemical properties, rheological behavior and morphology of pectin-pea protein isolate mixtures and conjugates in aqueous system and oil in water emulsion. *Food Hydrocolloids*, 56, 405–416, https://doi.org/10.1016/j.foodhyd.2015.12.033

Wijayanti, D., Hintono, A. & Yoyok, B. P. (2013). The protein content and tenderness of chicken nuggets with various level of broiler livers substitution. *Animal Agriculture Journal*, 2(1), 295–300.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 633.367:577.112
613.262:633.367

ID: 126711049

https://doi.org/10.18485/meattech.2023.64.2.85

Original scientific paper

Mitigating the allergenicity of lupin seeds through germination to enhance food safety

María López-Pedrouso[a], Ricard Bou[b], Roberto Bermúdez[c], Ruben Domínguez[c] and José Manuel Lorenzo[c]*

[a] *Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Santiago de Compostela, A Coruña, 15872, Spain*
[b] *Food Safety and Functionality Program, Institute of Agrifood Research and Technology (IRTA), Finca Camps i Armet s/n, Monells, 17121, Spain*
[c] *Centro Tecnológico de la Carne de Galicia, Rúa Galicia N° 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, Ourense, 32900, Spain*

ARTICLE INFO

Keywords:
Novel proteins
Mass spectrometry
Proteomics
Vegetable food
Seed storage proteins

ABSTRACT

The search for novel plant proteins presents a major challenge to the global food industry. Beyond the sustainability and nutritional value of seed proteins, there is a growing focus on exploring their potential health benefits for human consumption. However, the endeavour to evaluate the allergenic risks associated with plant-based proteins will require substantial efforts in the coming years. One key strategy to address this concern is the reduction of allergenicity in seeds through the process of germination, which triggers the strong proteolysis of storage proteins. In this study, *Lupinus luteus* L. seeds were germinated at 25ºC for 0, 3, 6 and 9 days under a constant humidity of 80%. Peptide extracts derived from sprouted lupin seeds were subsequently separated under reducing conditions by SDS-PAGE. The resulting protein bands were digested and subjected to mass spectrometry analysis (SWATH-MS) for accurate identification of allergenic proteins. This study's findings revealed the great impact of lupin seed germination on the protein patterns of hydrolysates, particularly noticeable after a germination period of 6 days. The main protein bands (90, 70, 64, 52, 46 and 37 kDa) exhibited a notable decrease in relative intensity as a result of germination. Furthermore, SWATH-MS analysis successfully identified two distinct isoforms (B0YJF7 and B8Q5G0) of the major allergen, β-conglutin, in all tested bands, both prior to and following germination. This study provides evidence for the crucial role of germination in the degradation of this allergen, leading to the elimination of epitopes and subsequently reducing its allergenicity.

1. Introduction

One of the major challenges for the global food industry is the search for plant proteins. Beyond the sustainability and nutritional value of seed proteins, there is an increasing emphasis on exploring potential benefits on human health (*López-Pedrouso, Lorenzo, Alché, Moreira & Franco, 2023)*novel food is becoming an emerging trend increasingly more demanding in developed countries. Food proteins from vegetables (pulses, legumes, cereals. White lupin (*Lupinus albus* L.), yellow lupin (*L. luteus* L.) and narrow-leafed lupin (*L. angustifolius* L.) are native European species known for their high protein content of up to 44%. These lupin varieties are commonly utilized in the food industry, particularly in the production of bakery and gluten-free products where lupin flour, protein isolates and concentrate are frequently employed (*Lucas et al*., 2015).

***Corresponding author:** María López Pedrouso, *mariadolores.lopez@usc.es*

Paper received July 13th 2023. Paper accepted July 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

In the case of lupin, three allergens were recognized by the WHO/IUIS Allergen Nomenclature Sub-Committee: Lup a 5 (profilin; 15 kDa), Lup an 1 (β-conglutin, 7S seed storage globulin, vicilin; 55–61 kDa) and Lup an 3 (non-specific lipid transfer protein; 11 kDa). Another potential risk arises from the cross-reactivity between lupin and peanuts, which can potentially trigger allergic reactions *(Mennini, Dahdah, Mazzina & Fiocchi*, 2016)but the identification of the involved individual allergens is still limited. The aim of this review is to describe new allergenic findings, of potential relevance for cross-reactivity among peanut and lupin. Recent Findings: Seventeen allergens of peanut have been included in the official allergen nomenclature database to date. Lupin sensitization has been observed in 15–20% of individuals with known peanut allergy, The majority of lupin seed proteins are comprised of α-conglutins (legumin-like. The immunoreactive properties of seeds can be reduced through germination and other processing conditions. Thus, proteins from cotyledons undergo hydrolysis and the removal of multiple epitopes takes place, with beneficial effects on seed consumption (*Ravindran & Ramaswamy*, 2023). This process has been proven to result in the suppression of epitopes and the reduction of allergenicity through protein degradation (*Sathe, Teuber & Roux*, 2005; *Pi, Sun, Fu, Wu & Cheng*, 2021). Moreover, the germination process of some seeds, including varieties such as beans, chickpeas, lentils and lupins, enhances their nutritional quality by increasing protein, ash and mineral (sodium, magnesium, zinc and iron) contents (*Atudorei, Stroe & Codină*, 2021).

2. Materials and methods

2.1. Germination of lupin seeds (Lupinus luteus L.) and protein extraction

The seeds of *Lupinus luteus* L. were germinated on filter paper at 25ºC, with a constant humidity of 80% maintained throughout the whole germination period. The germination process was conducted under dark conditions. A maximum germination time of 9 days was allowed to preserve the nutritional profile of the germinated seeds. Finally, freeze-drying was employed to store the seeds at –80ºC. Proteins from germinated and non-germinated seeds were prepared by homogenization using a TissueLyser II (Qiagen) in cold RIPA buffer [containing 200 Mmol/L Tris/HCl (pH 7.4), 130 Mmol/L NaCl, 10% (v/v) glycerol, 0.1% (v/v) SDS, 1% (v/v) Triton X-100, 10 Mmol/L $MgCl_2$] with anti-proteases and anti-phosphatases (Sigma-Aldrich). The tissue lysates were centrifuged for 20 minutes at 14,000 g in a microfuge at 4 °C. The protein quantification was assessed for each sample using RC DC™ kit (Bio-Rad) according to the manufacturer's recommendations.

2.2. SDS-Page analysis

Peptide extracts obtained from germinated and non-germinated lupin seeds were subjected to separation under reducing conditions using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Mini-Protean Tetra Cell equipment (Bio-Rad Lab, Hercules, CA, USA) was utilized to separate 15 µg amounts of the extracts onto pre-cast 10% gels. To dissolve and denature the samples, Laemmli buffer (62.5 mM TrisHCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue, 100 mM DTT) was employed and incubated at 95ºC for 5 minutes. Staining was performed using Coomassie Brilliant Blue G-250 solution, and the Gel Doc XR+ system (Bio-Rad Laboratories) was used to capture the images. The obtained images were subsequently analysed using the Image Lab™ software (Biorad Lab, Hercules, CA, USA).

2.3. Mass spectrometry: Digestion and analysis

The selected bands from 1-DE gels were excised and washed with a solution containing 50 mM ammonium bicarbonate (ambic) and 50% MeOH. Proteins were reduced with 10 mM DTT in 50 mM ambic and alkylated with 55 mM IAA in 50 mM ambic, and subsequently rinsed with 50 mM ambic in 50% MeOH, dehydrated through the addition of acetonitrile (ACN) and dried in a SpeedVac (Thermo Scientific, USA). Modified porcine trypsin was added to the dried gel slices at a final concentration of 20 ng/µL in 20 mM ambic, followed by incubation at 37°C for 16 h. The peptides were extracted three times by incubation in 40 µL of 60% ACN in 0.5% HCOOH for 20 min. The resulting peptide extracts were pooled, concentrated, and dried in a SpeedVac and stored at –20 °C until their analysis through LC-MS/MS.

A TripleTOF 6600 System (SCIEX, Foster City, CA) was employed for the data acquisition using a Data-dependent workflow. After MS/MS analysis, data files were processed using Pro-

teinPilot™ 5.0.1 software from SCIEX, which uses the algorithm Paragon™ for database search and Progroup™ for data grouping. Data were searched using a UniProt database. False discovery rate was calculated using a non-lineal fitting method displaying only those results that reported a 1% global false discovery rate or better.

3. Results and Discussion

3.1. Analysing SDS protein profile of lupin seeds during germination

The germination of lupin seeds had a significant impact on the protein banding patterns of hydrolysates over nine days, as illustrated in Figure 1. Protein analysis through SDS-PAGE revealed the presence of polypeptide bands with molecular weights below 90 kDa. Protein bands at 90, 70, 64, 52, 46 and 37 kDa were predominantly observed in non-germinated seeds. However, with longer germination periods, these storage proteins underwent degradation to supply amino acids for the synthesis of biomolecules, resulting in a remarkable decrease in the intensity and volume of protein bands after six days. Most edible pulse seeds, such as chickpea, fava bean, kidney bean, green lentil and yellow pea, germinate within three or five days. This process enhances *in vitro* digestion in the majority of cases, with the exception of kidney bean, suggesting an important bioprocess (*Ohanenye, Tsopmo, Ejike & Udenigwe*, 2020)major dietary sources in developed countries are of animal origin. However, the association of red meat consumption to the increased risks of some health conditions and its unsustainable pressure on the environment have increased the interest in plant proteins as healthier and sustainable alternatives. Of these, legumes have a great potential, but part of their proteins are indigestible due to interaction with other components such as phytate and polyphenols. As such, the quest to improve protein accessibility has become of interest to many researchers. Germination is proposed to be a bioprocess method to improve protein digestibility and protein biological properties. Scope and approach: This review discusses the importance of plant proteins and the hindrance of protein digestibility. This paper also highlights the role of germination in the deactivation of antinutritional factors, hydrolysis of indigestible proteins, and improvement of properties and content of proteins of different legume seeds. Key findings and conclusions: Protein digestibility is dependent on the nature of antinutritional factors (e.g. trypsin inhibitors and phytate. During ger-

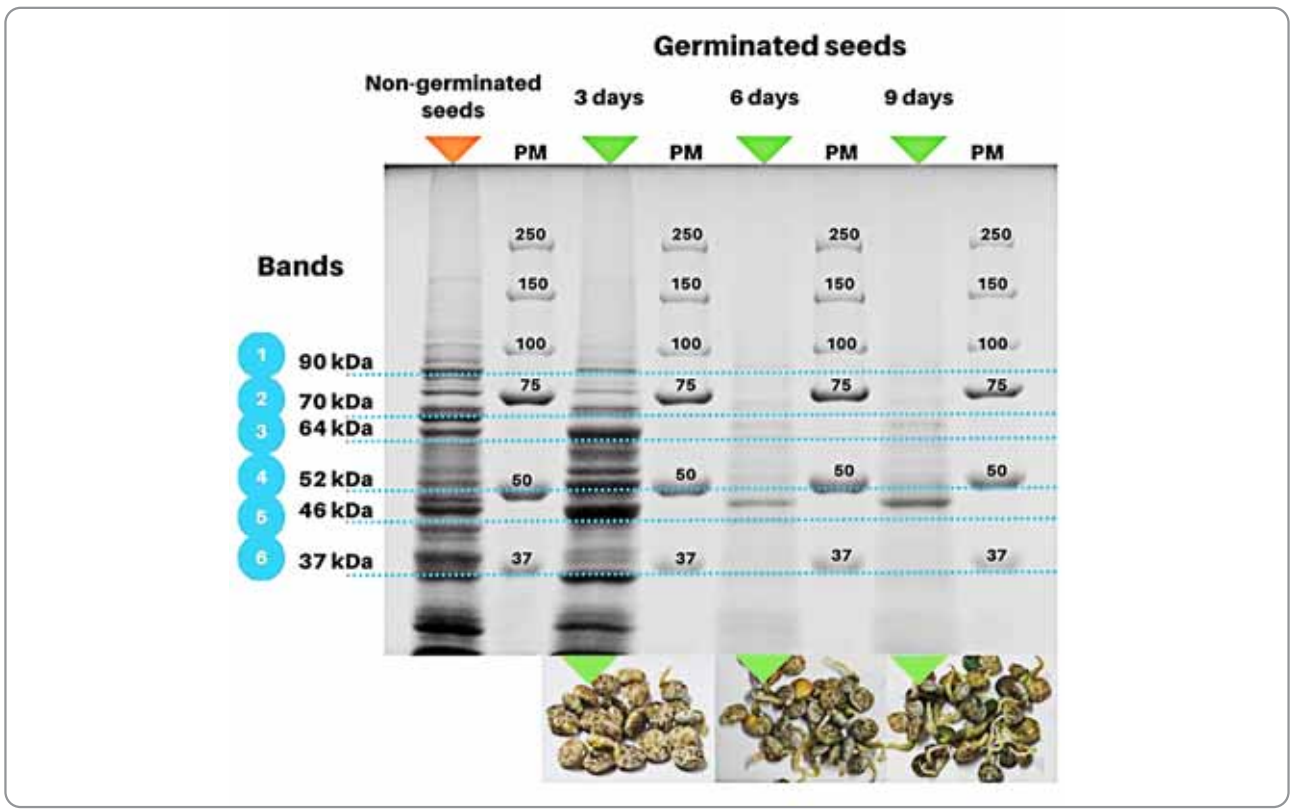


Figure 1. SDS-PAGE analysis of seed proteins from *Lupinus luteus* L seeds on different days of germination

mination, a significant protein transformation occurs through proteolysis increasing the content of free amino acids, which enhances digestibility and reduces the levels of anti-nutrient protease inhibitors. This enzymatic breakdown by protease activity leads to the degradation of storage proteins, including allergens (*Bera, O'Sullivan, Flynn & Shields*, 2023). In short, germination plays a crucial role in modifying the reactivity of seed allergens by effectively eliminating conformational and linear epitopes. This process reduces the allergenicity of lupin seed products. According to this strategy, germination significantly reduced the immunoreactivity of vicilin by 55% and 74% after ten days in black gram (*Vigna mungo*) and mung bean (*Vigna radiata*), respectively (*Gupta, Sathe, Su & Liu*, 2021). Similarly, in soybeans, the three major allergens (Gly m Bd 60K, Gly m Bd 30 K and Gly m Bd 28 K) underwent degradation earlier in the embryonic axes compared to cotyledons during the process of germination and seedling growth (*Wu et al*., 2012).

However, it is expected that each band will contain a complex mixture of polypeptides belonging to different seed proteins. A variety of proteins were present, resulting in albumin (water-soluble), globulin (salt-soluble), glutelin (alkaline-soluble) and prolamin (ethanol-soluble) being the most abundant. These proteins exhibit distinct polypeptide bands due to differences in their molecular weights. Among them, globulin constitutes the highest proportion (approximately 90% of the total protein) and is characterized by bands at 8, 11, 17, 23, 25, 30, 38, 40, 45 and 60 kDa (*Idowu, Alashi, Nwachukwu, Fagbemi & Aluko*, 2021).

3.2. Identification of allergens by mass spectrometry

Mass spectrometry analysis revealed the presence of two distinct isoforms of β-conglutin, B0YJF7 and B8Q5G0, in all tested bands both before and after germination (Table 1). These isoforms consisted of 521 and 611 amino acids, respectively, and exhibit great similarity (86.56% identity according to Uniprot). The major difference between them is the inclusion of an additional terminal sequence of 82 amino acids in the B8Q5G0 isoform. β-conglutin is a protein belonging to the cupin family. The cupin family comprises a diverse range of proteins sharing a common β-barrel structure. The two major storage proteins of lupin seed are α-conglutin (legumin-like or 11S globulin), and ß-conglutin (vicilin-like or 7S globulin). Vicilin proteins exhibit a characteristic structural arrangement comprising

Table 1. Identification of β-conglutin, the major allergen in lupin seeds, in the gel bands of non-germinated seeds (NGS) and geminated seeds at 6 days (GS)

Band	Status seed	Accession No. (Uniprot)	Name allergen	Mr (KDa) expected	Coverage (%)	No. of non-reductant peptides (95%)
1 (90 kDa)	NGS	B0YJF7	Cupin 1	61.47	13.44	3
	GS	B0YJF7	Cupin 1	61.47	17.47	5
2 (70 kDa)	NGS	B0YJF7	Cupin 1	61.47	32.44	23
	GS	B0YJF7	Cupin 1	61.47	70.63	147
		B8Q5G0	Lup an 1	71.9	53.36	117
3 (64 kDa)	NGS	B0YJF7	Cupin 1	61.47	33.40	27
	GS	B0YJF7	Cupin 1	61.47	57.01	21
4 (52 kDa)	NGS	B8Q5G0	Lup an 1	71.9	57.20	22
	GS	B8Q5G0	Lup an 1	71.9	38.95	146
5 (46 kDa)	NGS	B8Q5G0	Lup an 1	71.9	61.47	8
	GS	B0YJF7	Cupin 1	61.47	31.86	9
6 (37 kDa)	NGS	B0YJF7	Cupin 1	61.47	44.15	24
	GS	B8Q5G0	Lup an 1	71.9	41.73	22

two cupin domains, forming barrel-shaped structures composed of α-helices. Thus, β-conglutin, also referred to as vicilin-like globulins, is a 7S globulin with a trimeric structure. It is composed of polypeptides ranging from 16 to 70 kDa. Additionally, a glycosylated precursor of 64 kDa has been identified (*Duranti, Consonni, Magni, Sessa & Scarafoni*, 2008).

One of the most abundant allergen families identified was the cupin family. Among the food allergens within this group are 7S globulins, including soybean (β-conglycinin), peanut (conarachin; Ara h 1), walnut (Jug r 2) and lentil, as well as 11S globulins like peanut (arachin; Ara h 3) and soybean (glycinin) (*Mills et al*., 2002). In the case of lupin-derived foods, the main allergen of concern is β-conglutin, considered the most relevant issue associated with lupin consumption. Therefore, detecting and quantifying β-conglutin in processed products is a suitable strategy *(Lima-Cabello, Alché & Jimenez-Lopez*, 2019)sensitive and accurate ELISA method to detect, identify and quantify the lupin main allergen β-conglutin (Lup an 1, as is reducing their allergenicity, shown in this study. A massive degradation of β-conglutin isoforms (B0YJF7 and B8Q5G0) took place during the germination as shown in Figure 1, providing further evidence of their role as storage proteins. In most cases, germination led to the elimination of conformational and linear epitopes, thereby modifying the immunoreactivity, particularly after day 6 of germination.

4. Conclusions

The food safety of vegetal foods should be enhanced to facilitate these products into the market. It is crucial to develop methods for detection, identification and quantification of allergens, and to decrease allergenicity in fresh and processed foods. The most relevant allergen in seeds from *Lupinus luteus* L is β-conglutin, and particularly, two isoforms (B0YJF7 and B8Q5G0) were detected. This study demonstrates that germination plays a vital role in the degradation of this allergen, thereby indicating the elimination of epitopes and resulting in reduced allergenicity.

Disclosure statement: No potential conflict of interest was reported by the authors.

Acknowledgments: This work was supported by the project LUPIPROTECH (Project PID2020-114422RR) from the Spanish "Ministerio de Ciencia e Innovación" and the "Agencia Estatal de Investigación."

References

Atudorei, D., Stroe, S. G. & Codină, G. G. (2021). Impact of germination on the microstructural and physicochemical properties of different legume types. *Plants*, 10(3), 1–19, https://doi.org/10.3390/plants10030592

Bera, I., O'Sullivan, M., Flynn, D. & Shields, D. C. (2023). Relationship between Protein Digestibility and the Proteolysis of Legume Proteins during Seed Germination. *Molecules (Basel, Switzerland)*, 28(7), https://doi.org/10.3390/molecules28073204

Duranti, M., Consonni, A., Magni, C., Sessa, F. & Scarafoni, A. (2008). The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science and Technology*, 19(12), 624–633, https://doi.org/10.1016/j.tifs.2008.07.002

Gupta, S., Sathe, S. K., Su, M. & Liu, C. (2021). Germination reduces black gram (Vigna mungo) and mung bean (Vigna radiata) vicilin immunoreactivity. *LWT*, 135, 110217, https://doi.org/10.1016/j.lwt.2020.110217

Idowu, A. O., Alashi, A. M., Nwachukwu, I. D., Fagbemi, T. N. & Aluko, R. E. (2021). Functional properties of sesame (Sesamum indicum Linn) seed protein fractions. *Food Production, Processing and Nutrition*, 3(1), https://doi.org/10.1186/s43014-020-00047-5

Lima-Cabello, E., Alché, J. D. & Jimenez-Lopez, J. C. (2019). Narrow-leafed lupin main allergen β-conglutin (lup an 1) detection and quantification assessment in natural and processed foods. *Foods*, 8(10), 1–19, https://doi.org/10.3390/foods8100513

López-Pedrouso, M., Lorenzo, J. M., Alché, J. de D., Moreira, R. & Franco, D. (2023). Advanced Proteomic and Bioinformatic Tools for Predictive Analysis of Allergens in Novel Foods. *Biology*, 12(5), 1–14, https://doi.org/10.3390/biology12050714

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frías, J., Martínez-Villaluenga, C., Sussmann, D.…& Pueyo, J. J. (2015). The future of lupin as a protein crop in Europe. *Frontiers in Plant Science*, 6(September), 1–6, https://doi.org/10.3389/fpls.2015.00705

Mennini, M., Dahdah, L., Mazzina, O. & Fiocchi, A. (2016). Lupin and Other Potentially Cross-Reactive Allergens in Peanut Allergy. *Current Allergy and Asthma Reports*, 16(12), https://doi.org/10.1007/s11882-016-0668-8

Mills, E. N. C., Jenkins, J., Marigheto, N., Belton, P. S., Gunning, A. P. & Morris, V. J. (2002). Allergens of the cupin superfamily. *Biochemical Society Transactions*, 30(6), 925–929, https://doi.org/10.1042/BST0300925

Ohanenye, I. C., Tsopmo, A., Ejike, C. E. C. C. & Udenigwe, C. C. (2020). Germination as a bioprocess for enhancing

the quality and nutritional prospects of legume proteins. *Trends in Food Science and Technology*, 101(May), 213–222, https://doi.org/10.1016/j.tifs.2020.05.003

Pi, X., Sun, Y., Fu, G., Wu, Z. & Cheng, J. (2021). Effect of processing on soybean allergens and their allergenicity. *Trends in Food Science and Technology*, 118(PA), 316–327, https://doi.org/10.1016/j.tifs.2021.10.006

Ravindran, A. & Ramaswamy, H. S. (2023). *Effect of sonication — cooking on the immunoreactivity of soy slurry*.

Sathe, S. K., Teuber, S. S. & Roux, K. H. (2005). Effects of food processing on the stability of food allergens. *Biotechnology Advances*, 23(6), 423–429, https://doi.org/10.1016/j.biotechadv.2005.05.008

Wu, Y. M., Guan, R. X., Liu, Z. X., Li, R. Z., Chang, R. Z. & Qiu, L. J. (2012). Synthesis and degradation of the major allergens in developing and germinating soybean seed. *Journal of Integrative Plant Biology*, 54(1), 4–14, https://doi.org/10.1111/j.1744-7909.2011.01092.x

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523:664.8.036
664.022.3

ID: 126662665

https://doi.org/10.18485/meattech.2023.64.2.86

Original scientific paper

Validation of LC-MS/MS for food colors in foodstuffs and household products

Danka Spirić[a], Srđan Stefanović[a], Čaba Silađi[a], Radivoj Petronijević[a], Tamara Gerić[a], Nikola Borjan[a] and Silvana Stajković[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
LC-MS/MS
Food color
Additives

ABSTRACT

The aim of this study was to develop a quadrupole liquid chromatography mass spectrometry (LC-MS/MS) method suitable for quantification of synthetic food colors, used as additives or occurring as adulterants in the food or cosmetics industries. Ten colors were validated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, trueness and applicability. All tested parameters of the validation were within acceptable values, and the method was comparable to the existing high pressure liquid chromatography (HPLC UV-PDA) method.

1. Introduction

Food additives that are colorants, also known as food dyes, are used in the food industry as additives to homogenize color or to improve the visual quality of product. Sometimes they are also added to make color look more natural, since color stability fluctuates over time. Food colors can be synthetic, synthesized but equivalent to the natural color component, or naturally derived colorants. Their impact on human health is widely studied. Recent and older studies show that excessive exposure can cause cancer (*IARC,* 1975). The maximum levels of food colors in foods are defined in the European Union (*European Union Regulation*, 2023); this law is the update of the 2008 law on food additives. Certain colors have been allowed for use since December 2004 in meat products in Serbia, so in the following years, there was increased demand for development and use of adequate analytical methodology for determination of their presence and quantity in meat products (*Feng et al*, 2011). Different techniques are recommended for food analysis in terms of the presence and and amount of food colorants. One of the most convenient techniques is quadrupole liquid chromatography mass spectrometry (LC-MS/MS).

The aim of this study was to develop a LC-MS/MS method to quantify synthetic food colors, used as additives or occurring as adulterants in the food or cosmetics industries.

2. Materials and methods

Validation of the LC-MS/MS method for the determination of ten artificial colorants in food was performed on a triple quadrupole mass spectrometer, LCMS-8050 CL (Shimadzu Corporation, Japan).

***Corresponding author:** Danka Spirić, *danka.spiric@inmes.rs*

Paper received July 25th 2023. Paper accepted August 4th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Tartrazine, Patent Blue, Ponceau 4R, Indigo Carmine, Sunset Yellow FCF, Allura Red AC, Azorubine, Green S, Brilliant Blue FCF, and Brilliant black, were obtained from Sigma-Aldrich (St. Louis, MO, USA). All of the stock solutions (1000 μg/mL) prepared were dissolved in HPLC grade water. Methanol and acetonitrile were purchased from Sigma-Aldrich. The ESI ionization was executed in either negative or positive mode. Under certain ESI conditions, all compounds showed higher sensitivity in negative than in positive mode; the most abundant ion was $[M–H]^-$ for all analytes. Each target analyte (10 μg/mL, in HPLC grade water) was tuned individually in order to obtain stable precursor and product ion abundance. Mixtures of colors from working solutions were added to blank sweetened gelatin mass to obtain concentrations of 10 mg/kg, 20 mg/kg, 50 mg/kg and 200 mg/kg of each dye. These samples were prepared according our standard operating procedure (SOP), and were examined along with certified reference materials (CRMs), and foods/household products with declared dyes purchased from the retail market. Samples were extracted before analysis according to the SOP: minced, blended and/or homogenized, then dissolved in an ultrasound bath with a mixture of ethanol-ammonia-water 7.5-2.0-0.5/v-v-v. Extracts of the samples were filtered through membrane filters with a pore size of 0.45 μm into the vials for the spectrophotometer's autosampler.

3. Results

Limits of detection and quantification of the method were estimated following the IUPAC approach, which consisted of analyzing the blank sample to establish noise levels and then estimating limit of detection (LOD) and limit of quantification (LOQ) for signal/noise ratio, 3 and 10 respectively (*Feng et al*., 2011). The LOQs and LODs for the food dyes are listed in Table 1. Also, intermediate precision and trueness were measured using reference materials for artificial food dyes (FCFA CON-32QC and other products, Fapas®)

Linearity of results was determined for all standard colors in the range of 0 mg/kg to 200 mg/kg in both water and blank gelatin. The term linearity of signal in LC-MS is used for determination of the linear relationship between analyte signals and analyte concentrations in calibration samples and the linear relationship between analyte signals and analyte concentrations in samples due to the matrix effect (*Chia-Fen et al*., 2015). For all samples, linearity was between 0.97 and 1.00.

Within-laboratory reproducibility of the results was determined by analyzing the samples listed in Table 2. Samples used for analysis were fruit- and vegetable-based products, candy and cake products, mouth rinse (a personal hygiene liquid) and dishwashing detergent. Six replicates of each sample were prepared, and the results were analyzed with

Table 1. LOD and LOQ values, intermediate precision and trueness for the LC-MS/MS method for determining synthetic food dye content, * Fapas® artificial food dye in gelatin-based sweets

Name of additive	LOD (mg/kg)	LOQ (mg/kg)	Intermediate Precision. STDEV	Trueness (%)
Additive E 102 (Tartrazine)*	0.46	0.52	7.32	96.7
Additive E 131 (Patent Blue)	0.52	0.53		
Additive E 133 (Brilliant blue)*	0.38	0.45	8.64	87.6
Additive E 142 (Green S)*	1.43	1.52	8.45	85.32
Additive E 151 (Brilliant Black)	0.86	0.87		
Additive E110 (Sunset Yellow FCF)*	0.59	1.25	10.22	94.4
Additive E122 (Carmoisine. Azorubine)	0.42	0.94		
Additive E124 (Ponceau 4R)	0.68	1.20		
Additive E129 (Allura red AC)*	0.70	1.42	11.32	84.45
Additive E132 (Indigo carmine)	0.75	1.39		

Table 2. Validation parameters for LC-MS/MS to quantify synthetic food dyes, showing repeatability, reproducibility, and standard deviation

Sample	Analyte – synthetic food dye	Synthetic food dye content, measured by LC-MS/MS (mg/kg)	Within laboratory reproducibility	t_{crit}	|t|	STDEV (LC-MS/MS HPLC-UV PDA)
Surimi	E 120	5.67	0.36	2.57	0.71	9.10
Fruit dessert	E 102	47.55	1.26	2.57	0.36	8.38
Frozen peas	E 142	18.81	0.09	2.57	0.09	10.41
Fusilli	E 142	27.61	0.74	2.57	0.87	8.66
Butter biscuit	E 142	36.22	0.34	2.57	0.95	8.48
Lolly pop	E 102 E 124	40.71 58.74	0.17 0.48	2.57	1.41 0.38	6.47 9.87
Fruit yogurt	E 102	32.24	0.56	2.57	0.27	13.87
Tomato sauce	E 129	19.07	0.13	2.57	1.37	9.33
Baby food	E 102	20.08	1.08	2.57	0.84	7.17
Orange syrup	E 110	45.05	0.54	2.57	0.52	11.25
Mouth rinse	E 131 E151	32.69 24.29	0.29 0.34	2.57	1.52 1.29	8.03 11.45
Dishwashing detergent	E 102 E 133	37.28 7.51	0.27 0.91	2.57	0.38 2.07	6.37 9.74

T-test to compare the difference between replicates. All samples were commercially available on the local market and labeled by manufacturer regarding color content. Reproducibility for all products examined was far lower than the critical T-value, showing good reproducibility of the results by the LCMS method (Table 2).

Dwell time for all standards was 10 ms. Precursor ions, product ions and collision energy are listed in Table 3.

Table 3. LC-MS/MS chromatographic results and conditions

Food dye	Precursor m/z	Product ion m/z	Collision energy
Patent blue E131	559.2	435.3479.5	62.045.0
Brilliant blue E133	747.4	171.0561.0	79.061.0
Green S E142	553.3	416.0496.0	49.038.0
Azorubine E122	227.9	169.9220.9	22.018.0
Allura red E129	225.1	207.0136.0	22.034.0
Sunset yellow E110	407.1	207.1327.1	45.030.0
Ponceau 4R E124	267.9	301.9205.9	15.017.0
Indigo carmine E132	226.0	198.0105.0	27.053.0
Tartrazine	210.9	197.9170.9	15.015.0
Erythrosine E127	834.8	663.0537.0	52.054.0

Those values were initially transferred from existing methods but during the process of validation were changed, either undergoing a complete change or just slight shift of values (*Ntrallou et al*., 2020). Most of transitions that were dominant were chosen for further chromatographic conditions and used in validation process.

3.1 Comparison of LC-MS/MS to LC-UV-PDA validation results

LC-MS methods have the advantage of higher sensitivity, higher selectivity and higher throughput compared with LC-UV methods (*Kim et al*., 2018). Nevertheless, the previously developed existing high pressure liquid chromatography (HPLC UV-PDA) method was comparable to the new LC-MS/MS method, since standard deviations for samples analyzed with the two different methods were lower or about 10%.

4. Conclusion

The LC-MS/MS method developed and validated for determination of food colors is convenient and suitable for routine analysis of dyes in different food products and other household products that might affect human health. The method is comparable to our existing HPLC UV-PDA method, according to results obtained by analyzing different retail products from the market. Trueness and reproducibility are acceptable and reproducible compared to assigned values from reference materials.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supporte by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Chia-Fen, T., Ching-Hao, K. & Daniel Yang-Chih, S. (2015). Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry. *Journal of Food and Drug Analysis*, 23(3), 453–462, https://doi.org/10.1016/j.jfda.2014.09.003

European Union Regulation. (2023). Regulation 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives.

Feng, F., Yansheng, Z., Wei, Y., Li, S., Guibin, J. & Xiaogang, C. (2011). Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid chromatography–electrospray tandem mass spectrometry, *Journal of Chromatography B*, 879(20), 1813–1818, https://doi.org/10.1016/j.jchromb.2011.04.014

IARC, (1975). Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, vol. 8, IARC, Lyon, 1975, p. 125.

Kim, H. J., Lee, M. J., Park, H. J., Kim, H. J., Cho, S. K. & Jeong, M. H. (2018). Simultaneous determination of synthetic food additives in kimchi by liquid chromatography-electrospray tandem mass spectrometry. *Food Science and Biotechnology*,16, 27(3), 877–882, doi: 10.1007/s10068-018-0308-2

Ntrallou, K., Gika, H. & Tsochatzis, E. (2020). Analytical and sample preparation techniques for the determination of food colorants in food matrices. *Foods*, 7, 9(1), 58, doi: 10.3390/foods9010058

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.523:664.8.036

ID: 126635785

https://doi.org/10.18485/meattech.2023.64.2.87

Original scientific paper

Ensuring the safety of cooked and smoked sausages of a narrower diameter, in a cellulose casing, by heat treatment

Mladen Rašeta[a], Ivana Branković-Lazić[a], Boris Mrdović[a], Stefan Simunović[a], Sara Simunović[a], Jelena Jovanović[a] and Zsolt Becskei[b]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Pasteurization
Cooked susages
Pv value
Heat treatment
Food safety

ABSTRACT

The aim of this work is to ensure the safety of finely chopped cooked sausages stuffed in a cellulose casing, diameter 21 mm. Safety is ensured by thermal treatment of the level of pasteurization in a chamber under a defined thermal treatment program. By monitoring the temperature in the product's thermal center at different control positions in the chamber, temperatures of 75.26°C–77.11°C were determined with a thermocouple. In doing so, the products spent 8–9 minutes at a temperature higher than 70°C, and 18–20 minutes at a temperature higher than 55°C. Considering that it is a small diameter product, the heat intensively penetrates to the thermal center and thus ensures the safety of the product. However, due to the relatively short time of the heat treatment, the pasteurization values (pv) at the control points were in the range of 18.3–26 minutes. Therefore, we conclude that ensuring the safety of products with a narrower diameter is achieved only by controlling the temperature in the thermal center of the product, and not with pv values. Considering that this sausage is a perishable product, it is necessary to provide a proper cold chain (0–4°C) after manufacture so that the product remains suitable for consumption within the period defined in a shelf-life study.

1. Introduction

In recent years, there has been an increased demand for minimally processed, high-quality, convenient food products, with fewer additives and longer shelf life (*Balamurugan et al.*, 2018). In-package thermal pasteurization is widely used to eliminate pathogens in ready-to-serve foods, and factors affecting the inactivation of pathogens and shelf-life extension have been extensively studied (*McCormick et al.*, 2006). Pasteurization is a mild heat treatment in which food is heated to below 100°C. It is used to minimize health hazards from pathogenic micro-organisms in low-acid foods and to extend the shelf-life of acidic foods such as fruit juices for several days or weeks by the destruction of spoilage micro-organisms and/or enzyme inactivation. (*Fellows*, 2009). Pasteurization is a relatively mild heat treatment, compared to heat sterilization, and it causes smaller changes to the nutritional, functional, and sensory characteristics of most foods. However, the shelf life of pasteurized foods is usually only extended by a few days or weeks, compared to many months with the more severe heat sterilization treatments (*Fellows*, 2022). Pasteurized products include all those meat products which during production were exposed to the preservative effect of temperature below the boiling point of water, whereby a temperature of at least 70°C is achieved in the thermal

***Corresponding author:** Mladen Rašeta, *mladen.raseta@inmes.rs*

Paper received July 25th 2023. Paper accepted August 4th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

center, in accordance with the Regulations, or whereby the heat treatment procedure is such that a temperature of at least 65°C is reached in the thermal center of the product, over the time period required to reach a pasteurization value (pv) equal to or above 40 (*Official Gazette of the Republic of Serbia*, 2019). Temperatures of 72°C and 74°C are generally defined in the hazard analysis and critical control point (HACCP) documentation of food business operators as critical limits for the assessment of pasteurization for meat products (*Raseta et al.*, 2021).

Sausage is one of the earliest forms of food processing and became an art distinctive to particular locations during Middle Ages and a means of preserving meat (*Ebunoluwa et al*., 2022). Sausage is minced meat or a combination of meats blended with seasonings and spices stuffed into a casing or container (*Savell and Smith*, 2009). Cooked sausages are meat products that contain meat batter as a base and are preserved by heat treatment, usually pasteurization (*Vuković*, 2012). Smoked sausages are very popular and there are two types, uncooked and cooked; raw smoked sausages are made from cured or uncured meat that is ground and mixed with spices, salt, or other non-meat items and stuffed into casings to form sausages that are then smoked and refrigerated. Cooked smoked sausages include emulsion type and coarse ground sausages (*Topel et al.*, 2013).

In accordance with the current domestic legislation (*Official Gazette of the Republic of Serbia*, 2019) cooked sausages are meat products obtained from meat, fatty tissue, connective tissue, offal, blood products, and supplements, where part of the filling can be meat dough and which, after being filled into casings or molds, are heat-treated at the temperature of pasteurization, with or without smoking. Salt, brining salts, water, spices, spice extracts, sugars, and additives can be used as additives in the production of cooked sausages, smoke aromas, and natural aromas.

Cellulose casings are one of the most popular types of artificial casings that are used in meat processing, with the others being collagen and plastic (*Marchello and Garden-Robinson*, 2017). Cellulose casings have stronger mechanical properties than other sausage casings, making them the preferred skin material for cooked and/or smoked products (*Sreenath and Jeffries*, 2011). Cellulose is a fibrous material that plays a structural role in plants, where it withstands large osmotic pressure and has a load-bearing function (*Savić and Savić*, 2016). Cellulose is another glucan, but unlike α-amylose and amylopectin, the glucose units are linked by β(1–4) bonds. When heated, cellulose fibers break down at 180°C (without melting) and burn at temperatures exceeding 290°C. Cellulose does not dissolve in water, diluted acids, or in many organic solvents (*Thompson et al.*, 1995).

2. Materials and methods

Cooked sausages were produced by thermal treatment of the stuffed batter in an artificial cellulose casing (Figure 1). During filling, care was taken to ensure that the casing was well filled with stuffing, without damage, deformations, or creases.

