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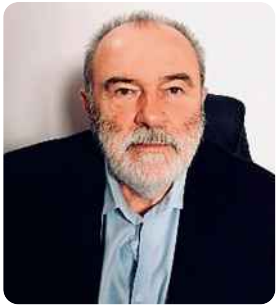

Milan Z. Baltic, University in Belgrade, Faculty of Veterinary Medicine, Department of Hygiene and Technology of Food Animal Origin, Belgrade, Republic of Serbia

Aurelija Spiric, Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

Subeditors of scientific journal "Meat Technology"
from Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

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CONTENT

FOUNDER AND PUBLISHER

Institute of Meat Hygiene and Technology

11000 Belgrade, Kacanskog 13
P.O. Box 33-49
Phone 381 11 2650-655
Fax 381 11 2651-825
e-mail: institut@inmes.rs
www.inmes.rs

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Review Paper

Meat safety: Risk based assurance systems and novel technologies

Ivan Nastasijević[1], Slavica Vesković[1], Milan Milijašević[1]*

A b s t r a c t: The meat industry has undergone substantial changes over the previous several decades due to development of new technologies in primary production (food animals on farm) — precision livestock farming, sensing systems; slaughter & dressing — automation and robotization; and meat processing — precision fermentation, 3-dimensional printed meat. The current, traditional meat inspection (ante-mortem and post-mortem), based on visual inspection, palpation and incision, had not been changed since the end of the nineteenth century. Although this traditional approach was effective at the time it was introduced for detection of classical zoonoses (brucellosis, tuberculosis, cysticercosis, anthrax infection), it was not fully efficient in terms of the current needs for consumer protection. Namely, public health hazards associated with meat are, nowadays, connected to zoonotic food (meat) borne pathogens (Salmonella, Campylobacter, Shiga toxin-producing E. coli, Listeria monocytogenes), faecally excreted by healthy animals, which are responsible for the majority of human illnesses attributed to meat consumption; traditional meat inspection cannot respond effectively to detect these food borne hazards, but can even increase cross-contamination due to palpation and/or incision procedures. Therefore, there is a need to develop a novel, modern meat inspection system which will be risk-and evidence-based — the meat safety assurance system or carcass safety assurance system. Such a modern system should be based on risk management and meat inspection protocols supported by analysis of Food Chain Information/Harmonised Epidemiological Indicators in the farm-to-chilled carcass continuum.

Keywords: *meat safety, assurance system, meat inspection, cultured meat.*

Introduction

Meat and high nutritional value. The consumption of meat is highly esteemed in most places in the world considering it as a food product with high nutritional value rich in highly bioavailable proteins, vitamins (B complex), essential amino acids and microelements (zinc, iron) (*Williams*, 2007; *McAfee et al.*, 2010; *Bohrer*, 2017). There is a relatively small percentage of people (2–10%) who choose not to consume meat, mainly in developed nations (*Corrin & Papadopoulos,* 2017). However, this percentage is still significant on a global level having in mind food markets and vegetarian and/or vegan diets. It is important to have the evidence-based data on nutritional content and bioavailability of diets based on meat versus vegetarian/vegan-based diets so that food choices from the public health level can be better evaluated. A number of scientific papers and reviews addressed the nutritional content of meat, e.g. red meat, poultry and seafood (*Pereira & Vicente*, 2013; *Williams*, 2007; *Wood et al.*, 2008; *Sikorski*, 2012) and non-meat products rich in proteins, e.g. crops, legumes (*Multari et al*., 2016). Most non-meat foods contain only 20–60% of the protein density of meat, and consideration needs to be made when replacing meat in the diet with non-meat foods. Additionally, when protein cost was evaluated, meat and non-meat foods had a similar cost when expressed as grams of protein/$US (*Bohrer*, 2017). The total amount of zinc and iron was similar in meat and some non-meat foods. Lastly, meat-based diet is also associated with a higher digestibility and availability of nutrients. For example, the digestibility index of meat (all animal flesh) is the highest: 1 (100%); followed by cooked beans 0.94, milk 0.93, cooked rice 0.92, eggs 0.91, wheat 0.85, boiled soybean 0.80, corn 0.66, baked potato 0.52 (*Ciuris et al.*, 2019).

Global meat production. Global meat production is projected to be 16% higher in 2025 than in the period up to 2015 (*OECD/FAO*, 2016). The major reason for this total increase of meat production is attributed to developing countries due to development of their economy and the purchasing power of consumers who demand meat as a protein-rich product. Poultry meat is the primary driver of the growth in total meat production in response to expanding

[1]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia.*

***Corresponding author:** Ivan Nastasijević, *ivan.nastasijevic@inmes.rs*

global demand for this more affordable animal protein compared to red meats (*OECD/FAO*, 2016). The main reasons that contribute to making poultry a meat of choice are low production costs and low product prices, as well as its multi-confessional dimension (poultry meat is equally accepted and consumed throughout the world by adherents of all major religions — Christians, Muslims, Buddhists, etc.). In the bovine meat sector, cow herd liquidation occurred in major producing regions which led to a decrease of beef production in 2015 (*OECD/FAO*, 2016). Beef production stabilised and increased from 2016 and onwards with higher carcass weight, thus neutralising the decline in cattle slaughter. Pig meat production increased from 2016, mainly driven by China, where herd size stabilised for a while after years of substantial reductions (i.e. a drop of 25 million pigs between 2012 and 2015). After a short period of the consolidation of the pork sector, a decrease in pig meat production on a global scale has been recorded from August 2018 due to the outbreak of African Swine Fever (ASF) in east Asia which predominantly affected Chinese pig meat production where several million pigs were culled in efforts to slow down and stop the spread of disease; estimations are that around 30% of the Chinese pig population (150–200 million pigs) has been infected by ASF by mid-2019 (*Mason-D`Croz et al.*, 2020). The sheep meat sector recorded growth of 2.1% per annum in the previous decade due to increased production in China, Pakistan, Sudan and Australia (OECD/FAO, 2016).

Global meat trade. World meat output comprising bovine, pig, poultry and ovine meat was estimated at 330 million tonnes in 2017, which was a 1% increase from the previous year (*FAO*, 2018). Considering the main meat producing countries, total meat output increased in Argentina (+4.8%), Russian Federation (+4%), Mexico (+3.5%), United States (+2.8%), India (+2.7%), Brazil (+2.1%); stagnation was recorded in the EU and China; meat output declined in South Africa (−2.5%). Poultry meat output was the most widely produced meat, reaching 120.5 million tonnes in 2017 (up 1.1% from 2016), which is around 36% of the total meat output on a global scale. This was followed by pig meat (118.7 million tonnes, +0.7%), which was around 35.9% of the global meat output; bovine meat (70.8 million tonnes, +1.5%), which comprised around 21.5% of the global meat production; and ovine meat (14.9 million tonnes, +1.3%), representing 4.5% of the total meat output volume on a global scale (*FAO,* 2018). World meat exports reached 32.7 million tonnes in 2017 (2.7% higher than in 2016). The highest increases in export were recorded in Turkey (+36.3%), Argentina (+22%), Thailand (+8.8%) and the United States (+5.6%). Declines in meat exports occurred in Chile (−9.5%), South Africa (−8.3%) and the EU (−3.4%). On the other hand meat imports increased in Angola (+25.3%), the Russian Federation (10.4%), Japan (+9.4%) and Viet Nam (+7.7%), while imports declined in Saudi Arabia (−11%), China (−6.3%), the EU (−4.2%) and Canada (−1.8%). In general, in 2017 the total meat trade output increased for bovine, poultry and ovine meat, while pig meat trade declined. With such development, poultry meat has become the most widely produced and internationally traded meat type worldwide (*FAO*, 2018).

Global meat safety issues. Meat safety is always at the forefront of public health and social-economic concerns (*Sofos*, 2008). Major meat safety challenges are associated with hazards that can be considered as a traditional, new or emerging, which can involve increased virulence and/or low infectious dose and with resistance to antibiotics or resistance to other food related stresses (*Sofos*, 2008). These hazards enter the meat chain in multiple points along the farm — abattoir — meat processing — distribution — retail — consumer continuum. Traditional microbiological/parasitic hazards are *Trichinella* spp., *Brucella* spp., *Mycobacterium bovis*, *Bacillus anthracis* and *Taenia solium/bovis* (cysticercosis). Emerging hazards are bacterial pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and non-O157, e.g. `big six`: O26, O45, O103, O111, O121, O145 (USDA FSIS, 2011) or O26, O103, O145, O111, O145 (EFSA, 2020), *Salmonella, e.g.* `big five`: *S. Typhimurium*, *S. Enteritidis*, *S. Infantis*, *S. Virchow*, *S. Hadar* (EFSA/ECDC, 2019), *Campylobacter jejuni, Yersinia enterocolitica* and *Toxoplasma gondii*, which are major pathogens affecting safety of raw meat and poultry, while *Listeria monocytogenes* remains a concern in ready-to-eat (RTE) processed meat products (*Sofos,* 2008). Chemical hazards are related to environmental contaminants which enter meat chain (mycotoxins, heavy metals, PCBs), veterinary drugs (antibiotics, sulphonamides), hormones and food additives (nitrites, polyphosphates). Other challenges include the need for development of rapid testing and pathogen detection methodologies with sufficient sensitivity and specificity, traceability systems (blockchain technology), agreement and allocation of responsibilities between veterinary and public health authorities regarding monitoring and surveillance systems for zoonotic diseases (including food

borne), establishment of government policy regarding maximum allowed contamination level-appropriate level of protection (MACL-ALOP) for food which should reach the consumer (*Nastasijevic et al.*, 2020), as well as establishment of risk-based food safety objectives in meat production/processing, together with complete and routine implementation of risk-based food safety management system, hazard analysis and critical control points (HACCP).

Integrated approach in the meat chain (farm-to-abattoir continuum/farm-to-chilled carcass). Significant changes, backed up with the technological development in modern food animal farming and meat production systems has led to a significant change in the public health threats that originate from meat in developed countries. Classical zoonoses, such as tuberculosis, trichinellosis, cysticercosis or anthrax infection became much less important (*Uzal et al.*, 2002; *Buncic et al.*, 2019), while bacterial agents carried and excreted (primarily via faeces) by food animals without symptoms or originated from environment, such as *Campylobacter*, *Salmonella*, STEC, *Listeria monocytogenes* and *Yersinia enterocolitica* have become the most relevant (Figure 1).

Materials and Methods

A literature review was performed by analysing published scientific papers and the major sources of information from scholarly databases such as Web of Science, EBSCO, PubMed Science Direct and Wiley. The scientific opinions and official web sites of inter-governmental organisations and agencies were also searched (EFSA, ECDC, FAO, WHO, OIE). This review identified relevant articles (research and review papers, technical reports by international organisations and databases), published in domains of meat inspection, zoonotic foodborne pathogens and meat safety assurance system, including the related public health impact. The selection criteria chosen to identify the relevant articles within the scope of this review and the objectives of this paper were as follows: 1) focus on the meat inspection protocols, traditional and novel approaches with well-established databases regarding meat safety assurance system; 2) focus on the potential for improvement of the current meat inspection and meat safety assurance system and the need for future research, and 3) novel and futuristic technologies in meat production. Search string included the following key words: meat, inspection, meat safety assurance system, zoonotic, food borne pathogens, public health, precision livestock farming, harmonised epidemiological indicators, food chain information, biosensors, automation, robotization, cultured meat, precision fermentation, 3D printing. However, some geographical restrictions were taken, by including selected countries with intensive experience and well-established, integrated meat safety assurance systems.

Biological meat-borne hazards

The main food (meat) borne hazards (mainly of bacterial origin) in the EU Member States (MS) in 2018 were, in decreasing order based on incidence, as follows*:* *Campylobacter*, *Salmonella*, STEC infections, *Yersinia*, *Listeria monocytogenes*, *Trichinella* spp. and *Toxoplasma gondii*.

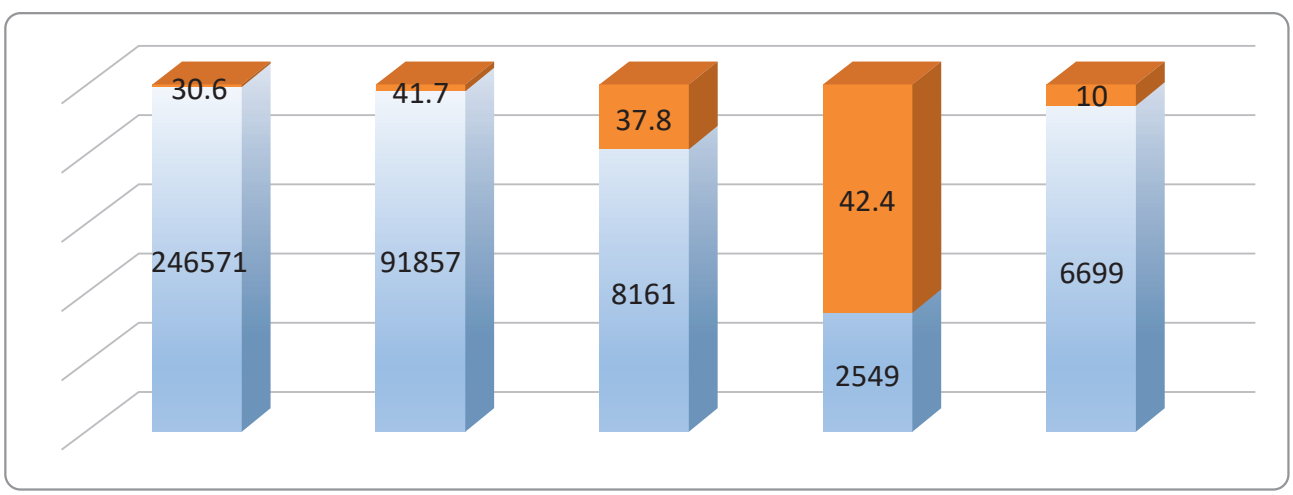

Figure 1. The relevance of the five major food borne pathogens in the meat chain in the EU, in 2018 (adapted from EFSA/ECDC, 2019)

Zoonotic food (meat) borne bacteria

Campylobacter. In the EU in 2018, there were 246,571 confirmed cases (64.1/100,000) of campylobacteriosis in MS, with 30.6% hospitalisation rate and 60 reported deaths (EFSA/ECDC, 2019). In the meat safety context, the most relevant are poultry meat and *Campylobacter jejuni* and *coli*. Overall, 37.5% of fresh broiler meat samples were positive in 2018 in the EU. *Campylobacter* was found in 34.6% of tested slaughter batches (neck skin from chilled broiler carcasses), 26% of tested broiler flocks and 71.6% of tested turkeys on farm. Strict implementation of biosecurity measures in primary production and GMP/HACCP during slaughter may reduce colonisation of broilers with *Campylobacter*, and contamination of carcasses (*EFSA/ECDC*, 2019; *Nastasijevic et al.*, 2020). In the abattoir, additional risk reduction can be achieved by using hot water/chemical decontamination or freezing of carcasses. At the consumer level, marination of poultry meat and adequate thermal processing can reduce the risk substantially (*Nastasijevic et al*., 2020). Campylobacteriosis is also associated with seasonality, with sharp increases during summer and early autumn. Recently, a new microbiological criterion was introduced in the EU to reduce the number of food borne outbreaks and improve public health; the criterion is for process hygiene at broiler slaughter, defining the maximum number of *Campylobacter* as 1,000 cfu/g in/on neck skin of chilled broiler carcasses (*EU*, 2017a). It is estimated that *Campylobacter* could be reduced by > 50% if no batches exceed this critical limit.

Salmonella. In the EU in 2018, 91,857 confirmed cases of food borne salmonellosis were reported in MS. Salmonellosis thus remained the second most commonly reported gastrointestinal infection with an incidence of 20.1/100,000, 41.7% hospitalisation rate and 119 reported deaths. Most *Salmonella* outbreaks were associated with *S.* Enteritidis and most outbreaks were linked with poultry meat intended to be cooked before consumption (*EFSA/ECDC*, 2019). *S.* Infantis was the most predominant serovar isolated in broilers (36.5%) and broiler meat (56.7%). On farm level, the most predominant food animals associated with *Salmonella* presence (in decreasing order) were fowl, pigs, turkeys, bovine and ducks and geese.

Monitoring of *Salmonella* is conducted during preharvest (feed, farm animals), harvest (abattoirs, cutting plants) and postharvest (retail, catering) (EU, 2003). Regulatory limits for food are set up in Regulation (EC) 2073/2005, defining process hygiene criteria (PHC) and food safety criteria (FSC); compliance with these criteria must be verified by the food business operator based on their self-monitoring plan. The reporting of food borne salmonellosis disease outbreaks in humans is mandatory according to the Zoonoses Directive (EU, 2003a). In the meat safety context, the most relevant are poultry meat (*S.* Enteritidis*, S.* Typhimurium *and S.* Infantis) and pork meat (*S.* Typhimurium).

Strict implementation of biosecurity measures in primary production and GMP/HACCP during slaughter can prevent/reduce colonisation of broilers with *Salmonella* and contamination of carcasses. In the abattoir, additional risk reduction can be achieved by using hot water/chemical decontamination. At the consumer level, marination of poultry meat and adequate thermal processing can reduce the risk substantially (Murphy et al., 2002). Salmonellosis is also associated with seasonality, with a sharp increase during summer months.

The EU MS are obliged to set up *Salmonella* National Control Programmes (NCP) in poultry with the aim to reduce the prevalence of serovars of major importance for public health, e.g. *S.* Enteritidis*, S.* Typhimurium*, S.* Infantis*, S.* Virchow*,* and *S.* Hadar (*EU*, 2003).