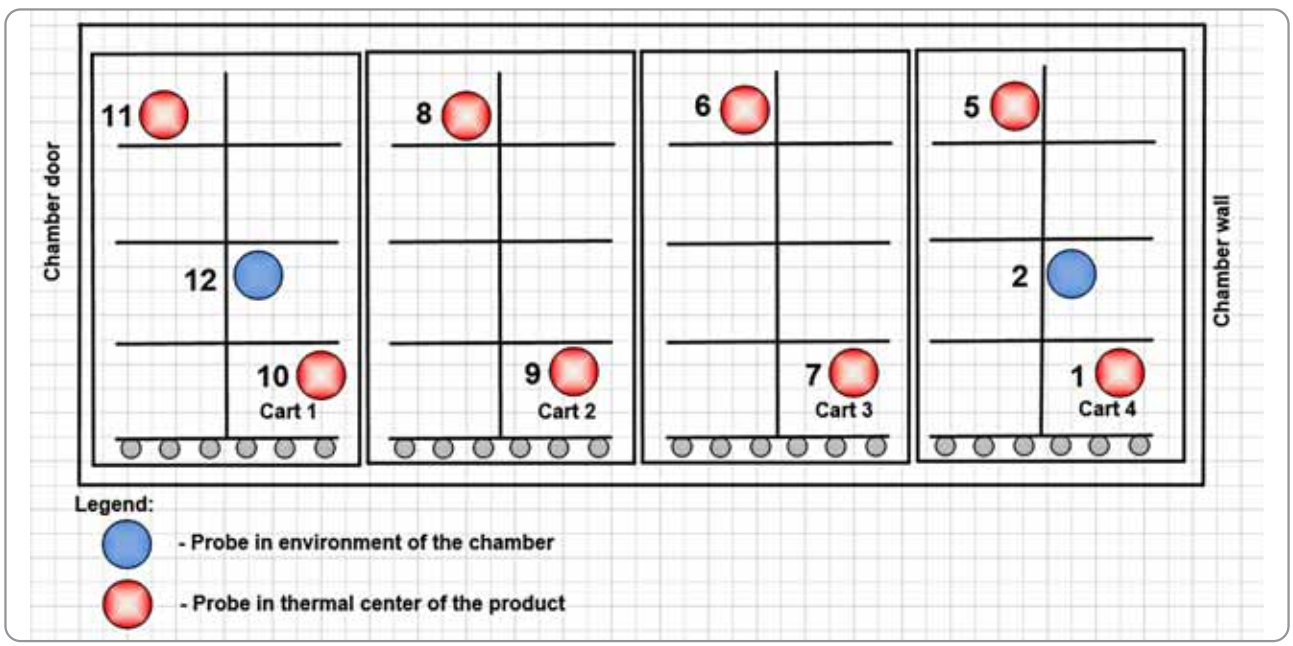


Figure 1. Stuffed batter in artificial cellulose casing placed on the cart before pasteurization

Figure 2. Cooked sausages on the cart after pasteurization

Figure 3. Plan of placement of probes in the thermal center of the product (red color) and in the autoclave medium — environment of the chamber (blue color), seen from the side

There were no air bubbles, gelatinous mass, or separated fat under the casing. The production of meat batter is the most sensitive operation in the production process of cooked sausages. The basic ingredient of cured sausages, in addition to the meat batter, is fat tissue. The sequence of adding the additives is of particular importance when making the filling for cooked sausages. First, phosphate is added (its role is to relax the myosin complex in the muscle tissue so that the proteins can bind water), then nitrite salt, and finally starch is added to draw water.

Figure 4. Arrangement of probes on the cart with the product in the chamber

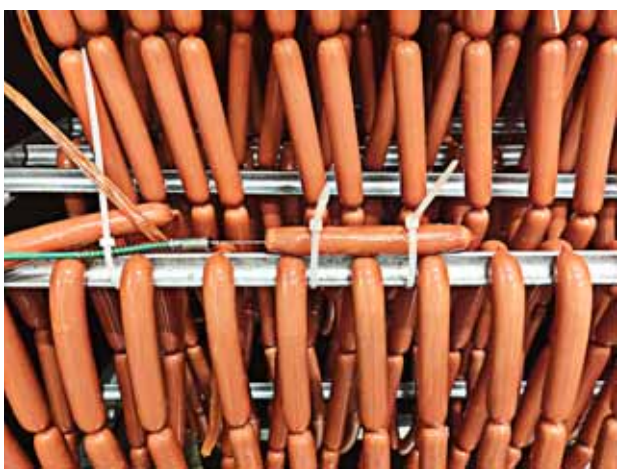


Figure 5. Probe placed in the thermal center of the product on a cart

The basis of quality is quality raw material. Fresh meat intended for the production of cured sausages retains its freshness if the number of microorganisms on its surface is small and if they cannot multiply in further processing. That is why the initial level of contamination in fresh meat as a raw material is decisive for the fresh and desirable taste of cured sausage (*Brauer*, 2010).

The heat treatment process consisted of several production steps, according to the previously set regime in chamber no. 1, Maurer type. The production steps consisted of the following stages:

1. Cooking up to 55°C, medium chamber, in an atmosphere of saturated water vapor for 10 minutes
2. Drying at 55°C for 35 minutes
3. Smoking and cooking at 55°C, medium chamber for 10 minutes
4. Drying at 55°C for 10 minutes
5. Cooking at 78°C, medium chamber in an atmosphere of saturated water vapor, until reaching 74°C in the thermal center of the product, for 15 minutes

The total time of heat treatment was 80 minutes.

Produced in this way, the cooked sausages were tender and juicy, with a pleasant characteristic taste that was complemented by the smell of smoke and spices (Figure 2)

Measurements were carried out in the heat treatment chamber during the regular production of finely chopped sausage in a cellulose casing. Measurements were performed with Ellab thermocouple (E-Val Pro, serial number 411982, validated software — US FDA, 21 CFR part 11, GMP, ver. 4.6.1.0), and the Ellab software, Val-Suite Prover, was used to prepare the technical report, 5.2.015.

Thermocouples with compensating cables were used, a total of 10 probes, of which 8 were placed in the thermal center of the product, in accordance with FAO recommendations (Channels 1, 5, 6, 7, 8, 9, 10, and 11), while 2 were placed in the medium (Channels 2 and 12) as depicted in Figures 3, 4, and 5.

By placing the probes in the manner shown in Figure 4, the possible falling out of the probe was

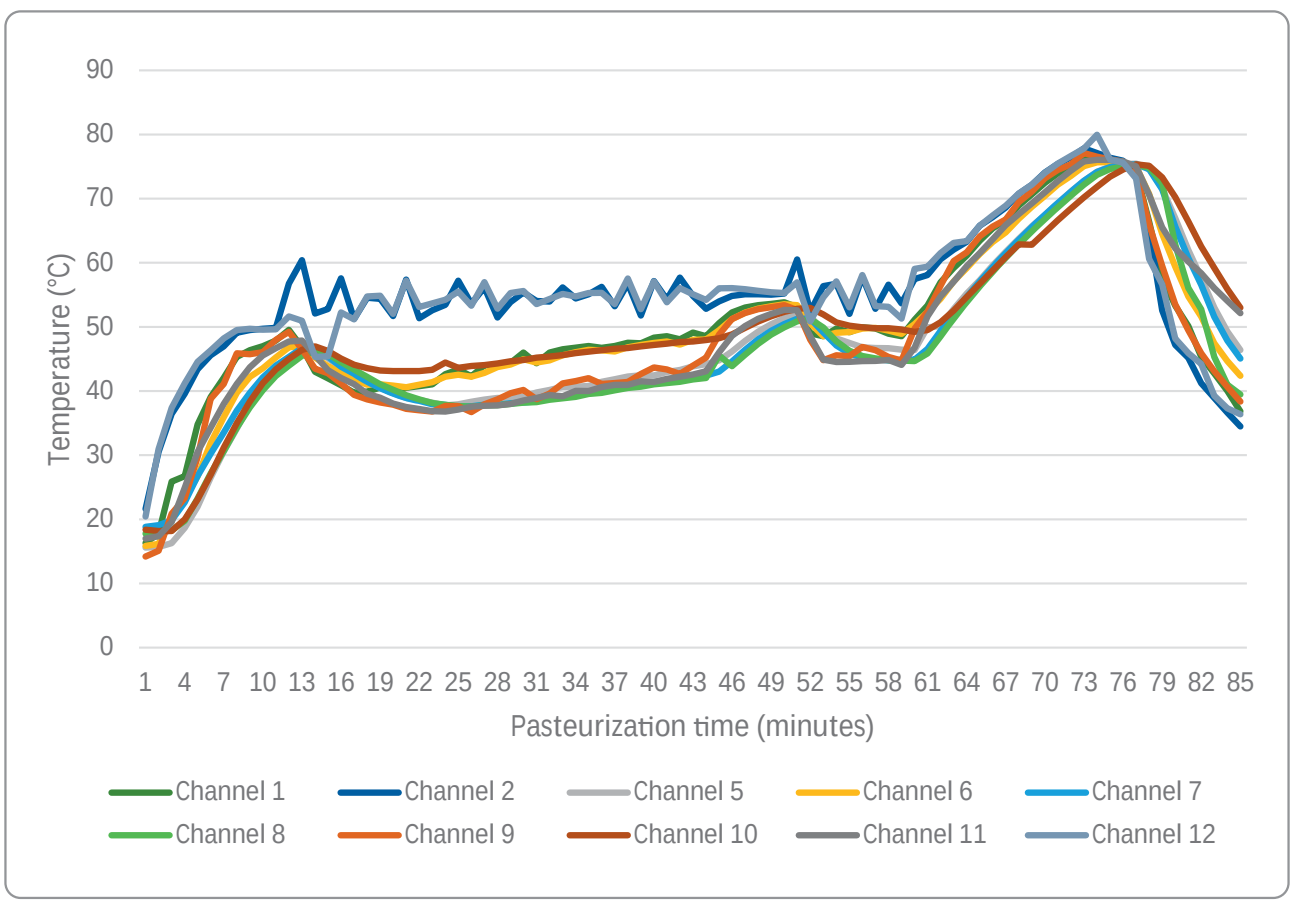


Figure 6. Temperature in the thermal center of the product and in the chamber environment during the pasteurization process

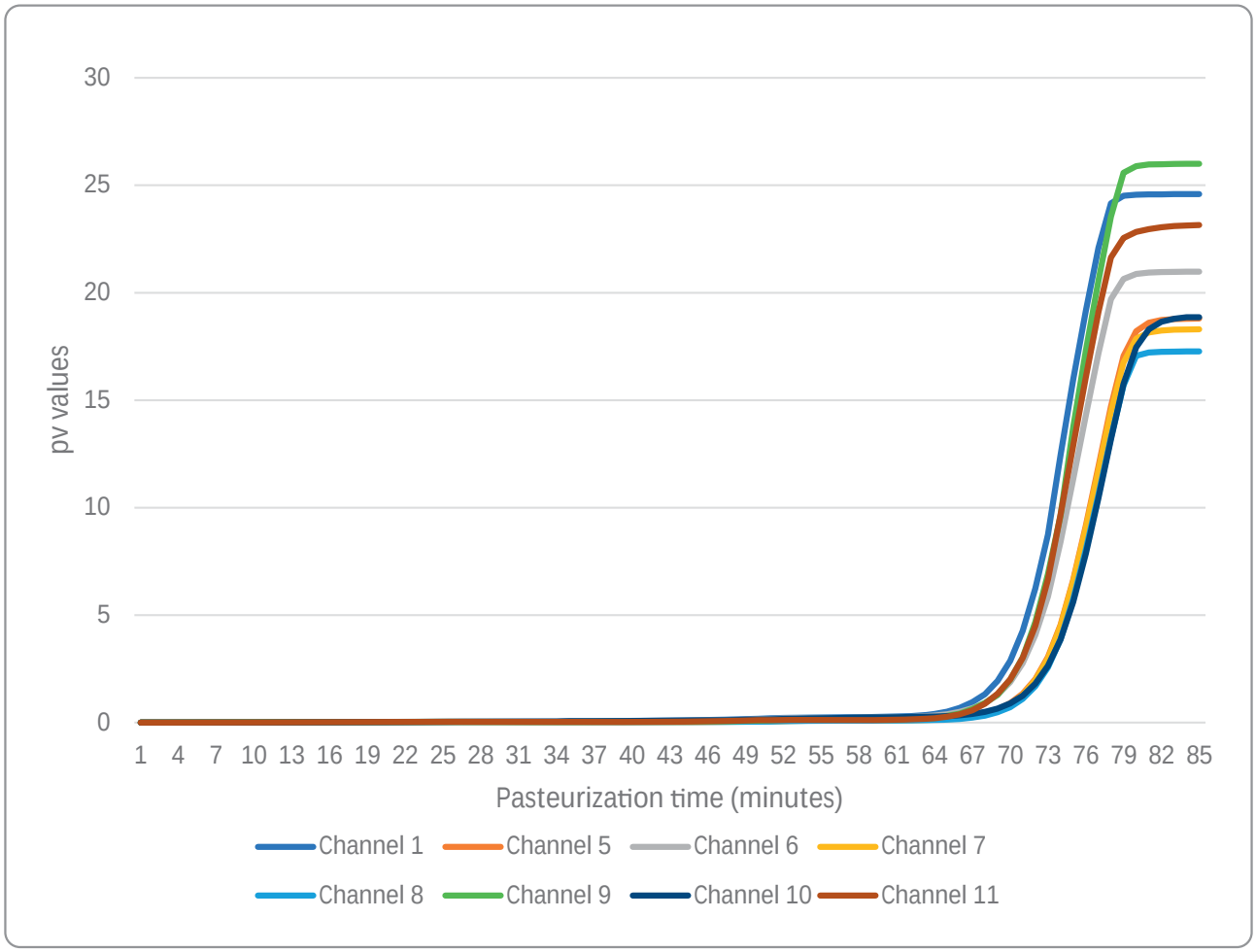


Figure 7. pv value during the pasteurization process

prevented; this can occur because the cellulose coating breaks very easily.

Temperature and pv values were recorded at one minute intervals.

3. Results and discussion

The process of monitoring the pasteurization of cooked sausages of a narrower diameter (21 mm) is presented Figures 6 and 7. Figure 6 presents the temperature in the thermal center of the product and in the chamber environment during the heat treatment. Figure 7 shows the pv values during the procedure monitoring.

The conclusions of monitoring the pasteurization process of cooked sausages of a narrower diameter (21 mm) are presented in Table 1.

Analyzing the obtained results, we can conclude that the process of heat treatment of finely ground sausages in a cellulose casing was uniform considering the obtained results (for 8–9 minutes, the thermal center of the product was at a temperature above 70°C and for 18–20 minutes, was at a temperature above 55°C). The maximum temperatures reached in the thermal center of the product were from 75.26°C to 77.11°C, which is in accordance with the legal regulations (*Official Gazette of the Republic of Serbia*, 2019). In this way, the safety of the product that was pasteurized under the given regime was ensured.

The use of p-value in the optimization of pasteurized sausages confirms and ensures the safety of the product in the defined storage conditions (*Raseta, et al.,* 2021). Given that the diameter of the sausage was small, 21 mm, heat penetration took place intensively during heat treatment, as did heat loss during cooling. Therefore, there was not enough time to generate a pv value for 40 minutes. The obtained pv values ranged from 18.8 minutes (Channel 5) to 26 minutes (Channel 9). This is the very reason the pv value is not suitable as a safety parameter for sausages with a narrower diameter. Also, the heat treatment regime included pasteurization of this product for 18 minutes (Channels 1, 7, 8, and 9), 19 min-

Table 1. Measurement results obtained by monitoring pasteurization in chamber no. 1. Maurer type

Probe channel	Position of the probe	Max. achieved core temperature (°C)	Time spent above 70°C (minutes)	Time spent above 55°C (minutes)	pv values (minutes)
1	Thermal center	76.69	9	18	24.59
2	Environment of the chamber	80	/	/	/
5	Thermal center	75.46	8	19	18.80
6	Thermal center	75.71	9	19	20.98
7	Thermal center	75.36	8	18	18.30
8	Thermal center	75.26	8	18	17.27
9	Thermal center	77.11	9	18	26.00
10	Thermal center	75.35	9	18	18.89
11	Thermal center	76.06	9	19	23.15
12	Environment of the chamber	80	/	/	/

utes (Channels 5, 6, and 10), and 20 minutes (Channel 11). Out of a total of 80 minutes of the heat treatment program, the finely chopped sausages spent only 18–20 minutes at pasteurization temperatures. Also, due to the porosity of the cellulose casing, during drying and smoking operations and during heat treatment, the product loses water and acquires a more desirable color. The cart with the product was weighed before being sent to heat treatment and after the pasteurization process was completed. The average weight of the cart was 95.36±0.25 kg before heat treatment and 85.52±0.24 kg after heat treatment. The measurements were made on a floor scale in the heat treatment room.

4. Conclusion

Thermal pasteurization is an important unit operation to the food industry for producing high-quality foods with extended shelf-life in refrigeration or cold storage

Ensuring the safety of products with a narrower diameter is achieved only by confirming the required temperature in the thermal center of the product, and not by confirming pv values.

To ensure the safety and quality of pasteurized cooked sausages within the defined shelf life, they need to be under cold chain conditions (from 0° to 4°C) during storage, transport, and trade.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Balamurugan, S., Inmanee, P., De Souza, J., Strange, P., Pirak, T. & Barbut, S. (2018). Effects of high pressure processing and hot water pasteurization of cooked sausages on inactivation of inoculated *Listeria monocytogenes*, natural populations of lactic acid bacteria, *pseudomonas* spp., and coliforms and their recovery during storage at 4 and 10°C. *Journal of Food Protection*, 81(8), 1245–1251, https://doi.org/10.4315/0362-028X.JFP-18-024

Brauer, H. (2010). Technology for Boiled Sausage Production. Allgemeine Fleischer Zeitung Deutscher Fachverlag, 23.

Ebunoluwa, A. S., Farell-Clarke, H., Lane, M. M., Cappello, P., Hairston, R., McCroskey, A., Prybolsky, L., Schnitzler, J., Van Winkle, J., Miller, D. & Armstrong, B. (2022). Flavour intensity and acceptability evaluation of smoked sausages. *Meat Technology*, 63(2), 79–84, https://doi.org/10.18485/meattech.2022.63.2.1

Fellows, P. J. (2009). Pasteurization. Food Processing Technology (Third edition) Principles and Practice Woodhead Publishing Series in Food Science, Technology and Nutrition, 381.

Fellows, P. J. (2022). Pasteurization. Food Processing Technology (Fifth edition) Principles and Practice Woodhead Publishing Series in Food Science, Technology and Nutrition 2009, 347.

McCormick, K. E., Han, Y. I., Acton, C. J., Sheldon, W. B. & Dawson, L. P. (2006). In package pasteurization combined with biocide impregnated films to inhibit *Listeria monocytogenes* and *Salmonella typhimurium* in turkey bologna. *Journal of Food Science*, 70(1), 52–57, https://doi.org/10.1111/j.1365-2621.2005.tb09046.x

Marchello, M. & Garden-Robinson, J. (2017). Food and Nutrition. In The art and practice of sausage making, 176, Fargo, North Dakota: NDSU Extension Service

Official Gazette of the Republic of Serbia (2019). Rulebook on the quality of minced meat, semifinished products and meat products 50.

Raseta, M., Jovanović, J., Becskei, Z., Branković-Lazić, I., Mrdović, B. & Djordjević, V. (2021). Optimization of pasteurization of meat products using pasteurization values (p-values). 61st International Meat Industry Conference IOP Conf. Series: Earth and Environmental Science 854, https://doi.org/10.1088/1755-1315/854/1/012079

Savell, J. W. & Smith, G. C. (2009). Meat Science Laboratory, Manual 8th Edition. American Press, Boston, M.A., USA, 109–128.

Savić, Z. & Savić, I. (2016). Sausage casings, Victus International GmbH Vienna, 2nd edition, chapter 7, 296.

Sreenath, H. K. & Jeffries, T. W. (2011). Interactions of fungi from fermented sausage with regenerated cellulose casings. *Journal of Industrial Microbiology and Biotechnology*, 38(11), 1793–1802, https://doi.org/10.1007/s10295-011-0966-z

Tompson, R. C., Susan, L. I., Mikos, G. A. & Langer, R. (1995). Polymers for biological Systems (in R.A. Meyers: Molecular Biology and Biotechnology: VCH Publishers, Inc.: New York-Weinheim-Cambridge).

Topel, D. G., Marple, D. N., Lonergan, S. M. & Parrish, Jr. F. C. (2013). The Science of Animal Growth and Meat Technology, 180–199.

Vuković, I. (2012). Osnove tehnologije mesa, 4. izdanje, Veterinarska komora Srbije, poglavlje 15, 223.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.514.5.065(497.11)

ID: 126633225

https://doi.org/10.18485/meattech.2023.64.2.88

Original scientific paper

Microbiological status of minced meat at retail in Belgrade district

Nikola Betić[a], Ivana Branković Lazić[a], Lazar Milojević[a], Ivan Vićić[b], Nedjeljko Karabasil[b], Nenad Parunović[a] and Vesna Đorđević[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13 11000 Belgrade, Republic of Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Minced meat
Retail
Pork
Beef
Belgrade

ABSTRACT

This study was conducted to determine the microbiological status of 390 beef and pork minced meat samples collected over three years from 52 retailers in the territory of Belgrade. The numbers of aerobic colony counts, *Escherichia coli*, and the presence of *Salmonella* spp. are prescribed criteria for this group of meat semi-products. *Salmonella* spp. was confirmed in one sample of minced beef meat (0.8%), while unsatisfactory *E. coli* counts were only determined in pork meat samples (2.7%). In 2021, all samples complied with the microbiological criteria for minced meat The highest occurrence of positive samples was observed during the III quarter of 2022 (P=0.04) with a frequency of 9.3%. The level of contamination of minced pork with *E. coli* bacteria in the same quarter was significantly higher compared to the II quarter of 2022 (627±75 vs. 292±9 cfu/g, P=0.009). Improvement of process hygiene and revision of process control, along with permanent education of food staff on the principles of GMP and GHP, are necessary for maintaining food safety and public health.

1. Introduction

Foodborne pathogens are a leading cause of disease and death in developing countries. According to the World Health Organization (WHO), the global burden of foodborne diseases is estimated at 600 million people (*WHO*, 2014). Foodborne diseases are preventable if food protection principles are followed from primary production to the consumer level. Changes in eating habits, mass catering, unsafe food storage conditions, and poor hygiene practices are significant contributors to foodborne illness. The cold chain must be cohesive for temperature-sensitive products and meat semi-products. If only one link is interrupted in the chain, that causes irreversible damage and these products or semi-products could be unsafe for consumers (*Betic*, 2019).

In Serbia, food business operators (FBOs) have been obliged, according to the Law of Food Safety (*Serbia*, 2019), to adhere to good hygiene and good production practices as prerequisites for hazard analysis and critical control point (HACCP) programs. Within the implemented HACCP, the FBOs must also have a self-control plan, proving that they control the system. HACCP, with its prerequisite programs, good manufacturing practice (GMP) and good hygiene practices (GHP), requires the FBOs to identify potential hazards that threaten to endanger the safety of the product in order to eliminate them and keep them under control.

***Corresponding author:** Nikola Betić, *nikola.betic@inmes.rs*

Paper received July 31st 2023. Paper accepted August 8th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

This study aimed to determine the microbiological risks and compliance with the applied HACCP in minced pork and beef purchased in retail stores and in front of the customer on the spot in the territory of the city of Belgrade.

2. Materials and methods

Sample collection was undertaken from January 2020 to December 2022 (Table 1). During this period, 390 samples were collected at 52 retailers throughout Belgrade, i.e., 127 samples of minced beef and 260 samples of minced pork. Samples were taken according to the self-control plan prescribed by FBOs; the dynamics ranged from monthly to quarterly, depending on the size of the facility. The raw meat came from two different slaughterhouses in Serbia. Minced meat samples (500 g from each cut and minced at the butchery) were also collected in 5 sterile plastic bags from each retailer.

After sampling, the samples were conditionally stored in hand refrigerators (4°C) and transported to the Institute of Meat Hygiene and Technology in Belgrade for immediate analysis. Minced meat was analysed using standard accredited methods, SRPS EN ISO 6579-1:2017 (*Salmonella* spp.), SRPS EN ISO 16649-2:2008 (*Escherichia coli*), SRPS EN ISO 4833-1:2014 (aerobic colony count). Each meat product placed on the market must, during its shelf life, meet the food safety criteria clearly defined by the Regulation in the EU (*EU*, 2005), with which the legal regulations in our country are harmonized (*Serbia*, 2010, 2018). Limits for food safety and process hygiene test results for minced meat are shown in Table 2.

The statistical analyses of the results were performed using SPSS 21. The frequency of positive results, considering meat type and sampling years and quarter periods, was calculated using a chi-square test. Differences in *E. coli* contamination levels were analysed with one-way ANOVA.

3. Results

During three years, 390 samples were collected from 52 retailers from the territory of the Belgrade district. The mandatory criteria for minced meat were not complied with for 2.1% (8/390) of investigated samples (Table 3). Of this, 0.8% (1/127) of beef minced meat was defective, while 2.7% (7/260) of pork samples tested positive. Considering the meat type (P=0.22) and sampling year (P=0.09), there was no difference in the occurrence of the non-compliant samples. In beef

Table 1. Sampling scheme during the three years

Year	I quarter	II quarter	III quarter	IV quarter	Total
2020	27	7	21	23	78
2021	25	31	28	30	114
2022	32	59	54	53	198
Total	**84**	**97**	**103**	**106**	**390**

Table 2. Limits for minced meat according Regulation EU and Serbian law

Microorganisms	Sampling plan*		Limits		Analytical reference method	Stage where the criterion applies
	N	c	M	M	Analytical reference method	Stage where the criterion applies
***Salmonella* spp.**	5	0	Absence in 10 g		EN ISO 6579	Products placed on the market during their shelf-life
Escherichia coli	5	2	50 cfu/g	500 cfu/g	EN ISO 16649-1 or EN ISO 16649-2	End of the manufacturing process
Aerobic colony count	5	2	$5x10^5$ cfu/g	$5x10^6$ cfu/g	EN ISO 4833	End of the manufacturing process

*n = number of units comprising the sample; c = number of sample units giving values between m and M.

minced meat, *Salmonella* spp. was determined during the III quarter of 2022, and was present in 3/5 of the tested meat samples. In contrast, the presence of *E. coli* bacteria in all non-compliant samples was predominant in minced pork meat, in all five examined units of these samples. It was observed that during 2021, all samples met the microbiological criteria for minced meat.

Concerning the results from the research by *Mrdovic et al.* (2019) in which the presence of *Salmonella* spp. was detected in 1.6% of the examined samples, as was the presence of *E. coli* in 5% of the tested samples, the results in this study showed a lower incidence of *Salmonella* spp. as well as of *E. coli* prevalence. On the other hand, the results obtained in this study showed a higher presence of pathogens than in Ireland, where the prevalence of *Salmonella* spp. was 0.1% and *E. coli* occurred in 0.2% of similar products (*Ireland,* 2013). Regarding data from 2010, published by EFSA and ECDC and originating in 12 countries in the EU, 2.8% of such meat preparations contained *Salmonella* spp., and 0.6% contained *E. coli* (*EFSA and ECDC*, 2012).

The frequency of positive samples during the quarter periods was statistically significant (P=0.01), with the highest occurrence during the III quarter of 2022 (Table 4). In the given period, all samples with

Table 3. The results of microbiological criteria testing for minced meat

	Satisfactory (%)	Unsatisfactory (%)	Total (100%)	*P* value
Pork meat	256 (97.3)	7 (2.7)	263	0.22
Beef meat	126 (99.2)	1 (0.8)	127	
2020	77 (98.7)	1 (1.3)	78	
2021	114 (100)	0 (0.0)	114	0.09
2022	191 (96.5)	7 (3.5)	198	
I quarter	84 (100)	0 (0.0)	84	
II quarter	95 (97.9)	2 (2.1)	97	0.01
III quarter	97 (94.2)	6 (5.8)	103	
IV quarter	106 (100)	0 (0.0)	106	
Total	**382 (97.9)**	**8 (2.1)**	**390**	

Table 4. The results of microbiological criteria testing of minced meat during the quarter periods of year

		Satisfactory (%)	Unsatisfactory (%)	Total (100%)	P value
2020	I quarter	27 (100)	0 (0.0)	27	0.43
	II quarter	7 (100)	0 (0.0)	7	
	III quarter	20 (95.2)	1 (4.8)	21	
	IV quarter	23 (100)	0 (0.0)	23	
2021	I quarter	25 (100)	0 (0.0)	25	-
	II quarter	31 (100)	0 (0.0)	31	
	III quarter	28 (100)	0 (0.0)	28	
	IV quarter	30 (100)	0 (0.0)	30	
2022	I quarter	32 (100)	0 (0.0)	32	0.04
	II quarter	57 (96.6)	2 (3.4)	59	
	III quarter	49 (90.7)	5 (9.3)	54	
	IV quarter	53 (100)	0 (0.0)	53	

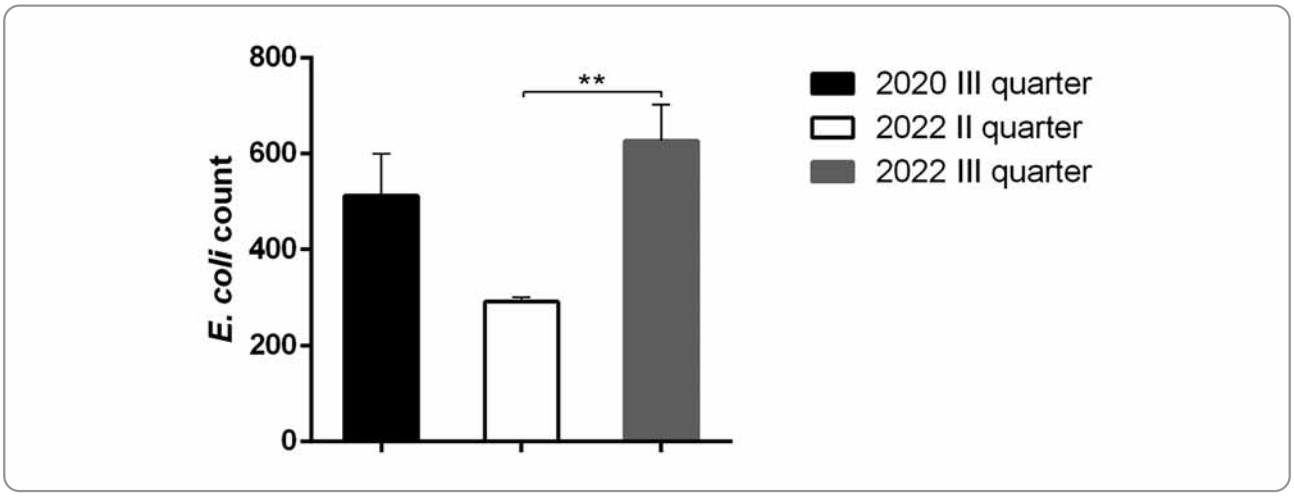


Figure 1. Level of contamination of minced pork with *Escherichia coli*

E. coli contamination were pork. Considering the contamination level of pork meat with *E. coli*, positive samples from the III quarter had a higher count than those from the II quarter of 2022 (627±75 vs. 292±9 cfu/g, P=0.009) (Figure 1). However, the same pork meat sampled in the III quarter of the 2020 year, in addition to a high mean *E. coli* contamination level (512±88 cfu/g), also had a high level of aerobic colony count (1.02±0.06 x 10^6 cfu/g). Consequently, most of the samples that did not comply with the required criteria were sampled during the III quarter, which was in the summer period of the year. The summer period is more challenging for maintaining the cold chain, which must not to be interrupted at any time along the meat distribution chain, in the continuum from slaughterhouse to consumer (*Nastasijević et al*., 2017).

4. Conclusion

This study showed the presence of *Salmonella* spp. in one sample of minced beef meat and *E. coli* in seven samples of minced pork meat. FBOs must improve process hygiene and review process control, and permanent education of food staff on the principles of GMP and GHP, like the pre-requisite programs of HACCP, is necessary. Particular attention should be given to the storage of foods. Fresh meat is highly perishable and has a short shelf life, which means the time available for product distribution is also short. If fresh meat and meat products are not kept at adequate, controlled temperatures, they can be excellent environments for pathogenic and other bacterial growth.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The work was supported by grants from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia to the Institute of Meat Hygiene and Technology (contract no. 451-03-47/2023-01/200050), and to the Faculty of Veterinary Medicine, Belgrade (contract no. 451-03-47/2023-01/200143).

References

Betic, N., Brankovic Lazic, I. & Nastasijevic, I. (2019). Biological hazards in the pork chain continuum: Risk mitigation strategy. *Meat Technology*, 60(2), 106–120.

EFSA and ECDC, (2012). *EFSA Journal*, 10(3), 2597, www.efsa.europa.eu/en/efsajournal/doc/2597.pdf

EU, (2005). Commission Regulation 2073/2005 on microbiological criteria for foodstuffs.

Ireland, (2013). Food safety authority of Ireland 2013, https://mail.inmes.rs/?_task=mail&_action=get&_mbox=INBOX&_uid=3326&_part=5&_fr ame=1&_extwin=1

Mrdovic, B., Nastasijevic, I., Lazic, I. B., Jovanovic, J., Nikolic, A., Petrovic, Z. & Raseta, M. (2019). Examination of meat preparations in order to control process hygiene in retail. In IOP Conference Series: Earth and Environmental Science, 333 (1).

Nastasijević, I., B. Lakićević & Z. Petrović. (2017). Cold chain management in meat storage, distribution and retail: A review. IOP Conference Series: Earth and Environmental Science, 85 (1).

Serbia, (2010). Ordinance on general and specific food hygiene requirements at any stage of production, processing and trade Official Gazette of the Republic of Serbia No 72/10.

Serbia, (2018). Amendments to the Ordinance on general and specific food hygiene requirements at any stage of production, processing and trade Official Gazette of the Republic of Serbia No 68/18.

Serbia, (2019). Law on Food Safety Official Gazette of the Republic of Serbia No 41/2009; 17/2019.

SRPS ISO 21528-2:2017, (2017). Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* — Part 2: Colony-count technique

SRPS ISO 4833-1:2014, (2014). Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique.

SRPS ISO 6579-1:2017, (2017). Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.

WHO, (2014). WHO estimates of the global burden of diseases, 46, 1–15.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.07
664.93.035

ID: 126626825

https://doi.org/10.18485/meattech.2023.64.2.89

Original scientific paper

Phosphate additives in meat products: analytical determination and interpretation of results

Radivoj Petronijević[a], Čaba Silađi[a], Danijela Vranić[a], Srđan Stefanović[a], Danka Spirić[a], Jelena Ćirić[a] and Aleksandar Bajčić[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Phosphate food additives
Ion chromatography
Analytical determination
Meat products

ABSTRACT

Analytical determination of phosphate additives in food presents some difficulties that are not only related to the applied analytical technique, but also to the ingredients used in production. Meat, by itself, contains organically bound phosphorus, but also inorganic phosphates. Even water used in production can contain certain amounts of inorganic phosphates. That is why a measurable quantity of phosphate exists in products even without the addition of permitted phosphate additives.

By applying ion chromatography to determine the total inorganic phosphate content in meat products, it was found that the greatest number of products that did not comply with the regulation were those in which phosphate additives are seldomly added. About one fifth of analysed fermented sausages and about one quarter of dried meat products contained phosphates at levels that exceeded the maximum permitted level, expressed as P2O5. The main reason may be that these products go through a drying phase during production, whereby, with the decrease in the amount of water in them, the share of other ingredients, as well as phosphate, increases.

1. Introduction

In humans and animals, phosphorus is involved in various physiological processes, such as acid-base balance regulation, it is a crucial part of the cell's structural and energy cycle, is a regulating and signalling component and has a significant role in the mineralization of teeth and bones. The versatility of the physiological aspects of phosphorus made it an essential nutrient with serious impact on metabolism of humans and, in general, all living beings (*EFSA*, 2015).

A wide range of physiological uses of phosphorus is possible because it exists in various inorganic and organic forms in the human body. Phosphorus is mainly present as inorganic phosphates in serum and intracellular fluids, while bones contain apatite forms, and soft tissues and extracellular fluids contain organic phosphates (*EFSA*, 2015).

Excess phosphorus/phosphate intake can have adverse effects, such as hyperphosphatemia, hyperparathyroidism, skeletal deformations, bone loss and ectopic calcification (*Ritz et al.*, 2012). Considering all relevant and available scientific data regarding phosphorus dietary intake, EFSA set the adequate intake (AI) of phosphorus to 160 mg/day for infants aged 7–11 months, between 250 and 640 mg/day for children, and 550 mg/day for adults (*EFSA*, 2015).

***Corresponding author:** Srđan Stefanović, *srdjan.stefanovic@inmes.rs*

Paper received July 31st 2023. Paper accepted August 8th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Along with naturally present forms of phosphorus, many processed foods contain several phosphate food additives. Phosphates are very important additives in the meat industry, because they improve water retention capacity, emulsification, oxidative and microbiological stability, softness and juiciness of meat products (*Pinton et al*., 2021). Current EU and national legislation on food additives (*EC 1333/2008*, 2008*; Sl. Glasnik RS 53/2018*, 2018), authorised the most relevant group of phosphate additives as "phosphoric acid — phosphates — di-, tri- and polyphosphates", and assigned E numbers 338–452. These additives are the most common source of inorganic phosphates, beside naturally present phosphates, in food. Legislation (*EC*, 1333/2008, 2008; *Sl. Glasnik RS 53/2018*, 2018) also establishes maximum permitted levels of phosphate additives in food. In general, levels of E338-452 additives in meat products are authorised up to 5000 mg per kg (0.5%). According to the legislation, these additives may be added individually or in combination, and their maximum level has to be expressed as P_2O_5.

Analytical determination of phosphates in meat products includes various physical and chemical techniques. Gravimetric, inductively coupled plasma (ICP) and spectrophotometric methods can only be used for direct determination of total phosphorus. Methods based on thin layer (TLC) and ion chromatography (IC), nuclear magnetic resonance (NMR) and electrophoresis have the ability to identify and quantify different inorganic phosphate ions, being more suitable for direct determination of phosphate additives in meat products (*EFSA*, 2019). On the other hand, methods for determination of total phosphorus were used for calculation and estimation of added phosphates in meat products in conjunction with determination of protein content (*ISO 937:1978*, 1978; *Dimitrovska et al.*, 2019). All mentioned techniques and methodologies have their advantages and flaws for the determination of the phosphate content in meat products. TLC, ion chromatography and capillary isotachophoresis measure phosphates directly, but may not be able to differentiate between added and naturally occurring phosphates. Other methods, such as NMR, thermo-differential-photometry and microwave dielectric spectroscopy, are not used for routine determination of added phosphates, and have the same limitation regarding phosphate origin (*Campden BRI Report*, 2012). Also, all reported instrumental techniques are unable to quantify polymerised phosphate higher than triphosphate. Of all available instrumental methods, IC is the most commonly used. However, IC cannot be successfully applied to the full range of foodstuffs permitted to contain phosphate additives (*EFSA*, 2019). For the simultaneous determination of phosphates and condensed phosphates using IC, systems employing gradient elution conditions with suppressed conductivity detection is needed (*Metrohm*, 2019). It is recommended that sample preparation times should be as short as possible and should include steps to deactivate phosphatase enzymes (*EFSA*, 2019).