STEC infections. In the EU in 2018, 8,161 confirmed cases of Shiga toxin-producing *E. Coli* (STEC) infections in humans were reported in MS. The incidence rate was 2.28/100,000, with 37.8% hospitalisation rate (411 Haemolytic Uremic Syndrome — HUS cases) and 11 reported deaths. A total of 48 food borne outbreaks were recorded and the major food sources were cheese, milk, bovine meat, vegetables and juices. In the meat safety context, bovine meat is considered as a major source of STEC-food borne infections (4% of bovine meat was STEC-positive in retail, 5.6% in the processing plant and 2.4% in the abattoir), followed by ovine meat (10.9% being STEC positive) and pork meat (4.8% STEC positive). Most STEC infections were associated with serogroup O157 due to this being the predominant testing method, while many others were linked with non-O157 serogroups. STEC serotypes associated with food borne outbreaks usually possessed distinctive virulence factors, e.g. *Stx*+ (shiga toxin) and *eae*+ (intimin, adherence factor for intestinal mucosa). In the EU, six major STEC serogroups of public health importance are recognised (O157, O26, O111, O103, O145, O104:H4). However, the only regulatory requirement is the food safety criterion for sprouts (sprouted seeds) at the retail level (EU, 2005; EU, 2013). In the US, the Food Safety and

Inspection Services of the U.S. Department of Agriculture (USDA FSIS, 2012) declared six non-O157 Shiga toxin-producing *Escherichia coli* (STEC) O groups (O26, O45, O103, O111, O121, and O145) to be adulterants in meat. These top six STEC O groups were associated with 75% to 80% of human infections (USDA FSIS, 2012). STEC infections also showed a seasonal trend and were associated with a sharp increase during summer months.

Listeria monocytogenes. In the EU, 2549 cases of food borne listeriosis were reported in 2018, with notification rate of 0.47/100,000 population. The highest number of reported cases was reported in Germany, Spain and France (684, 372 and 338, respectively), due to improved surveillance, while the lowest number was reported in Cyprus, Malta and Croatia (1, 1 and 4, respectively). During a four year period, a seasonal pattern was observed with high summer peaks and lower winter occurrence. The hospitalisation rate of all reported cases was 42.4%, with 229 reported deaths. This implies that although the notification rate and number of reported cases of listeriosis is lower then campylobacteriosis and salmonellosis, high hospitalisation and mortality rate mean *L. monocytogenes* is a pathogen which should be carefully monitored in the food chain, in particular in chains involving the age group over 64 years which is the vulnerable group of consumers and other vulnerable groups, e.g. pregnant women, immunocompromised persons and individuals with chronic diseases. Although the food vehicles causing listeriosis with strong evidence (in decreasing order) were category 'vegetables and juices' 'mixed food' 'fish and fish products', 'vegetables and juices` and `crustaceans, shellfish, molluscs`, an important portion of food borne listeriosis is also attributed to the consumption of ready-to-eat (RTE) meat products (*Lakicevic & Nastasijevic*, 2017; *EFSA/ECDC*, 2019). This is mainly related to fermented meat products with probable sources of infection being the raw material (meat used for manufacturing of fermented meat products). Therefore, understanding the presence and colonisation of this pathogen, and source tracking it in the meat production environment is of utmost importance for control and prevention of meat-borne listeriosis. An effective and potent food safety management tool for tracking of *L. monocytogenes* is whole genome sequencing (WGS), enabling the specific detection of *L. monocytogenes* strains in production environment and their tracking throughout the production lines (Nastasijevic et al., 2017). In addition, synergistic application of Good Agriculture Practice and Good Farming Practice at the farm level, along with Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP) and HACCP in abattoir and retail/catering are important for effectively controlling this pathogen (*Lakicevic and Nastasijevic*, 2017).

Yersinia. Yersiniosis was the fourth most commonly reported zoonosis in the EU MS during 2018, with 6,699 confirmed cases. The incidence rate was 1.6/100,000 with 27 hospitalisations and 1 reported death (*EFSA*, 2018; *ECDC*, 2019). *Yersinia enterocolitica* was the most relevant species for human infection. The main sources of *Yersinia* were bovine meat, pork meat and RTE meat products — 30.0%, 5.0% and 5.9%, respectively. On farm, the proportion of pigs with *Yersinia* was 0.4% and that of other domestic livestock (bovine, sheep, goats, farmed rabbits, farmed reindeers, etc.) was 1.7%. In the meat safety context, pork meat and meat products had the highest importance, having in mind that 26.7% of the total of 15 outbreaks in 2018 were linked to consumption of pig meat (*EFSA*, 2018; *ECDC*, 2019).

Zoonotic meat borne parasites

Trichinella spp. In 2018, 66 confirmed cases were reported in the EU (*EFSA/ECDC*, 2019). The incidence rate was 0.1/100,000, and that was the lowest rate ever recorded since the introduction of surveillance. The highest notification rate was recorded in Bulgaria followed by Romania. In 2018, 114 reported cases of food borne trichinellosis were reported with pig meat as the predominant source. A low prevalence of *Trichinella* was also confirmed in the EU in hunted wild boar (0.13%), in the period from 2014–2018 (*EFSA/ECDC*, 2019). The EU legislation requires testing of all *Trichinella*-susceptible animals intended for human consumption (*EU*, 2015), i.e. domestic pigs (fattening and breeding animals), farmed wild boar and solipeds.

Toxoplasma gondii. No food borne toxoplasmosis was recorded in the EU during 2018 (*EFSA/ECDC*, 2019). In addition, no single food borne outbreak has ever been reported to EFSA since the start of data collection in 2004. However, 194 confirmed cases of congenital toxoplasmosis were reported, with 78.9% of all registered cases in France. The highest prevalence of *Toxoplasma* infections in food animals were reported in cattle (27.8%) and in small ruminants (sheep and goats; 18.3%). Different diagnostic methods contributed to the bias in interpreting results from testing. Mainly blood samples and sometimes tissues and organs are tested with direct

methods — PCR or immunohistochemistry or indirect methods — ELISA, immunofluorescence assay, or complement fixation test, to detect antibodies (*EFSA/ECDC*, 2019). Results from different MS are not comparable due to differences in sampling strategy, sampling schemes and testing methods. Age of animals and production systems at farm level can influence the occurrence of *Toxoplasma* (*EFSA/ECDC,* 2019).

Zoonotic meat borne viruses

Among the foodborne viruses most important for public health, comprising Norovirus (NoV), Hepatitis A virus (HAV) and Hepatitis E virus (HEV), only HEV has also been identified as a zoonosis (*Koopmans*, 2012; *EFSA*, 2017; *O'Shea et al.*, 2019). It is associated primarily with pigs. In the EU, over the last 10 years more than 21,000 acute clinical cases with 28 fatalities have been notified with an overall 10-fold increase in reported HEV cases; the majority (80%) of cases were reported from France, Germany and the UK. However, as infection in humans is not notifiable in all MS, surveillance and number of reported cases differs between countries (*EFSA*, 2017).

The diagnosis of HEV infections in humans is not routinely conducted in most laboratories, and therefore, it is considerably under-diagnosed (*De Keukeleire & Reynders*, 2015). However, since HEV-associated cases have become more frequent in recent years, novel and improved diagnostic tools and screening strategies have been developed (*Abravanel et al.*, 2017). Main control options focus on prevention of HEV contamination. Also, high risk groups (underlying liver disease, immunocompromised, pregnant) should be advised against eating raw/undercooked meat and liver derived from wild boars and domestic pigs (*Buncic*, 2015). HEV is also considered as an occupational disease, with abattoir workers being the most frequently exposed.

Prions

Bovine spongiform encephalopathy (BSE) is a disease in cattle. It belongs to a group of fatal neurodegenerative diseases affecting humans and animals called transmissible spongiform encephalopathies (TSEs) (*Fernández-Borges et al.*, 2017; *Leemans*, 2019). They are caused by the abnormal form of a cell protein called prion protein (PrP). Since the discovery of BSE in cattle, only two cases have been confirmed in species other than cattle: one goat in France and one goat in the UK (*EFSA*, 2018). To date, among TSEs in animals (BSE, Classical scrapie, atypical scrapie, chronic wasting disease (CWD) and transmissible mink encephalopathy (TME)), only the classical BSE agent has been evidenced to cause TSE in humans (*EFSA*, 2018). BSE has three different presentations: classical BSE, H-type atypical BSE and L-type atypical BSE (*Ubagai et al*., 2020). Classical BSE is the only form that can be transmitted to humans through the consumption of contaminated meat, causing variant Creutzfeldt-Jakob disease (vCJD), which was first diagnosed in 1996. Although there is no epidemiological evidence that classical scrapie is zoonotic, the zoonotic potential of atypical scrapie agent needs further investigation (*Goldmann*, 2018). Nevertheless, transmission studies of human PrP in transgenic mice or primates suggest that some TSE agents other than the classical BSE agent in cattle (namely L-type atypical BSE, classical BSE in sheep, TME, CWD agents) might have zoonotic potential; and studies even indicate that the potential of the L-type atypical BSE agent appears similar or even higher than that of the classical BSE agent (*Buncic*, 2015). With regards to present risk mitigation measures, the current policy of removing specified risk material (SRM) in slaughtered ruminants from the food chain enables around one logarithm reduction of the relative infectivity associated with the carcass of an infected animal. This policy, along with controls of ruminant feeds in respect to SRM, remains the main BSE/TSE control strategy (*Buncic*, 2015).

Chemical hazards in the meat chain

Chemicals can occur in the meat chain due either to their existence in the environment through unintentional contamination of food, or to their intentional use somewhere along the meat production chain (*Nova & González-Schnake*, 2014). Industrial pollutants are unintentional contaminants of foods, but can be difficult to control, in spite the existing regulations. On the other hand, agricultural chemicals are deliberately applied to land or crops during production, so their use can be both regulated and controlled (*Meurillon et al.,* 2018). Some toxic chemical compounds can occur naturally in foods and in the environment (e.g. mycotoxins).

The rate of ingestion of chemical hazards by food animals can be either higher or lower than the rate of their excretion. In the former case, accumulation of chemicals occurs. In the latter case, animals have a 'decontaminating' effect from the public health perspective. Hazards that accumulate can be a greater public health risk than those which do

not accumulate, because if animals are exposed even only to low levels of accumulating hazards but over extended time, their tissues can finally contain levels that pose a risk to consumers (*EU*, 2017b). With chemical hazards that accumulate, older animals are a higher risk than younger animals due to prolonged time allowed for accumulation of contaminants in target tissues. In the EU, MS and third countries that export food of animal origin (meat and meat products) are obliged to implement national monitoring programme for residues in the food chain (EU, 2017b). The main chemical hazards are presented in Table 1.

Risk ranking and Harmonised Epidemiological Criteria

EFSA adopted scientific advice for the modernisation of meat inspection across the EU. Modern food producing animals and meat production systems went through significant changes over several previous decades due to technological and scientific development. The public health importance and attention gradually shifted from classical zoonoses (tuberculosis, brucellosis, trichinellosis, cysticercosis and anthrax) to zoonotic food borne pathogens (mainly) of bacterial origin, e.g. *Salmonella*, *Campylobacter*, STEC, *Listeria monocytogenes* (*Edwards et al.*, 1997; *Uzal et al.*, 2002; *Buncic et al.*, 2019). These, zoonotic meat borne hazards of bacterial origin cannot be detected by old-fashioned meat inspection (palpation, incision) and their presence on carcasses, due to cross-contamination during slaughter and dressing, can be only monitored through the control of process hygiene (self-control plan, as an integral component of Hazard Analysis Critical Control Point-HACCP system) based on carcass swabbing (mainly wet-dry, non-destructive method). Animals intended for slaughter can intermittently faecally shed zoonotic bacteria on farm, during transport, livestock markets and in the abattoir lairage. Cross-contamination can occur in all the

Table 1. Main groups of chemical hazards in the meat chain

Industrial pollutants	Agrochemicals	Growth promoters	Veterinary medicines	Natural substances	Food additives	Packaging compounds
Heavy metals Lead, Arsenic, Mercury, Cadmium, Copper, Fluorine, Selenium	*Insecticides* *Chlorinated hydrocarbons* Dihlor-difenil-trihloretan (DDT), Endrin, Aldrin/Dieldrin, β-Hexachlorocyclohexane (BHC) *Organophosphates* -- Coumaphos, Malathion, Diazinon *Herbicides*	*Hormones and hormone-like substances* Synthetic hormones (DES), Natural hormones (Oestradiol, Progesterone, Testosterone), Fungal oestrogens (Zearalenone)	*Antibiotics* Penicillins, Aminoglycosides, Tetracyclines, Cephalosporins, Macrolides, Quinolones, Nitro compounds (Nitroimidazoles, Nitrofurans)	*Mycotoxins* Aflatoxins, Ochratoxins	*Curing agents* Nitrites, Polyphosphates, Sodium chloride	*Plastics* VC-monomers, Plasticisers
Halogenated hydrocarbons Polychlorinated biphenyls (PCBs), Polychlorinated naphthalenes (PCNs), Dioxins		*ß-agonists* (Trenbolone),	*Sulphonamides* (Sulphametazines)	*Algal toxins* Paralytic Shellfish Poison (PSP), ASP (Amnesic Shellfish Poison), DSP (Diarrhetic Shellfish Poison), AZP (Azaspiracid Shellfish Poison)	*Antioxidants* Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Gallates	*Pigments/Inks*
		Thyreostatics			*Preservatives* Sulphite, Benzoate, Sorbic acid	
					Smoke	

Table 2. Ranking of main biological and chemical hazards identified for each animal species (EFSA, 2011; 2012; 2013a; 2013b)

Species	Biological hazards				Chemical hazards
	High	Medium	Low	Undetermined	
Cattle	STEC *Salmonella enterica*	N/A**	*Campylobacter spp. (thermophilic)* *Yersinia enterocolitica/ pseudotuberculosis* *ESBL/AmpC E. coli* *Cysticercus (Taenia saginata)* *Mycobacterium bovis*	*Toxoplasma gondii* *Trichinella spp.*	Dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs)
Sheep and goats	STEC *Toxoplasma gondii*	N/A	*Campylobacter spp. (thermophilic)* *Salmonella enterica* *Yersinia enterocolitica/ pseudotuberculosis* *ESBL/AmpC E. coli*	*Trichinella spp.*	Dioxins, Dioxin-like polychlorinated biphenyls (DL-PCBs)
Porcines	*Salmonella enterica*	*Yersinia enterocolitica/ pseudotuberculosis* *Toxoplasma gondii* *Trichinella spp.*	*Campylobacter spp. (thermophilic)* STEC *ESBL/AmpC E. coli* Cysticercus (Taenia solium) *Mycobacterium avium (hominissuis)*	N/A	Dioxins, Dioxin-like polychlorinated biphenyls (DL-PCBs)
Solipeds	*Trichinella*	N/A	*Campylobacter spp. (thermophilic)* *Salmonella enterica* *Yersinia enterocolitica/ pseudotuberculosis* STEC *ESBL/AmpC E. coli*	*Toxoplasma gondii*	Phenylbutazone*, Chemical elements (*cadmium)*
Poultry (broilers)	*Campylobacter spp. (thermophilic)* *Salmonella enterica*	*ESBL/AmpC E. coli*	N/A	*E. coli (process hygiene)*	Dioxins, Dioxin-like polychlorinated biphenyls (DL-PCBs), chloramphenicol, nitrofurans, nitroimidazoles
Farmed game (deer)	*Toxoplasma gondii*	N/A	N/A	N/A	N/A
Farmed game (wild boar)	*Salmonella enterica* *Toxoplasma gondii*	N/A	N/A	N/A	N/A
Farmed game (reindeer, ostriches, rabbits)			N/A		N/A

Legend: *EFSA recommended that phenylbutazone, which is not allowed in the food chain, be specifically included in the National Residue Control Plans (NRCPs) for solipeds. **N/A — not applicable

aforementioned phases along the meat chain related to animal-animal and animal-environment contact.

Based on this, EFSA issued a scientific opinion to provide identification and ranking of the major meat borne hazards according to their risk for public health (*EFSA*, 2013a, Table 2).

Biological hazards. The priority ranking was based on assessment of their impact according to incidence of disease, the severity of the disease in humans and source attribution (evidence that consumption of meat from the various species is an important risk factor for the disease).

Chemical hazards. Risk ranking of chemical hazards was based on the five-year outcomes of the National Residue Control Plans for 2005–2010 and other voluntary testing programs as well as substance-specific criteria, such as the chemical's toxicological profile.

EFSA also proposed harmonised epidemiological indicators (HEI). The indicators will be useful in the context of the proposed integrated meat safety assurance system, enabling the categorisation of farms, flocks or herds and abattoirs according to potential risk and the setting of microbiological targets for carcasses.

An epidemiological indicator is defined as "the prevalence or the concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates with the human health risk caused by the hazard" (*EFSA*, 2013b, Figure 2). The indicators can be used to consider improvement and modernisation of meat inspection methods and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the bovine/pig/poultry carcass meat safety assurance system to help categorise farms/herds and abattoirs according to the risk related to the hazards, as well as setting appropriate specific hazard-based targets in/on bovine/pig carcases and, when appropriate, in bovine/pig farms and herds. Risk managers should decide on the most appropriate indicator(s) to use, either alone or in combinations, at national, regional, abattoir or farm/herd level, depending on the purpose and the epidemiological situation. It is recommended that risk managers should define the harmonised requirements for the controlled housing

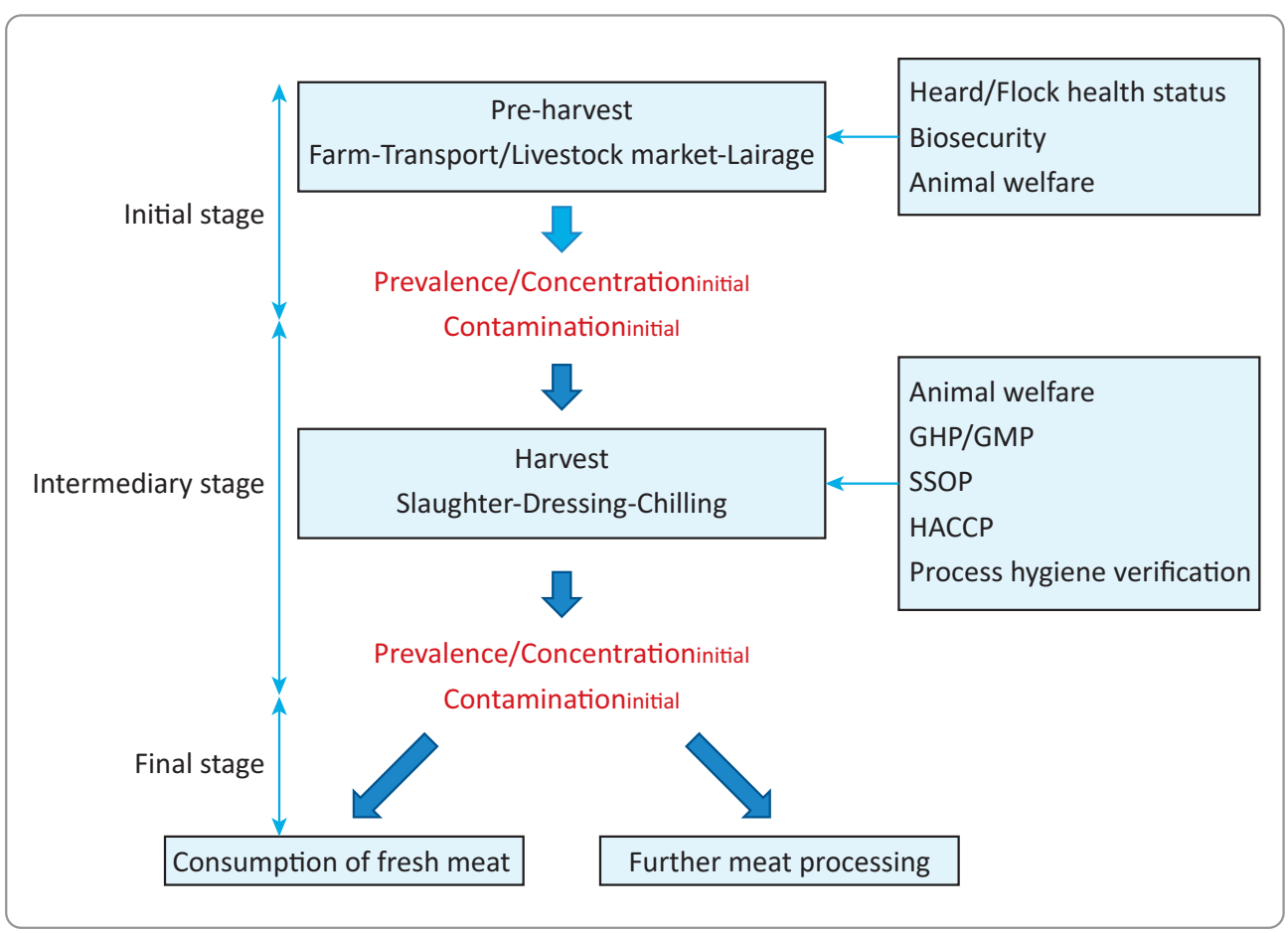

Figure 2. A model to set up harmonised epidemiological indicators for a meat safety assurance system based on the prevalence and/or level of the hazard in the farm-chilled carcass continuum

conditions of farms. In the EU, MS should plan to organise training regarding the implementation of the indicators and the reporting of data generated by the implementation of Directive 2003/99/EC (*EU*, 2003).

Risk based meat safety assurance system

In 2005, Codex Alimentarius Commission issued a Code of Hygienic Practice for Meat (*CAC*, 2005) and recommended integrated and risk-based approach to achieving meat safety. In this document, it is suggested that "hygiene measures should be applied at those points in the meat chain where they will be of greatest value in reducing food borne risks to consumers"; a greater emphasis on prevention and control of contamination during all aspects of producing and processing meat should be applied. Levels of hazard control in meat chain should correspond with required levels of consumer protection. In continuation with such approach, EFSA recently proposed a framework for a novel, flexible and dynamic risk-based meat safety assurance system (*EFSA*, 2011; 2012; 2013a; 2013b). The introduction and implementation of such a system is expected to be a slow and careful process, and it would evolve over time after collecting initial experience, fine tuning and verifying in practice. The modern risk-based meat inspection should be based on food chain information (FCI) from farm to abattoir (bottom-up) and vice versa (top-down), as well as HEI related to major meat borne pathogens and chemical contaminants. Risk managers will have the possibility to operate within the meat safety assurance system, taking into consideration FCI and HEI and making decisions based on the situation related to the level/type of meat inspection that should be applied, e.g. classical ante-mortem and post-mortem inspection (including palpation and incision) or visual-only inspection based on ante-mortem and post-mortem observation of the animal intended for slaughter. Visual-only inspection will be enabled when animals are sourced from farms with high levels of biosecurity and where animal health status and animal welfare are maintained at high levels (e.g. pathogen-free farms). Successful implementation of risk-based meat inspection should be carried out within the meat safety assurance system comprising several systems/elements/criteria in the farm-to-abattoir continuum (e.g. precision livestock farming, FCI, HEI, food safety management in abattoir, meat inspection — classical and/or modern, risk-based).

Precision livestock farming (PLF)

PLF applies principles of control engineering using electronic information transfer, e.g. from biosensors to optimise animal health, production and management processes on farm. PLF is a multidisciplinary science that requires close and effective collaboration among animal scientists, physiologists, veterinarians, ethologists, engineers, and information and communication technology (ICT) experts (*Berckmans*, 2017).

Since the global farm animal population will increase by 70% by 2050, a major problem in the next decades will be to ensure continuous monitoring of animal health within big groups of animals (*Berckmans*, 2017). Farms will hold more animals due to increasing numbers of animals and decreasing numbers of farmers. It is predicted that in the future a single farm (animal city) could have 25,000 milking cows, 200,000 fattening pigs or a few million broilers. Infections in such large conglomerations of food animals could have disastrous consequences, in particular when reduced antibiotic use is a priority due to prevent antimicrobial resistance (AMR). The alternative strategy could be development of vaccines, but this is time-consuming and their efficacy in big herds must be closely monitored to evaluate effectiveness (*Berckmans*, 2017). Therefore, potential for infections in these animal cities will be high and also related to the spread of zoonotic food borne agents to consumers via food, including meat. PLF supports intelligent management of animal health including rapid alert systems to meet growing human demand for animal proteins, while guaranteeing animal health and welfare, the future sustainability of animal farming, and improved food safety (*Berckmans*, 2017).

The main purpose of PLF is to obtain real-time, valid information regarding both (i) animal health (e.g. production diseases) and associated economic gains or losses, and (ii) food (meat) safety (e.g. zoonotic food borne pathogens — *Salmonella*, STEC, *Campylobacter*, *Yersinia*) and associated consumer health issues affecting public health. Therefore, PLF is currently considered as a state-of-the-art engineering endeavour towards sustainability in (primary) food production improving, consequently, consumers' health through more effective public health protection (*Nastasijevic et al.*, 2017). Another big issue is the environmental impact of the livestock sector. It is estimated that more than 90% of the NH_3, 37% of CH_4 and 65% of N_2O in the atmosphere comes from the livestock sector (FAO, 2013).

PLF offers a real-time monitoring and managing system for farmers, as follows: (i) a real-time warning is issued when something goes wrong so immediate action can be taken by the farmer to solve the problem, (ii) problems during animal rearing are detected, allowing immediate management action. Therefore, PLF is a powerful tool for measuring animal variables (good health, welfare, behavioural changes, good productive performance, good reproductive performance), modelling the acquired data to select information, and using these models in real time for monitoring and control purposes (*Berckmans*, 2017).

The main objectives of PLF are to manage individual animals by continuous real-time monitoring (24/7) regarding animal health, welfare, production, reproduction, environmental impact and food safety outcomes. PLF monitoring tools are:

(i) camera/real-time image analyses
(ii) microphone and real-time sound analyses
(iii) sensors around or on the animal (temperature detection)
(iv) biosensors (microfluidic) used for rapid tests (stress hormones, acute phase proteins, pathogen presence).

The point of these systems is to detect less-than-ideal conditions and provide an initial response regarding animals' behavioural changes. The first signs of problems picked up by the PLF sensing technology can be based on image analysis, sound analysis and sensors on the body (*Berckmans*, 2017).

A living organism is much more complex than any mechanical, electronic, or ICT system and is considered as a complex, individually different, time-varying, dynamic (**CITD**) system. Each living organism is individually different in responses to environmental stimuli or stressors (*Berckmans & Aerts*, 2016; *Quanten et al.*, 2006).

A good example of practical implementation of PLF image-based sensing systems is the early warning system for broiler houses, eYeNamic, to monitor general problems in broiler houses (> 30,000 animals), where it is very hard to observe such a high number of birds (Figure 3). The system is based on three or four cameras mounted on the ceiling of the house so that distribution of the birds can be monitored and the broilers' behaviour analysed in real time.

Another example of a sound-based PLF sensing system is monitoring animal health status on cattle farms via detection of calf cough episodes (*Berckmans*, 2017; *Carpentier et al*, 2018).

PLF can be also used to monitor behaviour of animals (ovines, cattle) in pasture during grazing by using the animal-borne accelerator, which has 24/7 monitoring capability. This PLF sensing system can detect animal movements during grazing, standing, walking and lying (*Barwick et al.*, 2020). Examples of PLF applications on pig farms include not only traditional environmental indicators (temperature, humidity, CO_2), but also direct measures of animal responses such as feed intake sensors, growth monitors,

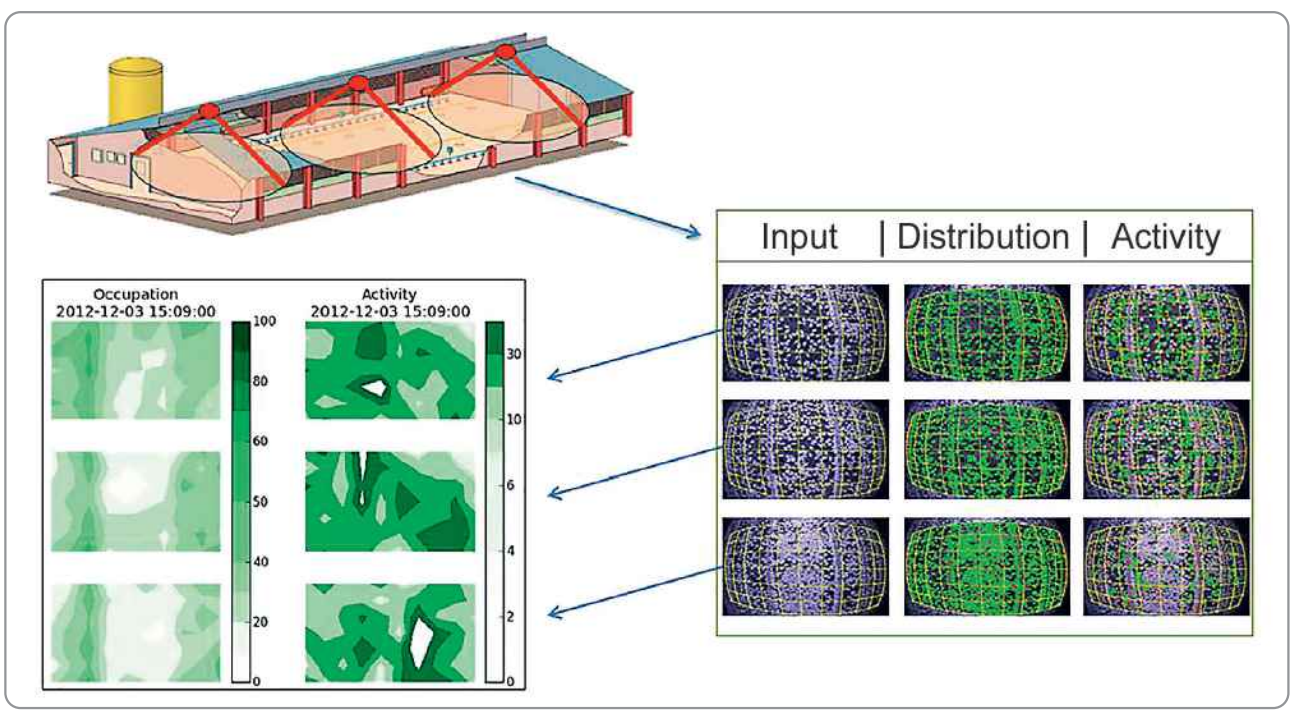

Figure 3. eYeNamic system — poultry farm (three top-view cameras) (adapted from *Berckmans*, 2017)

behaviour (cameras) and sound (microphones). The PLF concept is still rather new in the EU pig industry, and the number of farmers and companies engaged in pig farming businesses that are using PLF technology is increasing. A commercially available PLF sensing technology is associated with pig cough monitors, automatic weighing devices and camera systems. Furthermore, the business intelligence software is still under development and requires continuous improvements. The EU Commission recently supported a big project related to application of PLF in commercial farms in Europe, i.e. EU-PLF project (2012–2016). A database was created based on 20 fattening periods. Early warning tools for farmers were developed. In addition, automated welfare assessment based on electronic sensor output has been developed (*Vranken & Berckm*ans, 2017).

The application of PLF allows optimal use of knowledge and information in the monitoring and control of processes on farm. In addition, such an approach allows extension to the further step in the meat chain, helping to define the most effective control measures and risk mitigation strategies at the abattoir level. Therefore, PLF can be used strategically to support FCI flow in the farm-to-chilled carcass continuum and to facilitate decision-making by the risk managers, e.g. official veterinarian and/or authorised auxiliary appointed by the food business operator in terms of the scope and type of the ante-mortem and post-mortem inspection. Overall, PLF can serve effectively in supporting a risk-based meat safety assurance system (*Nastasijevic et al*., 2017).

Food Chain Information

Modern meat inspection should incorporate a more risk-based approach for protecting public health against food (meat) borne biological hazards than has been the case to date. Meat inspection should fulfil four major objectives: human health, animal health, animal welfare (ante-mortem inspection) and meat safety (post-mortem inspection) (*Felin et al.*, 2016; *EU*, 2019). Therefore, a comprehensive and integrated pork/beef/poultry carcass safety assurance system in the farm-abattoir continuum should be developed to ensure the effective control of major meatborne public health hazards, "with the primary production stage playing an essential role in managing these risks" (EFSA, 2011).

FCI should include data on the prevalence/concentration of major food borne hazards of public health importance at farm, transport and lairage, and abattoir (HEI). These data should be result from targeted sampling (pooled faeces on farm or carcass swabs at abattoir), microbiological detection (and serotyping) and auditing (animal welfare and biosecurity on farm; and GHP/HACCP at abattoir).

For example, in the EU, there is the intention to shift to visual-only post-mortem inspection of pigs. The official veterinarian (OV) (risk manager) decides on additional post-mortem inspection procedures, such as incisions and palpations, based on declarations in the food chain information (FCI) and ante-mortem inspection. However, it is of essential importance that the OV should be able to assess prior to slaughter which pigs are to be subjected for visual-only meat inspection and which need additional inspection procedures (*Felin et al*., 2016). The decision can be based on one or any combination of the FCI, ante-mortem inspection (including verification of animal welfare), post-mortem inspection or any other data regarding the animal that might, in the OV's opinion, indicate a possible risk to public health, animal health or animal welfare.

Meaningful FCI and collection & communication of inspection results (FCI/CCIR) interpreted and advised by the veterinarians can be a vehicle for positive change as a part of the modernisation of meat inspection (*FVE*, 2015). The most effective approach to control the main hazards in the context of meat inspection is an integrated meat safety assurance system for all animals, combining a range of available preventive and control measures applied in the farm-abattoir continuum.

Harmonised Epidemiological Indicators

For the most relevant foodborne biological hazards, EFSA has also proposed HEIs. The indicators will be useful in the context of the proposed comprehensive meat safety assurance system and risk based meat inspection, enabling the categorisation of farms, flocks or herds and abattoirs according to potential risk and the setting of microbiological targets for carcasses. The improvements to existing practices or alternative methods for meat inspection have been recommended, while the implications of the proposed changes to current practices for surveillance of animal health and welfare have been studied.

Bovine HEI. These indicators were defined to serve in developing a bovine carcass safety assurance system. By definition, an epidemiological indicator is defined as "the prevalence or the concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard (such as audits)

that correlates with the human health risk caused by the hazard" (*EFSA*, 2013b).

Indicators should help categorise farms/herds and abattoirs according to the risk related to the meat borne hazards of public health importance in the bovine meat chain, and be the basis of appropriate specific hazard-based targets in/on bovine carcases and in bovine farms/herds. These hazards are as follows: *Salmonella*, human pathogenic STEC, cysticercus (*Taenia saginata*) and *Mycobacterium bovis*; the last two are already covered by the current, traditional meat inspection process (*EFSA*, 2013b). The indicators can be applied at national, regional, abattoir and/or farm/herd level, depending on the purpose and the epidemiological situation of the country. Furthermore, the indicators can be used alone or in combination. For *Salmonella* and STEC, the proposed HEI include microbiology-based indicators, which will give specific information on *Salmonella* and STEC infection or contamination in the animal (on farm), hide or carcass (in abattoir). HEI based on audits at farm or transport conditions and visual inspection of bovine hide are also proposed, which will give a more general assessment of microbiological risk and, when used in combination with microbiological HEI, will support assessment and knowledge of the *Salmonella*/STEC risk. Lastly, the proposed indicators for *Salmonella*, STEC, cysticercus (*Taenia saginata*) and *Mycobacterium bovis* can also be applied to classify countries, regions, farms, abattoirs, slaughter batches and animals according to the infection status or risks related to the hazard. This approach will enable the comparability of data between the EU MS, as well as internationally (*EFSA*, 2013b).