The aim of this research was to point out the specific difficulties in interpreting results and discrepancies that occur during implementation of the current legislation regarding phosphate additives, based on results from applying the gradient ion chromatography method to determine the phosphate content of meat products.

2. Materials and methods

2.1. Chemicals

All standard chemicals and reagents were purchased from Merck KgaA, Darmstadt, Germany. Ultrapure water, ≥18 MΩ, were obtain by ELGA DV-25 and ELGA Ultrapure (LabWater, Lane End, High Wycombe, UK).

2.2. Samples

Meat products were part of regular controls of food quality and safety parameters, obtained from retail and from producers and importers. The research included 440 samples: 119 cooked sausages, 14 fresh sausages, 35 bacons, 117 fermented sausages, 90 dried and 65 smoked meat products.

2.2.1. Sample preparation

Samples for determination of total phosphorus were prepared according to the procedure described in the corresponding reference method (*ISO 13730:1996*, 1996). Preparation of samples for IC determination included extraction of phosphates from each homogenised sample with hot ultrapure water, centrifugation and filtration of the extract after cooling.

2.3. Methods

2.3.1. Total phosphorus content

Total phosphorus in the meat products was determined according to the spectrophotometric procedure described in the reference method (SRPS ISO 13730:1999, i.e. *ISO 13730:1996*, (1996)).

2.3.2. Ion chromatography

IC with conductometric detection was used for phosphate determination in the meat products. The IC system consisted of an 858 Professional Sample Processor, 930 Compact IC Flex with Oven/SeS/PP, and Conductivity Detector, all from Metrohm AG, Herisau, Switzerland. The separation column was Metrosep A Supp 7 250/4.0, also from Metrohm, and separation of anions was achieved by a mobile phase gradient in accordance with the original method provided by manufacturer (*Metrohm*, 2019).

2.4. Statistics

Preparation of data and statistical analysis were performed in MS Office 2016 Excel.

3. Results and discussion

The IC method, originally designed for determination of chlorates, thiocyanates, thiosulphates and perchlorates along with fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulphite and sulphate (*Metrohm*, 2019), was validated for determination of phosphate, diphosphate and triphosphate in meat products. Chromatograms of a meat product with declared added diphosphate and polyphosphate and the standard solution of phosphate, diphosphate and triphosphate, concentrations of 1 mg per litre each, are shown in Figure 1.

Several factors should be taken into consideration in interpretation of results. First of all, IC, as well as any other instrumental technique, cannot separate condensed phosphates higher than triphosphate (*EFSA*, 2019). However, according to the same source, polyphosphates (especially in aqueous solution such as the environment within meat products) are constantly degraded through their tri- and di-forms to phosphates, deeming measurement of individual ion species meaningless. This explains the legal requirement for the maximum permitted level of "phosphoric acid — phosphates — di- tri- and polyphosphates" to be expressed as P_2O_5, with the general remark that "The additives may be added individually or in combination". In that context, it should be noted that referring to this (or any other) analytical

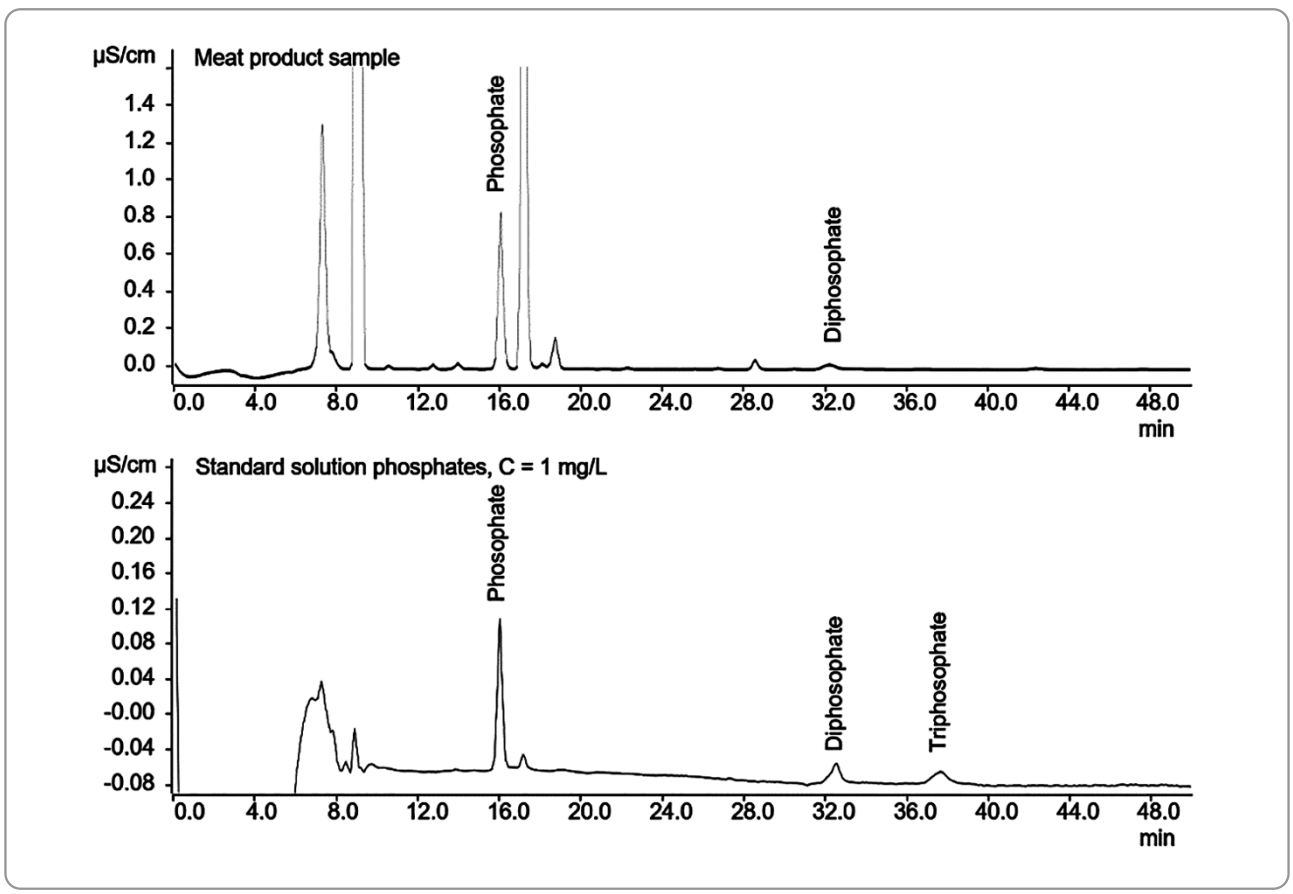


Figure 1. Chromatograms showing phosphate in a meat product and in the standard solution of mixed phosphate, diphosphate and triphosphate, each 1 mg per litre.

procedure for phosphate measurement as "determination of added phosphates", which can often be heard in both expert and lay circles, is misleading. Only total phosphate ions are measured. Their origin in the sample can be either from their natural presence in raw material (including, e.g., from water), or from utilization of phosphate-based food additives. However, the specific measurement of additive-originating phosphates (although desirable) is currently not required by the legislation, precisely for that reason. Maximum permitted levels are set at 5 g per kg for practically all groups of meat products taking into account both (relatively constant) natural presence and the technologically justified utilization of phosphate-based additives.

On the other hand, total phosphorus content, the parameter designed to monitor usage of phosphate-based additives, became less significant with the application of IC and was removed from the European Union legislation. In Serbia though, the national regulation on quality of meat products still sets the maximum permitted level of phosphorus at 8 g per kg (expressed as P_2O_5). This can explain the wrong perception of IC methods as tools for determination of "added" phosphates.

Results of determination of phosphates and total phosphorus content in meat products according to current legislation (*EC 1333/2008*, 2008; *Sl. Glasnik RS 53/2018,* 2018; *Sl. Glasnik RS 50/2019*, *34/2023*, 2023) are presented in Table 1.

As previously mentioned, usage of phosphates is authorised by EU and Serbian regulations on food additives (*EC, 1333/2008*, 2008; *Sl. Glasnik RS 53/2018*, 2018), and in Serbia, total phosphorus and protein contents were set by regulation on the quality of minced meat, meat semi products and meat products (*Sl. Glasnik RS 50/2019, 34/2023*, 2023). The maximum amount of total phosphorus in all meat products in Serbia was limited to 8 g per kg, expressed as P_2O_5.

Considering the number of non-compliant results for phosphates, the greatest number of such meat products were classified in two groups, fermented sausages and dried meat products. Current Serbian regulation (*Sl. Glasnik RS 50/2019, 34/2023*, 2023) does not require determination of total phosphorus in these categories, because these products have been dried in the process of manufacturing, and loss of moisture continues during the entire time they are stored. Consequently, the amounts of all other, non-water ingredients increase over time, including the phosphate content. Results of quality parameters determined in these products show that most of the non-compliant products were of good quality, but, according to Serbian and EU legal requirements (*EC, 1333/2008*, 2008; *Sl. Glasnik RS 53/2018*, 2018), the phosphate contents in the non-compliant products exceeded the maximum permitted level, regardless of the fact that di- and triphosphates were not detected.

4. Conclusion

Although IC is one of the most suitable instrumental methods for determining and quantifying inorganic phosphates in meat products, it has limitations that prevent its full application for the analysis of phosphate additives (*EFSA*, 2019). Unfortunately, other methods are also not able to fulfil the

Table 1. Phosphates and total phosphorus determined in meat products

Meat product	Total samples	Compliant		Non-compliant	
		Phosphates	Total Phosphorus	Phosphates	Total Phosphorus
Cooked sausages	119	114	115	5	4
Fresh sausages	14	14	14	0	0
Bacon	35	32	34	3	1
Fermented sausages	117	97	-	20	-
Dried meat products	90	68	-	22	-
Smoked meat	65	56	58	9	7
Total	440	381	221	59	12

requirements in this field (*EFSA*, 2019). Bearing in mind the method's limitations and advantages, it can be only said with certainty that IC can determine the total content of inorganic phosphates present in the product at the time of analysis.

From an analytical point of view, since almost every ingredient in a meat product contains some amount of inorganic phosphates (even the water used in production), it would be more suitable to speak of the total inorganic phosphates present in the product, rather than added phosphates.

In general, the category of non-heat-treated processed meats had a higher relative number of meat products that exceeded the maximum permitted level of 5000 mg of phosphate per kg compared to the cooked and fresh sausages (4.2% and 0%, respectively). This was despite the fact that phosphate additives are used less frequently in the production of non-heat-treated processed meats than in the cooked and fresh sausages. Considering that phosphate content naturally increases with drying and storage time, and these products are consumed less often than cooked sausages, it would be advisable to revise the Serbian legislation regarding the maximum permitted levels of phosphate additives in processed meats.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Campden BRI Report, (2012). Review of polyphosphates as additives and testing methods for them in scallops and prawns. BC-REP-125846-01 ISBN no 978-1-906634-60-5.

Dimitrovska, M., Ristovska, G., Chuleva, B. & Dimitrovski, D. (2019). Phosphates as food additives in meat and meat products in North Macedonia. The 60th International Meat Industry Conference MEATCON2019. IOP Conference Series: Earth and Environmental Science 333 (2019) 012054 doi: 10.1088/1755-1315/333/1/012054

EFSA, (2015). EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies) (2015) Scientific Opinion on Dietary Reference Values for phosphorus. *EFSA Journal,* 13(7), 4185, 54 pp. doi:10.2903/j.efsa.2015.4185

EFSA, (2019). EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Furst P, Gurtler R, Husøy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wolfle D, Aggett P, Cupisti A, Fortes C, Kuhnle G, Lillegaard IT, Scotter M, Giarola A, Rincon A, Tard A and Gundert-Remy U, (2019) Scientific Opinion on the re-evaluation of phosphoric acid–phosphates — di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. *EFSA Journal,* 17(6), 5674, 156 pp, https://doi.org/10.2903/j.efsa.2019.5674

ISO 937:1978, (1978). Meat and meat products — Determination of nitrogen content.

ISO 13730:1996, (1996). Meat and meat products — Determination of total phosphorus content — Spectrometric method.

Metrohm, (2019). IC Application Note No. S-305, Chlorate, thiosulfate, thiocyanate, and perchlorate besides standard anions applying a Dose-in Gradient.

Pinton, M. B., dos Santos, B. A., Lorenzo, J. M., Cichoski, A. J., Boeira, C. P. & Campagnol, P. C. B. (2021). Green technologies as a strategy to reduce NaCl and phosphate in meat products: an overview. *Current Opinion in Food Science*, 40, 1–5, Innovations in food science.

Sl. Glasnik RS 50/2019, 34/2023, (2023). Pravilnik o kvalitetu usitnjenog mesa, poluproizvoda od mesa i proizvoda od mesa. *Službeni Glasnik.*

Sl. Glasnik RS 53/2018, (2018). Pravilnik o prehrambenim aditivima. *Službeni Glasnik.*

EC 1333/2008, (2008). Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

Ritz, E., Hahn, K., Ketteler, M., Kuhlmann, M. & Mann, J. (2012). Phosphate additives in food-a health risk. *Deutsches Arzteblatt International*, 109(4), 49–55.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664:658.562.6]:582.28

ID: 126594569

https://doi.org/10.18485/meattech.2023.64.2.90

Review paper

Prevention of mycotoxins' effects — from field to table

Dejan Perić[a], Radmila Marković[a], Stamen Radulović[a], Svetlana Grdović[a], Dragoljub Jovanović[a] and Dragan Šefer[a]*

[a] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Animal nutrition
Production results
Mycotoxins
One health

ABSTRACT

It is assumed that mycotoxins have been present in feed and food since the beginning of eukaryotic fungi's life on Earth. With the recognition of the symptoms of the first intoxications, the so-called mycotoxicosis, there was a desire to find strategies in the fight against secondary metabolites of different types of fungi. The mycotoxins that most commonly contaminate feed are aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN) and fumonisin B1 (FB1). These mycotoxins can primarily cause hepatotoxicity, immunotoxicity, neurotoxicity and nephrotoxicity, and consequently cause adverse effects on animal health and performance. Today, in the 21st century, the need to find a multidisciplinary and integrated plan in the fight against mycotoxins has grown with the realization that mycotoxins cause large-scale damage in livestock. Physical, chemical, biological and nutritional strategies have been developed to combat mycotoxins in the feed industry. Meanwhile, the use of each of these strategies achieves benefits, but also has drawbacks, including being expensive or impractical to apply on a large scale.

1. Introduction

Mycotoxins are secondary metabolites of different species of fungi that can cause chronic or acute toxicity in animals. Although a large number of mycotoxins have been identified, those of feed safety importance are primarily produced by the five fungal genera *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria*. Aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN), and fumonisin B1 (FB1) are well known as the major mycotoxins that contaminate feed, such as corn, barley, wheat, and their by-products (*Zhao et al.*, 2021). The most toxic mycotoxin is AFB1, mainly produced by *Aspergillus*, which is classified as a group 1 carcinogen (*Zhang et al.*, 2019). It shows hepatotoxic, immunotoxic, mutagenic, carcinogenic and teratogenic properties in many animal species. DON and trichothecene type B cause anorexia and vomiting and can compromise intestinal and immune functions in all animal species by inhibiting nucleic acid and protein synthesis. ZEN has a similar structure to oestrogen and, therefore, competes with 17-β-oestradiol for binding to oestrogen receptors, which consequently leads to disruption of the reproductive capacity of animals. FB1 is the most abundant fumonisin, and which can cause hepatotoxicity, neurotoxicity, nephrotoxicity, immunotoxicity, developmental toxicity, and cancer in humans and animals (*Chen et al.*, 2021).

It has been proven that mycotoxins have a significant negative impact on animal health and performance, as well as on the quality and safety of food of animal origin, which has led to a great chal-

***Corresponding author:** Dejan Perić, *dperic@vet.bg.ac.rs*

Paper received August 1st 2023. Paper accepted August 10th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

lenge for the scientific and professional public. Many mycotoxins, especially *Fusarium* toxins (zearalenone, trichothecenes), can be synthesized during different stages of plant development. That process is often forced by unfavourable conditions for plant development, mechanical damage to plants, nutritional imbalances, temperatures unusual for the season, and frequent rainfall. As a preventive measure, it is necessary to implement good agricultural practices. After grain storage, physical, chemical and biological strategies have been developed to detoxify mycotoxins. The time between storage and potential application of the selected treatments should be as short as possible considering the very short time required for the synthesis of mycotoxins (sometimes only 2–3 days). When designing recipes, there is also a nutritional approach to reducing the harmful effects of mycotoxins (*Liu et al*., 2020). However, many techniques have been shown to be ineffective, expensive, or impractical to apply on a large scale.

2. Physical methods

Decontamination of mycotoxins by physical techniques in practical conditions is mostly cellar washing processes or solvent extraction, sorting or heating of grain.

According to the properties of water- or fat-soluble mycotoxins, cereals can be decontaminated by washing with water or by extraction with organic solvents. Flotation and water washing can remove 51–72%, 64–69%, 2–61% and 73–74%, respectively, of aflatoxin, trichothecenes, ZEN and fumonisins from grains (*Matumba et al*., 2015). Flotation and rinsing with an aqueous solution consisting of 10–30% NaCl, 30% sucrose, or 1 mol/L sodium carbonate can increase the rate of fumonisin removal from corn and wheat. A combination of washing technology and manual sorting together can reduce 84% of fumonisins (*Westhuizen et al*., 2011). Solvents commonly used for mycotoxin extraction include methanol, ethanol, hexane, acetonitrile, isopropanol, and aqueous acetone. However, these methods have major drawbacks, as they lead to loss of nutrients and are expensive due to drying and disposal of toxic extracts, which limits their large-scale application.

Mycotoxins are not evenly distributed in the grain and mostly occur in mouldy, broken and discoloured parts. Meanwhile, the specific gravity of grains contaminated with mycotoxins is relatively lower than normal. These characteristics allow sieving, aspiration, and gravity separation to be used to isolate grains contaminated with mycotoxins (*Tibola et al.*, 2016). Aspiration and gravity separation methods can reduce DON in wheat (*Salgado et al*., 2011).

Drying, as a treatment with the main goal of lowering the moisture in cereal grains, is still the most widely used method. Thermal treatment has been applied for the decontamination of mycotoxins in animal feed for many years. The effectiveness of this method depends on the chemical structure and concentration of mycotoxins, temperature, duration, moisture content, pH and ion concentration during heat treatment. AFB1, DON, ZEN and FB1 are thermally stable compounds with decomposition temperatures higher than 237, 175, 220, 150°C, respectively, which makes elimination by conventional heat treatment difficult. Conventional hydrothermal treatment (cooking) under pressure (0.10 MPa) at 160°C for 20 minutes can degrade AFB1 by 78–88% in rice, while heating under pressure (0.10 MPa) at 120°C for 4 hours can degrade AFB1 by 95% in wet peanut powder (*Fan*, 2003). However, thermal treatments use an excessive amount of energy, so the Maillard reaction caused by high temperature reduces the nutritional value of feed ingredients. This has led to limitations in the application of heat treatments in the feed industry.

3. Chemical methods

Chemical techniques can destroy the structure of mycotoxins, creating mildly toxic or non-toxic products. Decontamination of mycotoxins by chemical techniques primarily involves treatment with acids and bases, as well as other treatments with chemical agents (*Jalili & Son*, 2011).

Preventing the growth and development of mould is mainly based on the use of chemical agents that maintain low water activity (aw) in the substrate. It is imperative that none of the chemicals used be toxic to animals. Organic acids, such as sorbic, benzene, propionic, acetic and formic, are very often used as preservatives, especially for stored grain foods.

Given that salts are more soluble in water, suitable salts of potassium, sodium (sodium sorbate) or calcium (calcium propionate) are generally used. The mechanism of action of preservatives is based on inhibition of enzymatic activity in fungal cells (propionic and sorbic acid) or on damage to mould membranes (natamycin), but they cannot reduce the content of mycotoxins already present in feed. Propionic acid, which has pronounced antigerminative properties, is most often used in the animal feed industry. Alkaline chemicals, including ammo-

nia, sodium hydroxide, potassium hydroxide, and sodium carbonate, have been used to destroy various mycotoxins in mouldy feed (*Fang et al.*, 2020). Although the application of these chemical agents almost completely destroys mycotoxins, some agents lead to significant nutritional losses and a negative impact on palatability, which consequently causes worse feed consumption.

4. Biological methods

Screening and isolation of naturally occurring microorganisms that show the ability to biotransform against specific mycotoxins is a modern strategy to combat this problem. Mycotoxin biodegradation technology is a process by which the toxic group of mycotoxin molecules is broken down and destroyed by secondary metabolites produced by microorganisms or their secreted intracellular and extracellular enzymes, while non-toxic or less toxic degradation products are produced.

A number of different fungi have been shown to detoxify AFB1. Certain fungal strains such as *Saccharomyces cerevisiae* degrade AFB1 at levels of 69.0% (*Chlebicz & Śliżewska*, 2020). Similarly, some studies reported that different strains of *Aspergillus niger* showed the ability to degrade AFB1 at levels of between 88.6% and 98.7% (*Fang et al.*, 2020). Bacteria degraded aflatoxin mainly by secreting extracellular enzymes. Some strains of *Nocardia corynebacterioides*, *Flavobacterium aurantiacum* and *Bacillus* have been shown to degrade AFB1. Microorganisms metabolize ZEN mainly through conversion or degradation into α-zearalenol, β-zearalenol, sulfphate, and other secondary metabolites with low toxicity. *Bacillus natto* and *Bacillus subtilis* strains have been shown to remove ZEN from liquid media: more than 75% of ZEN can be biodegraded after incubation. Some fungal and bacterial microorganisms have been reported to be able to degrade fumonisins. *Styriak et al.* (2001) examined two strains of preserved laboratory yeast that were able to significantly degrade fumonisins in the culture medium. One is *Saccharomyces cerevisiae* IS1/1, which can degrade 45% of FB1 and 50% of a mixture of FB1 and FB2 in the culture medium, and the other is *Saccharomyces cerevisiae* SC82, which also degrades FB1 and a mixture of FB1 and FB2; the degradation rates were 22% and 25%, respectively. Together with the use of biotechnology, the activity of modern preparations in the fight against mycotoxins is based on these principles.

5. Nutritional approach to mitigating the effects of mycotoxins

It is possible to prevent the harmful effects of mycotoxins with an adequate correction of the feed recipe. Detoxification systems, including CIP450, ketoreductase and α-glutathione transferase, can degrade mycotoxins (*Zhang et al.*, 2016). Therefore, any nutrient that can promote the normal functioning of one of the above detoxification enzyme systems can be used as a strategy. Glutamate, cysteine and glycine can be used as substrates for glutathione synthesis. On the other hand, mycotoxins can reduce nutrient intake, so adding critical nutrients is one way to mitigate the adverse effects of mycotoxins (*Liu et al.*, 2020). Oxidative stress is an important mechanism of mycotoxin-induced cytotoxicity (*Zhang et al.,* 2016). Addition of antioxidants to feed contaminated with mycotoxins can improve the antioxidant capacity of the body and increase the resistance of animals to mycotoxins. Selenium and vitamins A, C and E and their precursors have pronounced antioxidant properties. Since most mycotoxins negatively affect the digestibility of proteins, and inhibit protein synthesis, as one of the mitigation methods recommended is to use feed with 1–2% more protein than usual levels. *Andretta et al.* (2012) suggested that methionine can moderate the adverse effects caused by DON in growing pigs. Dietary supplementation of glutamic acid, arginine, aspartate and lysine had positive effects on remission of DON-induced visceral disease, increase in antioxidant capacity and improvement of physiological and biochemical indices in the blood of fattening animals.

In practice, the most widely applied method of mitigating or eliminating the harmful effects of mycotoxins is the use of adsorbents. Adsorbents are substances that are not resorbed from the intestines, and have the ability to physically bind certain chemical substances, thus preventing their resorption. Any ideal mycotoxin absorbent should possess at least the following properties: high adsorption capacity against mycotoxins (especially mycotoxins with low hydrophobicity), low nonspecific binding to nutrients, as well as high safety, stability, and palatability (*Daković et al.*, 2007).

Medicinal charcoal is a carbon-containing substance obtained by pyrolysis of organic matter that is then subjected to activation processes in order to obtain a highly porous structure. Activated medicinal charcoal has high mycotoxin-adsorbent properties, being especially effective against AFB1 and ochratoxin A. The negative side of using medicinal char-

coal is the variable degree of adsorption of nutrients (vitamins and microelements), colouring of feed, but also a high proportion (>1%) in feed mixtures that significantly reduces the energy and nutritional value. Bentonites are adsorbents that have a lamellar crystal microstructure and a different chemical composition, and the adsorptive capacity depends on the presence of exchangeable cations (Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}) in the lattice. Bentonites bind AFB1 and moderate the toxic effects of the T-2 mycotoxin group.

Zeolites are crystalline, hydrated aluminosilicates of alkaline and alkaline earth cations. They have an infinite three-dimensional crystal structure. They have the ability to lose and receive water without major structural changes and to exchange some of their constitutional cations. Zeolites, precisely because of their structures, are applied in the adsorption of mycotoxins, since these crystalline compounds are used as molecular sieves and cation exchangers (*Daković et al.*, 2007). Zeolites are formed from an aluminosilicate network $(SiO_4)_4$ and $(AlO_4)_4$, in which the basic building unit is a tetrahedral structure (TO_4) with silicon or aluminium atoms at the centre, and oxygen atoms at the corners. The tetrahedra connect to each other in various ways, making the zeolite structure rich in channels and cavities. In this way, molecules are separated according to the molecular sieve system, a characteristic feature of most zeolite minerals. If the pore size is compatible with the mycotoxin molecule, a high degree of adsorption is observed. For natural zeolites to be effective in feed, a relatively high proportion in feed (about 1%) is needed, which means zeolites significantly negatively affect the amount of nutrients in the feed.

Latest-generation adsorbents have been developed from the cell wall components of microorganisms. Glucomannan is a common adsorbent that cannot be used by gut microbes. Mycotoxins can be adsorbed by esterified glucomannan, which is a type of broad-spectrum mycotoxin adsorbent with an effective binding capacity for aflatoxin, ZEN, fumonisin and DON of 95%, 75%, 59% and 12%, respectively. It has been proven that esterified glucomannan somewhat mitigates the harmful effects of mycotoxins when it comes to performance, immunity, haematological and biochemical indicators in the blood of broilers (*Vila-Donat et al.*, 2020). At the beginning of the era of microbial adsorbents, bacteria were shown to adsorb mycotoxins to form a complex and then excrete them together with the toxins, thereby reducing the hazard (*Liu et al*., 2020). Besides yeasts, lactic acid bacteria are the most studied microbial adsorbents. *Lactobacillus casei* can significantly reduce the absorption of aflatoxin in the intestinal tract. *Zeng et al.* (2009) reported that *Lactobacillus plantarum* F22 had a strong adsorption capacity for AFB1 and that the adsorption rate could reach 56.8%.

6. Conclusion

The occurrence of mycotoxins is a major concern and an unavoidable problem in the feed industry worldwide. Mycotoxins also threaten human health through the cycle of the food chain. This review summarizes a number of strategies for reducing mycotoxin contamination that are most commonly applied in terms of physical detoxification, chemical treatments, biological detoxification methods, and nutritional strategies. However, with growing awareness of environmental protection as well as feed and food safety, there is a growing expectation for more green and innovative technologies to control mycotoxin contamination.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Andretta, I., Kipper, M., Lehnen, C. R., Hauschild, L., Vale, M. M. & Lovatto, P. A. (2012). Meta-analytical study of productive and nutritional interactions of mycotoxins in growing pigs. *Animal*, 6(9), 1476–1482.

Chen, J., Wei, Z., Wang, Y., Long, M., Wu, W. D. & Kuca, K. (2021). Fumonisin B1: mechanisms of toxicity and biological detoxification progress in animals. *Food and Chemical Toxicology*, 149(3), 181–197.

Chlebicz, A. & Śliżewska, K. (2020). In vitro detoxification of aflatoxin B1, deoxynivalenol, fumonisins, T-2 toxin and zearalenone by probiotic bacteria from genus *Lactobacillus* and *Saccharomyces cerevisiae* yeast. *Probiotics and Antimicrobial Proteins*, 12(1), 289–301.

Fang, Q. A., Du, M. R., Chen, J. W., Liu, T., Zheng, Y. & Liao, Z. L. (2020). Degradation and detoxification of aflatoxin B1 by tea-derived *Aspergillus niger* RAF106. *Toxins* (Basel), 12(12), 777–787.

Fan, H. Y. (2003). Integrated control of aflatoxin in feed. *Hebei Animal Husbandry Veterinary*, 19(1), 40–47.

Jalili, M. & Son, S. (2011). The effect of chemical treatment on reduction of aflatoxins and ochratoxin A in black and white pepper during washing. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 28(4), 485–93.

Liu, M., Zhang, L., Chu, X. H., Ma, R., Wang, Y. W. & Liu, Q. (2020). Effects of deoxynivalenol on the porcine growth performance and intestinal microbiota and potential remediation by a modified HSCAS binder. *Food Chemical Toxicology*, 141(1), 143–173.

Liu, Y., Yamdeu, J. H., Gong, Y. Y. & Orfila, C. (2020). A review of postharvest approaches to reduce fungal and mycotoxin contamination of foods. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1521–1560.

Luo, L. X., Yuan, L. & Li, J. (2020). Complicated interactions between bio-adsorbents and mycotoxins during mycotoxin adsorption: Current research and future prospects. *Trends in Food Science and Technology*, 96,127–134.

Matumba, L., Poucke, C. V., Ediage, E. N., Jacobs, B. & Saeger, S. D. (2015). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 32(6), 960–969.

Salgado, J. D., Wallhead, M., Madden, L. V. & Paul, P. A. (2011). Grain harvesting strategies to minimize grain quality losses due to fusarium head blight in wheat. *Plant Disease*, 95(11), 1448–1457.

Styriak, I., Conková, E., Kmec, V., Böhm, J. & Razzazi, E. (2001). The use of yeast for microbial degradation of some selected mycotoxins. *Mycotoxin Research*, 17(1), 24–27.

Tibola, C. S., Fernandes, M. C. & Guarienti, E. M. (2016). Effect of cleaning, sorting and milling processes in wheat mycotoxin content. *Food Control*, 60, 174–179.

Vila-Donat, P., Marín, S., Sanchis, V. & Ramos, A. J. (2020). Tri-octahedral bentonites as potential technological feed additive for fusarium mycotoxin reduction. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 37(8), 1374–1387.

Westhuizen, L., Shephard, G. S., Rheeder, J. P., Burger, H. M., Gelderblom, W. C. & Wild, C. P. (2011). Optimising sorting and washing of home-grown maize to reduce fumonisin contamination under laboratory-controlled conditions. *Food Control*, 22, 396–400.

Zeng, D., Tang, Y. R., Ni, X. Q., Zhang, Z. L. & Ran, J. (2009). Absorption characteristics of *Lactobacillus plantarum* F22 to aflatoxin B1. *Food Science*, 30, 23.

Zhang, L. Y., Zhao, X. J., Liu, S. & Zhang, Y. G. (2019). Biological detoxification of aflatoxin for food and feed: a review. *Chinese Journal of Animal Science and Nutrition*, 31(2), 521–529.

Zhang, N. Y., Qi, M., Zhao, L., Zhu, M. K., Guo, J. & Liu, J. (2016). Curcumin prevents aflatoxin B1 hepatoxicity by inhibition of cytochrome P450 isozymes in chick liver. *Toxins* (Basel), 8(11), 327–332.

Zhao, L., Zhang, L., Xu, ZJ., Liu, X. D., Chen, L. Y. & Dai, J. F. (2021). Occurrence of aflatoxin B1, deoxynivalenol and zearalenone in feeds in China during 2018–2020. *Journal of Animal Science and Biotechnology*, 12, 74–82.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664]:658.562.4

ID: 126588681

https://doi.org/10.18485/meattech.2023.64.2.91

Original scientific paper

Performance evaluation of the ISO 18363-4:2021 method for quantitative determination of chloropropanediols and glycidol in fats and oils by GC-MS/MS

Srđan Stefanović[a], Saša Janković[a], Nikola Borjan[a], Stefan Simunović[a], Radivoj Petronijević[a], Dejana Trbović[a] and Čaba Silađi[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia*

ARTICLE INFO

Keywords:
MCPD
Glycidol
Ester
Oil
GC-MS/MS

ABSTRACT

A new GC-MS/MS method from the ISO 18363 series of standards for determination of fatty acid bound chloropropanediols and glycidol was assessed for its performance characteristics. This method enables indirect simultaneous determination of free 2-monochloropropane-1,3-diol (2-MCPD), 3-monochloropropane-1,2-diol (3-MCPD) and glycidol in a single sample preparation and analysis protocol. Results of the validation study show satisfactory performance characteristics, well within required limits. Recently held proficiency testing also showed the adequate performance of the laboratories employing this method. Taking into consideration the simpler sample preparation and stringent quality control inherent to this protocol, wider use of ISO 18363:4:2021 for routine applications can be expected in the foreseeable future.

1. Introduction

2-monochloropropane-1,3-diol (2-MCPD), 3-monochloropropane-1,2-diol (3-MCPD) and glycidol (GLY) belong to the chemically diverse group of processing contaminants, i.e. compounds that can be found in foods either through direct introduction into the food processing at some technological stage, or due to the chemical changes in natural food ingredients during, e.g., thermal treatment of food. The latter represents the formation mechanism of 2-MCPD, 3-MCPD and GLY in plant and animal-originating fats and oils, after they are exposed to prolonged high temperatures for refining purposes. These compounds are present in foods predominantly chemically bound to fatty acids (MCPD and glycidyl esters). However, upon entering the gastrointestinal tract, they readily hydrolyse into free compounds due to gut lipase activity (*Beekmann et al*, 2022). MCPD and GLY content in processed food is dependent on temperature, time and type of oil/fat; plant-originating fats have higher concentrations. Ubiquitous palm oil and fats are especially susceptible to MCPD/GLY synthesis, and having in mind their widespread use in numerous processed food commodities, they are among the most significant contributors to human exposure (*EFSA*, 2018).

From the toxicological perspective, MCPD/GLY gained significant attention in the last decade; the Joint FAO/WHO Expert Committee on Food Additives in 2016 (*JECFA*, 2017) established a provisional group maximum TDI of 4 µg/kg b.w.

Corresponding author: Srđan Stefanović, *srdjan.stefanovic@inmes.rs*

Paper received August 2nd 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

for 3-MCPD. The European Food Safety Authority established a tolerable daily intake (TDI) of 2.0 µg/kg b.w. per day for 3-MCPD and its esters, attributing potential renal toxicity to 3-MCPD. Furthermore, 3-MCPD is listed as a threshold genotoxic carcinogen (*EFSA*, 2016, 2018). GLY was classified as probably carcinogenic to humans, so in Group 2A according to the International Agency for Research on Cancer, suggesting ALARA (as low as reasonably achievable) as a risk management strategy (IARC, 2000). Both 3-MCPD and GLY are considered especially harmful for infants having in mind high exposures due to their low body weight. Full assessment of 2-MCPD is not currently possible due to insufficient data availability.

As a result, maximum levels (MLs) for 3-MCPD and GLY were set by the European Union *(EU*, 2023) ranging from as low as 6 µg/kg of GLY for liquid baby food to 2500 µg/kg of 3-MCPD for animal and vegetable fats and oils. These MLs are set for the sum of 3-MCPD and its esters, i.e. GLY and glycidyl esters expressed as 3-MCPD and GLY respectively.

Available analytical methods are numerous and can be divided into direct and indirect categories. Direct determination is based on detection and quantification of MCPD and glycidyl esters in the sample. However, according to *Zheng et al.* (2021), such protocols can lead to significant underestimation due to the limited availability of esters sold as commercial analytical standards. Indirect determination after transesterification and analysis of the main compounds (2-MCPD, 3-MCPD, GLY) is considered more suitable, since the entire quantity in the sample, regardless of where the ester originated from, is accounted for. Another difficulty is related to the pronounced instability of GLY after transesterification; this is alleviated by immediate derivatization step with sodium bromide, transforming GLY into 3-monobromopropanediol (3-MBPD).

The American Oil Chemists' Society (AOCS) has developed several methods for indirect determination of MCPD and GLY (*AOCS*, 2017a; *AOCS*, 2017b; *AOCS*, 2017c), adopted eventually by International Organization for Standardization (ISO) and integrated into the ISO 18363 series of standards (*ISO*, 2021). These methods, all based on gas chromatography with mass selective detection, differ by ester cleavage mechanisms (fast or slow transesterification), and quantitative determination of GLY. However, they all require division of each sample into two portions (for separate determination of MCPD and GLY), which doubles the time and resources necessary for measurement.

In 2021, the ISO 18363 family of standards was expanded with a new indirect analytical method for 2-MCPD, 3-MCPD and GLY determination in one unified preparation protocol and a single chromatographic run (*ISO*, 2021). This method employs fast alkaline cleavage of esters that is facilitated by sodium methoxide, immediate conversion of released GLY into 3-MBPD and subsequent derivatization of all three compounds with phenylboronic acid in order to make them suitable for GC analysis. Unlike previous methods that utilize GC/MS for measurement, ISO 18363-4 requires GC-MS/MS due to the specific quantification technique that has to be applied in order to accurately measure GLY concentration. Namely, during cleavage of esters in alkaline conditions, a certain amount of 3-MCPD is converted to GLY, leading to overestimation of GLY content (conversion of 2-MCPD is existent but negligible, hence not taken into account). In order to quantify the build-up of 3-MCPD-induced GLY, a specific internal standard ($^{13}C_{12}$ 3-MCPD) is used. The purpose of using isotopically labelled 3-MCPD (besides accurate quantification of ester-bound 3-MCPD) is to monitor formation of isotopically labelled GLY during transesterification (derivatized at a later stage to $^{13}C_{12}$ 3-MBPD). Using its concentration as a single calibration point, it is possible to quantify the amount of GLY originating from MCPD and subtracting this amount from glycidyl ester induced GLY compensates for the overestimation of GLY content. Quantification of 2-MCPD is performed using $^{13}C_{12}$ 3-MCPD as the internal standard as well, while GLY is quantified with its own labelled analogue (D5-GLY).

The aims of this study were to assess performance characteristics of the unmodified ISO 18363-4:2021 (*ISO*, 2021) method for quantitative determination of MCPD and glycidyl esters in fats and oils and to compare the results obtained with the data from the ISO collaborative study provided in the Standard, as well as with the performance criteria provided in the same document. For this purpose, authors utilised results from an in-house validation study. Additional confirmation of the method's fitness for purpose was judged by analysing the results of a proficiency test (PT) conducted in December of 2022. Having in mind that ISO 18363-4:2021 is a new method, the authors believe that assessment of the method's performance can encourage its wider

adoption, bearing in mind it's clear advantages over older and more established laboratory protocols.

2. Materials and methods

2.0.1. Reagents, standards and instrumentation

Methanol, isooctane, acetone, toluene, t-butyl methyl ether (TBME), sulphuric acid, sodium methoxide, sodium bromide were of p.a. quality. Analytical standards (1,3-distearoyl-2-chloropropanediol; 1,2-dipalmitoyl-3-chloropropanediol; glycidyl stearate; $^{13}C_{12}$ 1,2- dipalmitoyl-3-chloropropanediol and; glycidyl stearate-d5) were purchased from Toronto Research Chemicals (North York, ON, Canada). Blank matrix used for fortification was extra virgin olive oil purchased in a local supermarket and previously analysed for the absence of investigated analytes.

Analysis was performed on a Shimadzu GC-MS/MS system (Kyoto, Japan) consisting of GC 2030 oven, SPL 2030 split/splitless injector, AOC 20s auto sampler, AOC 20i auto injector and GCMS-TQ8050 NX triple quadrupole mass spectrometer using EI ionisation and operating in MRM mode. Separation was performed on Shimadzu SH-Rxi 5ms analytical column (30m × 0.25mm × 0.25 µm) equipped with a 1.5 m fused silica pre-column.

2.0.2. Analytical method

ISO (2021) provides a detailed analytical protocol for sample preparation and instrumental parameters. Briefly, 100 mg of fat or oil was weighted into a screw-capped glass vessel. After addition of internal standards, toluene and TBME, the mixtures were heated at 80°C. Vials were then cooled to 10°C prior to transesterification with sodium methoxide followed by vortex assisted homogenization and incubation at 10°C. The reaction was stopped by adding acidic sodium bromide. This also prevents decomposition of GLY, resulting in conversion to stable 3-MBPD. Removal of fatty acids and other co-extractives was accomplished by multiple liquid-liquid extractions with isooctane. After discarding the organic layer, phenylboronic acid was added for derivatization followed by another isooctane extraction. Non-polar derivatives are contained within organic layer, and so the isooctane fraction was transferred to GC auto sampler vial prior to chromatography.

The chromatographic program had the following parameters: splitless injection mode at 350°C, initial oven temperature 70°C, hold 1 min, ramp 15°C/min to 120°C, final ramp 40°C/min to 350°C, hold 2.5 min. The mass spectrometer operated in EI+ mode, and ion source and transfer line temperatures were 290°C and 315°C respectively. The following MRM transitions were monitored: 3-MCPD 196 > 147, 198 > 147; 2-MCPD 196 > 104, 198 > 104; 3-MBPD 240 > 147, 242 > 147; $^{13}C_3$ 3-MCPD: 199 > 149, 201 > 149; $^{13}C_3$ 3-MBPD: 243 > 149' D_5 3-MBPD 245 > 150, 247 > 150.

3. Results and discussion

3.1.1 Method validation

This validation study was conducted according to the principles provided in the IUPAC (International Union for Pure and Applied Chemistry) harmonised protocol (*Thompson et al*., 2002). A 10-point calibration was performed (0.003–0.790 mg/kg of oil), during a three-day experiment for linearity assessment. Accuracy and precision were determined by analysing six replicates of three certified reference materials (CRM), vegetable oils, one of which (T2672) was also previously used in the proficiency test, and all of which were obtained from the Food Analysis Performance Assessment Scheme (FAPAS T2664, T2669 and T2672). Table 1 shows certified values of these reference materials with the ranges of acceptable values for z-score < 2.

Intralaboratory reproducibility was expressed as RSDr and was determined in the same experiment used for bias assessment, since certified values of

Table 1. Certified values of MCPD/GLY reference materials

	T2664 (µg/kg)	T2669 (µg/kg)	T2672-PT (µg/kg)
3-MCPD esters	1285 (889–1681)	431 (275–588)	174 (101–246)
GLY esters	448 (286–610)	61.4 (34.4–88.4)	99.9 (56.0–143.9)
2-MCPD esters	651 (429–873)	176 (103–249)	62.0 (34.7–89.3)

the three CRMs were within the range of required MLs and encompassed both lower and higher concentrations for each compound. Limit of quantification (LoQ) was determined by analysing six blanks and determining S/N ratio at the retention times of interest.

3.1.2 Validation parameters

Table 2 summarizes the results of the single-laboratory validation study. Requirements specified in ISO 18363-4:2021 are provided for comparison.

The results show satisfactory degrees of accuracy and precision according to the requirements of the ISO and established criteria for analytical method performances. Between-day reproducibility was noticeably higher for GLY compared to 2-MCPD and 3-MCPD, which is in accordance with the requirements and can be explained by the unpredictability of the conversion of released 3-MCPD into 3-MBPD. Although this process is accounted for, through monitoring of the isotope-labelled 3-MCPD conversion, much attention should be paid to the calculation of results and analysis of at least one CRM in each sample batch, which is advisable for confirmation. Bearing in mind the stability of the esters and low sample weight, a single CRM can be used for a considerable time, significantly contributing to the quality of the results. The duration of transesterification and overall timing in sample preparation steps should be as precise and consistent as possible, since they are the key factors determining method performance.

3.1.3. Proficiency test analysis

A proficiency test for determination of MCPD/GLY esters in vegetable oil was conducted in October-December 2022. According to the PT report (FAPAS, PT 2672), only 4 out of 87 participating laboratories applied the ISO 18363-4:2021 method (4.6%). Such a low figure is expected, since the several older methods are very well established. However, the performance of this new method was satisfactory; according to the PT report, all four laboratories employing ISO 18363-4 performed adequately, with z-scores ranging from –0.4 to +0.9 for 3-MCPD, from –0.9 to +1.2 for GLY and from –0.6 to +1.6 for 2-MCPD. Considering the relatively wide distribution of acceptable results (Table 1) and overall overestimation of all compounds seen in the distribution of reported results, especially in the case of GLY, this new method clearly outperforms the average reported results.

4. Conclusion

ISO 18363-4:2021 (*IOS,* 2021) is a new analytical method for determination of MCPD/glycidyl esters in fats and oils, and based on validation results, demonstrates satisfactory performance.

Table 2. Results of the single-laboratory validation study

	3-MCPD esters expressed as free 3-MCPD	Glycidyl esters expressed as free GLY	2-MCPD esters expressed as free 2-MCPD	ISO 18363-4 requirements (*ISO*, 2021)
Calibration curve slope	0.995	1.005	0.999	3-MCPD: 0.95–1.05 GLY: 1–1.25 2-MCPD: 0.8–1
Repeatability (RSD, %)	4.6	5.1	2.8	3-MCPD: 9 GLY: 11 2-MCPD: 12
Between-day reproducibility (RSDr, %)	9.3	19.1	12.5	3-MCPD: 15 GLY: 38 2-MCPD: 27
Recovery (%)	95.8	91.3	102.2	80–120
LoQ (µg/kg)	20	18	21	100
MU (%)	20.4	38.4	25.4	n/a

Moreover, this new method also offers significant advantages regarding sample preparation time and resources, with an elaborate quantification scheme and stringent quality control due to the use of two internal standards and the GC-MS/MS technique. Having in mind all this, much wider use of ISO 18363-4:2021 in routine applications can be expected in the foreseeable future.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

AOCS, (2017a). 2- and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by acid transesterification and GC/MS. AOCS Official Method Cd-29a-13.

AOCS, (2017b). 2- and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by alkaline transesterification and GC/MS. AOCS Official Method Cd-29b-13.

AOCS, (2017c). 2- and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by GC/MS (difference method). AOCS Official Method. Cd-29c-13.

Beekmann, K., Sloot, S. J., Oey, S. B. & Leeuwen van, S. P. J. (2022). MCPD esters and glycidyl esters in food supplements of fish oils, algae oils, and krill oils. *Food Control*, 136, https://doi.org/10.1016/j.foodcont.2022.108865.

EFSA, (2016). Risks for human health related to the presence of 3-and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal*, 14(5), 4426.

EFSA, (2018). Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. *EFSA Journal,* 16(1), 5083.

EU, (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. OJ L 119, 103–157.

IARC, (2000). IARC monographs on the evaluation of carcinogenic risks to humans, some

industrial chemicals. *IARC Monographs*, 77, 469–486.

ISO, (2021). Animal and vegetable fats and oils-determination of fatty acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS — Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS. EN ISO 18363-4:2021.

JECFA, (2017). Evaluation of certain contaminants in food: Eighty-third report of the Joint FAO/WHO Expert committee on food Additives. In, 776. World Health Organization — Technical Report Series 74–106.

Thompson, M., Ellison, S. L. R. & Wood, R. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). *Pure Applied Chemistry*, 74 (5), 835–855.

Zheng, X., Fu, W., Zheng, K., Gao, B., Lin, L., Liu, W., Lin, Z. & Fang, Q. (2021). A novel method for simultaneous determination of esterified 2-/3-MCPD and glycidol in foods by GC-MS/MS. *Food Control*, 123, https://doi.org/10.1016/j.foodcont.2020.107766

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 316.64:663/664(497.113)

ID: 126582537

https://doi.org/10.18485/meattech.2023.64.2.92

Original scientific paper

Consumer attitudes and preferences toward traditional meat products in the autonomous province of Vojvodina

Tatjana Peulić[a], Predrag Ikonić[a], Jovana Delić[a], Bojana Kalenjuk Pivarski[c], Nikola Maravić[a], Aleksandar Marić[a] and Aleksandra Novaković[b]*

[a] *University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[b] *University of East Sarajevo, Faculty of Pedagogy, Semberski ratara, 76300 Bijeljina, Republic of Srpska, Bosnia and Herzegovina*
[c] *University of Novi Sad, Department of Geography, Tourism and Hotel Management, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*

ARTICLE INFO

Keywords:
Traditional food products
Traditional meat products
Traditional dry fermented sausages
Sremski kulen
Petrovská klobása
Lemeški kulen

ABSTRACT

The purposes of this survey were to identify consumer attitudes and preferences toward special groups of traditional food products and to identify the position of meat products among traditional food products. An online questionnaire surveyed 540 respondents in the autonomous province of Vojvodina, Serbia. The questionnaire was divided into two parts: 1) socio-demographic characteristics and 2) consumer attitudes and preferences toward traditional food products and the position of meat products within these foods. Traditional food products that were reported as mostly consumed in Vojvodina are meat and meat products (85.4%), milk and milk products (65.1%), honey (64.5%) and products from fruit and vegetables (53%). Dry fermented sausages are the most prevalent group among traditional meat products (57.3%), followed by dry cured meat products (23.6%) and bacon (9.9%). Five of sixteen selected meat products that are consumed in Vojvodina were registered as Geographical Indication (GI) labelled product in the Intellectual Property Office of the Republic of Serbia. All registered GI products belonged to the group of dry fermented sausages.

1. Introduction

Traditional food products are an integral part of a region's cultural heritage and identity. These products are often passed down through generations and are cherished for their unique and recognizable characteristics and quality (*European Commission*, 2007; *Trichopoulou*, 2007; *Ikonić*, 2021). In recent years, there has been a noticeable increase in consumer demands for traditional food products due to growing interest in authenticity and food origins, quality, nutritional value and health-consciousness, environmental sustainability, economic impact on local economies and culinary diversity (*Byrne*, 2013; *Feldmann*, 2015; *Donati*, 2021; *Kovács*, 2022).

The autonomous province of Vojvodina is a region located in the northern part of Serbia, famous for its diverse traditional food products and culinary heritage, reflecting a blend of different cultures from various ethnic groups that have inhabited this region throughout history. Traditional meat products play a significant role in Vojvodina's gastronomy (*Tasić,* 2015; *Ikonić* 2021; *Kalenjuk Pivarski*, 2022). Furthermore, meat products are important elements of

***Corresponding author:** Tatjana Peulić, *tatjana.peulic@fins.uns.ac.rs*

Paper received August 3rd 2023. Paper accepted August 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

diets worldwide, especially traditional meat products which are regarded as morehealthy than products from high-scale industrial processing.

The aims of this study were to identify consumer attitudes and preferences toward special groups of traditional food products and to identify the position of meat products among the traditional food products.

2. Materials and methods

In order to determine consumer attitudes and preferences, an online questionnaire was available from June 2022 to April 2023, to which responded a sample of 540 people currently living in Vojvodina.

2.1. Questionnaire

The questionnaire was divided into two parts: 1) socio-demographic characteristics (six questions) and 2) consumer attitudes and preferences toward traditional food products and the position of meat and meat products among these foods (two questions). In the first part, respondents answered questions about gender, age, place of living, employment, monthly income (€) and members in households. Further on, in the second part, respondents who consume traditional food products reported the most commonly consumed groups of products in their households and stated the names of those products. The following types of questions were used in the questionnaire: open questions, closed questions and multiple-choice questions.

2.2. Statistical analyses

The data was processed using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Descriptive statistics were employed to emphasize the characteristics of the study sample.

3. Results

Socio-demographic data shows that 69.1% of the respondents were females, 85% lived in the city, 55.6% were between 25 and 45 years old, 70.4% were employed and 65.6% lived in households with three or more members.

Consumer attitudes and preferences toward special groups of traditional food products are presented in Figure 1. As can be seen from Figure 1, the traditional food products that were reported as being most commonly consumed in Vojvodina are meat and meat products (462 respondents, 85.4 %), milk and milk products (352 respondents, 65.1%) honey (349 respondents, 64.5%) and products from fruit and vegetables (287 respondents, 53%).

Consumption of specific meat product groups within total traditional food products and within traditional meat products is shown in Figure 2. As can be seen Figure 2, dry fermented sausage is the most prevalent group both among traditional meat products (57.3%) and traditional food products (22.8%) in terms of consumption in Vojvodina. The second largest group reported was dry cured meat products, which amounted to 23.6% of traditional meat

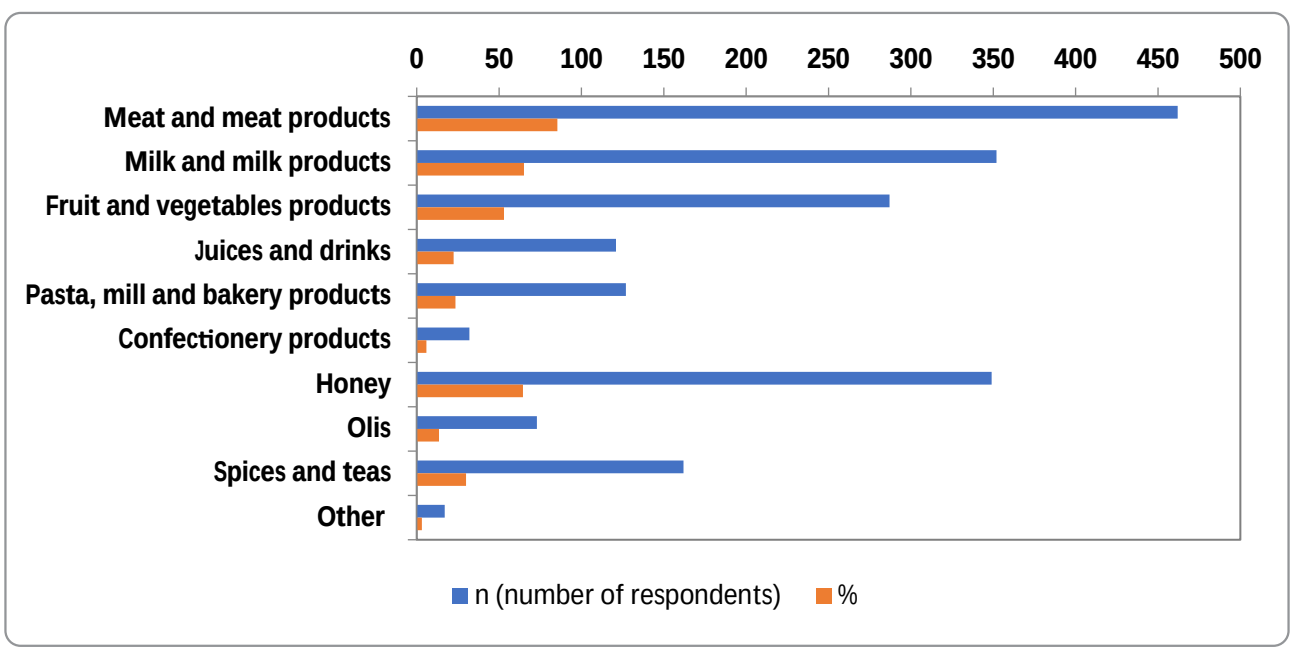


Figure 1. Consumer attitudes and preferences toward special groups of traditional food products

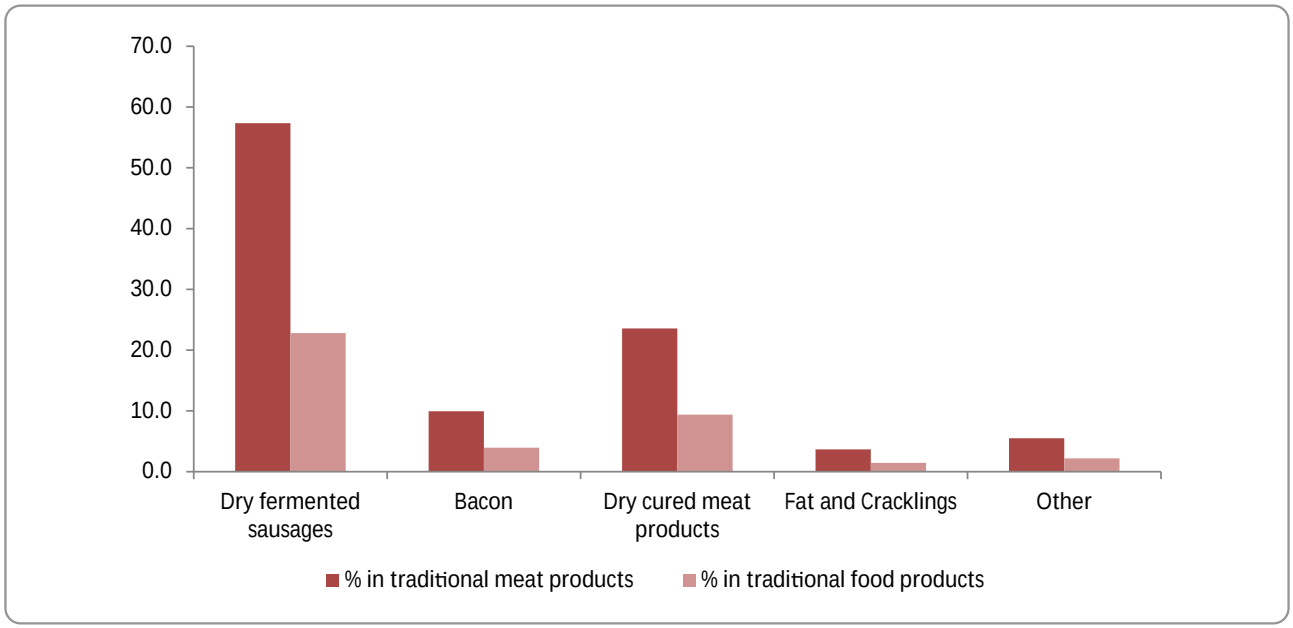


Figure 2. Consumption of specific meat product groups within traditional food products and within traditional meat products

products and to 9.4% of traditional food products. The third group in terms of consumption was bacon (9.9% of traditional meat products and 4.0% of traditional food products). Interestingly, this group comprises only one traditional product, which is dry bacon. In contrast, the group of dry fermented sausages included various varieties of *kulen* and sausages. Similarly, dry cured meat products offer con-

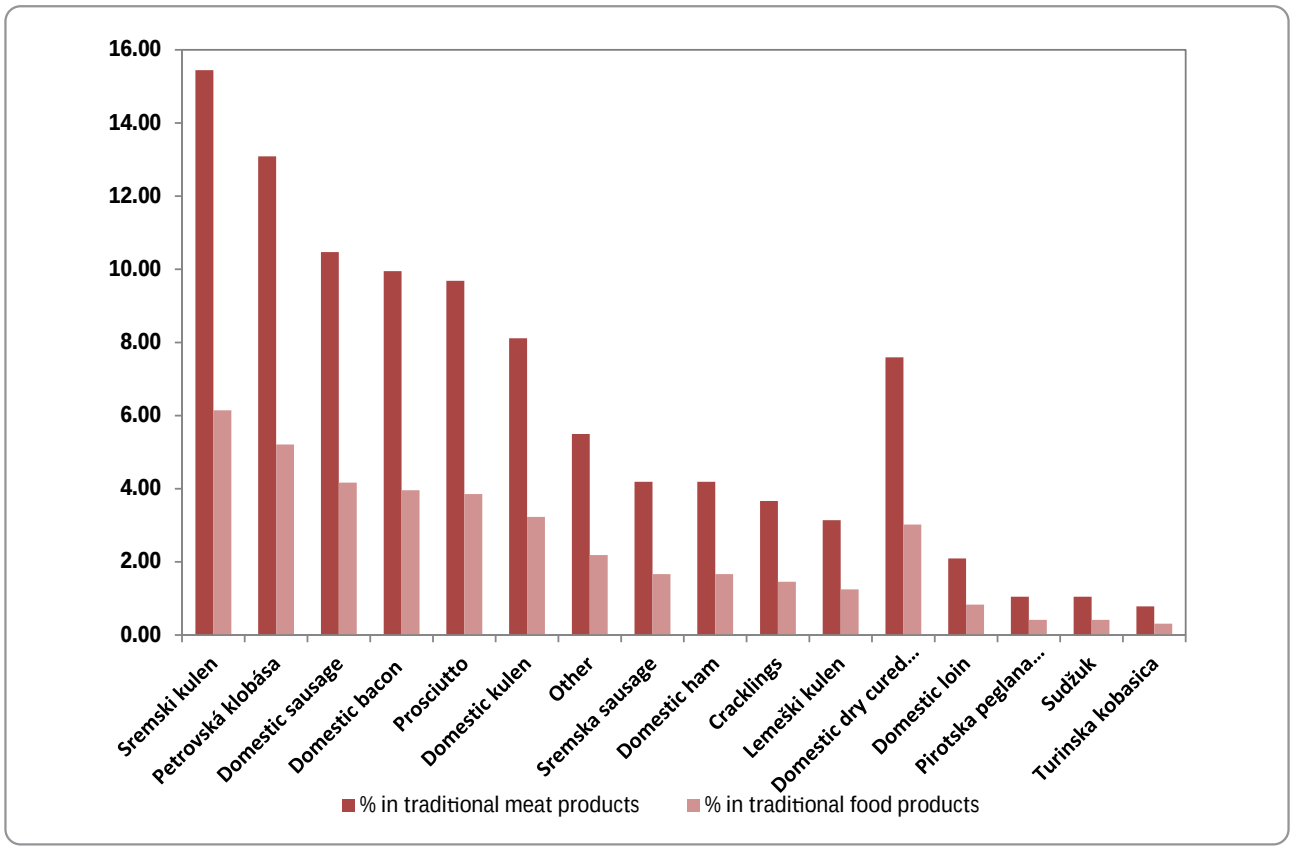


Figure 3. Consumption of specific, named meat products within traditional food products and within traditional meat products

sumers a diverse selection of *pršuta*, ham, loin, and more. On the European Union market, a similar situation, where sausages and hams are the most commonly consumed traditional meat products, was also observed (*eAmbrosia*, *Talone et al.*, 2007).

Consumption of specific, named meat products within traditional food products and within traditional meat products is presented in Figure 3. The results showed the following products were the most prevalent, as reported by consumers: "*Sremski kulen*", "*Petrovačka kobasica*", domestic sausage, domestic bacon, *pršuta*, domestic *kulen* and dry cured meat products. Here, it should be emphasized that the products "*Pirotska peglana kobasica*" and "*Sudžuk*" are characteristic for the southern part of Serbia, being particularly well-known and produced in that region. As a result, they are not widely consumed in Vojvodina, both due to the lack of their habitual consumption and their limited availability on the market in this northern region.

Five of 16 products, presented in Figure 3, were registered as Geographical Indication (GI) labelled products in the Intellectual Property Office (of the Republic of Serbia) namely "*Sremski kulen*", "*Petrovačka kobasica*", "*Lemeški kulen*", "*Sremska kobasica*" and "*Pirotska peglana kobasica".* All registered products belong to the group of dry fermented sausages.

4. Discussion and conclusion

The purposes of this survey were to identify consumer attitudes and preferences toward special groups of traditional food products and to identify the position of meat products among traditional food products. Based on the survey analysis, traditional meat products were the most commonly consumed products among all groups of traditional food products, and dried fermented sausages were the most prevalent group within the traditional meat products. *Lazzaroni et al.* (2013) stated that over the past five years, in Italy, protected designation of origin and protected geographical indication (PDO-PGI) products have accounted for approximately 15–20% of the total domestic purchases. Among these products, meat items constituted roughly 12%, while cheeses made up a more significant portion at around 32%. In their study, *Conter et al.* (2008) discovered that among various meat products, dry fermented sausages showed a positive consumption trend, and consumers demonstrated a greater willingness to pay higher prices for this specific type of product. Products protected in Serbia by the GI label, such as "*Sremski kulen*" and "*Petrovačka kobasica*", along with domestic sausages, domestic *kulen*, and *pršuta*, were the most commonly consumed traditional meat products among consumers in Vojvodina. Our findings suggest that a positive preference for high-quality food products is usually identified by consumers not based on commercial labels but by their name and then by the indication of origin labelling. *Cerjak et al.* (2008) in their study "What motivates consumers to buy traditional food products?" found that consumers perceive traditional food in a very positive way and that their most frequent associations with it are heritage, traditional recipes, health, support for local farmers, environmentally friendly production and sentimental hedonism (with perception of traditional food as a means to connect with childhood). Our results indicate that local brands were the most desirable for traditional meat products, and the GI label could be a potential tool for differentiating traditional meat products, especially for small and traditional farms.

Based on the results obtained in this study, it can be concluded that consumers' marked preference toward traditional food products, as well as traditional meat products and their specific sub-groups, could be useful for stakeholders, policymakers and certification bodies. Furthermore, in order to deeply understand consumers' attitudes and preferences toward traditional food products, their opinions about quality should be investigated in further research.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The research was funded by the Provincial Secretariat for Higher Education and Scientific Research (No. 142-451-3149/2022-03).

Acknowledgements: The authors wish to express their sincere gratitude to the Provincial Secretariat for Higher Education and Scientific Research (No. 142-451-3149/2022-03) and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no.: 451-03-47/2023-01/200222) for their financial support.

References

Byrne, D. V., Waehrens, S. S. & O'Sullivan, M. G. (2013). Future development, innovation and promotion of European unique food: An interdisciplinary research framework perspective. *Journal of the Science of Food and Agriculture*, 93, 3414–3419.

Cerjak, M., Haas, R., Brunner, F. & Tomić, M. (2014). What motivates consumers to buy traditional food products? Evidence from Croatia and Austria using word association and laddering interviews. *British Food Journal*, 116, 1726–1747.

Conter, M., Zanardi, E., Ghidini, S., Pennisi, L., Vergara, A., Campanini, G. & Ianieri, A. (2008). Consumers' behaviour toward typical Italian dry sausages. *Food Control*, 19, 609–615.

Donati, M., Wilkinson, A., Veneziani, M., Antonioli, F., Arfini, F., Bodini, A., Amilien, V., Csillag, P., Ferrer-Pérez, H., Gkatsikos, A., Gauvrit, L., Gil, C., Hòang, V., Steinnes, K. K., Lilavanichakul, A., Mattas, K., Napasintuwong, O., Nguyen, A., Nguyen, M., Papadopoulos, I., Ristic, B., Stojanovic, Z., Maksan, M.T., Török, Ä., Tsakiridou, E. & Bellassen, V. (2021). Economic spill-over of food quality schemes on their territory. *Journal of Agricultural & Food Industrial Organization*, 19, 95–111.

eAmbrosia — the EU geographical indications register, (2021). Retrieved from https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/geographical-indications-register/

European Commission, (2007). European research on traditional food. Website. ftp://ftp.cordis.europa.eu/pub/fp7/kbbe/docs/traditional-foods.pdf

Feldmann, C. & Hamm, U. (2015). Consumers' perceptions and preferences for local food: a review. *Food Quality and Preference*, 40, 152–164.

Ikonić, P., Peulić, T., Delić, J., Novaković, A., Dapcevic-Hadnadjev, T. & Škrobot, D. (2021). Quality standardization and certification of traditional food products. In IOP Conference Series: Earth and Environmental Science, Proceedings of the 61st International Meat Industry Conference, Zlatibor, Serbia, 26–29 September 2021. IOP Publishing: Bristol, UK, 2021; Volume 854, p. 012035.

Kalenjuk Pivarski, B., Šmugović, S., Tekić, D., Ivanović, V., Novaković, A., Tešanović, D., Banjac, M., Đerčan, B., Peulić, T., Mutavdžić, B., Lazarević, J. & Vukelić N. (2022). Characteristics of traditional food products as a segment of sustainable consumption in Vojvodina's hospitality industry. *Sustainability*, 14, 13553.

Kovács, I., Balázsné Lendvai, M. & Beke, J. (2022). The importance of food attributes and motivational factors for purchasing local food products: Segmentation of young local food consumers in Hungary. *Sustainability*, 14, 3224.

Lazzaroni, C., Iacurto, M., Vincenti, F. & Biagini, D. (2013). Consumer attitudes to food quality products of animal origin in Italy. In Consumer attitudes to food quality products: Emphasis on Southern Europe, 83–96.

Talon, R., Lebert, I., Lebert, A., Leroy, S., Garriga, M., Aymerich, T., Drosinos, E. H., Zanardi, E., Ianieri A., Fraqueza, M. J., Patarata, L. & Laukova, A. (2007). Traditional dry fermented sausages produced in small-scale processing units in Mediterranean countries and Slovakia. 1: Microbial ecosystems of processing environments. *Meat Science*, 77, 570–579.

Tasić T., Ikonić, P., Jokanović, M., Mandić, A., Tomovic, V., Šojić, B. & Škaljac, S. (2015). Content of vasoactive amines in Sremski kulen and Sremska kobasica traditional dry fermented sausages from Vojvodina, Proceedings of the 58th International Meat Industry Conference "Meat Safety and Quality: Where it goes?", Procedia Food Science 5, 2015, 282–284.

Trichopoulou, A., Soukara, S. & Vasilopoulou, E. (2007). Traditional foods: A science and society perspective. *Trends in Food Science & Technology*, 18, 420–427

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.54.043:636.592

ID: 126556681

https://doi.org/10.18485/meattech.2023.64.2.93

Original scientific paper

Indexing of fatty acids in raw turkey meat and products for their characterization in a healthy diet

Aleksandra Tasić[a], Ivan Pavlović[a], Tanja Bijelić[a], Jasna Kureljušić[a], Marija Pavlović[a] and Milan Baltić[b]*

[a] *Scientific Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, 8 Bulevar oslobođenja Street, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Fatty acid
GC/FID
Muscle
Turkey meat

ABSTRACT

The aim of this work was to determine the fatty acid profile and health lipid indices of fresh turkey meat, as well as products obtained from turkey meat, i.e. turkey sausage and pate. Turkey breast muscles were cut from the side of the carcass, separately vacuum packed and stored in a refrigerator before analysis. Sausages and pate were produced from turkey meat using a technological process. The fatty acid profile of the samples was determined by gas chromatography with a flame ionization detector (GC/FID), and lipid indices were calculated based on the composition. Turkey muscle had a higher percentage of C16:0 and C18:0 than turkey sausage and pate. A significantly higher proportion of saturated fatty acids (47.9%) than in sausage and pate, 27.2 and 8.9%, respectively, characterized turkey muscle. The lowest determined proportion of polyunsaturated fatty acids for turkey muscle was 12.0%. The atherogenicity index was satisfactory for all three tested products, while the thrombogenicity index was satisfactory only for the tested raw turkey muscle meat (1.656).

1. Introduction

A balanced diet affects the healthy growth and development of individuals throughout life, while otherwise imbalance can be the cause of chronic diseases and obesity. For this reason, part of food literacy includes awareness of nutritional value and information about the impact of food on the consumer's health status (*Guiné et al.*, 2023). Lipids are essential food components as they perform several physiological functions in the human body. Therefore, the modern consumer is increasingly concerned with their food and pays attention to its quality, nutritional composition and effects on human health. The profile of fatty acid composition in meat and meat products depends on their origin, their quality characteristics and oxidative stability. In addition, the ratios of PUFA/SFA and n-6/n-3 PUFA, the content of hypo/hypercholesterolemic fatty acids, and the atherogenicity and thrombogenicity indices have become important parameters for evaluating the nutritional value and health of foods (*Wo1oszyn et al.*, 2020).

In the scientific literature, there are many new improved methods for faster and more efficient preparation of samples for the determination of fatty acids in different matrices, one of them is single-phase preparation. One such method includes trans methylation of meat samples, using, e.g., 5% hydrochloric acid in methanol or 5% sulphuric acid in methanol plus 0.1 N sodium metal in methanol (0.5 ml), and then after that treatment by heating in an oven or by the effect of microwaves (*Perez-Palacios et al.,* 2022).

Turkey used to be considered a once-a-year delicacy, but today, more people know that turkey is an economical meat and low in fat compared to "red

***Corresponding author:** Aleksandra Tasić, *alekstasic79@gmail.com*

Paper received August 9th 2023. Paper accepted August 26th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

meat" and are making it part of their regular diet. Turkey is also a good source of protein and minerals such as Na, K and Fe (*Ferreira et al.*, 2000). In this paper, the aim was to compare the profile of fatty acids in turkey meat and turkey meat products, as well as to calculate the quality and health indices. Quality and healthy parameters were determined according to published calculations given by other authors (*Ulbritch and Southgate*, 1991; *Chen et al.*, 2016).

2. Materials and methods

Total lipids were extracted in triplicate from each 5 g of homogenized sample (raw turkey muscle (RTM), turkey extra sausage (TES) and turkey pate (TP)) by the Folch method and calculated gravimetrically (*Folch et al.*, 1957). Fatty acids were quantified as methyl esters (FAMEs) with a gas chromatography system Clarus 680 PerkinElmer (USA) equipped with a flame ionization detector and a long capillary columns 60 m × 0.25 mm ID, 0.25 μm df PerkinElmer Elite-WAX GC column. Individual FAME peaks were identified by comparison of their retention times with those of standards (37-component FAME mix, AccuStandard, USA). The sampling rate during the determination was 12.5 pts/s, the volume of injected sample was 1 μL, and the duration and recording time of the chromatogram was 45.0 minutes. The initial temperature of the GC oven was 140°C, which increased to 200°C at 5°C/min and was maintained at this temperature for 20 minutes; it was then increased to 280°C at 5°C/min and maintained for an additional 15 minutes. The average amount (n=5) of each fatty acid was used to calculate the total amount of SFAs, MUFAs, and PUFAs. Extracted fat (50 mg) was added to 1 ml of acetonitrile containing 0.4 mg/ml pentanoic acid ethyl ester (C5:0) (Dr Ehrenstorfer, LGC), as an internal standard (IS). Data were analyzed by one-way analysis of variance (ANOVA). The results were presented as the mean and pooled standard error of the mean, with $P<0.05$ considered statistically significant. Five representative samples from each group of tested products were used for testing.

3. Results

The determination methodology included the steps of solvent extraction of lipids and the step of derivatization of the FA in methyl esters (FAMEs) and quantification using the GC/FID method.

Table 1. The fatty acid profile (mean values ± standard deviation) of raw turkey muscle (RTM), turkey extra sausage (TES) and turkey pate (TP) (% of total fatty acids)

Fatty acid	Abbreviation	RTM	TES	TP	p -Value
Caprylic acid	C8:0	0.08±0.01	Nd	Nd	<0.001
Lauric acid	C12:0	Nd	Nd	0.07±0.02	<0.001
Myristic acid	C14:0	1.01±0.08	0.32±0.004	0.19±0.023	<0.001
Pentadecanoic acid	C15:0	0.14±0.019	Nd	Nd	<0.001
Palmitic acid	C16:0	33.24±0.35	20.89±0.26	6.33±0.15	<0.001
Palmitoleic acid	C16:1	3.16±0.24	2.94±0.25	0.96±0.10	<0.001
Heptadecanoic acid	C17:0	0.36±0.05	Nd	Nd	<0.001
cis-10-Heptadecenoic acid	C17:1	0.09±0.01	Nd	Nd	<0.001
Stearic acid	C18:0	12.84±0.51	5.87±0.32	1.95±0.26	<0.001
Oleic acid	C18:1n9c	34.62±0.32	40.15±0.35	56.72±0.33	<0.001
Elaidic acid	C18:1n9t	1.76±0.05	1.7±0.04	2.61±0.07	<0.001
Linolenic acid	C18:2n6c	10.75±0.95	25.22±1.17	22.31±1.22	<0.001
Linolelaidic acid	C18:2n6t	0.23±0.02	Nd	Nd	<0.001
α-Linolenic acid	C18:3n3	Nd	1.32±0.06	5.34±0.20	<0.001
Arachidic acid	C20:0	Nd	Nd	0.32±0.05	<0.001
cis-11-Eicosenoic acid	C20:1	0.18±0.02	0.08±0.03	0.83±0.11	<0.001
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3 (EPA)	0.92±0.045	Nd	Nd	<0.001
Behenic acid	C22:0	0.15±0.036	Nd	Nd	<0.001
Erucic acid	C22:1n9	0.31±0.05	0.66±0.10	0.13±0.02	<0.001
cis-13,16-Docosadienoic acid	C22:2n6	0.1±0.002	0.68±0.05	2.24±0.13	<0.001
Tricosanoic acid	C23:0	Nd	0.15±0.10	Nd	0.0018
Lignoceric acid	C24:0	0.05±0.05	Nd	Nd	0.0263

Nd – not detected, $p<0.05$ — statistically significant differences

Table 2. Quality and health parameters of detected fatty acids for raw turkey muscle (RTM), turkey extra sausage (TES) and turkey pate (TP) under the optimized GC/FID method (mean value, n=5)

Index	RTM	TES	TP
ΣSFA	47.87	27.23	8.86
ΣMUFA	40.12	45.53	61.25
ΣPUFA	12.00	27.22	29.89
Total n-6	11.08	25.90	24.55
Total n-3	0.92	1.32	5.34
Total n-9	36.87	42.59	60.29
n-6/n-3	12.04	19.62	4.60
PUFA/SFA	0.25	1.00	3.37
LA/ALA	10.98	25.22	22.31
EPA + DHA	0.92	0.00	0.00
AI	0.72	0.30	0.08
TI	1.656	0.682	0.143
HH	1.410	3.224	13.340
UI	66.880	101.290	126.370
NVI	1.48	2.28	9.68

Abbreviations: SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; LA/ALA – linoleic/α-linolenic acid; AI – atherogenic index; TI – thrombogenicity index; H/H, hypocholesterolaemic/hypercholesterolaemic index; NVI, nutritive value index; UI – unsaturation index.