For example, eight HEI were recommended for pathogenic STEC in bovine meat, within a bovine carcass safety assurance system in the farm-abattoir continuum (Table 3).

Pig HEI. The proposed HEI for pig meat, in the farm-abattoir continuum, encompasses the major meat borne hazards of public health importance, as follows: *Salmonella*, *Yersinia enterocolitica*, *Toxoplasma gondii*, *Trichinella*, Cysticercus (*Taenia*

Table 3. Harmonised epidemiological indicators for human pathogenic STEC in the bovine carcass safety assurance system (adapted from *EFSA*, 2013b)

Indicator (animal/food category)	Meat chain phase	Analytical/diagnostic method	Sample
HEI 1: Practices which increase the risk of introducing pathogenic STEC into the farm (purchase policy, mixing with other herds, access to pasture, access to surface water)	Farm	Auditing	N/A*
HEI 2: On-farm practices and conditions	Farm	Auditing	N/A
HEI 3: Pathogenic STEC status of the group(s) of bovine animals containing animals to be slaughtered within one month	Farm	Microbiology	Pooled (composite) faeces or floor samples
HEI 4: Transport and lairage conditions	Transport and lairage	Auditing	N/A
HEI 5: Visual inspection of hide conditions of animals at lairage (clean animal scoring system)	Abattoir	Visual inspection	N/A
HEI 6: Pathogenic STEC on incoming animals (after bleeding and before dehiding)	Abattoir	Microbiology	Hide swabs
HEI 7: Pathogenic VTEC on carcases pre-chilling	Abattoir	Microbiology	Carcass swabs
HEI 8: Pathogenic VTEC on carcases post-chilling	Abattoir	Microbiology	Carcass swabs

Legend: *Not applicable

Table 4. Harmonised epidemiological indicators for *Salmonella* in the pig carcass safety assurance system (adapted from *EFSA*, 2011)

Indicator (animal/food category)	Meat chain phase	Analytical/diagnostic method	Sample
HEI 1: *Salmonella* in breeding pigs	Farm	Microbiology (detection and serotyping)	Pooled (composite) faeces sample
HEI 2: *Salmonella* in fattening pigs prior to slaughter	Farm	Microbiology (detection and serotyping)	Pooled (composite) faeces sample
HEI 3: Controlled housing conditionsat farm	Farm	Auditing	N/A
HEI 4: Transport and lairage conditions	Transport and lairage	Auditing of time, mixing of batches and reuse of pens in lairage	N/A
HEI 5: *Salmonella* in fattening pigs –evisceration stage	Abattoir	Microbiology (detection and serotyping)	Ileal content
HEI 6: *Salmonella* in fattening pigs – carcases after slaughter process before chilling	Abattoir	Microbiology (detection and serotyping)	Carcass swabs
HEI 7: *Salmonella* in fattening pigs – carcases after slaughter process and after chilling	Abattoir	Microbiology (detection and serotyping)	Carcass swabs

*Not applicable

solium) and *Mycobacterium Avium*, subsp. *hominissuis*. For example, seven HEI are proposed for *Salmonella* in the context of pig carcass safety assurance system (Table 4), as follows: HEI 1 (on farm; *Salmonella* in breeding pigs), HEI 2 (on farm; *Salmonella* in fattening pigs prior to slaughter), HEI 3 (on farm; controlled housing conditions), HEI 4 (transport and lairage conditions), HEI 5 (in abattoir; *Salmonella* in fattening pigs at evisceration; ileal content), HEI 6 (in abattoir; *Salmonella* on pig carcasses, after dressing/before chilling), HEI 7 (in abattoir; *Salmonella* on pig carcasses, after chilling).

Poultry HEI. The poultry carcass safety assurance system should be based on HEI with regard to the most relevant food borne hazards of public health importance. These hazards are as follows: *Campylobacter*, *Salmonella*, and bacteria carrying extended spectrum β-lactamase (ESBL)/AmpC genes (*ESBL/AmpC E. coli*). Four HEI are proposed for the poultry carcass safety assurance system as follows (Table 5): HEI 1 (on farm; parent flock; *Salmonella* & *ESBL/AmpC E. coli*), HEI 2 (on farm; production flock; *Salmonella, Campylobacter* & *ESBL/AmpC E. coli*), HEI 3 (abattoir; incoming batches; *Campylobacter* & *ESBL/AmpC E. coli*) and HEI 4 (abattoir; carcass after chilling; *Salmonella, Campylobacter* & *ESBL/AmpC E. coli*).

Sampling of poultry carcasses should be based on the available FCI, including results from feed controls. The frequency of sampling for farms should be adjusted accordingly. The poultry meat inspection should be based on FCI (*EFSA*, 2012). It means that poultry flocks intended for slaughter should be classified into food safety risk categories, so that slaughter procedures and/or decisions on fitness for consumption can be adapted to the health status and food safety risk presented by the flock/batch (*Nastasijevic et al*. 2020). The main responsibility for such a system should be allocated to the FBO, whereby compliance is to be verified by the competent authority (i.e. veterinary inspection). Defined microbiological targets should be defined in primary production (on farm; prevalence/concentration of hazards at flock level) and in slaughter (in abattoir; prevalence/concentration of hazards on carcass).

Therefore, the HEI for poultry carcass safety assurance system should be monitored and used to categorise poultry production flocks (e.g. broilers) into specific risk categories (higher risk flocks and lower risk flocks). Such categorisation should be an integral component of FCI. Risk manager (i.e. competent authority and/or official auxiliary, designated FBO staff and/or abattoir worker) should make decisions accordingly and direct the incoming

Table 5. Harmonised epidemiological indicators for the poultry carcass safety assurance system (adapted from *EFSA*, 2012)

Indicator (animal/food category)	Meat chain phase	Analytical/diagnostic method	Sample
HEI 1: *Salmonella* & *ESBL/AmpC E. coli* in parent flock	Farm	Microbiology (detection and serotyping)	Pooled (composite) faeces sample
HEI 2: *Salmonella, Campylobacter* & *ESBL/AmpC E. coli* in production flock	Farm	Microbiology (detection and serotyping)	Pooled (composite) faeces sample
HEI 3: *Campylobacter* & *ESBL/AmpC E. coli* in incoming batches intended for slaughter	Abattoir	Microbiology (detection)	Ileal content
HEI 4: *Salmonella, Campylobacter* & *ESBL/AmpC E. coli* in carcasses after chilling	Abattoir	Microbiology (detection and serotyping)	Neck skin samples or carcass swabs

batches to the higher risk lines (slaughter lines with high GHP level, high risk reduction capacity, including decontamination of carcasses; intended for higher risk flocks) and lower risk lines (slaughter lines with lower/regular GHP level, lower risk reduction capacity, based on regular HACCP implementation and verification, testing — PHC and auditing). In addition, HEI defined at abattoir level should be used for risk classification of the abattoirs; this categorisation can be used for risk management purposes as described above (e.g. diverting high risk poultry flocks to abattoirs with higher risk lines).

Modern meat inspection in the context of carcass safety assurance system

Farm holdings and the meat industry have undergone substantial changes over recent decades due to improvements to and development of biosecurity, animal welfare, animal health, and slaughter/dressing and meat-processing technology. Meat as a potential source of food borne disease outbreaks has been studied over this time in numerous scientific projects. It is known that in the EU and other developed regions, within the meat chain, the public health focus has now shifted from classical zoonoses (brucellosis, tuberculosis, trichinellosis, anthrax) to food (meat) borne pathogens (*Salmonella*, *Campylobacter*, STEC O157/non-O157, *Yersinia*, *Listeria monocytogenes*) that are, nowadays, the major source of human food borne illness. These zoonotic food borne pathogens are usually faecally excreted (shed) by clinically healthy animals and can contaminate animal hides, skins or feathers which, in turn, leads to cross-contamination of carcasses (bovine, pig, poultry) during slaughter and dressing procedures. The current, traditional meat inspection system (observation, palpation, incision) is not fully effective in detecting these zoonotic food borne pathogens of public health importance. The risk-based meat inspection system needs to be developed and implemented to increase the level of control of food borne pathogens important for public health and to help ensure meat safety.

The prevention/control of cross-contamination at abattoir can be achieved with strict implementation of GHP and a risk-based food safety management system, e.g. HACCP (*Nastasijevic et al*., 2016), which can also encompass interventions (e.g. carcass decontamination). Therefore, it is of utmost importance to ensure the carcass microbiological safety before the meat will be distributed for final consumption (fresh chilled or fresh frozen meat) or further processing (fermented or pasteurised meat products). Since the slaughter and dressing procedures are to be completed with the final chilling at the abattoir (also slowing and preventing the growth of pathogens), the adopted approach means meat safety should be achieved only within the farm-to-chilled carcass continuum or with a carcass safety assurance system.

The modern and risk-based meat inspection system should be, therefore, based on FCI supported with defined HEI at three phases in the meat chain: (i) farm, (ii) transport & lairage, and (iii) abattoir. FCI should encompass data from farm holdings — categorisation of farms (biosecurity, animal welfare, animal health), transport and lairage (animal welfare, slaughter logistics) and abattoir — categorisation of abattoirs (GHP/HACCP, risk-reduction capacity of slaughter line — high risk versus low risk slaughter line). The HEI should provide data on prevalence/

level of major food (meat) borne hazards (*Salmonella*, *Campylobacter*, STEC, *ESBL/AmpC E.* coli) at different phases along the meat chain (farm, transport & lairage, abattoir) and are integral part of FCI. The risk manager (OV, designated staff with FBO, supported by the Official Auxiliary and abattoir staff) should make risk-based decisions about the level of ante-mortem and post-mortem inspection, e.g. whether it will encompass detailed clinical (ante-mortem) examination and detailed post-mortem inspection (including palpation and incision) or the inspection will be visual-only (*EFSA*, 2011; 2012; 2013b). As suggested, whenever possible, the palpation and incision should be omitted since these practices may increase the cross-contamination of carcasses. So, the visual-only meat inspection provided within the carcass safety assurance system should be based on FCI. This means that when animals/flocks intended for slaughter are sourced from farm holdings with low risk (based on HEI), they can be subjected to visual-only inspection and still provide the defined level of meat safety assurance (*Buncic et al*., 2019).

Novelties

Substantial changes have occurred in the global meat industry over the past century due to development of technology. The changes encompass increased automation and robotization, production of alternative meat using precision fermentation technology, and 3-dimensional (3D) printing of meat. These novel approaches to meat harvest/production should decrease the labour-dependant process (which can be of critical importance in emergency situations and crises such as the COVID-19 pandemic), while also providing climate change resilience and environmental sustainability.

Automation and robotization

Automation and robotization have led to significant increases in slaughter line (conveyer) speed for beef, pork, sheep, poultry and fish operations and have begun to take over the meat processing business. The meat industry is changing slaughter methods from conventional manual handling to an automated and robot-driven process. For example, the fastest line currently observed in broiler slaughter line enables speed at 13,500/h (*Barbut*, 2014). The automated pig slaughter/dressing lines include separation of the pelvic bone, carcass opening, breastbone splitting and neck clipping; these automated lines are now used in many pig abattoirs and run with capacities varying from 300–1280 pigs per hour (*Anonymous*, 2018). The automation and robotization in beef slaughter has certain limitations regarding development of technology for the slaughter process; this has been quite limited partly due to the biological variation in animals and the cost/benefit of applying complex technology (*Madsen et al*., 2006). Most of the development was recorded in the area of manually operated tools which have been improved to ease the physical work for operators or tools developed for improving the hygienic quality of slaughter. For example, in the USA there is a development allowing a high line speed in beef slaughter of 300 head/per hour; it is achieved by dividing slaughter and dressing processes across more meat handlers (operators) and by ensuring the animals slaughtered are relatively homogenous in size (uniformity of carcass conformation), as slaughter lines are usually specialised for steers or heifers. Several pieces of equipment are duplicated and processes are divided across several machines, e.g. hide pulling. These plants run in several shifts (*Madsen et al.*, 2006).

However, cattle are mainly slaughtered at lower line speeds worldwide. In the EU, most of large-scale abattoirs run in single shifts at line speeds from 30–75 head/per hour and these plants are seldom specialised, which means they operate with all types of cattle (*Madsen et al.*, 2006). This implies the new, automated technology must be flexible and should match the large biological variation of carcass dimensions. In addition, beef slaughter is usually carried out at regional level due to animal welfare and zoonotic issues as well as geographical constraints. Since individual abattoirs are too small to undertake a large research and development (R & D) task, it seems necessary to join investments in technology development between interested parties at international level. This will reduce the risk, as there will be lower individual financial contribution and it will also ensure a better match with the EU market requirements. Joint R & D should prioritise projects that can have a reasonable payback time and provide advantages regarding hygiene and food safety (*Madsen et al.*, 2006). The recommended development in beef slaughter is related to: automatic cleaning of dirty hides (including belly) prior to slaughter; automatic bung cutting, neck and breast opening; automatic hide pulling (critical for reducing carcass cross-contamination with food borne pathogens); removal of head and tail; automatic separation (cutting) of hind and forequarter; automatic

splitting and removal of the spinal cord (SRM) in one process. Automated deboning is more complex and cost-benefit analysis should be carried out to justify such automation (*Madsen et al.*, 2006).

On the other hand, modern technologies are now common in red meat (pork) and poultry meat harvest (slaughter/dressing, chilling). Shorter time is allowed for deboning; robots are designed to cut meat and they are replacing traditional manual operations. However, this can also be a challenge regarding meat safety because high speed equipment is not always equipped to respond to frequent variations in carcass size/conformation and, therefore, requires development and installation of tailor-made sensors and IT control systems. Automation and robotization requires progress in breeding and genetics to provide greater carcass uniformity, which would help in operating automated equipment (*Barbut*, 2014). Some alternative approaches have been also recently suggested, like the meat factory cell (MFC) (*Alvseike et al*., 2018). The MFC concept is different from the conventional slaughter and dressing approach that uses the conveyer system with workers positions along the slaughter line at numerous operational stations. MFC is based on individual cell stations instead of a conveyer; the slaughter and meat primal cutting is carried out in a way that carcass is disassembled from "outside-in", where limbs, neck, back and loin are removed before internal organs, so that primal cuts' cross-contamination is minimised (*Alvseike et al.*, 2018). However, this concept and its advantages related to improvement of hygiene, food safety and cost benefit are under development and consideration.

Novel development related to automation and robotization in the meat industry (i.e. slaughter and dressing) could have a substantial impact on improvement of meat safety due to reduced cross-contamination of carcasses and reduced human labour engagement. On the other hand, for effective outcome, it will require ongoing progress in genetics and breeding strategies to provide greater carcass uniformity, which is essential to allow efficient operation of automated equipment.

Precision fermentation

The advancement in technology based on meat that is comprised of animal cells grown outside an animal in a bioreactor is already ongoing and could come to fruition in the foreseeable future (*Reis et al*., 2020). Products such as "cell-based meat" are genetically identical to conventional meat products. Cell-based meat is also referred to by others as "clean meat", "lab-grown meat", "cultured meat" or "in-vitro meat". The production of cell-based meat is related to the technology called precision fermentation (*Anonymous*, 2019). Precision fermentation, through programming of microorganisms to produce desired complex organic molecules, will allow the production of protein tailored to the personal needs of a consumer — the "food as software" approach (where individual molecules engineered by scientists are uploaded to databases, and molecular cookbooks that food engineers anywhere in the world can use to design products in the same way that software developers design applications). This model will also enable constant improvement of the product, so each new version will be superior and cheaper than the last. It also ensures a production system that is completely decentralised and much more stable and resilient than industrial animal agriculture, with fermentation farms located in or close to towns and cities, strongly supporting development of peri-urban agriculture and providing a solid basis for food security worldwide (*Tubb & Seba*, 2019).

Growing muscle tissue in culture media from animal stem cells to produce meat theoretically eliminates the need to sacrifice animals. Cultured meat could in theory be constructed with a range of different characteristics and be produced faster and more efficiently than traditional meat. The technique to generate cultured muscle tissues from stem cells was described long ago, but only recently have commercially produced cultured meat products started to appear on the market (*Stephens et al.*, 2018). The technology is still at an early stage and prerequisites of implementation include a reasonably high level of consumer acceptance, and the development of commercially-viable means of large scale production. Recent advancements in tissue culture techniques suggest that production could be economically feasible, provided the final product has physical properties in terms of colour, flavour, aroma, texture and palatability that are comparable to conventional meat (*Kadim et al.*, 2015).

Such technological development will have a disruptive impact on traditional meat production (rearing food producing animals intended for slaughter and meat production) and the meat chain as a whole. As perceived, precision fermentation is the deepest, fastest and most consequential disruption in the agri-food sector since the first domestication of plants and animals ten thousand years ago. This means cell-based (meat) proteins will be five times cheaper by 2030 and ten times

cheaper by 2035 than existing animal proteins; they will also be superior in all key quality attributes, e.g. more nutritious, healthier and with better taste (*Tubb & Seba*, 2019). For example, in the US, the impact on industrial farming will be significant; by 2030, the number of cows will have fallen by 50% and the cattle farming industry will be faced will serious economic perspectives. In general, all businesses in the meat value chain (crop farmers, livestock farmers, meat processors) will be affected with this technological development. The disruptive changes will be economic, environmental, social and geopolitical. Economic changes are the forecasted collapses of farmland values (by 40–80%), of crop farming due to decreased need for animal feed and of meat processing businesses in countries with high GDP input related to animal farming. The environmental impact will be related to the fact that, by 2035, 60% of the land currently used for livestock and feed production will be freed for other uses and greenhouse gas emissions from cattle will drop by 60%, including the 50% decrease of drinking water consumption by cattle. Social changes will be related to the greater food (meat) quality, with more nutritious, better tasting meat, as well as cheaper and more accessible product for consumers; job losses are predicted, in particular, in beef and dairy production and associated industries of 90% by 2035 (*Tubb and Seba*, 2019). The geopolitical impact is the trading shift due to decentralised food (meat) production and decreased impact of climate change in comparison to traditional livestock farming; most likely, the major meat producers and exporters (US, Brazil, the EU) will lose their geopolitical advantage over countries that are currently dependant on importation of meat. Countries currently importing animal products will more easily produce these products domestically at a lower cost, using modern production methods (*Tubb and Seba*, 2019).