The total amount of fat in the tested samples was expressed as the mean value of five measurements for raw turkey meat (RTM), turkey extra sausage (TES) and turkey pate, finely chopped, sterilized (TP), and was 3.5%; 18.6% and 26.7% respectively. The fatty acid profiles of raw turkey meat and products from the turkey meat are summarized in Table 1. The products have different lipid contents and different percentages of fatty acids. Both muscle and turkey meat products contain the same acids out of 37 different acids, except that lower amounts of fattyacids were present in the muscle, while α-linolenic acid was present only in the products. Also, cis-5,8,11,14,17-eicosapentaenoic acid and behenic acid were not determined in the products but only in the muscle of turkey meat, 0.92% and 0.15%, respectively. Differences in fatty acids are expected due to the processing process, as well as the addition of salt and additives in the production process. The difference in fatty acids is evidently extremely significant ($p < 0.05$) for all measured acids with an obvious change in fatty acid quantity. The most noticeable change in fatty acid content was for palmitic acid, stearic acid, and linolenic acid (Table 1). PUFA/SFA is the most commonly used index to assess the impact of certain foods on cardiovascular health, due to the view that all PUFAs are able to reduce low-density lipoproteins, lipoprotein cholesterol, as well as serum cholesterol, while all SFA can contribute to an increase in serum cholesterol. As a result, this is a direct index: higher values indicate better (positive) effect, given a certain intake of meat or meat products. The recommended value is greater than 0.4 according to the requirements of the World Health Organization (WHO, 2003).

4. Discussion

The ratio of n-6/n-3 needs to be a lower ratio because this composition of fatty acids is preferable for reducing the risk of many chronic diseases of high prevalence in Western societies. According to health recommendations, the n-6/n-3 ratio should be less than the value of 4, which was not achieved by any of the tested products (Table 2). Poultry products are high in omega-3 and omega-6 fatty acids, with a favourable n-6/n-3 ratio, especially turkey meat (*Lalev et al.*, 2021). cis-5,8,11,14,17-Eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) are important acids because they improve vascular endothelial function and help lower blood pressure, the serum triglyceride level and platelet sensitivity. The presence of DHA was not determined in muscle and turkey meat products. The H/H index takes into account the known effects of certain fatty acids (especially oleic and linoleic acids) involved in cholesterol metabolism. A higher value of this index shows better effects on human health, which was also obtained

for sausage and pate (*Ulbritch and Southgate*, 1991; *Chen and Liu*, 2020). The high value of UI indicates a high degree of complete unsaturation; for pork and its products, the value ranges from 73 to 124 (*Chen and Liu*, 2020). Also, UI is of great importance in determining oxidative stability. The LA/ALA ratio is the highest for turkey sausages, while in the scientific literature, lambs had lower LA/ALA. With the growing popularity of processed meat products among consumers, meat scientists are investigating the potential applications and benefits of structured emulsions (emulsion and oleogels hydrogels) as fat replacers in a variety of applications. The addition of plant-based oil in animal fat leads to a decrease in SFA, and an increase in MUFA, PUFA and omega-3. For example, addition of bioactive components (extract of black chokeberry (*Aronia melanocarpa*) can increase the stability of turkey meat (*Pasichniy et al.*, 2022). Moreover, the results of our study confirm that adding additives and oil leads to a decrease in SFA and an increase in MUFA and PUFA in turkey meat products compared with turkey meat.

5. Conclusion

Products with higher levels of PUFA in their composition have a better cardiovascular prognosis. The chemical composition and fatty acid profile of the analyzed meat products were considerably impacted by differences in components and production technology. Meat with a lower content of saturated fatty acids is more indicative, from the point of view of consumer health, because lauric, myristic and palmitic fatty acids, when consumed in large quantities, increase the concentration of low-density lipoprotein (LDL) and total cholesterol in the plasma, increasing the risk of cardiovascular disease. In contrast to other saturated fatty acids, stearic acid, which was found at a level of 12.84% in turkey muscle, has a neutral or even lowering effect on blood cholesterol levels.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was funded by the Serbian Ministry of Science, Technological Development and Innovation (Contract No. 451-03-47/2023-01/200030).

Acknowledgements: This work was supported by Scientific Institute of Veterinary Medicine of Serbia, Belgrade.

References

Chen, Y., Qiao, Y., Xiao, Y., Chen, H., Zhao, L., Huang, M. & Zhou, G. (2016). Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers, and spent hens. *Asian-Australasian Journal of Animal Sciences*, 29(6), 855–864, http://dx.doi.org/10.5713/ajas.15.0840

Ferreira, M. M. C., Morgano, M. A., Queiroz, S. C. N. & Mantovani. D. M. B. (2000). Relationships of the minerals and fatty acid contents in processed turkey meat products. *Food Chemistry*, 69, 259–265.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, 226(1), 497–509.

Guiné, R. P. F., Florença, S. G. Aparício, G. Cardoso, A. P. & Ferreira, M. (2023). Food literacy scale: Validation through exploratory and confirmatory factor analysis in a sample of Portuguese university students. *Nutrients*, 15(1), 166, https://doi.org/10.3390/nu15010166

Lalev, M., Mincheva, N., Oblakova, M., Hristakieva, P., Ivanova, I., Atanassov, A. & Petrova, A. (2021). Insect-based diets effects on turkey meat quality. *Bulgarian Journal of Agricultural Science*, 27(5), 980–989.

Pasichniy, V., Tischenko, V., Bozhko, N., Koval, O. & Marynin, A. (2022). Use of bioactive properties of plant extracts to increase the storage stability of mechanically separated turkey meat. *Ukrainian Food Journal*, 11(4), 616–628.

Perez-Palacios, T., Solomando, J. C., Ruiz-Carrascal, J. & Antequera, T. (2022). Improvements in the methodology for fatty acids analysis in meat products: One-stage transmethylation and fast-GC method. *Food Chemistry*, 371, 130995, https://doi.org/10.1016/j.foodchem.2021.130995

Ulbricht, T. L. V. & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *The Lancet*, 338(877), 985–992.

Wo1oszyn, J., Haraf, G., Okruszek, A., Werenska, M., Goluch, Z. & Teleszko, M. (2020). Fatty acid profiles and health lipid indices in the breast muscles of local Polish goose varieties. *Poultry Science*, 99, 1216–1224, https://doi.org/10.1016/j.psj.2019.10.026

World Health Organization — WHO, (2003). Diet, Nutrition and the Prevention of Chronic Diseases Technical Report Series 916, Geneva: World Health Organization), 87–88. https://www.who.int/publications/i/item/924120916X

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.05

ID: 126504457

https://doi.org/10.18485/meattech.2023.64.2.94

Original scientific paper

Effects of different cooking methods of pork and beef on textural properties and sensory quality

Sara Simunović[a,b], Igor Tomasević[c,d], Vesna Đorđević[a], Tatjana Baltić[a], Jelena Ćirić[a], Stefan Simunović[a] and Ilija Đekić[b]

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040, Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Agriculture, Department of Food Safety and Quality Management, Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*
[c] *Department of Animal Source Food Technology, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*
[d] *German Institute of Food Technologies (DIL), Quakenbruck, Germany*

ARTICLE INFO

Keywords:
Cooking
Grilling
Frying
Texture
Pork
Beef

ABSTRACT

This study investigates the effects of different cooking methods and meat cuts on the textural and sensory properties of pork and beef. Meat samples were prepared according to guidelines and relevant standards using pan frying and grilling. Three types of pork cuts were included (pork ham, loin and neck) and three types of beef steak (chuck steak, round steak and sirloin steak). Textural properties such as hardness, springiness, cohesiveness, gumminess, and chewiness were analyzed. Sensory attributes that are the most common for assessing the palatability of cooked meat were used. In the comparison of cooking methods within textural parameters, grilled pork and beef samples were tougher than fried ones. The cooking methods of meat samples depending on types of cuts resulted in different sensory attributes.

1. Introduction

In Serbia from the data for 2023, the import of raw meat is increasing, especially when it comes to pork. The main reasons for that are declines in pork prices on the one hand, and on the other hand, increases in prices of all inputs required for farming. However, the availability for Serbian consumers of pork is at an acceptable level, with 90% of pork available to consumers being locally produced (*RZS*, 2023a).

The systematic changes and strategies are necessary for meat producers and farmers, in order to continue to produce meat continually and in a socially responsible manner. Regardless of the phase in the meat chain, all stakeholders from the livestock and feed production to retailers and consumers, need to be aware of possible price increases of meat. Recent governmental reports predicted 20% of meat price increase. However, meat is the second largest food category in Serbia that affects the average annual increase of food prices, accounting for approximately 23.9% of the total increase of food prices (*RZS*, 2023b). In this share, beef has the largest impact on food price increasing, while pork and chicken follow.

Meat production is mainly focused on the technological, environmental, ethical, nutritional and healthy aspects of meat and meat products (*Djekic et al.*, 2016; *Grunert et al.*, 2018). However, when observing any of these approaches, their impact on the quality of

***Corresponding author:** Sara Simunović, *sara.rajic@inmes.rs*

Paper received August 9th 2023. Paper accepted August 26th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

meat and meat products should be considered, as well as the changes in sensory and textural features (*Simunovic et al.*, 2020). In this case, additional quality controls are required for implementing new quality tools for monitoring. These changes and improvements are acquiring more human and financial resources, and consequently greater consumer satisfaction.

The objective of this research is to evaluate the effects of different thermic treatments and types of muscles on textural properties and sensory quality of meat. Previous studies showed influences of visual marbling score, carcass weight, cooking methods, aging technologies, etc. on the sensory quality and consumer's acceptance of fresh meat (*Cannata et al.*, 2010; *Gurinovich et al.*, 2023; *Hwang et al.*, 2019; *Hwang et al.*, 2020). To our knowledge, there are a limited number of studies that have examined the effect of cooking methods on fresh meat quality, especially beef (*Grujić et al.*, 2014; *Wołoszyn et al.*, 2020).

2. Materials and methods

2.1. Pork and beef samples

For the purpose of this research, three types of pork cuts (pork ham, loin and neck) and three types of beef steak (chuck steak, round steak and sirloin steak) were used. The samples were purchased locally at retail in Belgrade. After purchase, pork and beef were cut in uniform steaks, sealed in vacuum bags and stored in the refrigerator at 2 to 5°C. Before cooking, steaks were placed at room temperature for half an hour.

2.2. Cooking methods

Two cooking methods were used: pan frying and grilling. Pan frying is using a pan with additional oil placed on the direct heat of a conduction cook-top. On the other hand, treatment by open-heat electric grill is a popular method of cooking that has largely replaced others. A grill with a lid that closed on the meat and heated from top and bottom was used.

In both cases the heating procedure was the same, according to American Meat Science Association guidelines (*AMSA*, 2016). Firstly, appliances were preheated for at least 10 minutes. During that time, meat sample weights before cooking were recorded. Meat samples were placed on the pan and electric grill surfaces and removed when samples reached the desired internal temperature (71°C for all cuts of pork and beef).

Meat samples were cooled to room temperature, and prior to serving were sliced into cubes. The *AMSA* (2016) recommendations were used to achieve an appropriate and uniform thickness and size of samples. The final size of all samples for sensory analysis was 25.4 mm x 25.4 mm x 25.4 mm.

2.3. Instrumental texture analysis

Texture profile analysis (TPA) was performed using TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Vienna Court, UK) according to *Simunovic et al*. (2020) with some modifications. Uniform size cuts (approximate mass of 230 g) were prepared for each type of cut for pork and beef and both cooking methods. Rectangular samples (20 mm height, 15 × 15 mm base) were cut off along the axis of the muscle fibers and used for testing. Nine samples of pork and nine samples of beef from each type of cuts were measured, 54 per cooking method in total (2 x 9 x 3).

2.4. Sensory evaluation

2.4.1. Descriptive sensory analysis

The descriptive analysis was done by trained sensory panel in the Laboratory for Sensory Testing at the Institute of Meat Hygiene and Technology. The sensory panel consisted of 10 panelists (four male and six female members) experienced in sensory evaluation and of a good general health condition (*Djekic et al.*, 2021). Eight-point scales were used for evaluating tenderness, juiciness, and flavor intensity (*AMSA*, 2016). The points of scales were from 1 – extremely tough to 8 – extremely tender for tenderness, 1 – extremely dry to 8 – extremely juicy for juiciness, and 1 – none to 8 – extremely intense for flavor intensity.

2.4.2. Triangle test

The sensory panel consisted of 36 untrained panelists (consumers). They tested samples in laboratory conditions at the University of Belgrade, Faculty of Agriculture. Testing was carried out under conditions that prevented communication among assessors until all the evaluations had been completed. The panelists were informed that two samples were the same and that one was different. For the purpose of triangle test, grilled pork ham and pork loin were used, as these had been chosen as the best scored samples during descriptive analysis. The

selection of beef samples followed the same procedure; thus, grilled beef round steaks and sirloin steaks were included in triangle test.

2.4.3. Statistical analysis

In order to analyze results of textural properties and sensory analysis, descriptive statistics, testing of normal distribution (Shapiro–Wilk), homogeneity of variances (Levene Statistic), one-way analysis of variance (ANOVA), and post hoc test (Tukey) were performed using SPSS package (SPSS 23.0, Chicago, IL, USA).

3. Results and discussion

3.1. Influence on textural properties

According to Table 1, the hardness was not significantly different among all samples of cooked pork. However, grilled pork cuts were tougher, among which pork ham had the highest hardness value. This was also investigated by *Djekic et al.* (2021), where the high impact of culinary methods on both quality and oral processing parameters of pork ham was confirmed. On the other hand, grilled samples showed significant difference of cohesiveness, gumminess, and chewiness, among which pork ham and neck had the highest values ($p < 0.05$). Values for springiness were not significantly different in both cooking methods.

Contrary to pork samples, the hardness was significantly different for all beef cuts ($p < 0.05$; Table 2). Generally, grilled beef samples were tougher than fried ones. The tenderest beef cut was fried chuck steak, while the toughest one was grilled sirloin steak. Furthermore, gumminess was significantly different within fried and grilled beef samples. Similar to hardness, values of gumminess were the lowest for fried chuck steak, while the highest values were for sirloin steak. Chewiness was significantly different only for grilled beef samples, which is similar to pork results. On the other hand, values for springiness and cohesiveness were not significantly different.

Values (mean ± standard deviation) with different lowercase letters (a-c) in the same row differ significantly ($p < 0.05$).

Table 1. Textural properties of pork with different cooking methods

	Pan frying			Grilling		
Attributes	**Ham**	**Loin**	**Neck**	**Ham**	**Loin**	**Neck**
Hardness (N)	9.08 ± 3.33	13.31 ± 10.57	8.69 ± 2.12	17.92 ± 5.32	14.36 ± 2.69	17.61 ± 4.21
Springiness	0.72 ± 0.05	0.72 ± 0.08	0.72 ± 0.10	0.75 ± 0.07	0.69 ± 0.03	0.73 ± 0.09
Cohesiveness	0.67 ± 0.06	0.65 ± 0.06	0.67 ± 0.17	0.67 ± 0.04^{a}	0.60 ± 0.03^{b}	0.68 ± 0.05^{c}
Gumminess (N)	6.25 ± 2.34	8.21 ± 5.87	5.71 ± 2.05	12.78 ± 3.53^{a}	8.59 ± 1.52^{b}	13.75 ± 3.83^{c}
Chewiness (N)	4.57 ± 1.75	5.71 ± 3.88	5.8 ± 2.18	9.52 ± 2.69^{a}	5.96 ± 1.06^{b}	9.75 ± 2.20^{c}

Values (mean ± standard deviation) with different lowercase letters (a-c) in the same row differ significantly ($p < 0.05$).

Table 2. Textural properties of beef with different cooking methods

	Pan frying			Grilling		
Attributes	**Chuck steak**	**Round steak**	**Sirloin steak**	**Chuck steak**	**Round steak**	**Sirloin steak**
Hardness (N)	9.2 ± 6.47^{a}	16.03 ± 6.96^{b}	18.76 ± 10.5^{c}	22.13 ± 5.75^{a}	13.28 ± 5.33^{b}	30.81 ± 8.75^{c}
Springiness	0.66 ± 0.08	0.65 ± 0.08	0.69 ± 0.27	0.68 ± 0.03	0.62 ± 0.24	0.66 ± 0.11
Cohesiveness	0.59 ± 0.01	0.63 ± 0.05	0.67 ± 0.06	0.6 ± 0.06	0.55 ± 0.21	0.59 ± 0.03
Gumminess (N)	5.86 ± 4.68^{a}	10.89 ± 4.34^{b}	12.85 ± 8.3^{c}	13.47 ± 4.17^{a}	7.72 ± 4.33^{b}	18.56 ± 1.73^{c}
Chewiness (N)	3.73 ± 3.14	6.35 ± 2.58	5.57 ± 2.79	9.25 ± 3.02^{a}	5.42 ± 3.16^{b}	12.95 ± 9.2^{4c}

Values (mean ± standard deviation) with different lowercase letters (a-c) in the same row differ significantly ($p < 0.05$).

3.2. Influence on sensory quality

Firstly, the tenderness was not significantly different within both groups of cooked pork samples. This means the sensory panel was consistent in their responses. The lowest mean value for tenderness was evaluated for pork ham (Table 3), which means the sensory panel evaluated pork ham as the toughest sample. On the other hand, pork ham was scored as juicier than pork neck by the sensory panel.

However, juiciness of all treated pork samples was found to be significantly different within both cooking methods ($p < 0.05$). The pork loin was evaluated with the highest scores for all examined sensory attributes: tenderness, juiciness, and flavor in both cooking methods. That means pork loin had the best-scored sensory quality. On the other hand, pork ham and neck were scored similarly in general.

When it comes to flavor, the scores of fried pork samples were found to be significantly different ($p < 0.05$; Table 3). This is in accordance with the findings of *Peñaranda et al.* (2017) where it was revealed that fried pork meat had the highest intensity of flavor in comparison with grilled samples.

In the case of beef (Table 4), only tenderness was found to be significantly different ($p < 0.05$) and just within fried samples. The tenderness values of fried beef steaks were influenced by the cut, which was also confirmed in study of *Miller et al.* (2023). Round steak was evaluated as the most tender cooked sample. When it comes to juiciness and flavor, grilled sirloin steak was evaluated as the juiciest and the most characteristic in flavor. This was also confirmed by the study of (*Liu et al.,* 2020) where the premium beef steaks had the best scores for each sensory trait.

On the other hand, chuck steaks had the lowest scores for all sensory attributes, which is in accordance with results of *Miller et al.* (2022), where consumers rated chuck roasts lowest for overall, overall flavor, grilled flavor, and juiciness liking.

The objective of the triangle test was to analyze whether two samples are different. According to standard ISO 4120 (*ISO*, 2021), the following parameters were selected: $\alpha = 0.05$ (probability of concluding that a perceptible difference exists when one does not), $\beta = 0.05$ (probability of concluding that no perceptible difference exists when one does) and $p_d = 50\%$ (50% of assessors can detect difference). A total of 36 panelists were selected for both triangle tests. For pork meat, 14 panelists correctly identified the odd sample leading to the conclusion that pork samples were not perceived as different. On the contrary for beef, 20 assessors correctly

Table 3. Sensory evaluation of pork with different cooking methods

	Pan frying			Grilling		
Sensory Attributes	**Ham**	**Loin**	**Neck**	**Ham**	**Loin**	**Neck**
Tenderness	5.85 ± 1.36	6.25 ± 1.74	6.25 ± 1.74	6.03 ± 1.42	6.80 ± 1.61	6.15 ± 1.66
Juiciness	5.15 ± 1.66[a]	6.90 ± 0.77[b]	5.15 ± 1.57[c]	6.18 ± 1.42[a]	7.00 ± 1.37[b]	5.58 ± 1.59[c]
Flavor Intensity	6.18 ± 1.37[a]	7.00 ± 1.37[b]	5.58 ± 1.59c	6.30 ± 1.08	7.18 ± 1.15	6.35 ± 1.49

Values (mean ± standard deviation) with different lowercase letters (a-c) in the same row differ significantly ($p < 0.05$). Scale: "1 – extremely tough to 8 – extremely tender"; "1 – extremely dry to 8 – extremely juicy"; "1 – none to 8 – extremely intense".

Table 4. Sensory evaluation of beef with different cooking methods

	Pan frying			Grilling		
Sensory Attributes	**Chuck steak**	**Round steak**	**Sirloin steak**	**Chuck steak**	**Round steak**	**Sirloin steak**
Tenderness	4.05 ± 1.51[a]	5.23 ± 1.33[b]	4.13 ± 1.45[c]	4.33 ± 1.44	5.40 ± 1.49	4.73 ± 1.81
Juiciness	4.85 ± 1.73	5.30 ± 1.35	4.93 ± 1.52	4.98 ± 1.37	5.10 ± 1.34	5.49 ± 1.34
Flavor Intensity	5.37 ± 1.37	5.45 ± 1.44	5.47 ± 1.41	5.38 ± 1.2	5.43 ± 1.44	5.53 ± 1.51

Values (mean ± standard deviation) with different lowercase letters (a-c) in the same row differ significantly ($p < 0.05$). Scale: "1 – extremely tough to 8 – extremely tender"; "1 – extremely dry to 8 – extremely juicy"; "1 – none to 8 – extremely intense".

identified the odd sample, and this number was sufficient to conclude that the two beef samples were perceptibly different.

4. Conclusion

Results from this study regarding texture analyses indicate that cooking methods had impact on texture of meat, so more precisely, grilling makes pork and beef samples tougher than pan frying. Furthermore, hardness and gumminess values of beef samples are significantly different when comparing pan frying and grilling. Moreover, cooking methods influence the sensory quality of meat, especially when it comes to juiciness of pan-fried and grilled pork samples. Beside cooking methods, the influence of types of meat cuts was noted, identifying the toughest meat samples — pork ham and beef sirloin steak. Likewise, the sensory quality of pork and beef was influenced by the type of meat cut. The flavor intensity scores for different cuts of fried pork were significantly different. When it comes to different types of fried beef steaks, values of tenderness were significantly different across samples.

A limitation of the study may be the absence of consumer preference analysis related to pork and beef prepared with the more than two cooking methods. Future research into pork and beef could investigate influences of other cooking methods and/or other types of meat cuts.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

AMSA, (2016). Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (American Meat Science Association, Issue.

Cannata, S., Engle, T. E., Moeller, S. J., Zerby, H. N., Radunz, A. E., Green, M. D. . . . & Belk, K. E. (2010). Effect of visual marbling on sensory properties and quality traits of pork loin. *Meat Science*, 85(3), 428–434, https://doi.org/https://doi.org/10.1016/j.meatsci.2010.02.011

Djekic, I., Blagojevic, B., Antic, D., Cegar, S., Tomasevic, I. & Smigic, N. (2016). Assessment of environmental practices in Serbian meat companies. *Journal of Cleaner Production*, 112, 2495–2504, https://doi.org/https://doi.org/10.1016/j.jclepro.2015.10.126

Djekic, I., Ilic, J., Lorenzo, J. M. & Tomasevic, I. (2021). How do culinary methods affect quality and oral processing characteristics of pork ham? *Journal of Texture Studies*, 52(1), 36–44.

Djekic, I., Lorenzo, J. M., Munekata, P. E. S., Gagaoua, M. & Tomasevic, I. (2021). Review on characteristics of trained sensory panels in food science. *Journal of Texture Studies*, 52, 501–509.

Grujić, R. D., Vujadinović, D. P. & Tomović, V. M. (2014). Heat treatment influence on rheological properties of pork meat. *Journal of Hygienic Engineering and Design*, 6, 63–68.

Grunert, K. G., Sonntag, W. I., Glanz-Chanos, V. & Forum, S. (2018). Consumer interest in environmental impact, safety, health and animal welfare aspects of modern pig production: Results of a cross-national choice experiment. *Meat Science*, 137, 123–129, https://doi.org/https://doi.org/10.1016/j.meatsci.2017.11.022

Gurinovich, G., Khrenov, V., Patrakova, I. & Patshina, M. (2023). Studying an effect of thermal treatment methods on physico-chemical properties of beef depending on aging technology. *Food Systems*, 5(4), 376–382.

Hwang, S. I., Lee, E. J. & Hong, G. P. (2019). Effects of Temperature and Time on the Cookery Properties of Sous-vide Processed Pork Loin. *Food Science of Animal Resources*, 39(1), 65–72, https://doi.org/10.5851/kosfa.2019.e4

Hwang, Y. H., Lee, S. J., Lee, E. Y. & Joo, S. T. (2020). Effects of carcass weight increase on meat quality and sensory properties of pork loin. *Journal of Animal Science and Technology*, 62(5), 753–760, https://doi.org/10.5187/jast.2020.62.5.753

ISO, (2021). ISO 4120:2021 Sensory analysis — Methodology — Triangle test. https://www.iso.org/

Liu, J., Ellies-Oury, M-P., Chriki, S., Legrand, I., Pogorzelski, G., Wierzbicki, J., . . . & Hocquette, J-F. (2020). Contributions of tenderness, juiciness and flavor liking to overall liking of beef in Europe. *Meat Science*, 168, 108190, https://doi.org/https://doi.org/10.1016/j.meatsci.2020.108190

Miller, R. K., Luckemeyer, T. J., Kerth, C. R. & Adhikari, K. (2023). Descriptive beef flavor and texture attributes relationships with consumer acceptance of US light beef eaters. *Meat Science*, 204, 109252. https://doi.org/https://doi.org/10.1016/j.meatsci.2023.109252

Miller, R. K., Pena, C. A., Legako, J. F., Woerner, D. R., Brooks, C., Schilling, B., . . . &Wall, K. R. (2022). 2018 National Beef Flavor Audit: Consumer and Descriptive Sensory Attributes. *Meat and Muscle Biology*, 6(1), DOI: 10.22175/mmb.13017

Peñaranda, I., Garrido, M. D., Egea, M., Díaz, P., Álvarez, D., Oliver, M. A. & Linares, M. B. (2017). Sensory perception of meat from entire male pigs processed by different heating methods. *Meat Science*, 134, 98–102.

RZS, (2023a). Agriculture, forestry and fishery — Livestock breeding [Statistical release]. https://publikacije.stat.gov.rs/G2023/HtmlE/G20231033.html

RZS, (2023b). Monthly statistical bulletin Statistical Office of the Republic of Serbia Retrieved from https://publikacije.stat.gov.rs/G2023/Pdf/G20233005.pdf

Simunovic, S., Đorđević, V., Barba, F., Lorenzo, J. M., Rašeta, M., Janković, S. & Tomasevic, I. (2020). Characterisation of changes in physicochemical, textural and microbiological properties of Njeguška sausage during ripening. *Journal of Food Science and Technology -Mysore.* https://doi.org/10.1007/s13197-020-04862-z

Wołoszyn, J., Wereńska, M., Goluch, Z., Haraf, G., Okruszek, A., Teleszko, M. & Król, B. (2020). The selected goose meat quality traits in relation to various types of heat treatment. *Poultry Science*, 99(12), 7214–7224, https://doi.org/https://doi.org/10.1016/j.psj.2020.09.062

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664]:658.562.4

ID: 126500361

https://doi.org/10.18485/meattech.2023.64.2.95

Review paper

Flexibility and amendments of the Codex Alimentarius aimed towards small food business entities

Boris Mrdović[a], Branislav Baltić[a], Nikola Betić[a], Mladen Rašeta[a], Jelena Jovanović[a], Ivana Branković Lazić[a] and Aleksandar Bajčić[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Codex Alimentarius
FAO
WHO

ABSTRACT

The Codex Alimentarius represents a collection of international standards and guidelines concerning food safety. It was developed by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Encompassing various aspects of food production, from manufacturing to labeling, this comprehensive document primarily serves large industrial producers. However, the needs of small entrepreneurs in the food industry are increasingly being recognized. This paper explores potential adaptations of the Codex Alimentarius to better align with the requirements of micro- and small entities, with a particular focus on promoting flexibility and ensuring safety in their operations. Additionally, existing regulations that already allow for flexibility are examined, along with suggestions for further amendments aimed at small businesses in the food industry. Finally, this document addresses challenges related to the implementation of such measures, including enforcement, capacity-building, and raising awareness.

1. Introduction

The Codex Alimentarius is a globally recognized compilation of standards, guidelines, and codes of practice pertaining to food safety, quality control, and the production of consumable goods. With a history spanning over a century, it has emerged as one of the paramount references for ensuring global food safety and quality assurance. In recent years, however, the necessity to grant small entities in the food industry access to and benefits from the same protective mechanisms as larger enterprises has been increasingly understood. As a result, several flexibilities and additions have been introduced to the Codex Alimentarius, with a particular focus on catering to the needs of small entities. These flexibilities, such as exemptions from certain regulations or less stringent requirements, aim to ensure that small entities are not disadvantaged in comparison to their larger counterparts. This is especially significant considering the significant contribution that small businesses make to local economies, both in terms of job creation and economic growth. These additions also promote fair competition among food producers, enabling them to operate on an equal footing, regardless of their size or resources.

In addition to regulatory flexibility, the Codex Alimentarius provides valuable guidance to small businesses on a wide range of topics, including food safety and quality assurance. This encompasses instructions for developing appropriate systems and processes to ensure compliance with necessary

***Corresponding author:** Boris Mrdović, *boris.mrdovic@inmes.rs*

Paper received August 14th 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

standards, as well as advice on effective risk management related to the production of consumable goods. By following these guidelines, small enterprises can establish trust with customers and other stakeholders, thereby facilitating their sustainable growth and development. Consequently, it is imperative for small entities to familiarize themselves with the Codex Alimentarius and utilize it as a reliable resource during consumable goods production. Furthermore, the Codex Alimentarius offers crucial insights into emerging trends in food safety, such as the integration of digital technologies into quality assurance systems. This includes guidance on incorporating data collection and traceability, as well as advice on using predictive analytics to identify potential risks. By staying abreast of the latest advancements in the food industry through the Codex Alimentarius, small businesses can remain competitive and compliant in an ever-evolving environment.

Emphasizing the Importance of Leadership and FSMS: Recognizing the significance of leadership and food safety management system (FSMS) in maintaining safety and quality standards, the Codex Alimentarius has implemented a FSMS-based certification scheme (FCS). This scheme provides assurance to small food businesses regarding the high quality of their practices. It allows flexibility in the implementation of food safety management systems, along with the ability to incorporate new elements or modifications as needed. The Codex Alimentarius maintains a dynamic nature to cater to the needs of small food business entities. It has established a certification scheme for small processors and manufacturers, known as good manufacturing practices (GMP) principles. This enables these businesses to obtain product certification in line with GMP standards, overseen by an independent third party. Furthermore, the Codex Alimentarius encourages flexibility and additions to its code. It provides guidance to small processors and manufacturers on how to comply with food safety requirements without significant changes or modifications to their operations. Additionally, the Codex allows for the revision of guidelines and recommended practices as needed to ensure food safety maintenance. Moreover, the Codex promotes risk assessment approaches tailored to the specific nature of small business operations. It offers guidance and examples to small processors and manufacturers for the effective implementation of risk management plans. Additionally, the Codex recognizes the need to address the unique requirements of small food business entities, especially concerning labeling regulations. For instance, it allows simplified labeling requirements for small packages or containers, as well as exemptions from certain labeling requirements for small processors and manufacturers. Lastly, the Codex encourages the use of harmonized international standards and has formed partnerships with various organizations to provide technical assistance to small food businesses. This includes access to training materials on GMP principles and related topics, as well as advice and guidance on the implementation of good food safety practices. Such assistance helps small enterprises understand and comply with food safety regulations, ultimately safeguarding public health. The Codex Alimentarius serves as an invaluable tool in ensuring safe food production and distribution worldwide. By providing guidelines tailored to the needs of small businesses, it ensures that food safety standards are adhered to, even for those operating on a small scale. In this way, the Codex contributes to public health protection by promoting food safety for all. Beyond food safety guidelines, the Codex supports sustainable development and environmental protection. Through its standards and codes of practice, it encourages environmentally friendly practices in food production, distribution, and marketing. This helps ensure that food production occurs in a manner that minimizes environmental impacts, such as pollution and resource depletion. The Codex Alimentarius has proven to be an invaluable tool in promoting global food safety and sustainable development. Its standards and guidelines ensure that food produced worldwide meets acceptable levels of safety, quality, and sustainability. Moreover, its focus on small food businesses aids in protecting public health by ensuring compliance with good food safety practices in these industries.

Food Safety Culture: Food safety culture is widely recognized as a key element for the success of a food safety system. This culture encompasses the knowledge, attitudes, and behaviors shared among employees in the food industry. To cultivate this culture within small entities, it is crucial to provide them with flexibility in aligning with Codex Alimentarius standards. Given their limited resources and qualified personnel, these entities require additional support to ensure compliance with these standards. The Codex Alimentarius Commission has introduced several initiatives aimed at facilitating the adoption of food safety practices by small food business entities. These initiatives include providing

guidance documents for small producers, promoting access to validated systems and networks for designing customized food safety management plans, and developing simplified tools to facilitate the implementation of food safety programs. Furthermore, the Codex Alimentarius actively promotes the concept of "harmonized good practices", specifically tailored to small operations in developing countries. This approach seeks to promote safe production practices while considering the local context and specificities of each country. To support this endeavor, the Commission has developed a set of principles and guidelines for harmonized good practice, which have been adopted by various countries. Furthermore, the Codex Alimentarius aims to provide additional support to small food business entities. This includes offering training materials and programs, providing technical advice and assistance, and developing user-friendly tools to enhance understanding of food safety requirements and their implementation, particularly for less experienced personnel. Moreover, the Commission has established a network of regional centers and a global database containing risk profiles for various food items. These resources are intended to facilitate access to information for small entities, enabling them to better comprehend the applicable Codex Alimentarius standards. In other words, these efforts are geared towards aiding small entities in achieving food safety compliance and reducing the risk of associated health hazards. By providing access to resources, training, and support, the Codex Alimentarius strives to contribute to a safer global food supply. Simultaneously, the Commission is also working on promoting fair trade practices in the international food sector. Collaborating with various organizations enables the development and implementation of measures to establish fair pricing, enforce food safety standards, and regulate the global food supply chain. This includes the introduction of principles for accurate food labeling, providing consumers with essential information about origin, composition, and nutritional value. The Codex Alimentarius is also dedicated to reducing market disruptions that can lead to unfair practices regarding prices or the safety of food products. Overall, the Codex Alimentarius serves as a valuable resource for small food business entities by providing access to resources and guidelines on food safety requirements. The Commission's efforts contribute to ensuring the production of safe food in line with international standards, while also promoting equal access to goods and services across the global market. Through these initiatives, the Codex Alimentarius supports the development of healthier and more sustainable dietary systems worldwide (*Tanemura et al*., 2017).

Emphasized control measures: Emphasized control measures are needed to ensure that flexibility is embedded in the Codex Alimentarius, especially for small entities in the food business. Small producers, processors, and retailers often lack resources and technical expertise, making compliance with Codex guidelines challenging or even impossible. Therefore, it is crucial to establish a set of measures to ensure that these smaller players are given an opportunity to adhere to international standards. To this end, the Codex Alimentarius Commission should consider introducing additional measures to facilitate compliance for small entities in the food business. This could encompass streamlined procedures and documentation requirements, simplified hazard analysis and critical control point (HACCP) plans, streamlined certification processes, and more flexible labeling and packaging standards. Furthermore, steps should be taken to ensure that the implementation of Codex regulations is fair and equitable, regardless of the size of food enterprises. Moreover, it is essential that more resources are allocated to educating and training small entities in the food business about Codex Alimentarius requirements. National bodies should provide compliance guidelines with Codex standards, as well as access to information about new developments and initiatives related to Codex Alimentarius. Small entities should have access to technical assistance and capacity-building programs that would enable them to become aware of their responsibilities regarding food safety and Codex compliance (*Meulen*, 2019). Finally, it is important to assist and promote the development of innovative technologies for small entities in the food business. This could include mobile applications providing information about food safety regulations and certification requirements, as well as platforms for sharing best practices related to Codex Alimentarius regulations. Technologies like these could help small entities better understand their obligations and ensure they are capable of adhering to the international standards set within the Codex (*Price*, 2020).

Insistence on validation and verification: Validation and verification of Codex Alimentarius standards, particularly for small entities in the food industry, are of paramount importance. This ensures that national and international agencies are

fully informed about any potential issues related to small-scale food production or manufacturing. Furthermore, it is crucial for competent authorities to remain vigilant in detecting any violations that may pose risks to public health or other forms of harm. In order to facilitate compliance with Codex Alimentarius standards, governments should consider implementing adaptable control measures. This may involve reducing testing frequency for small-scale production facilities or granting exemptions from certain record-keeping or documentation requirements. Such measures would enable smaller entities in the food industry to optimize resource management and focus on ensuring food safety and quality. Moreover, it is important to recognize that small businesses face distinct challenges compared to larger enterprises. Therefore, governments should adopt additional measures that take into account the resources available to small entities in the food industry. This could include providing greater flexibility in compliance deadlines or offering technical support to operators for the effective implementation of Codex standards. Ultimately, governments must demonstrate a strong commitment to ensuring compliance with the standards and regulations established by Codex Alimentarius for small businesses in the food industry. This approach would create equal conditions for all stakeholders, while simultaneously preserving the safety and quality of food products in the market. Implementing such measures will not only benefit producers and manufacturers, but will also contribute to a safer and more secure food supply for all consumers (*Leprêtre*, 2018).

Allergens: The Codex Alimentarius has recently emphasized the importance of ensuring the safety of food products to protect consumer health. According to Codex standards, allergens and additives must be declared on food labels. However, there is a certain degree of flexibility within these standards for small entities, such as micro-businesses or small producers of traditional foods. Specifically, the Codex Alimentarius has outlined a series of recommendations for small producers with the aim of promoting flexibility and recognizing the unique circumstances of these micro-entities (see Annex III). These recommendations include the possibility of exemptions from certain labeling requirements or exceptions from specific standards applied to larger companies. Furthermore, the Codex Alimentarius can provide guidance for providing appropriate health warnings or advice, as well as for the use of precautionary statements and advisory labels *(Kruger et al*., 2020).

Traceability and Attention to Small FBOs: The Codex Alimentarius Commission has taken into consideration the needs of small entities in the food business, especially those operating with limited resources. They have sought to ensure that smaller businesses do not face undue additional burdens when striving to comply with international standards. To this end, greater flexibility and tailored solutions have been introduced for smaller FBOs, such as reduced testing and certification requirements. For instance, the commission has introduced a simplified version of the Codex HACCP system, designed to be more easily applicable for small enterprises. Food safety culture, allergen management, changes in HACCP principles, a new decision tree, definition and terminology changes, and the role of the Codex Alimentarius Commission — all these topics contribute to the dedication of the Codex Alimentarius Commission to ensuring food safety in global markets. With this dedication comes the recognition of the need for greater flexibility in the application of its standards and codes, particularly for small entities in the food business. Small entities encompass various stakeholders, including small food producers, importers, and processors. Due to limited resources and capacities, they often struggle to meet the standards outlined in the Codex Alimentarius. Therefore, it is crucial to provide increased flexibility in the implementation of these standards for small entities. To address this issue, the Codex Alimentarius Commission has introduced specific addenda and amendments tailored to small entities in the food business. This includes simplified risk-based classification, streamlined HACCP principle application, and a new decision tree to assist small enterprises in determining applicable standards within their specific circumstances (*WHO*, 2018).

Furthermore, the Commission has enhanced flexibility in the definitions and terminology used within its standards, ensuring better understanding and compliance for small entities. Encouraging the use of simple language further facilitates comprehension. By incorporating these additions and amendments, the Codex Alimentarius Commission enables the safe participation of small entities in global food markets without compromising safety or quality. This commitment supports food supply safety worldwide and promotes sustainable economic growth. These changes exemplify the Commission's dedication to providing guidance and support to all stakeholders in the food busi-

ness, regardless of size or resources. In the future, further adjustments are likely to be made to better tailor to small entities in the global food market. By offering additional support and guidance to small entities, the Codex Alimentarius Commission contributes to the availability of safe and quality food products for all stakeholders in the global food business. This, in turn, stimulates economic growth, reduces poverty, and enhances consumer nutrition worldwide. The Commission's work plays a vital role in ensuring a safe and secure global food system. Ultimately, the Codex Alimentarius Commission encourages small entities to familiarize themselves with standards and their application to their specific operations. Understanding these standards is essential for any small entity wishing to engage in international trade and ensure product safety. The Codex Alimentarius provides comprehensive guidance on this topic, along with other relevant information. With these guidelines and assistance, small businesses can confidently meet the highest safety standards for their products (*Ree et al*., 2021).

2. Conclusion

The flexibility and additions to the Codex Alimentarius have greatly benefited small businesses in the food industry. Regulatory requirements have been reduced, new labeling and safety provisions have been introduced, and recognition of regional differences has promoted fair resource distribution. These changes have enhanced global food safety standards, while improving cost-effectiveness and efficiency in food production and distribution. Undoubtedly, the Codex Alimentarius has proven to be an invaluable tool for small enterprises, allowing them to maintain competitiveness while adhering to safety and quality standards. As the Codex Alimentarius continues to evolve, its impact on the food industry is expected to further increase. The document remains a living framework, adapting to changing food safety regulations and ensuring that small businesses can thrive while upholding the highest levels of safety and quality. The ongoing flexibility and additions to the Codex Alimentarius will undoubtedly continue to benefit small businesses in the food industry in the years to come.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143) and Contract number — Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Kruger, J., Taylor, J. R., Ferruzzi, M. G. & Debelo, H. (2020). What is food-to-food fortification? A working definition and framework for evaluation of efficiency and implementation of best practices. *Comprehensive Reviews in Food Science and Food Safety*, 19(6), 3618–3658.

Leprêtre, C. & Merten-Lentz, K. (2018). Feature: The 50th session of the Codex Alimentarius Committee on Food Additives (CCFA50). *World Food Regulation Review*, 27(11), 19–29.

Meulen, B. (2019). Impact of the Codex Alimentarius. *European Food and Feed Law Review*, 14(1), 29–50.

Price, S. & World Health Organization, (2020). Codex and the SDGs: how participation in Codex Alimentarius supports the 2030 Agenda for Sustainable Development.

Ree, R., Sapiter Ballerda, D., Berin, M. C., Beuf, L., Chang, A., Gadermaier, G. & Bowman, C. (2021). The COMPARE database: a public resource for allergen identification, adapted for continuous improvement. *Frontiers in Allergy*, 2, 700533.

Tanemura, N., Hamadate, N. & Urushihara, H. (2017). The need for consumer science and regulatory science research on functional foods with health claims-What should we do to harmonize science and technology with society. *Trends in Food Science & Technology*, 67, 280–283.

World Health Organization, (2018). *Understanding the Codex Alimentarius*. Food & Agriculture Organization.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5(497.11)"2012/2021"
339.562:637.5(497.11)"2012/2021"

ID: 126494473

https://doi.org/10.18485/meattech.2023.64.2.96

Original scientific paper

Examination of the volume of meat production and the value of meat imports to Serbia from 2012 to 2021

Ivana Branković Lazić[a], Milan Ž. Baltić[b], Stefan Simunović[a], Jelena Ćirić[a], Tatjana Baltić[a], Jelena Jovanović[a] and Vesna Ž. Đorđević[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[b] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, Belgrade, Serbia*

ARTICLE INFO

Keywords:
Production volume
Meat value
Production trends

ABSTRACT

The total volume of meat production in Serbia from 2012 to 2021 was 550 thousand tons. In the overall meat production, pork accounted for over 50%. The trend in meat production during the observed period indicates an increase in production volume. The increased production volume is based on the fact that the slaughtering of cattle and the import of live animals to Serbia (pigs and poultry) for feeding and slaughtering as live animals from Serbia have both increased. The crisis in livestock farming in Serbia is evident from the data that the average value of meat and processed meat products imported and the value of live animal imports averaged over $US 150 million from 2012 to 2021.

1. Introduction

Serbia is a country where agriculture and food production have always had a special significance, not only for the food security of its population but also for exporting food surpluses. Even in the medieval Serbian states, they were exporters of livestock products (dried meat, bacon, cheese, honey, lard, leather, wool) (*Petrović-Garić* 2023, Đorđević *et al*., 2022). In the 19th century and until the beginning of World War I, Serbia was primarily an agrarian country (livestock and crop farming). Agricultural production relied heavily on livestock, particularly in pig, sheep, and cattle farming, and Serbia was a well-known exporter of livestock, especially to Austria-Hungary (*Baltić et al*., 2020). Between the two World Wars, the Kingdom of Yugoslavia did not have significant slaughter and processing capacities and continued to export live animals. From the 1960s, a larger number of modern slaughterhouses with meat processing facilities were established in Yugoslavia, and Serbia exported meat and meat products to several countries worldwide (*Anon*, 2019). The breakup of Yugoslavia, wars, sanctions, and societal changes (changes in ownership structures) affected the economic life of Serbia, including agriculture and the meat industry. Since 1990, the number of cattle has drastically decreased (by two-thirds), the number of pigs has been slightly declining or is stagnant, and the number of poultry has been decreasing. Only the number of small ruminants (sheep and goats) has seen a slight increase. In a little over 30 years, most of the industrial slaughterhouses have ceased to operate. There is no doubt that Serbia has good soil and climatic conditions, offering great opportunities for the production of plant and animal-based foods. However, it is undeniable that especially from the

***Corresponding author:** Ivana Branković Lazić, *ivana.brankovic@inmes.rs*

Paper received August 15th 2023. Paper accepted August 16th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

middle of the last century, there have been significant changes in the ratio between plant and animal-based production. Today, the main export articles are grain and fruit. For the past thirty years, this has led Serbia to transition from being an exporter to an importer of meat.

The aim of this paper is to highlight the changes in meat production and to emphasize that Serbia has shifted from being a major exporter of meat and meat products to becoming an importer of meat for processing as well as finished meat products.

2. Materials and methods

The data on the total volume of production and production of meat from individual animal species (cattle, pigs, poultry, and sheep), as well as data on the production of edible parts and fatty tissues in Serbia, and data on the volume of export and import of live animals from Serbia, along with data on the value of imported meat and live animals, were collected from the Statistical Yearbooks of Serbia from 2012 to 2021 (https://www.stat.gov.rs/). The results obtained were compared by statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The means and measures of variation for the volume of meat production, import, and export, as well as the value of meat imports and the value of live animal imports, were calculated. Trends in meat imports, meat production, and the values of meat and live animals have been calculated. All results are presented in tabular and graphical forms.

3. Results

The Serbian market is supplied with meat and meat products from the slaughter of animals raised or finished in domestic industrial and artisanal facilities, as well as partially from the slaughter and processing of meat in households (for personal consumption). Some meat intended for processing or already finished meat products are imported, while some meat for sale or processing comes from animals imported and partially fattened (finished)

Table 1. Meat production and import in Serbia from 2012 to 2021

Ordinal number	Parameters of the meat market	Unit of measurement	Means and measures of variation				
			$\bar{X}$	Sd ±	Min.	Max.	Cv %
1.	Production Serbia[1]	000 tons	550	26.76	513	578	4.86
2.	Imports Serbia[2]	000 tons	33.54	9.00	16.84	43.16	26.98
3.	Total 1+2	000 tons	583.54	32.97	53.84	62.16	5.65
4.	Import share [3]	%	5.75	1.50	3.20	7.34	26.08

Note: [1]Meat with bones, edible organs, and fatty tissues; [2]Frozen meat for processing and meat products; [3]Percentage of imports in the total meat quantity

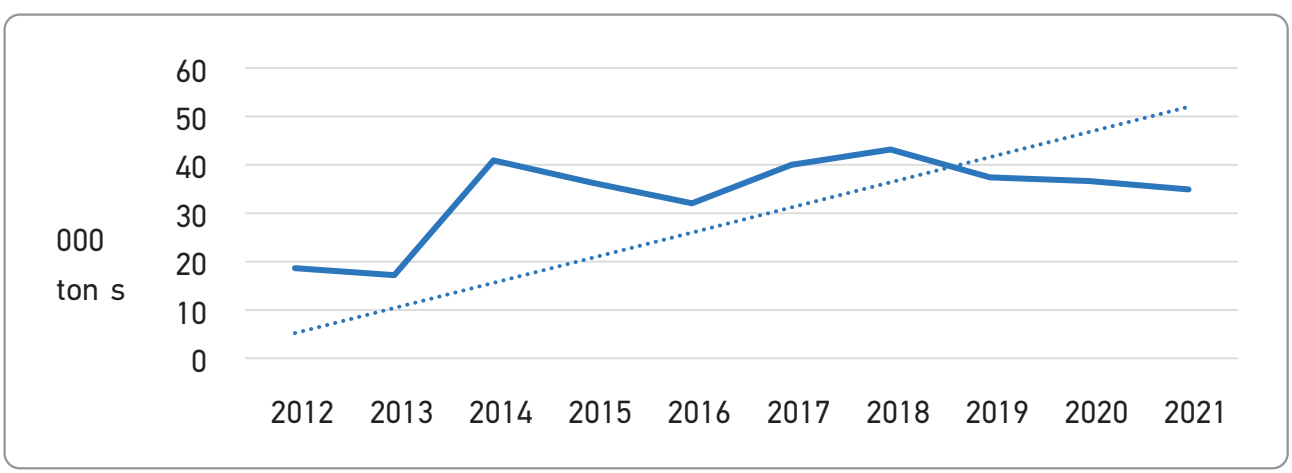


Figure 1. Trend of meat imports to Serbia from 2012 to 2021.

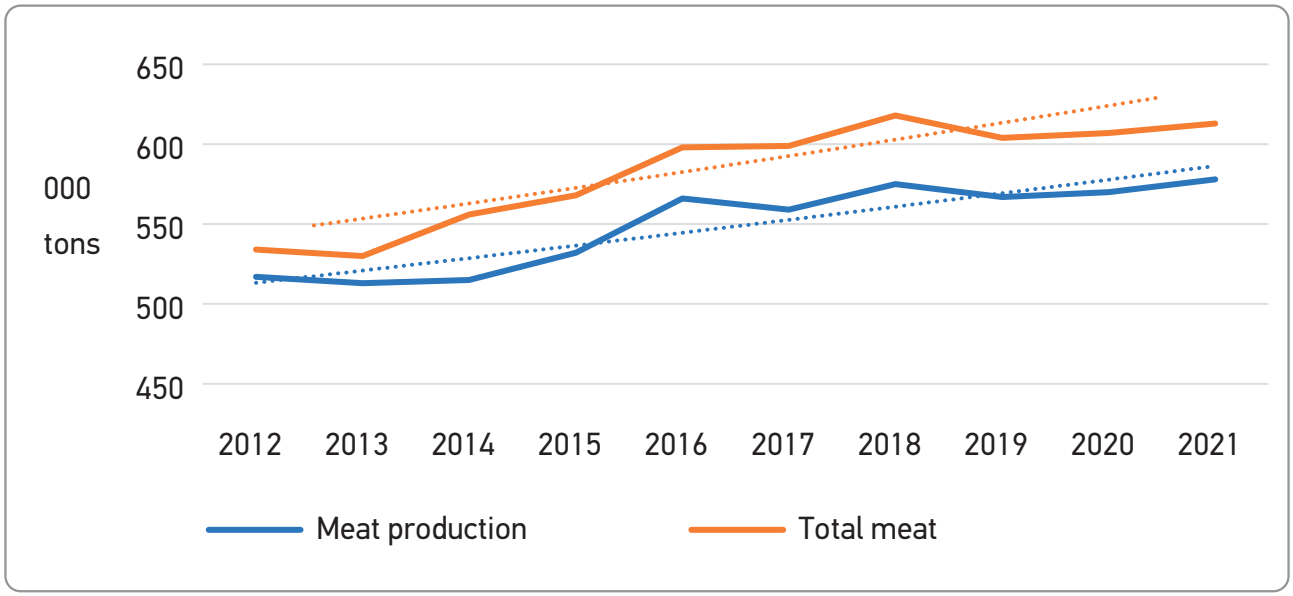


Figure 2. Comparative representation of the production trend and the total volume of meat on the Serbian market from 2012 to 2021.

in Serbia. Meat production in Serbia was, on average, 550±26.76 thousand tons annually from 2012 to 2021. During the same period, Serbia imported 33.54±9.00 thousand tons of meat annually for processing and finished meat products. On the Serbian market, from 2012 to 2021, there was an annual average of 583.54±32.97 thousand tons of meat (domestic production and imported meat and meat products). The average share of imports in the total volume of meat on the Serbian market was 5.75±1.50% (Table 1).

The trend of meat imports to Serbia from 2012 to 2021 is shown in Figure 1.

Comparative representation of the trend in meat production and the trend in the total volume of meat on the Serbian market for the same time period is shown in Figure 2.

Out of the total volume of meat produced in Serbia from 2012 to 2021, the largest share was pork meat (51.89%), followed by poultry meat (18.44%), beef meat (13.65%), sheep meat (6.15%), edible offal (5.45%), and fatty tissues (4.00%) (Figure 3).

The value of meat imports to Serbia from 2012 to 2021 was, on average, $US 104.83±24.00 million. The value per kilogram of imported meat and meat products to Serbia was on average $US 3.20±0.48

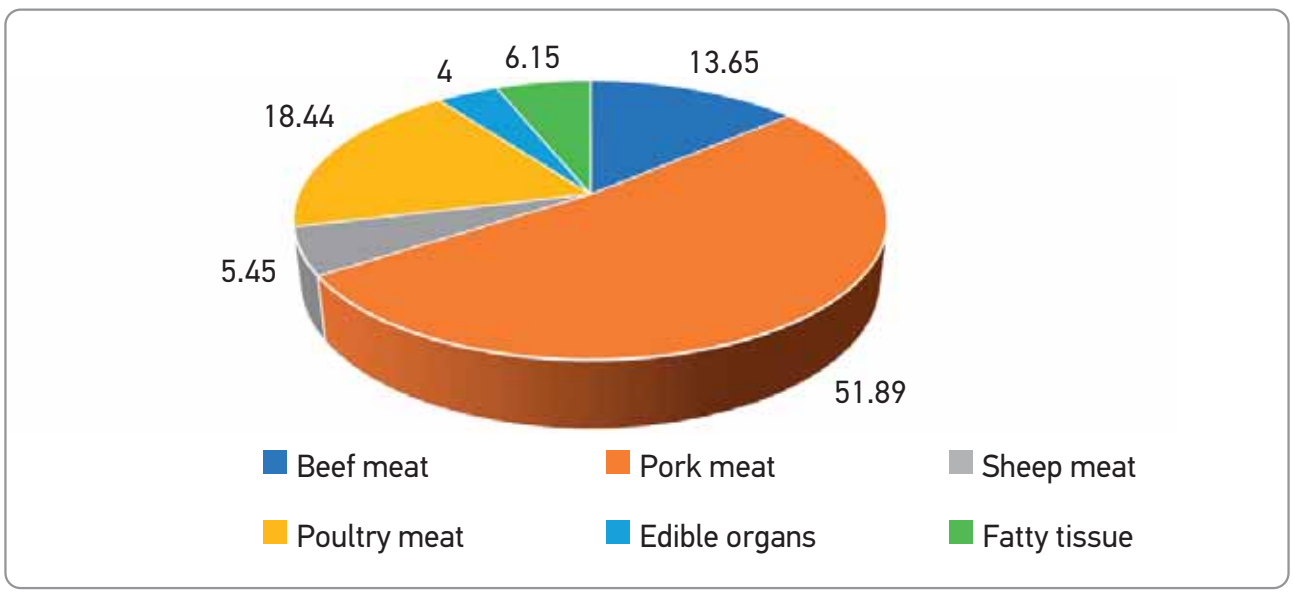
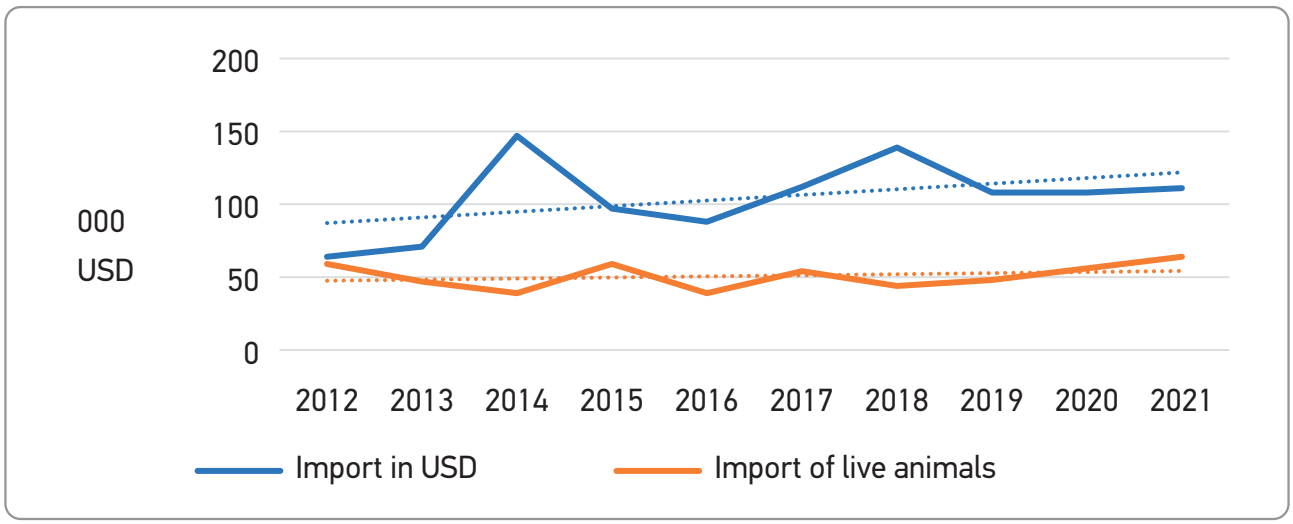


Figure 3. The share of different types of meat produced in Serbia (annual average from 2012 to 2021).

Table 2. The value of live animal meat imports to Serbia from 2012 to 2021.

Ordinal number	Parameters	Unit of measurement	Means and measures of variation.				
			$\bar{X}$	Sd ±	Min.	Max.	Cv %
1.	Meat import	milllion $US	104.83	24.00	66	147	22.77
2.	per kg/import	$US	3.20	0.48	2.68	4.12	15.00
3.	Import of animals	million $US	50.23	9.09	38.75	64.14	18.82



Graph 4. Comparative representation of the trends in the value ($US) of meat and meat product imports and live animal imports in Serbia from 2012 to 2021.

per year. Besides importing meat for processing and finished meat products, Serbia also imports live animals, mainly for the final fattening phase, which are then slaughtered as animals raised in Serbia. The average annual value of imported live animals to Serbia for the period 2012–2021 was $US 50.23±9.09 million (Table 2).

The trends of the value of meat and meat product imports, as well as live animal imports, from 2012 to 2021, are shown in Figure 4.

From 1912 to 2021, Serbia imported 4.7±0.33 thousand head of cattle, 174±86.29 thousand pigs, 2.3±0.21 thousand sheep, and 8.10 million poultry. During the same period, 43±9.57 thousand cattle, 25±5.66 thousand pigs, 87±4.20 thousand sheep, and 2.23±0.26 million poultry were exported, indicating a positive import balance for cattle and sheep, and a negative one for pigs and poultry.

4. Discussion

Food production has been an essential activity for humanity's survival, gradually transitioning from a hunter-gatherer lifestyle to a settled way of life. This task is challenging for the entire world, especially with the unexpected increase in the global population today (the "population bomb"). It was projected that the world's population would reach eight billion by 2026, up from seven billion in 2012, but this number was surpassed before the end of 2022. Providing sufficient quantities of animal-derived food, especially meat, which is a crucial part of human nutrition, poses a significant challenge. Meat is undoubtedly the most important animal-derived food (*Baltić et al*., 2022).

Global meat production in 2020 was 337 million tons (MT), comprising 133 MT (39.47%) poultry meat, 110 MT (32.64%) pork, 68 MT (20.18%) beef, and 26 MT (7.71%) small ruminant and horse meat. In Serbia, pork makes up most of the meat produced, accounting for over 50% of the total meat production, followed by poultry (18.44%) and beef (13.65%). Over the first 20 years of this century (2000 to 2020), global meat production increased by 45%, poultry meat by 94%, pork by 22%, beef by 22%, and other types of meat by 39%. The demand for meat is projected to reach 400 MT in 2030 and nearly 500 MT in 2050 (*Putnik, Bursać Kovačević*, 2021).

While global meat production continues to rise, Serbia's meat production has been declining since

1990 (*Dokmanović et al*., 2017). Total meat production in Serbia was 616 thousand tons in 1990, decreased to 518 thousand tons in 2015, and slightly increased to 527 thousand tons in 2021. The most significant decline was observed in beef production, dropping from 155 thousand tons in 1990 to 71 thousand tons in 2021. This is mainly due to the reduced number of cattle, from around two million in 1990 to 878 thousand in 2021, caused by increased slaughtering (due to the unprofitability of keeping cows for milk production) and the fact that from 2012 to 2021, 43±9.57 thousand live cattle were exported, likely due to favourable prices. Pork production has not significantly changed from 1990 to 2021 (286 thousand tons in 1990, 283 thousand tons in 2000, and 298 thousand tons in 2021), despite the decline in the number of pigs from 4.3 million in 1990 to 2.8 million in 2021. This can be explained by the fact that Serbia imported an average of 174±86.29 thousand live pigs annually from 2012 to 2021. Poultry meat production was 112 thousand tons in 1990, decreased to 67 thousand tons by 2000, and slightly increased to 114 thousand tons in 2021. This increase can also be attributed to the import of live poultry (an average of 8.10±1.16 million head per year from 2012 to 2021). Sheep and goat meat production in Serbia increased from 24 to 34 thousand tons from 1990 to 2021. During the same period, the number of sheep and goats increased from 1.49 million in 2000 to 1.71 million in 2021. Meat production would have been even higher if an annual average of 87±4.20 thousand live sheep had not been exported from 2012 to 2021. From the analysis of meat production in Serbia from 2012 to 2020, it can be concluded that meat production is increasing, mainly based on the increased slaughtering of cattle, as their numbers drastically decrease. The production is also reliant on meat imports for processing (with an average annual value of $US 104.83±24.00 million for the mentioned period) and imports of live animals (with an average annual value of $US 50.23±9.09 million) (https://www.stat.gov.rs/).

5. Conclusion

Since 1990, Serbia has transitioned from being an exporter of live animals, meat, and meat products to becoming an importer. The reasons behind this change are numerous, complex, and multifactorial, and they are not justified. Serbia has sufficient quantities of roughage for ruminants and an adequate supply of concentrated feed (such as grains, soy, and sunflower) for animal nutrition, which are being used for the export animals. It would be much more beneficial to utilize these resources for feeding animals and exporting meat and live animals instead.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143) and Contract number — Grant No. 451-03-47/2023-01/200050 from 03.02.2023.

References

Anon, (2019*).* *Monografija, 60 Konferencija struke i nauke u industriji mesa* (Urednici: Vesna Ž. **Đorđević** i Milan Ž. Baltić), Institut za higijenu i tehnologiju mesa, Beograd, Srbija, 3–8.

Baltić, M. Ž., Bošković Cabrol, M., Dokmanović, M., Janjić, J., Glišić, M., Branković Lazić, I. & Dimitrijević, M. (2022). Meso in vitro-ante portas. *33. Savetovanje Veterinara Srbije, Zlatibor, 08–11. Septembar 2022*, 379–391.

Baltić, M. Ž., Janjic, J., Popovic, M., Baltic, T., Jankovic, V., Starcevic, M. & Sarcevic, D. (2018). Meat in traditional Serbian cuisine. *Meat Technology,* 59(1), 54– 62.

Dokmanović, M., M. Tešić, V. Teodorović, N. Karabasil, Radmila Marković, Milica Todorović & Đurić, J. (2013). Ispitivanje mesnatosti trupova svinja u Srbiji. *Veterinarski Glasnik,* 67(3–4), 227–236.

Đorđević, V., Mirilović, M., Katanić, N., Janjić, J., Nedić ,D., Šarčević, D. & Baltić Ž. M., (2022). Food production and security in the area of Serbia: historical background and current situation**.** *Meat Technology,* 63(1), 1–10.

Garić Petrović, G., (2017). Unapređenje stočarstva u Kraljevini Srbiji: selekcija, uvođenje novih sojeva i ukrštanje. *The historical review,* LXVI, 15–34.

Putnik, P. & Bursać Kovačević, D. (2021). Meat consumption: theory, practice and future prospects. *Theory and Practice of Meat Processing,* 6, 4, 335–342.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.54.046:636.5]:546.46

ID: 126480649

https://doi.org/10.18485/meattech.2023.64.2.97

Original scientific paper

Magnesium content in chicken meat — share in food intake

Saša Janković[a], Stefan Simunović[a], Srđan Stefanović[a], Zoran Petrović[a], Jasna Đinović-Stojanović[a], Sanin Tanković[b] and Nikola Borjan[a]*

[a] *Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[b] *Veterinary Office of Bosnia and Herzegovina, Maršala Tita 9a/II, 71000 Sarajevo, Bosnia and Herzegovina*

ARTICLE INFO

Keywords:
Magnesium
Content
Chicken meat
Food
ICP MS

ABSTRACT

The aim of this study was to determine magnesium content in two types of chicken meat samples and to calculate average intake of magnesium through chicken by adults in Serbia. Meat and meat products are important sources of many nutrients, including protein, minerals, vitamins and fats. One of the minerals necessary for the normal functioning of the human body is magnesium. Considering the price, availability and consumption, chicken meat is one of the most important foods in the human diet. A total of 81 samples were analysed using inductively coupled plasma with mass detection. The results showed that the average magnesium content in chicken meat was 297.8 mg/kg. The intake of magnesium by chicken meat per adult in Serbia was found to be 51.48 g per day, which represents 5% of the recommended daily intake. Taking into account the wide spectrum of other nutrients that we consume through chicken meat, we can conclude that chicken plays an important role in overall magnesium intake by adults in Serbia.

1. Introduction

Magnesium is alkaline earth metal that occurs in nature as a cation in water or as a mineral part of a large number of compounds. Magnesium plays an extremely important role in the body. It is part of many enzyme systems that, among other things, participate in protein synthesis and normal muscle work. In addition, it is important for maintaining normal blood pressure and functions of the central nervous system. Magnesium deficiency is becoming increasingly common today, due to refined magnesium-poor foods. Symptoms of magnesium deficiency include muscle tremors, weakness, nausea, vomiting and cramps. Magnesium deficiency is associated with the development of diseases like coronary heart disease, malignant tumours, osteoporosis and hypertension (*Ryan,* 1991; *Swaminathan*, 2003). Stone fruits, leafy vegetables, fish, eggs and meat are the most important sources of magnesium in human diet. Daily recommended intake of magnesium ranges from 320 mg per day for woman and 420 mg per day for men (*DRIs*, 2011).

Adequate intake of magnesium ensures normal functioning of the nervous system and facilitates the body's reaction to stress. Lack of magnesium in the body is associated with symptoms of depression and irritability. The chemical imbalance of magnesium and other electrolytes in the cell, which is caused by exposure to stress and a magnesium-deficient diet, is responsible for disturbances in the work of the

***Corresponding author:** Saša Janković, sasa.jankovic@inmes.rs

Paper received August 17th 2023. Paper accepted August 20th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

nervous system and various psychological manifestations. In addition, magnesium contributes to the maintenance of normal bones and teeth and has a mild laxative effect.

Humans take in about 10% of the total amount of magnesium with water. Foods rich in chlorophyll, such as green vegetables and spinach, contain high amounts of magnesium. Nuts, seeds and unprocessed grains are known as good sources of magnesium. Lower levels of magnesium are also found in legumes, fruits, fish and meat. Based on the recommendations of several relevant institutions around the world, EFSA recommends magnesium intake of 300 mg for women and 350 mg for men.

The aim of this study was to determine magnesium content in two types of chicken meat and to calculate average intake of magnesium through chicken by adults in Serbia.

2. Materials and methods

2.1. Samples

A total of 81 chicken meat cuts (red meat and breast meat) were collected in Serbian markets from January 2022 to May 2023. After collection, meats were labelled and stored in polyethylene bags and frozen at –18°C prior to analysis. Frozen samples were thawed at 4 ºC for a day before analysis and then homogenized. An amount, approximately 0.5 g, of each homogenized sample, was transferred into a Teflon vessel with 5 mL nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL hydrogen peroxide (30% analytical grade, Sigma- Aldrich, St. Louis, MA, USA) for microwave digestion.

2.2. Extraction

The microwave (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from room temperature to 180°C, 10 min hold at 180°C, 20 min vent. After cooling, the digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 mL with deionized water produced by a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK).

2.3. Quantification

Analysis of magnesium content was performed by inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). The most abundant isotope ^{24}Mg was used for quantification. Torch position, ion optics and detector settings were adjusted daily using tuning solution (Tune B, Thermo Scientific), in order to optimize mechanical and electrical parameters and minimize possible interference. Basic operating conditions of the instrument were: RF power (1550 W); cooling gas flow (14 L/min); nebulizer flow (1 L/min); collision gas flow (1 mL/min); operating mode (Kinetic Energy Discrimination — KED); dwell time (10 ms).

Standard stock solution containing 1000 mg/L of magnesium was purchased from CPAchem (Bogomilovo, Bulgaria). These solutions were used to prepare standards for five-point calibration curves (including zero). Multielement internal standard (6 Li, 45Sc, 71Ga, 89Y, 209Bi) was introduced online by an additional line through the peristaltic pump, and covered a wide mass range. All solutions (standards, internal standards and samples) were prepared in 2% nitric acid. The analytical method was validated by Guidelines for Single Laboratory Validation (SLV) of Chemical Methods for Metals in Food (AOAC) and accredited according to ISO 17025. The quality of the analytical procedure was verified by analysis of the certified reference material ERM — BB384 (lyophilised pork muscle, ERM, Geel, Belgium). Reference material was prepared using an identical procedure as for the samples using microwave digestion. The measured concentration was within the range of the certified value.

3. Results and discussion

Table 1. show magnesium levels found in chicken red and breast meat. On average, a higher concentration of magnesium was detected in chicken breast (335.1 mg/kg) than in chicken red meat (279.1 mg/kg). However, using the Student's-t test, a statistically significant difference in Mg concentrations in the two investigated groups of chicken meat was not found ($P<0.05$).

The values obtained in our study are significantly lower than the published results of *Batista et al.* (2012) who reported an average of 1182 mg/kg, while they are slightly higher compared to the study by *Zand et al.* (2012) who reported average value of 190 mg/kg of magnesium. Magnesium levels in chicken cuts in our study were similar to the levels found in broiler breast meat (223–291 mg/kg) reported by *Ahmed et al.* (2015). The levels of magnesium in the same cuts were lower than reported by *McCance and Widdowson* (2015), who found average of 240 mg/kg of mag-

Table 1. Magnesium levels in analysed samples of chicken (mg/kg)

Type of sample	Number of samples	Magnesium content (mg/kg)
Chicken red meat	54	279.1±41,2[a]
Chicken breast meat	27	335.1±42,3[a]

P<0.05; [a] Values in the same column followed by the same letter were not found to be significantly different

nesium. Therefore, levels of magnesium determined in our samples were in range of USDA reported data for different chicken products. According to the USDA, 4 oz (113.4 g) contains 25 mg of Mg, which corresponds to 220.5 mg/kg. (*USDA*, 2016). In addition, the average content of magnesium in red chicken meat according to the Technical University of Denmark database is 242 mg/kg (range 185–330 mg/kg) and in white — 250 mg/kg.

When it comes to consumption calculation, according to the EFSA total diet study (available on https://www.efsa.europa.eu/en/microstrategy/food-ex2-level-3), the intake of chicken meat by an average adult in Serbia is 51.48 g/day, which means that by consuming chicken meat, the average intake is 15.3 mg of magnesium, which represents 5 % of the recommended daily intake this metal.

4. Conclusion

The results of our study showed that chicken meat contains a respectable level of magnesium. With the average consumption of chicken meat, we consume about 5% of our daily magnesium needs. The results of our study indicate the importance of consuming chicken in regards to magnesium intake. It is very important to indicate that, besides magnesium, chicken meat contains other essential nutrients, which is why it represents an important part of human diet.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, according to the provisions of the Contract on research financing 2023. No 451-03-47/2023-01/200050 dated from 03.02.2023.

References

Ahmed, S. T., Islam M. M., Bostami, A. B. M. R., Mun, H., Kim, Y. & Yang, C. (2015). Meat composition: fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. *Food Chemistry*, 188, 481–488.

Batista, B. L., Grotto, D., Hornos Carneiro, M. F. & Barbosa Jr., F., (2012). Evaluation of the concentration of non-essential and essential elements in chicken, pork, and beef samples produced in Brazil. *Journal of Toxicology and Environmental Health A,* 75, 1269–1279.

McCance, R. A. & Widdowson, E. W., 2015. The 7th Edition of McCance and Widdowson's the Composition of Foods. Public Health England, Wellington House, London, pp. 1–550.

Mohammed, H., Lichao H, Nawaz, A., Guofeng, J., Xi, H., Meihu, M., Warda S. A., Elgasim A. E. & Khalifa, M. (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. *Meat Science*, 175,108453.

Ryan, M. F. (1991). The Role of Magnesium in Clinical Biochemistry: An Overview. *Annals of Clinical Biochemistry,* 28(1),19–26, doi:10.1177/000456329102800103

EFSA (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)). (2015). Scientific Opinion on Dietary Reference Values for magnesium**.** *EFSA Journal,*13(7), 4186.

Swaminathan, R. (2003). Magnesium metabolism and its disorders. *The Clinical Biochemist Reviews*, 24(2), 47.

Technical University of Denmark, DTU Foods public food database, https://frida.fooddata.dk/.

USDA, (2016). National Nutrient Database for Standard Reference. Release 28. (accessed 23.05.2016.) http://fnic.nal.usda.gov/food-composition/.

Zand, N., Chowdhry, B.Z., Wray, D.S., Pullen, F.S. & Snowden, M. J., (2012). Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chemistry*, 135, 2796–2801.

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.5.065
614.31

ID: 126459657

https://doi.org/10.18485/meattech.2023.64.2.98

Review paper

Microbial biofilms in a meat processing environment

Viera Ducková[a], Miroslav Kročko[a] and Jana Tkáčová[a]*

[a] *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

ARTICLE INFO

Keywords:
Biofilm
Meat environment
Microbial contamination

ABSTRACT

The presence of pathogenic or spoilage microorganisms on surfaces in meat processing plants poses a risk of contamination of meat products by these microorganisms and the spread of foodborne diseases, as well as a reduction in the shelf life of products, even more so if these microorganisms are already attached to the surfaces in the form of biofilms. Biofilm is considered one of the major challenges for public health and food safety. The paper focuses on the characteristics of biofilms and their presence in the meat processing environment from the point of view of the representation of individual groups of microorganisms that were isolated from these biofilms during operation or after the sanitation process in meat processing plants.

1. Introduction

The consumption of meat constantly raises questions and concerns among consumers about the hygiene and safety of meat. These concerns are mostly of a biological nature and relate to the presence of pathogenic and spoilage microorganisms. In recent decades, it has become increasingly clear that bacteria including foodborne pathogens, such as *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli*, and *Campylobacter*, together with common meat spoilage microorganisms, such as *Pseudomonas* spp., *Brochothrix thermosphacta*, *Lactobacillus* spp. and others, mostly grow in biofilms rather than in planktonic form (*Frank*, 2001; *Lindsay & von Holy*, 2006; *Sofos and Georgnaras*, 2010, *Giaouris et al*., 2014; *Gaillac et al*., 2022; *Yang et al*., 2023).

The attachment of potentially pathogenic and spoilage bacteria to the surfaces of food contact machines and equipment and the subsequent formation of biofilms are serious problems for the meat industry as they can lead to cross-contamination of products, resulting in reduced shelf life and the spread of foodborne illnesses. In meat processing and meat product manufacturing environments, the microorganisms present can adhere to food contact surfaces in complex multi-species communities. Bacterial interactions are known to play a key role in the attachment and detachment of microorganism cells from biofilms, as well as in the resistance of biofilm community members to antimicrobial agents (*Sofos and Georgnaras*, 2010). Disinfection of food contact surfaces in such environments is a challenging task, which is exacerbated by the high antimicrobial resistance of bacteria associated with biofilms.

This paper focuses on the characteristics of biofilms and their occurrence in meat processing environments.

***Corresponding author:** Viera Ducková, *viera.duckova@uniag.sk*

Paper received August 9th 2023. Paper accepted August 30th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

2. Biofilm characteristics

Biofilm is not a recently discovered phenomenon, but it has been studied in the food industry for several decades. One of the first theories describing the formation of biofilms was formulated by *Costerton* in 1978, stating that most microorganisms prefer to grow as communities attached to solid surfaces. These attached bacterial populations exhibit different characteristics from their planktonic (free-swimming) forms and can exist in all nutrient-rich aquatic ecosystems, both on the body and within living organisms or on artificial surfaces (*Costerton et al.*, 1978).

There are several definitions of biofilms, with most definitions referring to biofilms as aggregations of microbial cells connected by extracellular polymeric substances (EPS or glycocalyx or extracellular matrix) that rapidly proliferate and grow on the surfaces of various materials (*Coghlan*, 1996; *Frank*, 2001; *Shi and Zhu*, 2009; *Satpathy et al.*, 2016; *Muhammad et al.*, 2020). Biofilms have been detected in various areas of food producing plants, including, e.g., floors, walls, pipes, and drains, as well as food contact surfaces and production equipment made of various materials including stainless steel, plastic, rubber, Teflon, nylon, glass, etc. (*Sofos and Georgnaras*, 2010; *Wang*, 2019). The extracellular matrix of biofilms consists of polymeric compounds synthesized by microorganisms, such as extracellular polysaccharides, proteins, phospholipids or even extracellular DNA (eDNA) (*Lemon et al*, 2008; *Davies and Marques* 2009; *Rahman et al*., 2022).