The rapid development of cell-based meat will have striking and disruptive impact on the current understanding of the livestock and meat chain, as a whole, including the meat safety assurance system. The upcoming development and new reality of this novel technology will not only dramatically change the profile of the meat value chain, but also will change consumer perception because cultured meat is supposed to be pathogen-free since it is produced under precisely controlled conditions. A new paradigm for a meat safety assurance system associated with this novel technology should be developed in the foreseeable future.

3D Printing of meat

3D printing is an emerging technology for the food (meat) industry, providing an excellent opportunity to utilise meat by-products for the manufacturing of customised meat products. This technology uses computer-aided design (CAD) software assisting a digital manufacture machine in the generation of three-dimensional objects without any additional tool (*Noorani*, 2017). The combination of nutritionally balanced ingredients and novel internal structures can be integrated into a multi-material 3D model that meets specific individual needs, such as chewing and swallowing difficulties (Dick et al., 2019). This is important, in particular, for elderly consumers dealing with swallowing and mastication difficulties; the PERFORMANCE project was dedicated to solving these issues and improving 3D printing according to the needs of special categories of consumers (*RTDS Group*, 2014).

3D printing, also known as "additive manufacturing" (AM), is a process that generates freeform structures by introducing a prototype into CAD software; the prototype is then converted by slicing software into a suitable file form that can be recognised and processed by 3D printers (*Noorani*, 2017). The technology is based on layer-by-layer deposition with predetermined thickness to create complex 3D objects from different materials used like inks; the minimum necessary amount of materials is strictly used to consolidate the shape of the printed objects.

When it comes to food design/manufacturing using 3D printing, three categories were identified as a raw materials, based on the printability of food ingredients (*Sun et al*., 2015), as follows: (i) native printable food materials (cheese, vegemite and marmite, chocolate) that have enough flow ability to be easily extruded, (ii) non-native printable traditional food materials (meat, fish & seafood, fruits & vegetables) that require addition of flow enhancers to ease the extrusion and post-cooking process, and (iii) alternative ingredients, which are novel sources of functional constituents allowing customisation of nutrition (proteins and fibres isolated from insects, algae, bacteria and fungi (*Sun et al*., 2015). Meat and meat by-products are non-printable by nature due to their fibrous structures. Therefore, such raw materials require modification of their rheological and mechanical properties via addition of flow enhancers to obtain an extrudable paste-like material (*Liu et al*., 2018).

In general, 3D printing is considered as a novel technology with broad spectrum of applications in the medical field (tissue engineering), automotive and aerospace fields (component design), fashion,

and lastly, food design (*Gross et al*., 2014). 3D printing is a relevant technology with sustainable benefits such as reduced demand for raw materials, workforce, energy and transportation (*Peng*, 2016; *Sher & Tuto*, 2015). However, some issues still need to be improved and require intensive research and optimisation for 3D printing, such as time consumption for initial inversion, limited printable materials, accuracy level and surface finish (*Noorani*, 2017).

3D printing of meat is a novel technology that is still undergoing intensive research and needs substantial improvements to comply with technological processes and satisfy consumer demands. The meat safety assurance system will need to be adapted to allow effective control of the process, specifically addressing potential public health issues related to additives which are used to obtained meat in the form of paste-like materials suitable for extrusion.

Conclusion

The meat industry has undergone substantial changes over the previous several decades due to development of new technologies in primary production and meat processing. The current, traditional meat inspection protocols (ante-mortem and post-mortem), based on visual inspection, palpation and incision, had not been changed since the end of the nineteenth century. Although the traditional inspection approach was effective at the time it was introduced, with regard to detection of classical zoonoses (brucellosis, tuberculosis, cysticercosis, anthrax), it is not fully efficient in terms of the current needs for consumer protection. Namely, public health hazards associated with meat are, nowadays, the zoonotic food (meat) borne pathogens (*Salmonella*, *Campylobacter*, STEC, *Listeria monocytogenes*) that are responsible for the majority of human illnesses attributed to meat consumption; traditional meat inspection cannot respond effectively to detection of such food borne hazards, but can even increase cross-contamination due to palpation and/or incision procedures. Therefore, there is a need to develop a novel, modern meat inspection system which will be risk-and evidence-based and will cover the farm-chilled carcass continuum — this is the meat safety assurance system or carcass safety assurance system. The risk managers (OV/Official Auxiliary, FBO designated staff/abattoir workers), who are responsible for decision-making within the meat safety assurance system, should decide on the level and type of ante-mortem and post-mortem inspection, based on FCI/HEI. When FCI/HEI reflect high levels of farm biosecurity, animal health and animal welfare, risk managers can decide to apply visual-only inspection, without compromising the meat safety. EFSA recently recommended this approach to the EU MS. The process of introducing and scaling up the meat safety assurance system to full implementation will be gradual, flexible and carefully tuned to avoid unnecessary disruption of meat production chain and to allow stakeholders in the meat chain (farmers, meat processors, competent authorities and consumers) to achieve their public health and economic goals successfully. Lastly, novel technologies to be introduced in livestock chains and meat value chains are in the scope of rearing food producing animals on farm (PLF, sensing systems), slaughter & dressing (automation and robotization) and meat processing (precision fermentation, 3D printing). In the foreseeable future, these novel technologies will also have a disruptive and substantial impact on the meat value chains; this will require further and continuous adaptation or even thorough transformation of the meat safety assurance system to comply with meat safety and consumer protection regulations.

Bezbednost mesa: Sistem osiguranja baziran na oceni rizika i nove tehnologije

Ivan Nastasijević, Slavica Vesković, Milan Milijašević

A b s t r a k t: Industrija mesa je prošla kroz suštinske promene tokom prethodnih nekoliko decenija usled razvoja novih tehnologija u primarnoj proizvodnji (životinje u farmskom uzgoju) — precizan farmski uzgoj, senzorski sistemi; klanje i obrada — automatizacija i robotizacija; i prerada mesa — precizna fermentacija, trodimenzionalno štampanje mesa. Sadašnja, tradicionalna inspekcija mesa (ante-mortem i post-mortem), bazirana na vizuelnom pregledu, palpaciji i inciziji, nije bila promenjena još od kraja devetnaestog veka. Premda je takav tradicionalni pristup bio efektivan u vreme kada je usvojen, u kontekstu detekcije klasičnih zoonoza (bruceloza, tuberkuloza, cisticerkoza, antraks infekcije), on nije u potpunosti efikasan u kontekstu sadašnjih potreba u vezi sa zaštitom potrošača. Naime, opasnosti po javno zdravlje koje potiču od mesa u današnje vreme su povezane sa zoonotskim alimentarnim patogenima (Salmonella, Campylobacter, Shiga toxin-producing E. coli, Listeria monocytogenes) koje fekalno izlučuju zdrave životinje, a koji su odgovorni za većinu oboljenja ljudi povezanih sa konzumacijom mesa; tradicionalna inspekcija mesa ne može da odgovori efektivno u vezi sa detekcijom takvih alimentarnih opasnosti, već čak može da dovede i do uvećanja unakrsne kontaminacije mesa usled primene procedura palpacije i incizije. Stoga, postoji potreba da se razvije novi, moderan sistem inspekcije mesa koji će biti baziran na analizi rizika i zasnovan na naučnoj evidenciji — ‚sistem za osiguranje bezbednosti mesa' ili ‚sistem za osiguranje bezbednosti trupa'. Takav moderan sistem treba da bude baziran na upravljanju rizikom i protokolima za inspekciju mesa shodno analizama informacija iz lanca hrane/Harmonizovanih epidemioloških indikatora u kontinuumu farma-ohlađen trup.

Ključne reči: *bezbednost mesa, sistem osiguranja, inspekcija mesa, automatizacija, kultivisano meso.*

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Original scientific paper

Evaluation of effects of electronarcosis stunning on broiler chickens' welfare and meat quality

Guilherme Maroldi Kida[1], Guilherme Baú Torezan[2], Ana Maria Bridi[2], Alexandre Oba[2], Ana Paula Ayub da Costa Barbon[1], Caio Abércio da Silva[1], Rafael Humberto de Carvalho[1,2]*

A b s t r a c t: This study aimed to evaluate the electrical parameters during stunning by electronarcosis and their influence on broiler chicken welfare and meat quality. The research was carried out on 500 broilers, divided into 5 treatments with 100 broilers each. After unloading for slaughter, the birds' haematomas and fractures were evaluated. Subsequent to the groups' evaluation and separation, the electrical parameters were adjusted and the broilers were hung and electrically stunned in a water bath at a commercial slaughterhouse. The five different electrical parameters were: T0 = No electrical stunning (Halal); T1 = 95V, 600Hz and 2.4A; T2 = 125V, 1200Hz and 2.88A; T3 = 129V, 1500Hz and 2.88A and T4 = 216V, 1500Hz and 2.88A. Following the slaughter line, the birds were submitted to bleeding, scalding and feather removal. Carcasses were removed from the line and evaluated individually, recording both the carcass sites where haematomas and fractures were found and pH values (pH_{15min}). Carcasses followed the industrial process, and at the end, the breast fillets were removed and stored (4°C) for 24 h for pH, colouration (L, a* and b*) and water holding capacity analysis. The different electrical parameters used in stunning through the electronarcosis method had a direct influence on the haematoma and fracture levels, since T0 (63.8%) and T2 (61.7%) had high levels of haematomas and T0 (5.8%) a high level of fractures. The parameters of pH, colouration and water holding capacity showed differences between the various treatments used. The method of slaughter without electrical stunning presented the worst rates of these parameters among the evaluated electrical stunning methods.*

Keywords: *fracture, Halal, haematoma, poultry, slaughterhouse.*

Introduction

World population growth is associated with increased demand for food, especially animal proteins that are widely produced and consumed around the world. Due to the high number of poultry animals slauFghtered for human consumption, requirements such as birds' welfare during the slaughter process and product quality have become a matter of concern to consumers (*USDA*, 2018).

According to the World Society for the Protection of Animals (*WSPA*, 2010), factors such as product quality, biosecurity and sustainability are important questions for the continued production of broiler chickens, but not less important is the humanitarian slaughter that has been increasingly gaining the consumers' attention. In accordance with the World Organization for Animal Health (*OIE*, 2004), the humanitarian slaughtering procedure is the set of scientific and technical guidelines that ensure poultry welfare, from birds' reception in the slaughter premises until the bleeding operation. In the context of humanitarian slaughter, the best-known chicken stunning method used by commercial slaughterhouses is electrical stunning (electronarcosis) (*Sirri* et al., 2017). Stunning is a process responsible for leading the animal to a state of immediate loss of consciousness caused by the inhibition of impulses in reticular activating and somatosensory systems (*Heath* et al., 1994). In this process, enough electric current to induce convulsion and insensitivity to pain must reach the bird's encephalon, while maintaining the vital functions until the bleeding stage (*Gregory and Wotton*, 1989). The insensitivity period enables the animal to be slaughtered without suffering pain or affliction, thus reducing the bird's response to stress at the time of slaughter. In addition, it promotes the birds' immobilisation and facilitates cutting of the main neck vessels (*OIE*, 2004).

The electrical stunning methods can lead to pain and suffering, higher incidence of fractures, haemorrhagic spots and meat defects, such as the appearance of pale, soft and exudative meat (PSE), resulting in

[1]*Philadelphia University Center, Department of Veterinary Medicine, Londrina, Paraná, Brazil;*
[2]*Londrina State University, Agricultural Sciences Center, Graduate Program in Animal Science, Department of Animal Science, Londrina, Paraná, Brazil.*

***Corresponding author:** Rafael Carvalho, *rafael.carvalho@uel.br*

significant losses to the poultry industry (*WSPA*, 2010; *Savenije et al.*, 2002). All broilers must be stunned before slaughter, except in cases of religious precepts, which are required by a particular religious community or when meat is destined for commercial regions that require this exigency (*Girasole et al.*, 2015).

In this regard, according to the Gulf Standardization Organization (*GSO*, 2015), which establishes the requirements for animal slaughter according to Islamic rules (Halal), electric shock and any other forms of shock should not be used in the process of slaughtering birds. Halal slaughter, which follows the most traditional Islamic precepts, such as Saudi Arabia's, has been questioned by animal rights activists who claim that sacrifice without stunning can cause pain and suffering (*Shahdan et al*., 2016). Nevertheless, to export to more tolerant countries, Halal slaughter allows electronarcosis, as long as the stunning process does not cause cardiac arrest induction of the bird (*Fuseini et al*., 2018). Therefore, due to its importance in animal welfare, religious precepts and product quality, the objective of this study was to evaluate and compare the effects of non-stunning and stunning with different electrical parameters in broilers, and the influence of the stunning methods on bruises, fractures and final meat quality, following the precepts of animal welfare.

Material and Methods

All procedures adopted in this research were previously approved by the Animal Use Ethics Committee of the Philadelphia University Center, (CEUA 000/2019).

Pre-slaughtering broilers

The 48-day-old Cobb® broiler chickens (n = 500) of both sexes were conventionally prepared for slaughter with a 10-hour fast before hanging. The broilers were manually caught, respecting the capacity of 22 kg per cage, and transported in trucks. In the slaughterhouse, on the resting platform, the broilers were bathed with sprinkling water at ambient temperature immediately before slaughter. The ambient temperature and the relative humidity varied from 20.8 to 28.6°C and 55 to 78%, respectively, during the experiment. Before slaughter, a total of 125 broilers were separated and divided into 25 broilers per treatment for each day (n = 4) analysed.

Experimental procedure and sample collection

The broilers were evaluated after unloading the cages in the slaughterhouse to register the presence of bruises and fractures and the respective affected sites. The evaluation sites for recording bruises were wing tip, mid wing, wing drumstick, breast and thigh, divided into left and right sides (Figure 1A). The sites evaluated for recording fractures were wing tip, mid wing, wing drumstick and thigh, divided into left and right sides (Figure 1B). Both bruises and fractures were counted as a total value per animal. This procedure was performed to identify the animals that had bruises and fractures derived from before the slaughter process.

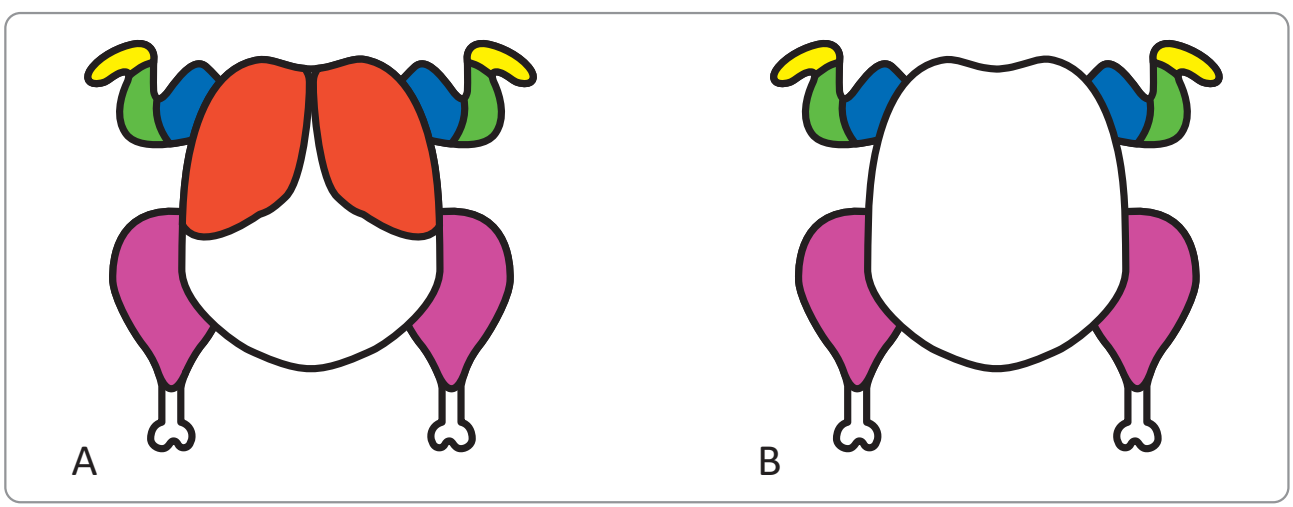

Figure 1. (A) Bruising evaluation sites: wing tip (yellow), mid wing (green), wing drumstick (blue), breast (red) and thigh (purple). (B) Fracture evaluation sites: wing tip (yellow), mid wing (green), wing drumstick (blue) and thigh (purple).

Table 1. Electrical desensitisation parameters: voltage, frequency and amperage for different treatments.

Treatments	T0*	T1	T2	T3	T4
Voltage (V)	–	95 V	125 V	129 V	216 V
Frequency (Hz)	–	600 Hz	1200 Hz	1500 Hz	1500 Hz
Amperage (A/24 broilers)	–	2.4 A	2.88 A	2.88 A	2.88 A

Legend: * Broilers that did not go through the electric stunning process, religious precept (*Shahdan et al., 2016*).

At the end of the individual evaluation of each broiler, they received an identification seal on their thigh. After the electrical parameter was properly adjusted in the equipment, the group of broilers (n=125), previously inspected, identified and separated, was hung on the slaughter line in order to immerse the broilers in the electric stunner machine.

Previous to slaughter, the broilers were divided into five treatments (Table 1), based on the application of the electrical stunning in an immersion bath or without electrical stunning. One hundred broilers were used per treatment, and they were divided into four replications on different days.

Electrical sensitisation was performed in a stunning Fluxo 3.0®, with variable electric current, submerging the broilers' heads in salted potable water. The electrical parameters used contained different variations of voltage (V), amperage (A) and frequency (Hz), but the type of electric current, square alternating with duty cycle 50%, and the broilers' exposure period in the electric stunning machine were kept the same for all groups. The broilers were stunned for ten seconds, and the stunning settings were based on the terrestrial animal health code (*OIE*, 2004). The humanitarian bird slaughter manual (*WSPA*, 2010) was used with adaptations.

After stunning, the broilers were evaluated for the efficiency parameters in the electronarcosis process and for welfare parameters, assuring that the examined methods would not cause death or injuries to the animals. All broilers were assessed immediately after leaving the desensitisation bowl, using visual analysis and a digital chronometer to evaluate the absence of rhythmic cloacal breathing, presence of body tremors, absence of coordinated wing beat, arched neck, lack of vocalisation, wings close to the body with tremors and absence of eyelid reflex, which indicate an efficient stunning process that does not hurt the animals, inducing neither pain nor discomfort at the time of slaughter.

Manual bleeding was performed within 10 seconds after stunning. The bleeding period was approximately 3.5 minutes for all treatments.

After slaughter, following standard industrial practices, carcasses were scalded at 52°C for two minutes and the feathers were removed automatically by machine. At this point, the carcasses were removed from the slaughter line and the same sites for bruises and fractures (Figure 1) were re-examined. The carcasses' pH value (15 min) was also measured, then they were returned to the line they followed for automatic evisceration, pre-chiller (4 to 16°C/28 min) and chiller (0 to 4°C/54 min). Breast fillets (*Pectoralis major*) were collected approximately 1h and 40 minutes *postmortem* and stored at 4°C for 24h for pH, colour (L*, a* and b*) and drip loss analysis.

Colour and pH measurement

The pH values (15 min and 24h) were determined by introducing the electrode directly into the breast muscle with the contact potentiometer (Testo, Model 205). The analyses were performed in 24h *postmortem* triplicates as described by *Carvalho et al*. (2017).

The Colorimeter (Minolta CR 400) was used to evaluate the colour parameters of L* (luminosity), a* (red component) and b* (yellow component) on the fillet's ventral surface, taking five different recognition points per sample, according to the methodology described by *Carvalho et al*. (2017).

Water holding capacity (WHC)

The analysis was performed as described by *Honikel* (1998). To determine weight loss during storage, approximately 2 g amounts of breast fillet were weighed before and after storage (4°C). The WHC was expressed as a percentage derived from the ratio differences between the samples' initial and final weights, as shown below:

$$WHC\,(\%) = \frac{Pf\,(g) \times 100}{Pi\,(g)}$$

where Pf is final weight and Pi is initial weight.

Chicken fillet classification

Chicken fillets were classified as either PSE or normal meats, based on pH and L* values as described by *Carvalho et al*. (2017). Therefore, the fillets with L* 24h ≥ 53.0 and pH 15 min ≤ 5.80 values were classified as PSE, while fillets under 44.0 < L*24h < 53.0 and 5.80 < pH 15 min < 6.00 values were considered normal. For incidences of PSE meat, the binary variation (1 and 0) was used, with 1 indicating PSE meat and 0 normal meat.

Statistical analysis

The Statistica software for Windows 13.0 (StatSoft, Tulsa, USA) was used. Tukey's test at 1% probability ($p<0.01$) was used for comparing the differences among the five treatments.

Results and discussion

Individual analysis of 500 broilers before and after slaughter allowed the number of bruises and fractures already obtained in the pre-hanging stages to be discarded, and isolation of the injuries caused at the slaughterhouse. Thus, the influence of each stunning method used was quantified. The bruise and fracture rates for each type of treatment applied is shown in Table 2.

The results show the broilers which were not submitted to electrical stunning (T0) and the T2 group presented with higher incidences of bruising ($p<0.01$) when compared to the other treatments. The T4 group presented a lower bruising incidence, and thus, better results than treatments T0 and T2, which corresponded to differences of 32.4% and 30.3%, respectively.

The non-stunning method caused a higher incidence of fractures than did the other treatments ($p<0.01$), since T0 presented with 5.7%, 4.8%, 5.4% and 5.3% higher fracture incidence than T1, T2, T3 and T4 treatments, respectively.

The differences between T0 without stunning, which presented higher haematoma and fracture rates, and the other groups analysed are due to the broilers' agitation during the bleeding process. *Wilkins* (1998) and *Cuadrado* (2012) indicate the negative effects caused by stunning can be reduced through higher frequencies, since the muscle contraction strength caused by the broiler's electrical stimulation is reduced, resulting in fewer bone fractures and muscle bruises. This effect was verified in our study by comparing the T2 and T3 treatments, in which the percentage of haematoma was significantly higher when the frequency was 1500 Hz (T3) than when the frequency was 1200 Hz (T2).

The combination of high voltage and high frequency, as performed in T4, produced lower haematoma rates due to the better stunning process, since the use of high voltage promotes greater efficiency of stun, as it rejects the resistance value according to Ohm's law represented by the formula V = R.I; where V is the voltage measured in volt (V), R is the electrical resistance measured in Ohm (Ω), and I is the intensity of electric current measured in ampere (A) (*Parks*, 2007).

According to *Scheuermann* (2017), broilers' electric desensitisation with approximate electric current of 100 mA per broiler, frequency of 600 Hz and voltage at 96 V, enables the passage of electric current in the broiler's brain in greater magnitude than is normally used for neurological activity, so constitutes the minimum electrical premises for an effective stunning process. This parameter, utilised in T1, resulted in low haematoma and fracture rates, due to this low electric tension producing relatively little muscle contraction.

The pH values at 15 minutes and after 24 hours, the colouration (L*), (a*) and (b*) and WHC (Table 3) show the poultry submitted to slaughter without stunning presented a lower pH15min value ($p<0.01$) than did the other treatments.

Anaerobic glycolysis occurs over time after slaughter and results in lactate formation and accumulation in the muscle, which reduces meat pH, as

Table 2. Incidences of bruise (%) and fracture (%) in broilers according to stunning method.

Treatments	T0	T1	T2	T3	T4
Bruises (%)	$63.80^{a}\pm12.66$	$41.80^{c}\pm12.57$	$61.70^{a}\pm8.19$	$51.20^{b}\pm11.15$	$31.40^{d}\pm7.05$
Fractures (%)	$5.80^{a}\pm1.75$	$0.01^{b}\pm0.03$	$1.00^{b}\pm2.21$	$0.40^{b}\pm0.51$	$0.50^{b}\pm0.05$

Legend: Different superscript letters in the same row represent statistically different averages by Tukey's analysis with 1% significance. T0 = No electrical stunning (Halal); T1 = 95 V, 600 Hz and 2.4 A; T2 = 125 V, 1200 Hz and 2.88 A; T3 = 129 V, 1500 Hz, 2.88 A and T4 = 216 V, 1500 Hz and 2.88 A.

Table 3. pH (15min and 24h), colouration (L *, a * and b *) and water holding capacity (WHC) values for different sensitisation parameters.

	T0	T1	T2	T3	T4
pH15min	$5.99^{d}\pm0.12$	$6.39^{a}\pm0.17$	$6.27^{ab}\pm0.12$	$6.21^{bc}\pm0.18$	$6.31^{ab}\pm0.12$
pH24h	$5.76^{a}\pm0.12$	$5.83^{a}\pm0.17$	$5.78^{a}\pm0.12$	$5.86^{a}\pm0.18$	$5.81^{a}\pm0.12$
L*	$53.29^{a}\pm1.71$	$49.90^{b}\pm1.69$	$50.39^{b}\pm1.89$	$50.71^{b}\pm1.72$	$49.18^{b}\pm2.01$
a*	$2.64^{a}\pm0.92$	$1.42^{b}\pm0.99$	$1.79^{ab}\pm0.85$	$1.14^{b}\pm0.58$	$1.53^{b}\pm0.87$
b*	$5.24^{a}\pm1.66$	$6.12^{a}\pm1.94$	$6.31^{a}\pm0.88$	$6.20^{a}\pm2.08$	$5.70^{a}\pm1.55$
WHC (%)	$93.32^{b}\pm1.11$	$95.52^{a}\pm1.30$	$94.60^{ab}\pm1.0$	$95.25^{a}\pm1.35$	$95.86^{a}\pm1.18$

Legend: Different superscript letters in the same row represent statistically different average results by Tukey's test with 1% significance ($p<0.01$). T0 = No electrical stunning (Halal); T1 = 95 V, 600 Hz and 2.4 A; T2 = 125 V, 1200 Hz and 2.88 A; T3 = 129 V, 1500 Hz and 2.88 A and T4 = 216 V, 1500 Hz and 2.88 A.

shown by the lower pH at 24h after slaughter than pH at 15 mins post-slaughter in all treatments (*Lawrie* 2005).

The stress caused at the time of slaughter can be noticed by the accentuated pH decrease in the first 15 minutes in non-stunned poultry (T0). Acute stress in birds causes release of catecholamines and glucocorticoids that accelerate the animal's metabolism, so anaerobic glycolysis occurs at a much higher rate in non-stunned than in stunned broilers. When the carcass temperature is close to physiological (40ºC), low pH values occur due to myofibrillar and sarcoplasmic protein denaturation, as evidenced by the pH after 15 mins in the T0 group (*Olivo et al*., 2001; *Ali et al*., 2008; *Carvalho et al*., 2017).

According to *Carvalho et al*., (2017), pH is closely related to colouration and WHC.

The T0 group without stunning presented higher L* values ($p<0.01$) than the other treatments. The a* value for the group without stunning was higher ($p<0.01$) than those of treatments T2 and T3, being 0.85 and 1.5 units greater, respectively. B* values did not differ between the analysed groups ($p>0.01$).

The L* value relates to luminosity, varying from white (100) to black (0). These values were higher ($p<0.01$) in the treatment without stunning compared to the others, indicating paler meat. Protein denaturation derived from the acute stress process, as found in T0, promotes pale meat, due to higher birefringence with less light transmitted by the fibres. Thus, a greater amount of light is scattered and the meat appears lighter in colour (*Bendall and Swatland*, 1988; *Swatland*, 1995).

The data related to the treatment without stunning denote the stress the poultry went through, being identified by the low pH value observed in the first minutes after slaughter, the high L* rate in the luminosity evaluation and the low WHC.

a* values, related to green to red shades, indicated higher red shades for T0 and T2, due to the greater agitation of broilers during slaughter and occasionally higher bruising and splattering rates. According to *Kranen et al*. (2000), with *pectoralis* muscle haemorrhages, histological studies show their morphological appearance and blood leakage is determined by the structure of the tissue and the amount of blood leaving the circulation. The diversity of type and bleeding location indicates that bleeding is caused by several different mechanisms including electronarcosis.

According to *Fernandes* (2004), WHC is defined as meat's ability to retain its moisture or water during the application of external forces. The highest WHC values were seen in the T4 group ($p<0.01$), with broilers submitted to high frequency and high voltage desensitisation, being 2.54 percentage points and 1.26 percentage points higher than groups T0 and T2, respectively.

The WHC of meat is a very important quality attribute that influences the productivity of meat product, which in turn has economic implications, but is also important in terms of product quality and sensory quality. WHC is directly involved with meat cooking and cooling procedures, more specifically the heating and cooling rates that can influence the palatability and succulence of the final product (*Cheng*, 2008).

Increased lactic acid production with consequent pH decrease (Table 3), associated with high body temperature immediately after slaughter, cause denaturation and loss of muscle protein solubility, leading to decreased WHC (*Mckee and Sams,* 1998; *Van Laak et al*., 2000; *Carvalho et al.,* 2017), a fact indicated in the T0 group when compared to the other treatments.

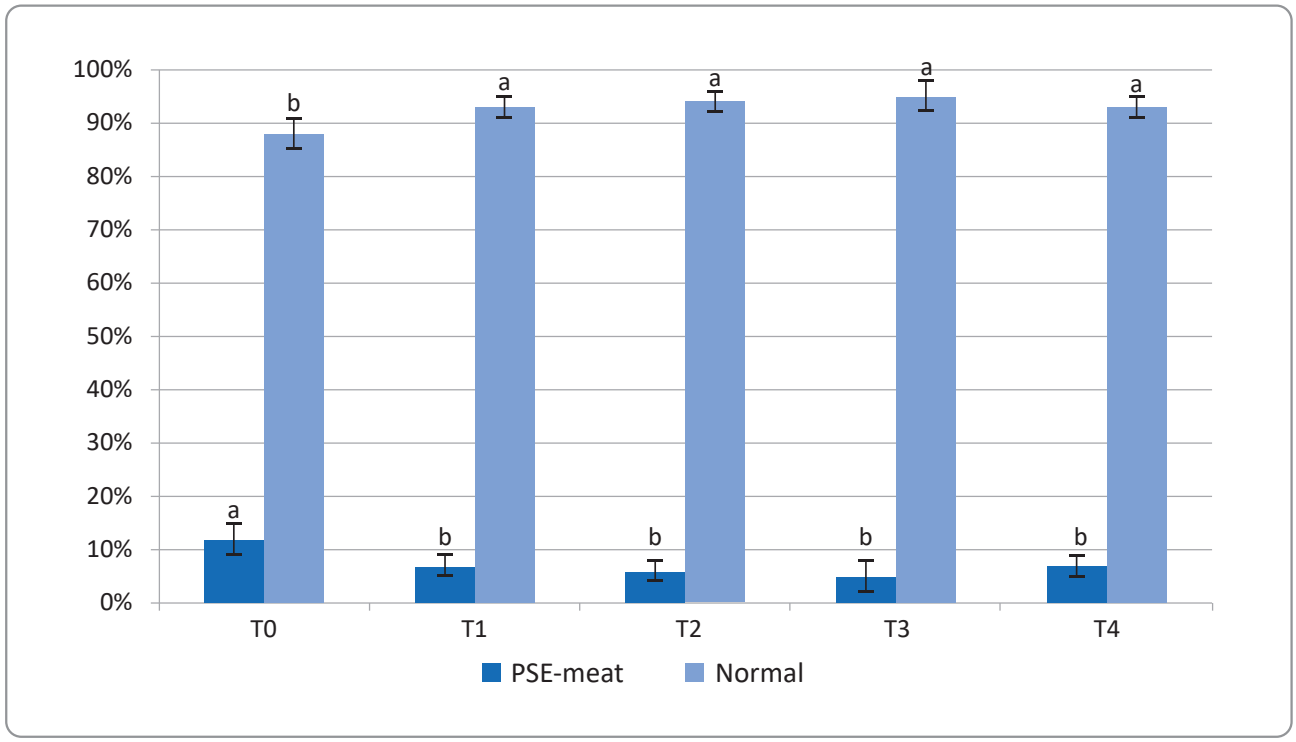

Figure 2. Percentages of PSE and normal meat for five different stunning procedures.

Legend: T0 = No electrical stunning (Halal); T1 = 95 V, 600 Hz and 2.4 A; T2 = 125 V, 1200 Hz and 2.88 A; T3 = 129 V, 1500 Hz and 2.88 A and T4 = 216 V, 1500 Hz and 2.88 A. Standard deviation bars are indicated at the top of the bars. Significant differences presented by Tukey's test at 1% ($p<0.01$) are demonstrated at the tops of the bars for each stunning procedure. ns = not significant. n = 25 per treatment.

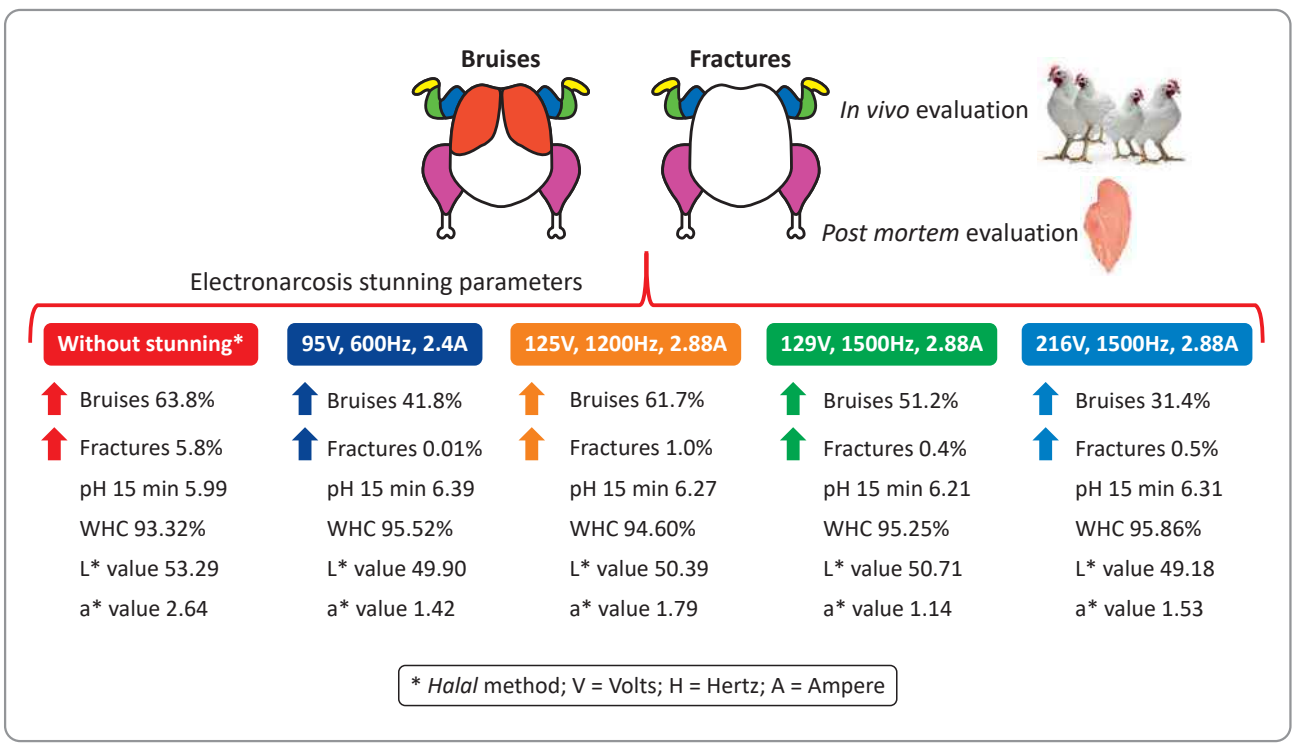

Figure 3. Proposed mechanisms for the observed effects of electronarcosis stunning on meat quality of chicken thigh meat.