The most important characteristic of biofilms in relation to the food or meat processing environment is their increased resistance to adverse conditions (mechanical damage during sanitation, UV radiation, biocides, etc.) compared to their planktonic counterparts (*Simões et al*., 2010; *Rahman et al.*, 2022; *Yang et al.,* 2023). This resistance is mediated by the physical barrier provided by EPS, efflux systems, differentiation of bacterial cells to an inactive state or modification of the microenvironment that may make a particular sanitizer less effective (*Giaouris et al.,* 2014; *Yang et al.,* 2023). In addition, bacteria growing in biofilms have an increased exchange of genetic information, which can result in the rapid spread of genes encoding, e.g., antibiotic resistance, between bacterial populations (*Hausner and Wuertz*, 1999; *Ch´ng et al.*, 2019; *Nikolaev et al.*, 2022a).

Another important characteristic is that biofilm microorganisms communicate with each other (*Donlan*, 2002). In the extracellular matrix of the biofilm, signal molecules can accumulate in high enough concentrations to be effective for intercellular communication and community-wide behavior (quorum sensing system) (*Sutherland*, 2001). The quorum sensing system is based on the process of autoinduction. The system provides a mechanism for self-organization and regulation of microbial cells. Bacteria excrete signals molecules (auto-inducers) into the surrounding environment, and where they accumulate during bacteria growth (*Fuqua and Greenberg*, 2002). The high cell density of microorganisms leads to an increase in the concentration of signals and induces the expression of certain genes or physiological changes in neighboring cells (*Parsek and Greenberg*, 2005). Oligopeptides and N-acylhomoserine lactones (AHLs) are the main autoinducer molecules involved in intraspecific communication in G^+ and G^- bacteria, respectively (*Fuqua and Greenberg*, 2002; *Parsek and Greenberg*, 2005). The quorum sensing system is known to be involved in a significant amount of important microbial activities. In addition to biofilm formation and synthesis of extracellular polymeric compounds, these activities include, for example, the biosynthesis of extracellular enzymes, antibiotic biosynthesis, production of biosurfactants, and extracellular virulence factors in G^- bacteria (*Daniels et al*., 2004; *Fux et al*., 2005). The quorum quenching system, a strategy for blocking the quorum sensing system and inhibiting the production of virulence factors, is also currently known. This strategy reduces virulence without killing pathogenic microorganisms. This system can also be called a mechanism by which bacterial communication can be interrupted, with the potential for preventing biofilm formation and to produce microbiologically safer foods. In recent years, there have been significant advances in the study of quorum sensing and quorum quenching mechanisms (*Zhang et al.*, 2019).

A microbial biofilm lives as a community of microorganisms with simple homeostasis, a simple circulatory system, and metabolic cooperation, and the response of each cell of this community is completely different from that of planktonic cells of the same species. Because it is a complex, differentiated community, its formation can be considered unique in biology, due to the coordinated activities of the relatively small genomes of prokaryotes (*Dunsmore et al*., 1981).

3. What does it look like with biofilms in a meat processing environment?

The persistence of organic soil residues in food processing environments can lead to the formation of microbial harborages, biofilms, and niches that can serve as a source of cross-contamination (*Sofos and Geornaras*, 2010). Daily cleaning and disinfection of equipment in meat processing plants is therefore required. Sanitation is a multi-step process that aims to achieve two main objectives: a visibly clean facility (removal of food residues that support the growth of microorganisms) and a reduction of microorganism counts to an acceptable level. The goal of sanitation in food processing plants is not to achieve sterility of surfaces, and therefore, different types of microorganisms may be present on cleaned surfaces (*Langsrud et al.*, 2016; *Wang et al.*, 2018). *Jessen and Lammert* (2003) compared the effectiveness of acid and alkaline sanitation on a production line for sliced cooked ham products. Their results showed that even on visually clean surfaces after regularly performed sanitation, aerobic bacterial counts varied from < 1 CFU cm^{-2} to $3.7x10^4$ CFU cm^{-2}. These authors report that sanitization was more effective when an alkaline sanitizer with a chlorine-based disinfectant component was used compared to an acid sanitizer containing peracetic acid. Consistent with the above are the results of *Rossini and Gaylarde* (2000). On the contrary, *Fatemi and Frank* (1999) found higher efficacy with acid disinfectants composed of hydrogen peroxide and peracetic acid compared to chlorine compounds when tested in meat system. According to *Wang et al.* (2018), effective sanitation can reduce the number of indicator organisms by up to 3 log units on food contact surfaces and 1 log unit for non-food contact conveyor surfaces in a meat processing plant. The authors of this study report that the genus *Pseudomonas* was dominant among bacteria isolated from surfaces of a beef plant conveyor belt after sanitation. Among other genera, they also isolated *Comamonas, Acinetobacter, Flavobacterium, Pseudarcobacter, Bacteroides, Janthinobacterium* and *Aeromonas*. *Wagner et al.* (2020) identified ten biofilm hotspots (7 sampled during processing and 3 after sanitation) in beef, pork, and poultry meat processing plants. Five biofilms were from food contact surfaces (slicers and associated equipment and screw conveyor) and five were from non-food contact surfaces (drains and water hoses). From these biofilms, 29 different genera of bacteria were isolated. The most frequently isolated strains were from the genera *Brochothrix* (present in 80% of biofilms), *Pseudomonas* and (isolated from 70% of biofilms). The authors of this study reported that they isolated representatives of 4 to 12 different genera from each biofilm, indicating the presence of multi-species biofilms. According to *Giaouris et al.* (2014), pathogenic bacteria such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter jejuni* and spoilage bacteria, e.g., *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Brochothrix thermosphacta*, *Shewanella putrefaciens*, *Lactobacillus* or *Leuconostoc*, form robust biofilms on the surfaces of food contact equipment in meat processing environments. Also, *Wang* (2019) reported that *Escherichia coli, Salmonella, Staphylococcus, Bacillus*, and *Pseudomonas* species can coexist and form biofilms in meat processing plants. In the context of pork, *Grudlewska-Buda et al.* (2023), based on biofilm analysis, pointed out that all vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains tested in their study showed a higher ability to form biofilms compared to susceptible strains. The taxonomic composition and structural organization of microbial biofilms at meat-processing plants (poultry, pork, and mixed materials) were also studied by *Nikolaev et al.* (2022a, b). Bacteria identified in these biofilms included *Pseudomonas, Flavobacterium, Arcobacter, Vagococcus, Chryseobacterium, Carnobacterium, Corynebacterium, Kocuria*, etc.

4. Conclusion

It can be concluded that in meat processing plants, even after the regular sanitation process, biofilm hotspots have been found, from which a wide variety of different types of microorganisms, both pathogenic and spoilage, have been isolated. It is, therefore, necessary to monitor the effectiveness of sanitation on a regular basis and at the same time look for new alternative methods (e.g., use of essential oils, bacteriophages, etc.) to minimise the spread of microorganisms. It is also important to continue to study biofilms and better understand their functioning, which may reveal new strategies for their elimination, such as the quorum quenching system.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This work was supported by KEGA 034SPU-4/2021, VEGA 1/0402/23 and Visegrad+ Grants 22230075.

References

Coghlan, A. (1996). Slime city. *New Scientist*, 2045(15), 32–36.

Costerton, J. W., Geesey, G. G. & Cheng, G. K. (1978). How bacteria stick. *Scientific American*, 238(1), 86–95.

Daniels, R., Vanderleyden, J. & Michiels, J. (2004). Quorum sensing and swarming migration in bacteria. *FEMS Microbiology Reviews*, 28, 261–289.

Davies, D. G. & Marques, C. N. H. (2009). A fatty acid messenger is responsible for including dispersion in microbial biofilm. *Journal of Bacteriology*, 191(5), 1393–1403.

Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases,* 8(9), 881–890.

Dunsmore, D. G., Twomey, A., Whittlestone, W. G. & Morgan, H. W. (1981). Design and performance of system for cleaning product-contact surfaces of food equipment: a review. *Journal of Food Protection*, 44, 220–240.

Fatemi, P. & Frank, J. F. (1999). Inactivation of *Listeria monocytogenes* / *Pseudomonas* biofilm by peracid sanitizers. *Journal of Food Protection*, 62(7), 761–765.

Frank, J. F. (2001). Microbial attachment to food and food contact surfaces. *Advances in Food and Nutrition Research*, 43, 319– 370.

Fuqua, C. & Greenberg, E. P. (2002). Listening in on bacteria acyl-homoserine lactone signalling. *Nature Reviews in Molecular Cell Biology*, 3, 385–395.

Fux, C. A., Costerton, J. W., Stewart, P. S. & Stoodley, P. (2005). Survival strategies of infectious biofilms. *Trends in Microbiology*, 13, 34–40.

Gaillac, A., Briandet, R., Delahaye, E., Deshamps, J., Vigneau, E., Courcoux, P., Jaffres, E. & Prévost, H. (2022). Exploring the diversity of biofilm formation by the food spoiler *Brochothrix thermosphacta*. *Microorganisms*, 10, 2474, https://doi.org/10.3390/microorganisms10122474

Giaouris, E., Heir, E., Hébraud, M., Chorianopoulos, N., Langsrud, S., Moretro, T., Habimana, O., Desvaux, M., Reiner, S. & Nychas, G. J. (2014). Attachment and biofilm by foodborne bacteria in a meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel method. *Meat Science*, 97, 298–309, http://dx.doi.org/10.1016/j.meatsci.2013.05.023

Grudlewska-Buda, K., Skowron, K., Bauza-Kaszewska, J., Budzynska, A., Wiktorczyk-Kapischke, N., Wilk, M., Wujak, M. & Paluszak, Z. 2023. Assessment of antibiotic resistance and biofilm formation of *Enterococcus* species isolated from different pig farm environments in Poland. *BMC Microbiology*, 23, 89, https://doi.org/10.1186/s12866-023-02834-9

Hausner, M. & Wuertz, S. (1999). High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Applied and Environmental Microbiology*, 65(8), 3710–3713.

Ch´ng, J. H., Chong, K., Lam, L. N., Wong, J. J. & Kline, K. A. (2019). Biofilm-associated infection by enterococci. *Nature Reviews Microbiology*, 17(2), 82–94.

Jessen, B. & Lammert, L. (2003). Biofilm and disinfection in meat processing plants. *International Biodeterioration and Biodegradation*. 51, 265–269, https://doi.org/10.1016/S0964-8305(03)00046-5

Langsrud, S., Moen, B., Moretro, T., Loype, M. & Heir, E. (2016). Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. *Journal of Applied Microbiology*, 120, 366–378.

Lemon, K. P., Earl, A. M., Vlamakis, H. C., Aguilar, C. & Kolter, R. (2008). Biofilm development with emphasis on *Bacillus subtilis*. *Current Topics in Microbiology and Immunology*, 322, 1–16.

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X. & Huang, T. (2020). Beyond risk: bacterial biofilm and regulating approaches. *Frontier Microbiology,* 11, 928.

Nikolaev, Y., Yushina, Y., Mardanov, A., Gruzdev, E., Tikhonova, E., El-Registan, G., Beletskiy, A., Semenova, A., Zaiko, E., Bataeva, D. & Polishchuk, E. (2022a). Microbial biofilms at meat-processing plant as possible places of bacterial survival. *Microorganisms*, 10, 1583, https://doi.org./10.3390/microorganisms10081583

Nikolaev, Y. A., Tikhonova, E. N., El-Registan, G. I., Zhurina, M. V., Plakunov, V. K., Demkina, E. V., Zaiko, E. V., Bataeva, D. S., Nasyrov & Yushina, Y. K. (2022b). Comparative investigation of the composition and structure of microbial biofilms retrieved at meat-processing plants using different raw materials. *Microbiology,* 91(5), 577–592.

Parsek, M. R. & Greenberg, E. P. (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends in Microbiology*, 13, 27–33.

Rahman, S. M. E., Islam, S. M. A, Xi, Q., Han, R., Oh, D. H. & Wang, J. (2022). Control of bacterial biofilms in red meat — A systematic review. *Meat Science*, 192, 108870, https://doi.org/10.1016/j.meatsci.2022.108870

Rossini, E. M. M. & Gaylarde, C. C. (2000). Comparison of sodium hypochlorite and peracetic acid as sanitising agents for stainless steel processing surfaces using epifluorescence microscopy. *International Journal of Food Microbiology*, 61, 81–85.

Satpathy, S., Sen, S. K., Pattanaik, S. & Raut, S. (2016). Review on bacterial biofilm: an universal cause of contamination. *Biocatalysis and Agricultural Biotechnology*, 7, 56–66.

Shi, X. & Zhu, X. (2009). Biofilm formation and food safety in food industries. *Trends in Food Science & Technology,* 20, 407–413.

Simões, M., Simões, L. C. & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT — Food Science and Technology*, 43, 573–583.

Sofos, J. N. & Geornaras, I. (2010). Overview of current meat hygiene and safety risk and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. *Meat Science*, 86, 2–14. DOI: 10.1016/j.meatsci.2010.04.015

Sutherland, I. W. (2001). The biofilm matrix — an immobilized but dynamic microbial environment. *Trends in Microbiology*, 9, 222–227.

Wagner, E. M., Pracser, N., Thalguter, S., Fischel, K., Rammer, N., Pospíšilová, L., Alispahic, M., Wagner, M. & Rychli, K. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. International *Journal of Food Microbiology*, 328, 108668, https://doi.org/10.1016/j.ijfoodmicro.2020.108668

Wang, H., He, A. & Yang, X. (2018). Dynamics of microflora on conveyor belts in a beef fabrication facility during sanitation. *Food Control*, 85, 42–47.

Wang, R. (2019). Biofilms and Meat Safety: A mini-review. Biofilm-related contamination in meat industry. *International Association for Food Protection,* 120–127, https://doi.org/10.4315/0362-028X.JFP-18-311

Yang, X., Wang, H., Hrycauk, S., Holman, D. B. & Ells, T. C. (2023). Microbial dynamics in mixed-culture biofilms of *Salmonella Typhimurium* and *Escherichia coli* O157:H7 and bacteria surviving of conveyor belts of meat processing plants. *Microorganisms*, 11(2), 421, https://doi.org/10.3390/microorganisms11020421

Zhang, J., Feng, T., Wang, J. & Wang, Y. (2019). The mechanisms and applications of quorum sensing (QS) and quorum quenching (QQ). *Journal of Ocean University of China*, 8, 1427–1442, https://doi.org/10.1007/s11802-019-4073-5

Content is avaliable at SCOPUS

Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 636.46.09:616.988.75
637.5:658.562.4

ID: 126444297

https://doi.org/10.18485/meattech.2023.64.2.99

Review paper

Tackling African swine fever and highly pathogenic animal diseases for sustainable meat production and food security

Budimir Plavšić[a]

[a] *World Organisation for Animal Health, 12, rue de Prony, 75017 Paris, France*

ARTICLE INFO

Keywords:
ASF
Control
Biosecurity
Food security
WOAH

ABSTRACT

The purpose of this article is to review the impact of the current international geographical distribution of African Swine Fever (ASF) and other transboundary swine diseases and their consequences. Moreover, the aim is to discuss the importance and modalities of their prevention and control, based on international standards of the World Organisation for Animal Health (WOAH, founded as OIE in 1924) and the Resolution on Global Control of ASF, adopted by 182 Member countries in 2019.

Highly pathogenic swine diseases have multifaceted impacts on food security and rural development. These impacts are driven by factors such as reduced meat production, price volatility, trade disruptions, economic losses in rural areas, decreased livelihoods, investment uncertainty, changes in rural infrastructure, and, for some of them, potential zoonotic characteristics. Understanding these dynamics is crucial for creating effective preventive and control strategies and measures to mitigate such consequences on food security and rural development in affected regions. Scientific research and evidence-based policy measures, as promoted by WOAH, are essential to address these challenges comprehensively.

Regional and international collaboration for identifying and coordinating the management of ASF is crucial for their effective prevention and control. In Europe, the WOAH plays a vital role in this mechanism, collaborating with 53 Members and partners to support the control and eradication of ASF and promote the implementation of international standards and best practices. National ASF strategies should follow these examples, with a focus on legislation enforcement and capacity building, in close collaboration with all stakeholders, to introduce effective prevention and biosecurity measures, official controls, traceability of swine commodities, wild pig management, and awareness programs.

Collaboration, cooperation and learning from best practices will contribute to better tackling these challenges and ensure sustainable disease control, meat production and food security.

1. Introduction

Livestock production is vital for human health and nutrition, food security, rural development, poverty reduction and sustainable agriculture (*Randolph et al.*, 2007). However, livestock diseases significantly affect the sustainability of livelihoods among impoverished communities while a key challenge is the limited access to quality veterinary and other services, technical capacities, funds, and relevant information about preventing and treating these diseases (*Heffernan*, 2009). The growth of the human population has multiple influences on swine health and its management, leading to a higher pork demand among consumers, consequently boosting the global pig population. More and more people are com-

***Corresponding author:** Budimir Plavšić, *b.plavsic@woah.org*

Paper received August 5th 2023. Paper accepted August 15th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

ing into direct or indirect contact with livestock and wildlife, either in proximity or in terms of geographical presence, thereby amplifying the potential for disease transmission, including those with zoonotic potential (*Vonderohe et al.*, 2022). These instances underscore the necessity of constructing a comprehensive global perspective on swine pathogens to bolster preparedness and comprehend the dynamics of their emergence and dissemination.

The expanding, intensifying, and centralized nature of pig production has given rise to unique challenges in porcine health management and public health, potentially impacting the sustainability of swine farming (*Maes et al.*, 2020). These challenges predominantly arise from the sheer volume of pigs confined within limited spaces, providing fertile ground for pathogen proliferation. Viral and bacterial propagation risks are compounded by environmental and physiological stressors, like heat stress or weaning, which can weaken the immune system, overall health, and welfare of animals. The existing disease issues are further complicated by escalating antibiotic resistance levels, making it more challenging to manage bacterial diseases that were once treatable with antibiotics (*Davies*, 2012; *Mathew et al.*, 1999; *Muurinen et al.*, 2021; *Vonderohe et al.*, 2022).

Infectious diseases represent a significant obstacle to pig production, and the global expansion of the swine industry has played a role in the emergence and dissemination of pathogens. The pig sector, characterized by its vast size and varying biosecurity levels, is vulnerable to transboundary animal diseases (TADs) like African swine fever (ASF), Classical swine fever (CSF), foot-and-mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS), porcine epidemic diarrhea (PED), with devastating economic impact (*De Vos et al.*, 2003; *Meuwissen et al.*, 1999; *Nedić et al.*, 2011; *Plavsic et al.*, 2009; *VanderWaal & Deen*, 2018). These diseases, facilitated by legal and illegal international trade, perpetually endanger swine health, diminishing livestock productivity and quality. Such outcomes extend beyond producers, impacting broader socioeconomic well-being. Even if some infections have low mortality, they negatively impact the immune system of animals, which might be more susceptible to bacterial coinfections, many of which have harmful potential (*Saade et al.*, 2020). Trade restrictions, often accompanying disease cases, impose significant economic burdens, particularly on exporting nations. Veterinary control measures, including depopulations, compound these costs, bearing additional economic, social and political consequences.

Moreover, some pig diseases have impact on public health, while various factors contribute to the rise of zoonotic infections in humans, such as the density of pig farms and population, inadequate hygiene and biosecurity conditions on farms, in transport or hunting practice, the pathogen's characteristics, professional contact with pigs, inadequate sanitation practices. The significance of livestock pathogens for public health was emphasized by the H1N1 "swine flu" pandemic in 2009, originating from influenza A viruses discovered in swine populations *(Smith et al.*, 2009). Other highly pathogenic zoonotic viral pathogens, such as Ebola and Nipah viruses, are limited to specific geographical localization, but have potential for more severe consequences (*Haddock et al.*, 2021; *Uddin Khan et al.*, 2013). The intensified interaction among humans, livestock, and wildlife will likely lead to new zoonotic viral pathogens that could threaten both swine and human health. Swine-related zoonoses frequently result in significant economic consequences due to the potential threat posed by new pathogens to humans, decreased public interest in pork consumption, mandatory culling of swine herds, and the imposition of international trade restrictions (*Uddin Khan et al.*, 2013).

The more significant the impact of animal diseases on agricultural production, the more evident the need for rigorous prevention and preparedness measures *(Brown et al., 2021; Plavšić et al.*, 2019). TADs, including ASF, with their potential to cause economic, trade, and food security crises, necessitate international cooperation for their control and management *(Plavšić et al.*, 2019). A comprehensive epidemiological understanding of entry mechanisms of these diseases is vital to ensure stakeholder preparedness, prevent pathogen introduction to farms via diverse pathways, and enable effective outbreak detection, rapid response, and effective control. Unfortunately, numerous countries, particularly those in the developing world, face a deficiency in crucial veterinary and diagnostic capacities, leading to a lack of clarity concerning the origins of primary outbreaks, the transmission pathways, and the inability for rapid containment. Recent cases of ASF spreading in Europe and Aasia, and outbreaks of FMD, CSF and PED in other regions, underscore the challenge of identifying index cases despite heightened risk and alert levels (*Huang et al.*, 2013; *Moennig et al.*, 2003; *Pharo*, 2002; *VanderWaal & Deen*, 2018; *Zhou et al.*, 2018).

2. Pig production

The worldwide pig industry plays a crucial role in providing animal protein, with pork being the most consumed terrestrial meat, making up over 36% of global meat consumption and showing steady growth (*Drew*, 2011; *FAO*, 2014). An apparent contrast marks the pig production landscape: on the one hand, there are traditional small-scale farms driven by subsistence, and on the other hand, there are industrialized operations that are vertically integrated (*Plavšić et al.*, 2019). Despite the growing importance of large-scale pig farming in meeting the demand for pork, a significant number of pig producers (around 43% of the worldwide pig population), are involved in small-scale, backyard type of farms, producing pork products mainly for own consumptions (*Robinson et al.*, 2011). In rural settings, pig farming is an essential source of meat and cash income, having an efficiency in using household food waste, providing manure for fertilization, and serving as a financial safety net. However, smallholder pig farming is typically not a primary source of income, leading to limited investment in housing facilities, biosecurity measures, and adoption of new technologies. This disparity between commercial and backyard farmers is especially evident in efforts to prevent and control diseases. The backyard sector, which is characterized by low biosecurity levels, outdated practices, and poor adherence to animal health regulations, plays a significant role in the introduction, spread, and persistence of most pig diseases, particularly ASF, CSF and FMD, including in countries with a considerable share of world's pig population (*Costard et al.*, 2013a; *Kedkovid et al.*, 2020; *Postel et al.*, 2019; *Vergne et al.*, 2017).

3. African swine fever

ASF, a severe haemorrhagic disease with a nearly 100% fatality rate in infected domestic pigs, has recently emerged as a global concern for food security following its devastating impact on swine production in several countries (*Dixon et al.*, 2020; *Kukielka et al.*, 2017; *Plavšić et al.*, 2019; *Turlewicz-Podbielska et al.*, 2021; *VanderWaal & Deen*, 2018; *Vergne et al.*, 2017; *Zhou et al.*, 2018). It poses an immense challenge for control due to its ability to cause asymptomatic infections, resulting in carrier animals capable of spreading the virus to new herds and wild boar. Additionally, it can persist in tick vectors for over five years and is difficult to diagnose. Efforts to create a safe and efficient vaccine have proven unsuccessful thus far.

ASF poses a complex epidemiological challenge, given its persistent adaptation and global spreading across diverse ecosystems, affecting different swine populations, while utilizing various transmission mechanisms. The survival of the ASF virus (ASFV) within a given ecosystem depends on various ecological factors, including the dynamics of wild host populations, vectors, soil conditions, and the characteristics of livestock production systems. These factors collectively influence the density of host and vector species and the nature of their interactions (*Costard et al*., 2013b).

ASF represents the most significant threat to the potential sustainability of global swine production and will probably continue to do so until an effective control strategy, supported by the availability of an efficient, potent, and safe vaccine, is developed. In the battle against ASF, it is imperative to recognise that control efforts must extend beyond the major responsibilities of specific sectors and encompass all key players, ideally using a "whole-of-society approach" (WoS), promoted by public health sectors during Covid-19 crisis (*Ortenzi et al*., 2022). This approach means that ASF control is not solely a technical challenge but a collective responsibility that requires active participation and commitment of practically all stakeholders, from farmers, food business operators, and professional services to consumers, hunters, and the overall community. By uniting efforts across all segments of society, a robust defence system against ASF should be built to safeguard both swine populations and the broader ecosystem in which they exist (*Maes et al.*, 2020; *Ortenzi et al.*, 2022; *Turlewicz-Podbielska et al.*, 2021; *VanderWaal & Deen*, 2018; *Vonderohe et al.,* 2022).

4. Control of African swine fever

4.1 International standards

In the context of ASF prevention and control, strict adherence to international standards is imperative. Although control of ASF is feasible, success requires regional and global coordination (*Park et al.*, 2020; *Plavšić et al*., 2019). WOAH Terrestrial Animal Health Code provides the harmonised international standards for mitigation of zoosanitary risks, including through zoning, compartmentalisation and application of risk-based trade measures *(WOAH Resolution No. 33: Global Control of African Swine Fever*, 2019; *WOAH Terrestrial Animal Health Code*, 2023) .

4.2. Global initiatives and international collaboration

In 2019, WOAH proposed its Membership the development of a strategic framework for the sustainable control of ASF, while ensuring economic growth, food security, and safe trade of swine commodities. All 182 Members unanimously adopted Resolution No. 33 on Global Control of African Swine Fever at the 87th General Session of the World Organisation for Animal Health (WOAH) after the comprehensive presentation and profound discussions among Delegates. It acknowledged the complexity of ASF and the need for multisectoral cooperation and highlighted the importance of collaboration between the Food and Agriculture Organization of the United Nations (FAO) and the WOAH in managing animal health risks related to ASF under GF-TADs mechanism (General Framework for Progressive Control of Transboundary Animal Diseases). The Resolution also emphasized the roles of Member countries, FAO, and WOAH in global ASF control, emphasizing the importance of adopting and implementing national control programs, risk communication, transparency in disease notification, and adherence to international standards (*WOAH Resolution No. 33: Global Control of African Swine Fever*, 2019).

4.2.1 GF-TADs Framework — global level

The GF-TADs is a joint initiative of the WOAH, and FAO, which combines the strengths of both organisations to achieve agreed common objectives. Initiated in May 2004, GF-TADs serves as a facilitative platform dedicated to strengthening global and regional collaborations in combating TADs. Its core objectives include enhancing capacity-building efforts and supporting establishing programs tailored to address specific TAD control priorities at both global and regional levels.

The GF-TADs initiative for the Global control of African Swine Fever is designed to address strategic challenges, foster partnerships, enhance prevention and preparedness measures, and reduce the negative impacts of ASF. The GF-TADs platform encourages regional alliances and synergizes with existing control strategies for other transboundary animal diseases. The initiative provides a structure for global ASF control, establishing a theory of change translated into a logic framework. This framework outlines outputs and indicators for three objectives: i) improve the capability of countries to control ASF using WOAH standards based on the latest scientific knowledge, ii) establish an effective coordination and cooperation framework for the global control of ASF, and iii) facilitate business continuity.

4.2.1 GF-TADs Framework — regional level

Following leadership from global level, regional offices of WOAH and FAO, in collaboration with relevant stakeholders, developed an efficient regional mechanism, to identify and coordinate management of regional activities, including priority TADs. In Europe, WOAH is providing role of Secretariat of this mechanism, in close cooperation with FAO and EC, current elected chairmanship. Among other priority animal diseases, ASF is recognized as one with critical importance for its Membership, covering 53 Member countries and partners in Europe. Since 2014, an operational Standing Group of Experts for ASF (SGE ASF) has been in place. It aims to build up closer cooperation among countries affected by African swine fever, thereby addressing the disease in a more collaborative and harmonized manner across Europe. The SGE ASF is a unique opportunity to engage affected countries into a fruitful regional dialogue and increased transparency. The GF-TADs offers the ideal framework to discuss common and harmonized mitigation measures based on scientific and technical grounds only. On top of the formal members of the SGE ASF (the countries in Europe affected by ASF), representatives from any other country in Europe and beyond are welcome to attend as observers.

4.3 National control strategies

National ASF strategies should follow these standards and best practices, enforce legislation, and collaborate with all stakeholders. Members should enhance technical capabilities, use scientific knowledge, and engage national stakeholders, since a collaborative approach is crucial to prevent further spread of ASF. To formulate robust and effective control strategies, it is essential to understand the potential for ASFV transmission within the domestic pig population, and within a wild boar reservoir, as well as at the interface between wildlife and domestic pig production. Currently, the primary methods for ASF control, proven in many regions, involve containment measures and stringent biosecurity practices (*Turlewicz-Podbielska et al.*, 2021).

Nonetheless, it is of utmost importance to carefully assess the risk factors associated with ASF and tailor control strategies accordingly, a task that presented significant challenges in certain regions during the current epizootics in Europe and Asia. A comprehensive categorization of risk factors associated with ASF was proposed, classified into twelve distinct categories, encompassing various aspects of ASF epidemiology. These categories include ASF virus characteristics, biosecurity, disease control measures, environmental factors, pig husbandry practices, movements of animals and associated materials, network connections within the swine industry, pig-related attributes, societal influences, surveillance activities, vaccination considerations, and wildlife management practices. By systematically organizing these risk factors, authors offered a valuable resource for understanding, assessing, and mitigating the spread of ASF, facilitating more informed decision-making and improved preparedness strategies within the swine industry and stakeholders (*Bergmann et al*., 2022).

4.3.1. Control strategies in domestic pigs

Effective prevention and control of ASFV is a complex task involving numerous stakeholders within the pork food system. National Veterinary Authorities (VA) are crucial in designing and implementing policy instruments, such as legislation, regulations and guidelines, disease surveillance programmes, and outbreak response procedures. These policies often need to pay more attention to socioeconomic, cultural, behavioural, or political factors, leading to limited stakeholder acceptance and reduced effectiveness. Consideration of stakeholder behavioural responses and feedback loops between policy instruments is essential. For instance, prohibiting food waste feeding may drive farmers to seek alternative protein sources, potentially from illegal means, thereby impacting the effectiveness of control measures. It is noted that well-resourced Veterinary Services (VS) can more effectively enforce compliance and the pork value chain stakeholders more readily accept and implement control measures. Achieving this requires a deep understanding of relevant socioeconomic factors and knowledge about effective incentives, often needing more social science research and technical capacity-building. Additionally, the availability of specific tools and information, such as farm registration, animal identification and traceability and the spatial distribution of farms and pig flows, will support VA in developing effective control and prevention policies for ASF (*Barnes et al.*, 2015a; *Dixon et al.*, 2020; *Hidano et al.*, 2018; *WOAH Terrestrial Animal Health Code*, 2023).

The prevention of ASFV introduction is a critical focus for VS and the pork industry due to the challenges in controlling and eradicating ASF in affected areas. This prevention strategy targets countries, regions, farms, and local wild boar populations. It involves implementing farm-level biosecurity measures, movement control and inspection activities to deter the legal and illegal importation of infected pigs, pork products, or food waste. Awareness campaigns are vital to reduce risky behaviours among travellers between affected and non-affected areas. The effectiveness of biosecurity measures depends on the local socioeconomic context and the policy instruments. Cooperation with hunters and adaptation of gaming methods involving local hunting grounds and forestry authorities may provide insights into achieving adequate knowledge to impose preventive measures (*FAO Anim. Prod. Health*, 2010; *Jurado et al.*, 2018; *Merrill et al.*, 2019).

Surveillance is paramount in the control of the spreading of ASFV, aiming to early detect outbreaks at sources, and enable swift responses to prevent its spread. Extended survival period of the virus in the environment and pork products, necessitates early detection to contain its transmission effectively. Passive and active surveillance components play roles in early detection, with passive surveillance being crucial for domestic and wild pigs. It relies on farmers and other stakeholders in the pork food system to report suspect cases. Farmers, in particular, must be capable of recognizing clinical ASF symptoms and be willing to report them promptly. Detection often occurs when multiple pigs exhibit symptoms, usually two weeks or more after the initial case. Active surveillance involves diagnostic testing of live or dead pigs for the virus, primarily, and antibodies, secondarily. Routine virus testing in slaughterhouses and at large pig farms can increase ASFV detection probabilities *(Dixon et al., 2020; Guinat et al., 2016; Halasa et al.*, 2016; *Hoinville et al.*, 2013; *Stärk et al.*, 2006).

Responding to an ASF outbreak is multifaceted, particularly in complex pork food systems. Recommendations for response strategies emphasize the importance of tracing potentially infected contacts both forwards and backwards to identify the source and potential spread of the virus. However, conducting these activities can be highly challenging, especially in some countries and regions, which might be amplified when some of the contacts involved in transmission have illegal or informal backgrounds. Establishing

protection and surveillance zones with strict restrictions on pig movement and ASFV investigation activities around an outbreak is essential. Decision-making regarding preventive culling pigs at risk of infection is intricate, involving considerations about farms in direct or indirect contact, whether to cull only those on the affected farm, a part thereof, or those on neighbouring farms within a certain radius. These decisions must account for various epidemiological, economic, cultural or social factors, as extensive culling and carcass disposal can spread the infection further if not implemented properly (*Guinat et al.,* 2016; *Halasa et al.,* 2016; *Honhold*, 2011; *te Beest et al.*, 2011).

Moreover, the mental distress experienced by farmers, veterinarians and field staff during culling operations adds another layer of complexity. Developing an integrated, locally adapted perspective based on best international practices can ensure effective outbreak response policies. Still, it is a significant challenge for national and local disease control authorities, particularly in low- to middle-income countries (*Hall et al.*, 2004; *Makita et al.*, 2015).

The role of vaccination in ASF control and prevention policies is a critical consideration. Future ASF vaccines should be integrated into comprehensive control and prevention strategies tailored to national or local pig production system considering economic and social contexts, human behaviour-related risk factors and responses to control measures. However, vaccines alone cannot replace the necessity for high-level biosecurity measures and behavioural change among all swine value chain stakeholders to restrain ASFV transmission effectively. A significant concern is the premature use of vaccine candidates driven by ASF's severe socioeconomic impact, highlighting the need for thorough effectiveness evaluation before their deployment (*Borca et al.*, 2020; *Brake, 2022a*; *Chen et al.*, 2020; *Dixon et al.*, 2020; *Urbano & Ferreira*, 2022).

Financial compensation to farmers following culling constitutes a pivotal policy instrument, positively influencing the inclination of farmers to notify disease cases. This instrument ranks among the most crucial tools accessible to Veterinary Authorities. It holds particular significance not only in effective prevention but also in the early reporting of ASF cases. A comprehensive socioeconomic analysis must underpin financial compensation schemes to ensure their effectiveness; otherwise, they could incentivise farmers to tolerate outbreaks or deter them from reporting suspected ASF cases due to concerns about potential financial losses (*Barnes et al.*, 2015b).

4.3.2. Control strategies in wild boar population

In ASF-affected countries within the European Union, most ASFV incursions have been traced back to the introduction of the virus into wild boar populations, either through anthropogenic sources or infected wild boar movement. These infected wild boar populations serve as a reservoir of infection for domestic pigs and result in trade restrictions. To achieve ASF-free status by WOAH, based on self-declaration, Belgium and the Czech Republic successfully applied control and eradication strategies since the virus introductions primarily affected wild boar populations at specific points. These strategies included the establishment of zones, including infected, buffer, and control zones, as quickly as possible. In the Czech Republic, the infected zone with fences was physically isolated to reduce the risk of natural disease spread among free-ranging wild boars and demarcate restricted areas. While using fences for ASF control in wild boar populations is subject to debate, they can likely limit wild boar movements, acting as a barrier to virus spread. Feeding and hunting bans were imposed in the infected and buffer zones to minimize disruptions to affected and at-risk populations. Efficient wild boar carcass surveillance systems were developed to detect and remove infected carcasses effectively. In the control zone, stringent wild boar depopulation strategies were recommended to reduce wild boar densities with minimal disturbances. Collaboration with hunting communities and relevant authorities played a pivotal role in achieving successful outcomes. The European Commission (EC) is now advocating for adopting these measures in other EU countries. However, it is essential to note that their application may require significant adjustments in accordance to ecological, epidemiological, and social contexts (*Abrahantes et al.,* 2017; *Dixon et al.,* 2020; *Jori & Bastos*, 2009; *More et al.*, 2018; *Nielsen et al.*, 2021).

5. The impact of ASF on the swine industry

The ongoing epidemiological situation with ASF poses a real threat to the global pig industry (*Costard et al.*, 2013a; *De Vos et al.*, 2003; *Zhou et al.*, 2018). Effective strategies for managing ASF outbreaks are paramount to safeguard the future of the pig industry and prevent adverse consequences on a local, regional, or global scale. Existing guidelines and recommendations for developing ASF control strategies, namely those adopted by WOAH Member countries, are available. As the disease has

already spread across many European and Asian countries, this section addresses critical issues related to ASF control based on the Global Framework for ASF control under the GF-TADs umbrella (*Dixon et al.*, 2020; Park et al., 2020; *Plavšić et al.*, 2019).

The success of ASF control largely depends on how farmers and stakeholders perceive and manage risk, highlighting its crucial role. Firstly, there is a critical need to closely monitor small-scale farms practicing swill feeding and lacking adequate biosecurity measures or even prohibiting them in the highest-risk areas. Backyard holdings can be significant sources of ASF infections in previously unaffected areas. The pathways for ASFV transmission to these holdings are diverse and may involve swill feeding, contaminated fomites, vehicles or other equipment, accompanied with movement of humans and animals. Although the risk of ASFV introduction to larger farms implementing robust biosecurity measures is generally low, it escalates when the viral circulation in the surrounding farms and environment increases, particularly in the case of backyard pig holdings. Therefore, it is highly recommended that affected countries implement policies to standardize biosecurity level on pig farms, properly control those with inadequate biosecurity, and consider compartmentalization or zoning to mitigate the risk. With complementary awareness-raising campaigns, availability of governmental subsidies to improve biosecurity measures could significantly decrease risks of spreading ASF and other diseases (*Kedkovid et al.,* 2020).

Efforts to prevent the introduction of viruses into farms through feed and feed ingredients represent a important aspect of biosecurity management, even for commercial pig farms (*Dee et al.*, 2018). Previous studies have demonstrated the extended survival of various swine viruses in these products. For instance, ASFV's half-life in different feed or feed ingredients ranged from 9.6 to 14.2 days under conditions simulating trans-Atlantic shipment (*Stoian et al.,* 2019). Recent reviews have outlined key principles to mitigate virus transmission through pig feed, including heat treatment (e.g., pelleting feed at higher temperatures), chemical mitigation (e.g., treating feed with formaldehyde and propionic acid), and managing storage periods based on virus half-life data. The effectiveness and practicality of these strategies for various swine viruses warrant further investigation and clarification (*Kedkovid et al.*, 2020).

Although many countries have moved beyond the peak of severe ASF outbreaks and are now in the endemic stage with increasing pork prices (e.g. in Asia), the temptation to expand pig production should not overshadow the persistent effects of ASF. The importance of maintaining biosecurity cannot be overstated, and there is a continuous need to educate farmers and those involved in the supply chain to prevent a reintroduction of ASF (*Bergmann et al.,* 2022; *Kedkovid et al.,* 2020).