Legend: WHC = Water holding capacity

The incidence of PSE meat did not significantly differ between the desensitised groups ($p>0.01$), but differed significantly from that of the T0 group without stunning ($p<0.01$). The non-stunning of broilers (T0) resulted in 88% of meat presenting normal characteristics and 12% presenting PSE characteristics, due to the acute stress caused at the time of slaughter without any electrical stunning. This immediately increased the birds' metabolism, seen by rapid muscle glycogen depletion and meat pH reduction caused by lactic acid accumulation, while carcass temperatures were still in physiological patterns (*Carvalho et al*., 2017). This biotransformation denatures myofibrillar and sarcoplasmic proteins that are directly involved with meat tenderness and pale colouration in the final product.

The other treatments presented rates of 93% of normal meat and 7% PSE in T1 treatment; 94% normal meat and 6% PSE in T2; 95% normal meat and 5% PSE in T3 and 93% normal meat and 7% PSE in T4 ($p>0.01$).

The results show the stunning stage should be submitted to greater control during poultry processing, since it interferes with the final product quality and the utilisation of the cuts obtained. The reduction of haematoma is importantly influenced by stunning, as there was 30.3 percentage points less bruising in the desensitised groups (T4 and T2), and 32.4 percentage points less bruising in the T4 group (with stunning) than in the T0 group (without stunning). The WHC of chicken fillets differed insignificantly ($p>0.01$) between the desensitised groups, unlike the non-desensitised group (T0) that had a lower WHC, resulting in product with higher exudative characteristics and of lower quality.

All electrical configurations tested effectively stunned the broilers, keeping them in a state of unconsciousness until the moment of slaughter and not causing the death of the animals during the electronarcosis process. Figure 3 summarises the proposed mechanisms by which electronarcosis stunning can influence the birds' welfare and chicken meat quality.

Conclusion

Not using any stunning caused more bruising and fractures due to greater movement of broilers at the time of slaughter. Otherwise, stunning with high voltage and frequency (216V, 1500 Hz and 120 mA per broiler) caused significantly fewer bruises and fractures by causing better stunning with fewer unwanted effects. Clearly, the parameters used in electrical desensitisation have a direct influence on meat quality and animal welfare, but deeper research is needed on the parameters to stipulate an ideal configuration for both the animal and the industry.

Ocena uticaja omamljivanja elektronarkozom na dobrobit i kvalitet mesa pilića

Guilherme Maroldi Kida, Guilherme Baú Torezan, Ana Maria Bridi, Alexandre Oba, Ana Paula Ayub da Costa Barbon, Caio Abércio da Silva, Rafael Humberto de Carvalho

A p s t r a k t: Cilj ovog istraživanja je bio da se procene električne parametri tokom omamljivanja elektronarkozom i njihov uticaj na dobrobit pilića brojlera i kvalitet mesa. Istraživanje je sprovedeno na 500 brojlera, podeljenih u 5 tretmana sa po 100 brojlera. Nakon istovara za klanje, urađena je procena ptica na prisustvo hematoma i preloma. Nakon procene i razdvajanja grupa, električni parametri su prilagođeni, a brojleri su okačeni i omamljeni pomoću struje u vodenom kupatilu u komercijalnoj klanici. Pet različitih električnih parametara je korišćeno u istraživanju: T0 = bez električnog omamljivanja (Halal); T1 = 95V, 600Hz i 2,4A; T2 = 125V, 1200Hz i 2,88A; T3 = 129V, 1500Hz i 2,88A i T4 = 216V, 1500Hz i 2,88A. Nakon linije klanja, ptice su podvrgnute iskrvarenju, oparivanju/šurenju i uklanjanju perja. Trupovi su skidani sa linije klanja i pojedinačno procenjivani, beležeći i mesta na trupu na kojima su pronađeni hematomi i prelomi, kao i vrednosti pH (pH15min). Trupovi su pratili industrijski proces, gde su na kraju fileti grudi pilića odvojeni i čuvani (4°C) tokom 24 sata za analizu pH, boje (L, a* i b*) i kapaciteta zadržavanja vode. Različiti električni parametri koji se koriste za omamljivanje metodom elektronarkoze imali su direktan uticaj na nivo hematoma i preloma, budući da su T0 (63,8%) i T2 (61,7%) pokazali visok nivo hematoma, a T0 (5,8%) visok nivo preloma . Parametri pH, boje i sposobnosti zadržavanja vode pokazali su razlike između različitih tretmana. Metoda klanja bez električnog omamljivanja pokazala je najgore stope ovih parametara među ocenjenim metodama električnog omamljivanja.*

Ključne reči: *prelom, halal, hematom, živina, klanica.*

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Review paper

Health hazards associated with ready-to-eat-meat in Nigeria: A call for public concern and critical interventions

Earnest Erhirhie[1], Chuka Nwosu[2], Tedwins Emudainohwo[3], Chidimma Chukwunwejim[4] Peter Eze[5], Daniel Ajaghaku[6]*

A b s t r a c t: Scientific investigations on the type of ready-to-eat (RTE) meat popularly called Suya and sold in Nigeria are generating scientific and public concern due to microbial and chemical hazards associated with the products. This review evaluated the safety profile of Nigerian RTE meats, with special focus on Suya as a potential source of microbial, heavy metal and polycyclic aromatic hydrocarbon (PAH) food hazards. Assessments of outcomes of research articles on safety of RTE meat published from 1984 to 2019 were carried out using electronic databases and key word searches. Research outcomes were categorised into six sections representing the six geopolitical zones (South-south, South-east, South-west, North-east, North-west and North-central) of Nigeria. Virtually all research findings in various zones revealed microbial, heavy metal or PAH levels on RTE meat were higher than permitted limits and acceptable standards. The unhygienic activities of most meat slaughterers (sources of raw meat), processors (who prepare and package RTE meat) and vendors (involved in display and hawking processes) are major contributing factors to microbial and chemical hazards. To this end, adequate safety and sanitary measures are suggested and other essentials should be implemented by designated authorities and relevant stakeholders to ensure the menace posed by unhygienic RTE meat is curtailed drastically.

Keywords: *RTE meat, safety, microbiological contamination, chemical contamination, Nigeria.*

Introduction

Meat refers to animals' flesh (skeletal muscles) and other parts such as fats, liver kidney, heart, lung, brain, intestine, and connective tissue that serve as food (*Olayinka and Sani*, 2014). Worldwide, including in Africa and Nigeria, meat is considered a rich source of protein and essential micronutrients that are needed for growth and good health for people in various socio-demographic categories, including the young, old, rich and poor (*Olayinka and Sani*, 2014). The majority of the Nigerian populace depends on livestock for food and livelihood (*Elelu et al.*, 2019). Meat requires adequate preservation due to its short shelf life (*Olaoye et al.*, 2016). On the other hand, meat has essential nutrients that support microbial growth and metabolism when adequate preservation and hygiene is not maintained (*May et al.*, 2003; *Eke et al.*, 2013; *Nwakanma et al.*, 2015).

Globally, it has been assessed that about 600 million (1 in 10) persons annually are predisposed to foodborne disease, resulting in about 420,000 deaths every year and foodborne diseases consume about US$ 3.6 billion yearly (*Ezirigwe*, 2018; *WHO*, 2015). From the World Health Organization (WHO) assessment, about 200,000 deaths annually due to diarrhoea result from food poisoning (*Afolabi and Odubanjo*, 2015*; WHO*, 2019).

Nigeria is the most densely populated nation in Africa, with a population of about 185 million distributed over 250 ethnic groups (*WHO*, 2019). The greater

[1]*Chukwuemeka Odumegwu Ojukwu University, Faculty of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, Igbariam, Anambra State, Nigeria;*
[2]*National Agency for Food and Drug Administration and Control Zonal Laboratory, Agulu, Anaocha, Anambra State, Nigeria;*
[3]*University of Benin, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Benin City, Nigeria;*
[4]*Enugu State University of Science and Technology, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Microbiology and Biotechnology, Enugu, Nigeria;*
[5]*Nnamdi Azikiwe University, Faculty of Health Sciences and Technology, Department of Environmental Health Science, Nnewi Campus, Anambra State, Nigeria;*
[6]*Enugu State University of Science and Technology Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State, Nigeria.*

***Corresponding author:** Earnest Erhirhie, *erhirhieochuko@yahoo.com*

proportion, 52% of the populace, resides in rural areas while 48% reside in the cities (*Akinlua*, 2015). The country has six geopolitical zones, North-west (NW), North-east (NE), North-central (NC), South-west (SW), South-east (SE) and South-south (SS), which are distributed over 36 states (*Akinlua et al.*, 2015).

In Nigeria, waterborne diseases such as diarrhoea, typhoid and cholera resulting from food poisoning are a major public health concern. The Nigerian Federal Ministry of Health reported roughly 90,000 cases of food poisoning in 2007 (*Osakue et al.*, 2016). This is substantiated by the increasing human population, rural-urban migration and industrialisation, environmental pollution, poverty (*Osakue et al.*, 2016) and over-reliance on ready-to-eat (RTE) food, due to individuals' busy schedules and lack of time to properly prepare their meals. This is supported by an increase in the number of outlets selling RTE food in various locations (*Izah et al.*, 2017). In the interest of saving money for a rainy day, most of the populace, including travellers, school children and low-income earners, patronise RTE food vendors, but pay scarce attention to safety issues, quality and hygiene (*Ezirigwe*, 2018).

Presently, the nutritional standard of most RTE meat, especially Suya, is low due to poor handling conditions leading to contamination with air microbiota, other microorganisms and chemicals in excess of safe limits during butchering, processing, packaging and vending (*Osakue*, 2016).

Studies by authors in various locations in Nigeria have revealed that heavy metals, polycyclic hydrocarbons (PAHs) and microorganisms are major sources of contaminants on RTE Suya meat, and this is of public health significance in initiating reductions of foodborne diseases (*Nwakanma et al.*, 2015*; Folorunso et al.*, 2018).

To this end, this review attempts to provide an update on the safety status of RTE meat sold in Nigeria. The outcome of this review will provide useful information to the general public and health institutions on the risks associated with the intake of bacteria- and heavy metal-contaminated Suya meat. Also, improved strategies to prepare hygienic Suya for human consumption and avoid the public health menace of highly contaminated product are suggested.

Methodology

The study constitutes a literature survey of the microbial, heavy metal and PAH contamination of RTE meat sold in various geographic zones in Nigeria published in scientific journals between 1984 to June 2019. Electronic databases (Google scholar, Pubmed, Science direct, and Medline) were accessed using the following search terms; Suya, ready to eat meat, heavy metal contamination of Nigeria Suya meat, bacterial contamination of Suya meat. Manual searches of reference lists from papers downloaded on these related topics were also performed to uncover additional related studies missed by the search engines. The last search was carried out on 28 December, 2019. Probable health implications of microbial and heavy metal contamination and measures to prevent contamination were also searched for using search engines. Relevant measures to curtail contamination and improve the quality of RTE meat in Nigeria are suggested.

Description of RTE meat

RTE meat can be described as well-prepared animal tissue which does not require further preparation before consumption (*Okoli et al.*, 2018). RTE meats can be formulated to be street delicacies prepared from boneless or entire beef, lamb or pork meat with added spices, salts, flavours and vegetables followed by roasting under charcoal fire (*Eke et al 2013; Olaoye et al.*, 2016). In Nigeria, these meats occur in various forms: skewer meat or Suya (tsire, boneless, spiced and barbequed), kilishi (dried meat similar to Suya but un-spiced), pork as well as chicken and goat meat (chevon) (*Fasoyiro 2012; Shamsuddeen and Puma*, 2016). Besides the aforementioned, kanda (Igbo) and tinko (Yoruba), which are dried and unspiced meats obtained from carcasses of rejected or dead animals (buffalo, donkey, cattle etc.), though not used in RTE meats, are widely consumed in Africa (*Adeyeye 2016; Ribah et al.*, 2018). Balangu guru, Kilishi, balangu, kundi, Jirga, ndako and dambunama are Hausa names for processed smoked, roasted or dried meats eaten in Northern Nigeria (*Ogbonna et al 2012; Yusuf et al.*, 2012*; Folorunso*, 2018). RTE meats serve as good sources of proteins, vitamins and minerals for growth, repair and maintenance of tissue cells (*Nwakanma et al.*, 2015*; Adeyeye*, 2016).

Suya, the most popular RTE meat in Nigeria

Suya mostly prepared from beef (or bovine meat) is the most commonly sought after RTE meat in every geographic location in Nigeria, including rural and urban areas. The history of Nigerian Suya meat can be traced to the Hausa/Fulani people of Northern Nigeria and other neighbouring countries, including Cameroon, Niger and Sudan (*Garba et al.*,

2017), where 80% of Nigerian cattle rearing occurs and is a major source of their livelihood (*Ogbonna et al.*, 2012*; Egbebi and Muhammad 2016, Falegan et al.*, 2017).

Suya meat, popular in Northern Nigeria, has spread in popularity to villages, town and cities in other parts of Nigeria, where Suya is now sold in many outlets (*Inyang et al.*, 2005). This is confirmed by the prevalence of Suya vendors in big cities and small towns, where their busy schedules occur between 12 noon until night (*Ogbu et al.*, 2016).

Suya is purchased by people in the street, clubs, restaurants, fast food outlets, picnics, beaches, hospitality venues, hotels and other institutions (*Ahmadu and Aduwa*, 2015). It is also served in homes, at parties and for ceremonies as substitutes for fish and other forms of meat (*Nwakanma et al.*, 2015*; Okoli et al.*, 2018).

The study by *Ahmadu and Aduwa* (2015) on the economic analysis of Suya production in Benin City, Edo State, Nigeria, revealed that small scale Suya production is a profitable venture, where every naira invested could yield a net return of 58,000 naira. A similar study was also conducted by *Iliyasu et al*. (2013) in Northern Nigeria, where there are growing livestock and human populations. Thus, with the increase in population, it is predictable and evident that demand for Suya will increase.

In Suya preparation, carcass parts used include beef meat, kidney, liver and intestine (*Garba et al.*, 2017). Suya meat is prepared by roasting and spicing boneless portions of edible tissues and muscle tissues of animals, and can be eaten alone or in combination with onions, herbs or vegetables. Grinding peanuts into a powder is the first step in the preparation of Suya. After this stage, the peanut powder is thoroughly mixed with ground pepper, garlic and ginger. Meat is then cut into small sizes or thin sheets and rolled in the peanut-spice mixture. In order for the peanut cake to stick together, the pulverised meat is left standing in the peanut-spice mixture for about 40–60 minutes. Thereafter, the meat portions are pushed onto skewers and brushed with vegetable oil. Skewered meat is barbecued or roasted on a charcoal fire for about fifteen to twenty minutes, depending on the intensity of the fire (*Olaoye et al.*, 2016*; Nwakanma et al.*, 2015*; Onuorah et al.*, 2015*; Konne et al.*, 2018). Drying of boneless tissues during this slow roasting aids the loss of moisture and helps prevent spoilage (*Ogbonna et al 2012; Onuorah et al.*, 2015). After processing, Suya is packaged in aluminium foil, newspaper, cellophane or other materials (*Alonge et al.*, 2017).

Studies on heavy metals, PAHs and microbial contamination in RTE meat in various geo-political zones in Nigeria

This study covers our investigation and findings from the six geo-political zones in Nigeria. As highlighted below, the microbial, heavy metal and PAH contents of RTE meat were investigated in various locations in Nigeria.

South-south Nigeria

This geopolitical zone encompasses six states: Delta, Edo, Bayelsa, Rivers, Akwa-Ibon and Cross River (*Akinlua et al.*, 2015). In a study carried out in Delta State, levels of heavy metals (lead, cadmium and mercury) were studied on Suya procured from Warri, Ughelli and Ozoro (three samples from each location). Cadmium and mercury levels in all the Suya were below the maximum levels permitted by WHO. On the other hand, lead content was higher than expected values in Suya procured from Warri (0.125 mg/kg), but lower in Suya from Ughelli (0.060 mg/kg) and Ozoro (0.085 mg/kg). The authors suggested Suya from the selected locations should be monitored to avoid adverse effects (*Ojebah and Ewhre*, 2015).

A comparative bacteriological analysis of Suya meats, hawked and from barbeque stands, was carried out in Ozoro, Delta State. *Enterobacter aerogenes and Bacillus subtilis* were the most prevalent bacteria isolated, while *Staphylococcus aureus* and *Lactobacter* were the least prevalent in the meats. Hawked Suya were more contaminated with pathogenic bacteria, *Bacillus subtilis Enterobacter aerogenes*, *Staphylococcus aureus, Lactobacter* species and *Escherichia coli* than were Suya meats sold on barbeque stands (*Orogu and Oshilim*, 2017).