Cooperation is a crucial factor in shaping the future of the pig industry. Given the suspected transmission of transboundary diseases through the illegal trade of pigs and pork products, improved cooperation among neighbouring countries is essential (*Nilubol et al.*, 2012). This type of transmission can occur across geographically isolated areas or over large distances, as demonstrated by the recent introduction of the CSF virus into Japan and the transmission of PED virus from Asia to North America via contaminated fomites (*Davies*, 2015; *Postel et al.*, 2019). Concerning ASF, we should understand that unless the problem in Africa and Asia is properly addressed, the virus may re-emerge. Therefore, cooperation should not be limited to neighbouring countries but extend further, potentially facilitated by initiatives from international organizations like the GF-TADs mechanism (*Park et al.,* 2020; *Plavšić et al.,* 2019).

An efficacious ASF vaccines, urgently required and coupled with robust biosecurity practices, are pivotal for the future of pig production. Recent advancements in the development of vaccine candidates demonstrate promising results in safety and efficacy (*Borca et al.*, 2020; *Chen et al.*, 2020). There are several reasons for tempered optimism that current and forthcoming ASF vaccine candidates can progress from discovery research to product development while effectively fulfilling the stringent regulatory criteria: vaccine purity, potency, safety, and efficacy. Among the five primary approaches to developing ASF vaccines, namely inactivated, naturally attenuated, laboratory-passaged attenuated, recombinant subunit, and recombinant gene-deleted modified live vaccines (MLV), the prospects for first-generation vaccine product licensure are most promising with ASF recombinant gene-deleted MLV candidates soon (*Brake*, 2022b). Ideal vaccine candidates may serve as marker vaccines, valuable for disease control programs like DIVA (differentiating infected from vaccinated animals). However, avoiding overemphasizing vaccination at the expense of other essential control measures, notably biosecurity and animal movement restrictions, is crucial. Lessons from previous disease control efforts in some countries, such as those targeting CSF or pseudorabies

virus (PRV), highlight the pitfalls of relying solely on vaccination. Although effective vaccines for some diseases exist, inadequate surveillance and control measures have allowed endemic diseases to spread (*Luo et al.*, 2014; *Sun et al.*, 2016). Furthermore, the potential rise in non-standardized vaccine production units due to increased demand raises concerns about vaccine safety and efficacy, mirroring challenges seen in CSF control (*Luo et al.,* 2014). Therefore, a holistic approach is imperative for effective disease management in pig production combining vaccination with stringent biosecurity and surveillance.

6. Discussion

Learning from the experience with pandemic diseases and extracting it to the case of ASF control, national, regional, and global policymakers should advocate for comprehensive control strategies encompassing the entirety of government and society. The WoS approach in ASF control, extends its scope beyond governmental bodies, involving a wide array of relevant stakeholders, including professional health services (veterinary, environmental, forestry and agriculture authorities), farmers, hunters, and traders, individuals, families, communities, intergovernmental organizations, religious institutions, civil society, academia, the media, voluntary associations, and, notably, the private sector and industry.

Establishing an efficient regional coordination mechanism for the management of priority TADs is crucial for their effective prevention and control. In Europe, the WOAH plays a vital role in this mechanism, collaborating with 53 Members and partners, namely EC and FAO, to support the control and eradication of ASF and promote the implementation of international standards and best practices. National ASF strategies should adhere to these examples, focusing on the enforcement of legislation, collaboration with all stakeholders, and enhancing technical capabilities, scientific knowledge, and risk communication. A collaborative approach is crucial to prevent the spread of ASF, with Member Countries enforcing WOAH's international standards at the local level. This includes risk-based prevention, biosecurity measures, traceability, official controls, wild pig management, and awareness programs.

Effective prevention and control of ASF require the involvement of various stakeholders within the pork production system. National VA play a crucial role in designing and implementing policy instruments, which should consider socioeconomic, cultural, behavioural, and political factors to ensure stakeholder acceptance and effectiveness. Strict adherence to WOAH international standards is imperative in ASF prevention and control. Regional success stories, like those in Belgium and the Czech Republic, underscore the importance of coordinated efforts and tailored strategies to combat ASF effectively.

Impact of ASF on food security, include several major changes: *1. Decline in meat production*: ASF substantially affect meat production, with a pronounced impact on the swine industry. The disease's high mortality rate among swine herds frequently results in a significant reduction in pork availability, a critical source of protein in many regions. Sometimes, entire herds must be culled to mitigate the outbreak, exacerbating the supply shortage. *2. Price fluctuations*: Diminished meat production reduction because of ASF outbreaks can lead to price volatility, rendering this protein source less accessible to vulnerable populations, especially those with limited incomes. Such price volatility can engender food insecurity among poor communities. *3. Disruption of trade*: ASF outbreaks often trigger trade constraints on pork and pork-related products, locally and internationally, which disproportionately affect exporting nations. Trade barriers impede the flow of pork into international markets, potentially disrupting global food supply chains and affecting food security in both exporting and importing countries. *4. Transition in consumer preferences toward alternative protein sources*: In response to the reduced availability of pork or because of ASF epidemics, consumers may shift their preferences toward alternative protein sources, such as poultry, fish, or plant-based proteins. However, heightened demand for these alternatives could increase prices, undermining food security through various channels.

African Swine Fever and similar TADs have far-reaching consequences on rural development, including various aspects, such as: *i) the economic consequences* (rural areas heavily reliant on swine production suffer substantial economic losses due to disease outbreaks, encompassing mortality, reduced production, and surge effects on related industries such as feed production, transportation, and processing); *ii) decreased livelihoods* (swine farming is a crucial livelihood source for numerous small-scale farmers in rural regions where disease outbreaks jeopardize these livelihoods, compelling individuals and families to seek alternative income sources, often challenging to find in rural settings): *iii) investment uncertainty* (ASF introduce uncertainty into the swine industry, making it less

appealing for investment, while the fear of recurrent outbreaks and potential financial losses discourages both local and foreign investors from engaging in the swine sector, hindering rural community growth and development) and *iv) infrastructure and services* (regions heavily dependent on swine farming often modify rural infrastructure and services to support this industry; when outbreaks occur, causing industry contraction, infrastructure and services like processing facilities, transportation networks, and veterinary services may become underutilized or obsolete, impacting overall rural development); *v) social impact* (swine farming plays a pivotal role in the social fabric of rural communities, tightly interwoven with their way of life; the stress and social disruption resulting from disease outbreaks can have enduring psychological and social consequences on individuals and rural communities, further affecting rural development).

7. Conclusion

The ongoing global epidemiological situation with ASF presents a significant and persistent threat to the swine industry worldwide. The consequences of uncontrolled ASF outbreaks are profound locally, regionally, and globally. Addressing global challenges in animal health and welfare, particularly highly pathogenic diseases like ASF, requires a collaborative effort on an international scale.

The GF-TADs represent an efficient mechanism to address the challenges ASF and other TADs, for its Membership, particularly in Europe. The establishment of the SGE ASF in Europe in 2014 has facilitated closer cooperation among countries grappling with disease, promoting a harmonized and transparent response to disease across Europe. Through cooperation, coordination, and scientific collaboration, this framework represents a significant step toward achieving effective control and prevention strategies for ASF on a global scale.

Developing a national control strategy for the control of ASF and other TADs, requires a multifaceted approach, which should include: i) Implementing robust surveillance systems to monitor disease prevalence and promptly detect outbreaks, involving both passive and active surveillance, ii) Promoting stringent biosecurity measures within the swine production system to minimize disease introduction and spread, including practices such as controlled access, disinfection, and quarantine protocols, iii) Evaluating and regulating hunting practices, which can contribute to disease transmission, which particularly covers biosecurity hunting practice, and iv) Developing and implementing effective control measures, including rapid alerts and response system, culling infected animals, and movement restrictions. The development of a high-quality vaccine for ASF is a high-priority research area.

Activities relevant to control measures include training and education of farm staff, hunters, and veterinary personnel, including disease recognition, passive surveillance and particularly biosecurity measures. In addition, rapid response teams and specialized units should be ready to contain outbreaks through culling, disinfection, and safe disposal of infected animals. International trade policies should be designed to support disease-free meat and animal product exchanges while ensuring compliance with WOAH standards.

Practical strategies for managing ASF outbreaks, based on WOAH standards and best practices shared by GF-TADs, are imperative to safeguard the future of the pig industry and mitigate these adverse effects. One of the key priorities is the close monitoring of small-scale farms, especially those employing risky practices such as swill feeding and lacking adequate biosecurity measures. Backyard holdings can be significant sources for initiating ASF outbreaks in previously unaffected areas. Affected countries should standardize farm practices, regulate farms with inadequate biosecurity, and consider implementing compartmentalization or zoning measures to mitigate risks.

Farmers' and stakeholders' perceptions and risk management play a pivotal role in the success of ASF control efforts. While some countries have moved past the peak of severe ASF outbreaks and are experiencing increasing pork prices, the importance of maintaining biosecurity practices must not be underestimated. Continuous education and awareness campaigns are essential to prevent the resurgence of ASF. Given the potential transmission of transboundary diseases through the illegal trade of pigs and pork products, enhanced cooperation among neighbouring countries is imperative.

By working together and learning from successful experiences, the global community can better tackle these challenges and ensure sustainable meat production and food security for all.

Disclosure statement: No conflict of interest was reported by authors

References

Abrahantes, J. C., Gogin, A., Richardson, J. & Gervelmeyer, A. (2017). Epidemiological analyses on African swine fever in the Baltic countries and Poland. *EFSA Journal. European Food Safety Authority*, 15(3), https://doi.org/10.2903/J.EFSA.2017.4732

Barnes, A. P., Moxey, A. P., Vosough Ahmadi, B. & Borthwick, F. A. (2015a). The effect of animal health compensation on "positive" behaviours towards exotic disease reporting and implementing biosecurity: A review, a synthesis and a research agenda. *Preventive Veterinary Medicine*, 122(1–2), 42–52, https://doi.org/10.1016/J.PREVETMED.2015.09.003

Barnes, A. P., Moxey, A. P., Vosough Ahmadi, B. & Borthwick, F. A. (2015b). The effect of animal health compensation on "positive" behaviours towards exotic disease reporting and implementing biosecurity: A review, a synthesis and a research agenda. *Preventive Veterinary Medicine*, 122(1–2), 42–52, https://doi.org/10.1016/J.PREVETMED.2015.09.003

Bergmann, H., Dups-Bergmann, J., Schulz, K., Probst, C., Zani, L., Fischer, M., Gethmann, J., Denzin, N., Blome, S., Conraths, F. J. & Sauter-Louis, C. (2022). Identification of Risk Factors for African Swine Fever: A Systematic Review. In *Viruses* (Vol. 14, Issue 10). MDPI. https://doi.org/10.3390/v14102107

Borca, M. V., Ramirez-Medina, E., Silva, E., Vuono, E., Rai, A., Pruitt, S., Holinka, L. G., Velazquez-Salinas, L., Zhu, J. & Gladue, D. P. (2020). Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain. *Journal of Virology*, 94(7), https://doi.org/10.1128/JVI.02017-19

Brake, D. A. (2022a). African Swine Fever Modified Live Vaccine Candidates: Transitioning from Discovery to Product Development through Harmonized Standards and Guidelines. In *Viruses* (14, Issue 12). MDPI. https://doi.org/10.3390/v14122619

Brake, D. A. (2022b). African Swine Fever Modified Live Vaccine Candidates: Transitioning from Discovery to Product Development through Harmonized Standards and Guidelines. *Viruses*, 14(12). https://doi.org/10.3390/V14122619

Brown, V. R., Miller, R. S., McKee, S. C., Ernst, K. H., Didero, N. M., Maison, R. M., Grady, M. J., & Shwiff, S. A. (2021). Risks of introduction and economic consequences associated with African swine fever, classical swine fever and foot-and-mouth disease: A review of the literature. *Transboundary and Emerging Diseases*, 68(4), 1910–1965, https://doi.org/10.1111/TBED.13919

Chen, W., Zhao, D., He, X., Liu, R., Wang, Z., Zhang, X., Li, F., Shan, D., Chen, H., Zhang, J., Wang, L., Wen, Z., Wang, X., Guan, Y., Liu, J. & Bu, Z. (2020). A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs. *Science China. Life Sciences*, 63(5), 623–634, https://doi.org/10.1007/S11427-020-1657-9

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M., & Pfeiffer, D. U. (2013a). Epidemiology of African swine fever virus. *Virus Research*, 173(1), 191–197, https://doi.org/10.1016/J.VIRUSRES.2012.10.030

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M., & Pfeiffer, D. U. (2013b). Epidemiology of African swine fever virus. *Virus Research*, 173(1), 191–197, https://doi.org/10.1016/J.VIRUSRES.2012.10.030

Davies, P. R. (2012). One world, one health: the threat of emerging swine diseases. A North American perspective. *Transboundary and Emerging Diseases*, 59(SUPPL. 1), 18–26, https://doi.org/10.1111/J.1865-1682.2012.01312.X

Davies, P. R. (2015). The dilemma of rare events: Porcine epidemic diarrhea virus in North America. *Preventive Veterinary Medicine*, 122(1–2), 235–241, https://doi.org/10.1016/J.PREVETMED.2015.08.006

De Vos, C. J., Saatkamp, H. W., Huirne, R. B. M. & Dijkhuizen, A. A. (2003). The risk of the introduction of classical swine fever virus at regional level in the European Union: a conceptual framework. *Revue Scientifique et Technique (International Office of Epizootics)*, 22(3), 795–810, https://doi.org/10.20506/RST.22.3.1433

Dee, S. A., Bauermann, F. V., Niederwerder, M. C., Singrey, A., Clement, T., De Lima, M., Long, C., Patterson, G., Sheahan, M. A., Stoian, A. M. M., Petrovan, V., Jones, C. K., De Jong, J., Ji, J., Spronk, G. D., Minion, L., Christopher-Hennings, J., Zimmerman, J. J., Rowland, R. R. R.… & Diel, D. G. (2018). Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PloS One*, 13(3), https://doi.org/10.1371/JOURNAL.PONE.0194509

Dixon, L. K., Stahl, K., Jori, F., Vial, L. & Pfeiffer, Di. U. (2020). African Swine Fever Epidemiology and Control. *Annual Review of Animal Biosciences*, 8, 221–246. https://doi.org/10.1146/ANNUREV-ANIMAL-021419-083741

Drew, T. W. (2011). The emergence and evolution of swine viral diseases: to what extent have husbandry systems and global trade contributed to their distribution and diversity? *Revue Scientifique et Technique (International Office of Epizootics)*, 30(1), 95–106, https://doi.org/10.20506/RST.30.1.2020

FAO Anim. Prod. Health, R. I. (2010). *Good practices for biosecurity in the pig sector: issues and options in developing and transition countries.* https://www.fao.org/3/i1435e/i1435e00.pdf

FAO, (2014). *Sources of meat*. http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html

Guinat, C., Wall, B., Dixon, L. & Pfeiffer, D. U. (2016). English Pig Farmers' Knowledge and Behaviour towards African Swine Fever Suspicion and Reporting. *PloS One*, 11(9). https://doi.org/10.1371/JOURNAL.PONE.0161431

Haddock, E., Saturday, G., Feldmann, F., Hanley, P. W., Okumura, A., Lovaglio, J., Long, D., Thomas, T., Scott, D. P., Pulliam, M., Richt, J. A., de Wit, E. & Feldmann, H. (2021). Reston virus causes severe respiratory disease in young domestic pigs. *Proceedings of the National Academy of Sciences of the United States of America*, 118(2). https://doi.org/10.1073/PNAS.2015657118

Halasa, T., Bøtner, A., Mortensen, S., Christensen, H., Toft, N. & Boklund, A. (2016). Control of African swine fever epidemics in industrialized swine populations. *Veterinary Microbiology*, 197, 142–150, https://doi.org/10.1016/J.VETMIC.2016.11.023

Hall, M. J., Ng, A., Ursano, R. J., Holloway, H., Fullerton, C. & Casper, J. (2004). Psychological impact of the animal-human bond in disaster preparedness and response. *Journal of Psychiatric Practice*, 10(6), 368–374, https://doi.org/10.1097/00131746-200411000-00005

Heffernan, C. (2009). Panzootics and the poor: Devising a global livestock disease prioritisation framework for poverty alleviation. *OIE Revue Scientifique et Technique*, 28(3), 897–907, https://doi.org/10.20506/RST.28.3.1934

Hidano, A., Enticott, G., Christley, R. M. & Gates, M. C. (2018). Modeling Dynamic Human Behavioral Changes in Animal Disease Models: Challenges and Opportunities for Addressing Bias. *Frontiers in Veterinary Science*, 5(JUN). https://doi.org/10.3389/FVETS.2018.00137

Hoinville, L. J., Alban, L., Drewe, J. A., Gibbens, J. C., Gustafson, L., Häsler, B., Saegerman, C., Salman, M. & Stärk, K. D. C. (2013). Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Preventive Veterinary Medicine*, 112(1–2), 1–12, https://doi.org/10.1016/J.PREVETMED.2013.06.006

Honhold N, D. I. G. W. S. A. L. J. (2011). Good Emergency Management Practices: The Essentials. *Rome, Italy: Anim. Prod. Health, Food Agric. Organ,* https://www.fao.org/publications/card/fr/c/68b14f27-5234-51f3-b46e-8ecea0029d9b

Huang, Y. W., Dickerman, A. W., Piñeyro, P., Li, L., Fang, L., Kiehne, R., Opriessnig, T. & Meng, X. J. (2013). Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *MBio*, 4(5), https://doi.org/10.1128/MBIO.00737-13

Jori, F. & Bastos, A. D. S. (2009). Role of wild suids in the epidemiology of African swine fever. *EcoHealth*, 6(2), 296–310, https://doi.org/10.1007/S10393-009-0248-7

Jurado, C., Martínez-Avilés, M., Torre, A. D. La, Štukelj, M., Ferreira, H. C. de C., Cerioli, M., Sánchez-Vizcaíno, J. M. & Bellini, S. (2018). Relevant Measures to Prevent the Spread of African Swine Fever in the European Union Domestic Pig Sector. *Frontiers in Veterinary Science*, 5(APR), https://doi.org/10.3389/FVETS.2018.00077

Kedkovid, R., Sirisereewan, C. & Thanawongnuwech, R. (2020). Major swine viral diseases: an Asian perspective after the African swine fever introduction. In *Porcine Health Management* (Vol. 6, Issue 1). BioMed Central. https://doi.org/10.1186/s40813-020-00159-x

Kukielka, E. A., Martínez-López, B. & Beltrán-Alcrudo, D. (2017). Modeling the live-pig trade network in Georgia: Implications for disease prevention and control. *PloS One*, 12(6), https://doi.org/10.1371/JOURNAL.PONE.0178904

Luo, Y., Li, S., Sun, Y. & Qiu, H. J. (2014). Classical swine fever in China: a minireview. *Veterinary Microbiology*, 172(1–2), 1–6, https://doi.org/10.1016/J.VETMIC.2014.04.004

Maes, D. G. D., Dewulf, J., Piñeiro, C., Edwards, S. & Kyriazakis, I. (2020). A critical reflection on intensive pork production with an emphasis on animal health and welfare. *Journal of Animal Science*, 98(Suppl 1), S15–S26, https://doi.org/10.1093/JAS/SKZ362

Makita, K., Tsuji, A., Iki, Y., Kurosawa, A., Kadowaki, H., Tsutsumi, A., Nogami, T. & Watari, M. (2015). Mental and physical distress of field veterinarians during and soon after the 2010 foot and mouth disease outbreak in Miyazaki, Japan. *Revue Scientifique et Technique (International Office of Epizootics)*, 34(3), 699–712, https://doi.org/10.20506/RST.34.3.2387

Mathew, A. G., Saxton, A. M., Upchurch, W. G. & Chattin, S. E. (1999). Multiple antibiotic resistance patterns of Escherichia coli isolates from swine farms. *Applied and Environmental Microbiology*, 65(6), 2770–2772, https://doi.org/10.1128/AEM.65.6.2770-2772.1999

Merrill, S. C., Koliba, C. J., Moegenburg, S. M., Zia, A., Parker, J., Sellnow, T., Wiltshire, S., Bucini, G., Danehy, C. & Smith, J. M. (2019). Decision-making in livestock biosecurity practices amidst environmental and social uncertainty: Evidence from an experimental game. *PloS One*, 14(4), https://doi.org/10.1371/JOURNAL.PONE.0214500

Meuwissen, M. P. M., Horst, S. H., Huirne, R. B. M. & Dijkhuizen, A. A. (1999). A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Preventive Veterinary Medicine*, 42(3–4), 249–270, https://doi.org/10.1016/S0167-5877(99)00079-3

Moennig, V., Floegel-Niesmann, G. & Greiser-Wilke, I. (2003). Clinical signs and epidemiology of classical swine fever: A review of new knowledge. *Veterinary Journal*, 165(1), 11–20, https://doi.org/10.1016/S1090-0233(02)00112-0

More, S., Miranda, M. A., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Edwards, S., Garin-Bastuji, B., Good, M., Michel, V., Raj, M., Nielsen, S. S., Sihvonen, L., Spoolder, H., Stegeman, J. A., Velarde, A., Willeberg, P., Winckler, C., Depner, K.… & Gortázar Schmidt, C. (2018). African swine fever in wild boar. *EFSA Journal. European Food Safety Authority*, 16(7), https://doi.org/10.2903/J.EFSA.2018.5344

Muurinen, J., Richert, J., Wickware, C. L., Richert, B. & Johnson, T. A. (2021). Swine growth promotion with antibiotics or alternatives can increase antibiotic resistance gene mobility potential. *Scientific Reports*, 11(1), https://doi.org/10.1038/S41598-021-84759-9

Nedić, D., Tešič, M., Baltic, M., Plavšić, B., Nada, T., Mirilović, M., & Rajković, M. (2011). Management and control program for suppression and eradication of classical swine fever in Serbia. *Acta Veterinaria*, 61(2–3), https://doi.org/10.2298/AVB1103295N

Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Schmidt, C., Herskin, M., Michel, V., Pasquali, P., Roberts, H. C., Sihvonen, L. H., Spoolder, H., Stahl, K., Velarde, A., Winckler, C., Blome, S., …& Miranda Chueca, M. A. (2021). Research objectives to fill knowledge gaps in African swine fever virus survival in the environment and carcasses, which could improve the control of African swine fever virus in wild boar populations. *EFSA Journal. European Food Safety Authority*, 19(6), https://doi.org/10.2903/J.EFSA.2021.6675

Nilubol, D., Tripipat, T., Hoonsuwan, T. & Kortheerakul, K. (2012). Porcine reproductive and respiratory syndrome virus, Thailand, 2010–2011. *Emerging Infectious Diseases*, 18(12), 2039–2043. https://doi.org/10.3201/EID1812.111105

Ortenzi, F., Marten, R., Valentine, N. B., Kwamie, A. & Rasanathan, K. (2022). Whole of government and whole of society approaches: call for further research to improve population health and health equity. *BMJ Global Health*, 7(7), https://doi.org/10.1136/BMJGH-2022-009972

Park, J.Y., Torres, G., Plavsic, B. & Rozstalnyy, A. (2020). *FAO and OIE. 2020. Global control of African swine fever: A GF-TADs initiative. 2020–2025. Paris.* https://www.gf-tads.org/asf/the-global-initiative-for-the-control-of-asf/en/#:~:text=The%20GF%2DTADs%20initiative%20for,the%20adverse%20impacts%20of%20ASF.

Pharo, H. J. (2002). Foot-and-mouth disease: an assessment of the risks facing New Zealand. *New Zealand Veterinary Journal*, *50*(2), 46–55. https://doi.org/10.1080/00480169.2002.36250

Plavsic, B., Nedic, D., Micovic, Z., Tesic, M., Stanojevic, S., Ruzica, A., Krnjaic, D., Nada, T., & Milanovic, S. (2009). Veterinary information management system (vims) in the process of notification and management of animal diseases. *Acta Veterinaria*, ***59***(1). https://doi.org/10.2298/AVB0901099P

Plavšić, B., Rozstalnyy, A., Park, J. Y., Guberti, V., Depner, K. & Torres, G. (2019). *OIE • 12, rue de Prony.* Strategic challenges to global control of African Swine fever.

Postel, A., Nishi, T., Kameyama, K. I., Meyer, D., Suckstorff, O., Fukai, K. & Becher, P. (2019). Reemergence of Classical Swine Fever, Japan, 2018. *Emerging Infectious Diseases*, 25(6), 1228–1231, https://doi.org/10.3201/EID2506.181578

Randolph, T. F., Schelling, E., Grace, D., Nicholson, C. F., Leroy, J. L., Cole, D. C., Demment, M. W., Omore, A., Zinsstag, J. & Ruel, M. (2007). Invited Review: Role of livestock in human nutrition and health for poverty reduction in developing countries1,2,3. *Journal of Animal Science*, 85(11), 2788–2800, https://doi.org/10.2527/jas.2007-0467

Robinson, T. P., Thornton, P. K., Franceschini, G., Kruska, R. L., Chiozza, F., Notenbaert, A. M., Cecchi, G., Herrero, M., Epprecht, M., Fritz, S. & You, L. (2011). Global livestock production systems. FAO.

Saade, G., Deblanc, C., Bougon, J., Marois-Créhan, C., Fablet, C., Auray, G., Belloc, C., Leblanc-Maridor, M., Gagnon, C. A., Zhu, J., Gottschalk, M., Summerfield, A., Simon, G., Bertho, N. & Meurens, F. (2020). Coinfections and their molecular consequences in the porcine respiratory tract. *Veterinary Research*, 51(1), https://doi.org/10.1186/S13567-020-00807-8

Smith, G. J. D., Vijaykrishna, D., Bahl, J., Lycett, S. J., Worobey, M., Pybus, O. G., Ma, S. K., Cheung, C. L., Raghwani, J., Bhatt, S., Peiris, J. S. M., Guan, Y. & Rambaut, A. (2009). Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature*, 459(7250), 1122–1125, https://doi.org/10.1038/NATURE08182

Stärk, K. D. C., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R. S. & Davies, P. (2006). Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: review of current approaches. *BMC Health Services Research*, 6, https://doi.org/10.1186/1472-6963-6-20

Stoian, A. M. M., Zimmerman, J., Ji, J., Hefley, T. J., Dee, S., Diel, D. G., Rowland, R. R. R., & Niederwerder, M. C. (2019). Half-Life of African Swine Fever Virus in Shipped Feed. *Emerging Infectious Diseases*, 25(12), 2261–2263, https://doi.org/10.3201/EID2512.191002

Sun, Y., Luo, Y., Wang, C. H., Yuan, J., Li, N., Song, K. & Qiu, H. J. (2016). Control of swine pseudorabies in China: Opportunities and limitations. *Veterinary Microbiology*, 183, 119–124, https://doi.org/10.1016/J.VETMIC.2015.12.008

te Beest, D. E., Hagenaars, T. J., Stegeman, J. A., Koopmans, M. P. G. & van Boven, M. (2011). Risk based culling for highly infectious diseases of livestock. *Veterinary Research*, 42(1), 81, https://doi.org/10.1186/1297-9716-42–81

Turlewicz-Podbielska, H., Kuriga, A., Niemyjski, R., Tarasiuk, G. & Pomorska-Mól, M. (2021). African Swine Fever Virus as a Difficult Opponent in the Fight for a Vaccine—Current Data. *Viruses*, 13(7), 1212. https://doi.org/10.3390/v13071212

Uddin Khan, S., Atanasova, K. R., Krueger, W. S., Ramirez, A. & Gray, G. C. (2013). Epidemiology, geographical distribution, and economic consequences of swine zoonoses: a narrative review. *Emerging Microbes & Infections*, 2(12). https://doi.org/10.1038/EMI.2013.87

Urbano, A. C. & Ferreira, F. (2022). African swine fever control and prevention: an update on vaccine development. In *Emerging Microbes and Infections,* 11(1), pp. 2021–2033, Taylor and Francis Ltd. https://doi.org/10.1080/22221751.2022.2108342

VanderWaal, K. & Deen, J. (2018). Global trends in infectious diseases of swine. *Proceedings of the National Academy of Sciences of the United States of America*, 115(45), 11495–11500, https://doi.org/10.1073/PNAS.1806068115/-/DCSUPPLEMENTAL

Vergne, T., Chen-Fu, C., Li, S., Cappelle, J., Edwards, J., Martin, V., Pfeiffer, D. U., Fusheng, G. & Roger, F. L. (2017). Pig empire under infectious threat: risk of African swine fever introduction into the People's Republic of China. *The Veterinary Record*, 181(5), 117, https://doi.org/10.1136/VR.103950

Vonderohe, C. E., Brizgys, L. A., Richert, J. A. & Radcliffe, J. S. (2022). Swine production: how sustainable is sustainability? *Animal Frontiers: The Review Magazine of Animal Agriculture*, 12(6), 7, https://doi.org/10.1093/AF/VFAC085

WOAH Resolution No. 33: Global Control of African Swine Fever, (2019). RESOLUTION No. 33: Global Control of African Swine Fever. *87 GS/FR — PARIS, May 2019*, 181–183, https://rr-europe.woah.org/wp-content/uploads/2020/03/87-gs_recom33_asf.pdf

WOAH Terrestrial Animal Health Code, (2023). *The World Organisation for Animal Health (WOAH) Terrestrial Animal Health Code*. The World Organisation for Animal Health (WOAH). https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/

Zhou, X., Li, N., Luo, Y., Liu, Y., Miao, F., Chen, T., Zhang, S., Cao, P., Li, X., Tian, K., Qiu, H. J. & Hu, R. (2018). Emergence of African Swine Fever in China, 2018. *Transboundary and Emerging Diseases*, 65(6), 1482–1484, https://doi.org/10.1111/TBED.12989


carnex
svaki dan je dobar dan

Koraci naše tradicije odjekuju kvalitetom

Kompanija Zlatiborac je osnovana s idejom da se kroz neprekidnu modernizaciju i primenu savremenih tehnologija zadrži tradicionalni koncept proizvodnje zlatiborskih suvomesnatih proizvoda. Specifičnost regije i klimatskih uslova sela Mačkat, prirodan proces sušenja mesa na ruži vetrova i dimljenja na bukovom drvetu, uz primenu jedinstvene recepture, doprineli su da se izdvojimo ne samo na domaćem, već i na inostranim tržištima.

Poslovni proces se odvija u okviru savremenih kapaciteta i opreme, na 40.000 m^2 proizvodnog prostora, uz ispunjavanje najvažnijih standarda i sertifikata za kvalitet i bezbednost hrane: HACCP, IFS, EAC. Proizvodni portfolio se neprekidno unapređuje, a sadržan je u dve kategorije, trajnih i polutrajnih suvomesnatih proizvoda. Proizvodi su dostupni u tri vrste pakovanja, vakuumu, rinfuzu i zaštitnoj atmosferi.

Zlatiborac brend se posebno izdvaja po komparativnim prednostima u odnosu na konkurenciju, te u segmentu „slajs-pakovanja", o čijim funkcionalnim benefitima neprekidno edukuje svoje potrošače, zauzima lidersku poziciju na tržištu. Kao proizvođač, „Zlatiborac" je jedini koji svoje suvomesnate proizvode seče tanko i koso, pod uglom od 38 stepeni.

Sveobuhvatnu pažnju poklanjamo čistoći i higijeni, a u prostoru „bela soba" u kome nastaju upravo ovi slajs-proizvodi vlada apsolutni princip kontrolisanja strujanja vazduha koji eliminiše svako prisustvo neželjenih čestica, baš kao u hirurškoj sali. Besprekorne uslove obezbeđujemo filtracijom vazduha, merenjem i regulacijom prečišćavanja, dok se vazduh u našim „belim sobama" u potpunosti promeni čak 18 puta u toku jednog sata.

Godinama unazad, „Nemačko poljoprivredno društvo" (DLG), kao vodeća evropska institucija za bezbednost i unapređenje kvaliteta hrane nagrađuje zlatiborac proizvode najvišim ocenama i medaljama za kvalitet. Ova posebna nagrada dodeljuje se proizvođačima koji su u prethodnih pet godina ostvarili natprosečan uspeh u takmičenju, a „Zlatiborac" je prva kompanija s naših prostora koja je dobila ovo važno priznanje. Kontinuirana posvećenost kvalitetu proizvoda i stalna ulaganja u tehnološki proces koji prati važne svetske standarde u prehrambenoj industriji doneli su „Zlatiborcu" ovo svetsko priznanje koje jasno govori da su tradicionalne recepture sušenja suvomesnatih delikatesa sinonim za kvalitet i na svetskom tržištu. Do kraja 2022. godine dodeljeno je ukupno 95 zlatnih i 40 srebrnih DLG medalja.

U „Zlatiborcu" danas radi više od 650 ljudi koji svojom marljivošću i posvećenošću doprinose ostvarivanju korporativne misije, negujući vrednosti integriteta, doslednosti, usmerenosti na rezultate i kreativnosti. U dodiru tradicije i jedinstvenog prirodnog ambijenta nastali su izvorni proizvodi, a u kompaniji ponosno ističemo da je tradicija garancija našeg umeća, a priroda izvor naše snage. Život u skladu s prirodom je jedna od osnovnih odrednica naše korporativne kulture.

U godinama koje dolaze, nastavićemo da ostvarujemo naše ciljeve u povećanju obima proizvodnje, povećanju udela na domaćem i inostranom tržištu, u osvajanju novih tržišta, unapređenju kvaliteta i bezbednosti proizvoda, uz očuvanje životne sredine.

Misija „Zlatiborca" je stvaranje stabilne, organizovane i snažne kompanije, koja će stalnim inovacijama, ulaganjem u kvalitet, tehnologiju, opremu, edukaciju i obuku zaposlenih, stvoriti proizvod koji će svojim istaknutim karakteristikama odgovoriti potrebama i željama zahtevnih savremenih potrošača.

Hvala vam na poverenju.

Matijević

INDUSTRIJA MESA NOVI SAD

Kukuruz,
pšenica, ječam
soja, suncokret,
šećerna repa...

Agronom
KONTROLA
- Rodnost
- Kvalitet
- NON GMO

KONTROLA
tehnolog stočarstva
Vlaga ·
Toksini ·
Proteini ·

STOČNA HRANA

SA NAŠIH FARMI
ušna markica A-01
000001

GODIŠNJE KLANJE
300.000 Tovljenika
10.000 Junadi
7.000.000 Pilića

INDUSTRIJA MESA MATIJEVIĆ

Veterinar
KONTROLA

Tehnolog proizvodnje
KONTROLA
kvaliteta proizvoda

100 KAMIONA
Godišnja otprema
robe: 45.000 t

162 PRODAJNA OBJEKTA

matijevic.rs
Industrija mesa "Matijević" doo, 21 000 Novi Sad, Rumenački put 86

NOUO

OD PILEĆEG FILEA I BATAKA

Tako fina Patelina


Natur
ZA MAMU
ZA BATU
ZA DEKU
ZA SEKU
ZA TATU
ZA BAKU
PROIZVEDENO U SRBIJI
SVI VOLE
Domaće
Perutnina Ptuj

ANALYSIS
LABORATORY EQUIPMENT

Since it was founded in 1996, **Analysis d.o.o.** offers high-quality instruments and reliable solutions for your laboratory.

SOLUTIONS FOR MEAT QUALITY AND SAFETY ANALYSIS

Vanquish Core HPLC

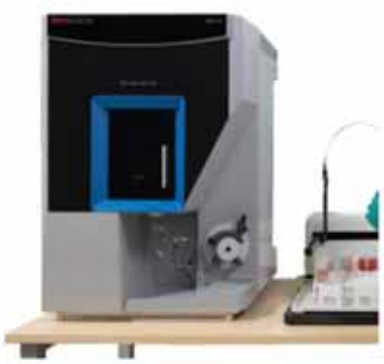
TSQ Quantis Plus triple quadrupole MS

Orbitrap Exploris GC MS

thermo scientific
Authorized Distributor

EA IsoLink IRMS system

Evolution One Plus UV-Vis spectrophotometer



iCAP PRO ICP-OES

iCAP RQplus ICP-MS

ZM300 ultra cenrtifugal mill

TU500 laboratory turbidimeter and complete online & laboratory water analysis equipment


YUHOR
Još od 1902.
YUUHOOR!
Dobar ukus
na dobrom glasu

Super vet

ELEVATE YOUR LABORATORY SOLUTIONS

Your trusted partner for Laboratory Consumables and Reagents.

QUALITY DRIVEN SOLUTIONS

RELIABLE & TRUSTWORTHY

GLOBAL PARTNERSHIPS

GOLD STANDARD DIAGNOSTICS

Kits for Mycotoxins, GMO, Allergens, Water and Environmental Testing, Veterinary Residues

ThermoFisher SCIENTIFIC

Culture Media, Quality Control, Precise Method (Listeria, Salmonella..)

BIOREBA
Your Partner in Agro-Diagnostics

Plant Pathogen Diagnostic Ragents, Sample Preparation Devices

TESTVERITAS

Proficiency Testing Schemes

CONTACT INFORMATION

+381-63-694-063

+381-11-2694-877

supervet.bg@gmail.com

Aleksinackih rudara 75
11000
Belgrade, Serbia

www.supervet.rs

Naši partneri u okviru laboratorijskog programa:

SIGMA-ALDRICH
MERCK

CARL ROTH®

ROMER
Romer Labs®

Honeywell

LGC Standards

CPA chem

CORNING

Crnotravska 27,
11000 Beograd

www.uni-chem.rs
info@uni-chem.rs

Telefon: 011/3281-986
Fax: 011/3281-205

"Meat Technology" is the scientific journal, founded in year 1961, that publishes results of basic and applied research in the field of biotechnical sciences i.e. the following subcategories: veterinary sciences, food engineering and biotechnology.

Journal "Meat Technology" is abstracted in FSTA (Food Science and Technology Abstract). Full text is available in SCOPUS, CABI Database, EBSCO publishing, DOAJ, AGRIS, www.ifocus.my Database and www.inmes.rs. "Meat Technology" is an open access journal. All articles can be downloaded free and used in accordance with the Creative Commons Attribution 4.0 International (CC BY 4.0).

Honorable Editors

Editorial Board Members

Advisory Board Members

Subeditors from Institute of Meat Hygiene and Technology

FOUNDER AND PUBLISHER

Institute of Meat Hygiene and Technology

11000 Belgrade, Kaćanskog 13

P.O. Box 33-49

Phone 381 11 2650-655

e-mail: institut@inmes.rs

www.inmes.rs

DIRECTOR

Vesna Z. Đorđević, PhD

EDITOR IN CHIEF

Vesna Z. Đorđević, PhD

PROOFREADER FOR ENGLISH LANGUAGE

Sheryl Avery, PhD

TECHNICAL EDITION

Danijela Šarčević, PhD

Slaviša Šobot

PUBLICATION OF THIS JOURNAL IS FINNANCIALLY SUPPORTED BY:

Ministry of Science, Technological Development and Innovations of the Republic of Serbia

www.journalmeattechnology.com