In 2018, Akpoghelie identified 16 PAHs on smoked fish and grilled Suya meat procured from open markets in the Effurun, Igbudu, Jugbale, Olomoro, Oleh and Ozoro markets, Delta State. The study revealed the levels of PAHs in smoked fish and grilled Suya meats were significantly higher than PAHs in these products when they had been soaked in boiled water. The author concluded the skin or outer layer of fish could serve to bioaccumulate PAHs. Thus, soaking these foods in boiling water for a few minutes could drastically reduce or eliminate PAHs (*Akpoghelie*, 2018).

Eke et al. (2013) assessed the microbiological status of commercial Suya products in Ekpoma, Esan West local government area, Edo State. A total

of 40 Suya samples were collected from 20 randomly selected Suya spots (two samples from each spot). Six bacteria genera (*Staphylococcus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Bacillus* and *Paracoccus*), two moulds and two yeasts were isolated, with total viable counts ranging from 1.0×10^3 to 4.8×10^3 cfu/g. From the study, Suya sold in Ekpoma were potentially contaminated with microorganisms. Eke and co-workers called for the attention of relevant food regulatory authorities on Suya sold in the study locations.

In another study, the microbiological quality and proximate analyses of RTE fried chicken parts sold in eateries and roadsides in three local government areas (Egor, Oredo, and Ikpoba Okha) in Benin City, Edo State, were determined (*Osakue et al.*, 2016). From the study, 13 genera of bacteria and 7 genera of fungi were isolated, with *Proteus* (9.9%)*, Staphylococcus aureus* (9.9%), *Enterobacter* (8.5%) *and Micrococcus* (8.5%) having the highest prevalences. There was no *E. coli* or other hazardous food-borne pathogens in samples procured from eateries. Although, the highest microbial counts were recorded in meats from Ikpoba Okha, aerobic colony counts of bacteria and fungi in all meats were greater than allowed in the international food standards (which call for $<10^5$ cfu/g). The authors recommended the quality of fried chicken sold in these vending sites be improved (*Osakue et al.*, 2016).

In recent times, *Inobeme et al.* (2018) investigated the heavy metal contents of smoked fresh chicken, beef and goat meats from selected areas in Auchi, Edo State. The study revealed the content of metals, (except lead in smoked fish and iron in all samples) was within the safe limits as recommended by WHO. The authors suggested that meat sellers should be advised of the dangers associated with open smoking of public food.

Suya beef alongside samples of frozen and roasted Atlantic mackerel (*Scomber scombrus,* Scombridae), and plantain (*Musa paradiasca*) were randomly selected from three sales points in Amassoma town, Bayelsa State, and screened for the presence of 15 PAHs (*Amos-Tautua et al.*, 2015). Benzo[*a*]anthracene with mean level of 7.23 µg/g was detected in Suya beef, while a significant amount of benzo[*a*]pyrene (2.41 µg/g) and benzo[*b*] fluoranthene (4.51 µg/g) were found in roasted mackerel fish. PAH was not detected in roasted plantain or in the raw food items. The authors concluded that the levels of PAHs in roasted fish and Suya in Amassoma were above the permissible limits (*Amos-Tautua et al.*, 2015). They also suggested that people should not eat the charred skin of roasted fish, meat or poultry so as to reduce their intake of chemical hazards.

In Yenagoa city, Bayelsa State, 18 Suya meats from six communities (three meats from each) were assessed for microbiological quality. Although there was no significant difference in microorganisms from the six communities, six bacteria and four moulds were identified: *Aspergillus niger* (39.7%) was the most prevalent, followed by S*taphylococcus aureus* (28.1%), *Mucor* (11.8%) and *Proteus* (9.3%). *Escherichia coli, Bacillus, Micrococcus, Pseudomonas*, *Aspergillus flavus* and *Penicillium* were the other bacteria and fungi isolated (*Kigigha et al.*, 2016).

The bacterial status and antibacterial susceptibility profiles of selected pathogenic bacteria from eight Suya outlets in Bori city, Port Harcourt, River State, was assessed by Amadi and co-workers. The study revealed that RTE Suya meats were contaminated with a variety of bacterial species. Among 10 bacterial species (comprising six gram-positive and four gram-negative bacteria) isolated, *Staphylococcus aureus* and *Escherichia coli* were the most prevalent (*Amadi et al.*, 2015).

Recently, *Dibofori-Orji and ThankGod (2018)* evaluated heavy metals (iron, lead, cadmium, chromium and nickel) in raw and roasted Suya, sold and consumed in Iwofe, Trans Amadi and Port Harcourt city, River State. The study revealed the Suya studied contained lower than the permitted levels of iron (FAO/WHO permitted level for iron), chromium and cadmium (USDA permitted levels of 1.0 mg/kg and 0.5 mg/kg, respectively), levels of lead and nickel significantly exceeded the FAO/WHO permitted levels of 0.2 mg/kg for these metals.

Konne et al. (*2018*) determined the levels of bacterial contamination in 30 Suya samples from six locations in Bonny local government area, River State. The study revealed the Suya were contaminated with various bacteria, including pathogens: *Bacillus cereus* (10, 34%), *Salmonella* (5, 17%), *Staphylococcus aureus* (1, 3.4%)*, Klebsiella* (2, 6.8%), *Enterococcus* (6, 20%) and *Proteus.*

Microbiological analyses of RTE fish sold in three locations, Ozuoba, Rumuokoro and Ada-George in Port Harcourt revealed the total viable counts, *Staphylococcus* counts, and total coliform counts exceeded the acceptable microbiological standard levels. The authors concluded the locations for roasting the fish, the fish handling and vendors' personal hygiene could have contributed to the microbial loads of the roasted fish (*Odu and Ameweiye*, 2013).

Hazard analyses on two street-vended meat products — Suya and fried clam — popular in Akwa Ibom and Cross River States was carried out by visiting several street vending operations. Categories of foods collected were: raw meats, final products just after processing, after reheating and during holding. The study revealed that aerobic plate counts of the foods increased during handling, processing, storage, and reheating when compared to raw meat procured from the abattoir (*Ekanem*, 2000).

Another study evaluated the microbiological safety of 10 duplicates of different parts of fresh beef meat sold in two major markets (Watt and Marian) in Calabar city, Cross River State. High numbers of pathogenic *Klebsiella pneumoniae*, *Salmonella* and *Escherichia coli*, among others, were found in the fresh meats, which could be health risks for food poisoning. They recommended the populace should adequately cook fresh meat before consumption and the National Agency for Food and Drug Administration and Control (NAFDAC) should also ensure strict compliance of RTE meat producers to the food standard (*Ukut*, 2010).

South-east Nigeria

States constituting this zone are Enugu, Imo, Abia, Ebonyi and Ananbra (*Akinlua et al.*, 2015). In 1990, Sokari and Anozie investigated the occurrence of enterotoxin-producing strains of *Staphylococcus aureus* in 530 meat samples randomly selected from itinerant hawkers in traditional markets and some streets in Port Harcourt (Rivers State) and Enugu State. The study revealed the presence of coagulase-positive *Staphylococcus aureus* on 449 (84.7%) of the products, and among these *S. aureus* strains, 243 (54.1%) produced various enterotoxins, the majority of which were detected on fried beef and Suya rather than on fresh beef. The authors suggested the high level of contamination observed could have resulted from cross contamination due to excessive hand contact (*Sokari and Anozie*, 1990).

In 2002, Chukwura and Mojekwu evaluated the microbiological safety profile of Suya meat sold in various Suya spots in Awka urban area, Anambra State. The study revealed the Suya were contaminated with several genera of bacteria (*Bacillus*, *Staphylococcus aureus, S. epidermidis, Proteus*, *Micrococcus* and *Serratia*) and fungi (*Aspergillus flavus, A. niger, A. fumigatus* and *Fusarium*). A Suya meat collected from Tracas Station had the highest total viable count, of 95.5×10^4 cfu/ml while a lower count of 37.5×10 cfu/ml was recorded for Suya from beside the Ubatel Hotel (*Chukwura and Mojekwu*, 2002).

The bacteriological quality of Suya meats randomly selected from five Suya spots (Eke-Awka, Temporary site, Aroma, Okpuno and Ifite Awka) in Anambra State was evaluated. The following bacteria were isolated from Suya meats: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The coliform levels were above the approved limits due to unhygienic preparation and handling, indicating that such Suya meats could pose food safety risks to consumers (*Onuorah et al.*, 2015).

In another study, the bacteriological status of 12 roasted Suya meat samples procured from various roadside marketers in Enugu city, Enugu State was investigated. Bacterial isolates were identified as *Staphylococcus aureus* (35%), *Pseudomonas* (35%). *Staphylococcus aureus* (35%) and *Pseudomonas* (35%) were the most commonly isolated organisms followed by *Escherichia coli* (15%) and *Streptococcus* (15%). The sanitary condition of the Suya sold in those locations was below the required standard for human consumption. The authors suggested that handling by butchers and the use of contaminated water and equipment could be major sources of microbial contamination of the Suya meat (*Nwakanma et al.*, 2015).

The application of seasonings/spices and heating/processing methods on the levels of PAHs were evaluated on fried, roasted and cooked meats in Enugu city. Higher molecular weight PAHs were detected at toxic levels benzo[*a*]anthracene, benzo[*a*] pyrene, chryene and pyrene. Although, cadmium, copper, zinc, lead, chromium and iron present were within permissible limits, cooked meats had the least PAHs followed by fried and roasted meat respectively, indicating that the application of seasonings/spices in meat causes significant increases in the heavy metal content (*Okeke et al.*, 2018).

A recent study by *Okoli et al. (2018)*, on the prevalence, toxigenic potential and antimicrobial susceptibility profile of *Staphylococcus* isolated from roasted and spiced RTE beef, pork, chicken and goat meats in Enugu State, revealed that 9.4% of selected meats were contaminated with *Staphylococcus*. Of these, 79.2% were resistant to fusidic acid, but none were resistant to chloramphenicol, ciprofloxacin, linezolid or teicoplanin. Most of the contaminated samples were from open markets and motor parks rather than from a mechanic village.

In another study, the presence and levels of 11 PAHs in Suya alongside other three commonly consumed roasted foods (freshly roasted plantain, yam and fish) in Owerri municipality were assessed. The study revealed that Suya had the second highest level of PAHs (0.0372 mg/kg) after roasted plantain (0.0465 mg/kg) while roasted fish had the lowest level (0.0135 mg/kg). The authors concluded the levels of PAHs detected in the foods were above the WHO permissible limits and could predispose consumers to potential health risks (*Ogbuagu and Ayoade*, 2012).

The level of 10 PAHs in raw cow hide (ponmo) and lean beef (charcoal grilled Suya meats) obtained from Umuahia main market, Abia State were determined. The study revealed a greater concentration of three PAHs in Suya meats due to direct smoking over open-flame charcoal. The authors suggested adequate measures should be put in place to avoid the carcinogenic effect of PAHs from the smoking on consumers (*Ogbonna and Nwaocha*, 2015).

South-west Nigeria

Locations in this zone are Oyo, Osun, Ekiti, Ogun, Ondo, and Lagos States (*Akinlua et al.*, 2015). In 2008, Edema and co-workers carried out an 8-month microbiological safety survey on Suya samples from six Suya spots in South-west Nigeria between November 2005 and June 2006, with a total of 144 samples (24 replicates per sample). The study revealed microbial contamination in processing water, meat processing slabs, utensils, spices and raw meat. *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* and aflatoxigenic moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) were potential pathogens isolated from utensils and hands of the producers during slicing, staking onto skewers, spicing and holding at ambient temperature (28±2^{o}C). Based on this study, a critical limit for the critical control points was proposed by the authors (*Edema et al.*, 2008).

Samples of raw meat prior to roasting and Tsire-Suya samples were collected from five locations (University of Lagos, Bariga, Allen avenue round about, Ikeja) in Lagos State and were examined for total viable counts, coliform count, *Staphylococcus* count*,* and *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli.* From the study, lower bacterial counts were found in Tsire-Suya than in raw meat. Isolated organisms, except *P. aeruginosa*, were susceptible to the spices (*Afromomum melegueta*, *Piper guinense* and *Capsicum frutescence*) used for Suya preparation. The authors opined that the presence of isolates in Tsire-Suya could be due to post-processing contamination or poor processing (*Apata et al.*, 2013).

A comparison of physical, chemical, microbiological and organoleptic characteristics of Suya meats prepared in the laboratory as well as Suya meats from four locations (Yewa, Egba, Remo and Ijebu) in Ogun State were evaluated. The results showed that Suya prepared in the laboratory were more hygienic than Suya from the four other locations (*Iweala et al.*, 2014).

In 2014, Adebiyi and co-workers investigated heavy metal contamination of food, including Suya and drinks in Ota, Ogun State. The study revealed that most roasted food, including Suya were contaminated with nickel at levels above the FAO and WHO tolerable limits (*Adebiyi et al.*, 2008).

As potential bio-indicator of metal exposure, Suya meat and raw meat (serving as a control) sold in the open market, roadsides and motor parks in Lagos, Ile-Ife, Ogbomoso and Ibadan were investigated. Concentrations of iron, zinc, lead, manganese and copper were above the control levels in the raw meat and were above the recommended tolerable upper intake levels as supported by high values of pollution index (PI>1) (*Ologhobo et al.*, 2010).

A study was carried out on different varieties of chicken and beef Suya sourced from three locations in Ibadan city, Oyo State. Varieties studied were raw; spiced; spiced and roasted; leftover, unheated, spiced and roasted Suya from the previous day; as well as leftovers, heated, spiced and roasted Suya from the previous day. Chicken and beef Suya had microbial counts that could pose health risks to consumers. The authors advised of the need to educate Suya vendors in personal hygiene and environmental sanitation practices during their handling of products, as improved practices would prevent cross contamination (*Egbebi and Seidu*, 2011).

In a related study, the microbiological quality (total viable count) of RTE chicken and beef Suya selected from various spots within Oyo town, Oyo State, was investigated. The following bacteria and fungi were isolated from chicken and beef Suya meats: *Bacillus*, *Escherichia*, *Pseudomonas, Staphylococcus, Aspergillus* and *Penicillium*. The authors suggested sterile conditions should be employed in the meat industry to avoid food-borne diseases and infections (*Afolabi and Odubanjo*, 2015).

The quality and safety of 50 sun-dried meat products (kundi) from 10 major markets in Ibadan, Oyo State were assessed by *Adeyeye (2016)* for proximate composition, rancidity indices and the

presence of aflatoxigenic fungi and mycotoxins. Besides high proximal protein content, nine fungal strains, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamarri*, *Fusarium compacticum*, *Fusarium oxysporum*, *Fusarium sacchari*, *Penicillium chrysogenum*, *Penicillium citrinin* and *Penicillium oxalicum* were isolated from the kundi. The majority of kundi were contaminated with mycotoxigenic fungi and mycotoxins. The authors recommended proper monitoring of sun-dried meat sold in major markets in Ibadan.

The microbiological quality of 32 Suya meats sold in four locations within Ado-Ekiti and Akure was studied. Out of 15 genera identified on the products, 8 were bacteria, 4 were moulds and 3 were yeast. *Staphylococcus*, coliforms and *Aspergillus* were the most prevalent. The authors solicited for proper education of processors and consumers on good sanitary practices (*Egbebi and Muhammad*, 2016).

The safety of 20 Suya meats collected from 10 randomly selected Suya spots (two samples at different locations) within Ado Ekiti, Ekiti State, was evaluated. Among the five bacterial genera (*Escherichia coli, Enterobacter*, *Streptococcus*, *Staphylococcus aureus* and *Bacillus*) isolated, *Staphylococcus aureus* was the most prevalent (on 13; 65%), followed by *Streptococcus* (3; 15%), *Bacillus* (2; 10%), *Escherichia coli* and *Enterobacter*. The authors concluded that Suya meats from these study locations were unhygienic and called for the need to instruct Suya vendors on proper sanitation practices and safety hazards associated with improper food handling (*Falegan et al.*, 2017).

Microbiological analysis of 20 skewers of Suya meat obtained from four popular Suya spots in Owo, Ondo State, was carried out. The study revealed *Staphylococcus aureus* and *Pseudomonas* as the most prevalent isolates, followed by *Escherichia coli* and *Streptococcus*. The authors concluded the Suya meats sold in these locations were below the safety standard (*Egbebi and Seidu*, 2011).

The microbiological safety profiles of Suya meats procured from 10 locations, Mushin, Oshodi, Ikorodu, Shomolu, Ketu, Ojota, Surulere, Ikeja, Ebute-meta and Island in Lagos city, were investigated. The following bacteria were isolated: *Escherichia coli*, *Staphylococcus*, *Pseudomonas*, *Clostridium septicum*, *Micrococcus* and *Bacillus alvei* as well as fungi: *Mucor racmosios, Geomyces panorus, Penicillium* and many *Aspergillus*. As concluded by the authors, the presence of these organisms revealed the unhygienic condition of the meat sold in the study locations (*Hassan et al.*, 2014).

North-central Nigeria

Locations and states constituting this geopolitical zone are Federal Capital Territory (FCT), and Niger, Kwara, Nassarawa, Plateau, Kogi, and Benue States (*Akinlua et al.*, 2015). Daminabo and co-workers investigated the microbiological toxicity of 60 kilishi (dried beef cracker) randomly selected from five sale outlets in Abuja city. All bacterial isolates were resistant to cotrimoxazole and streptomycin, while 50% of them were sensitive to ampicillin. All isolates were sensitive to gentamicin while all *E. faecalis* isolates were susceptible to nitrofurantoin. On the other hand, enterococci isolates were resistant to more than one antibiotic. The authors suggested good hygiene practices among kilishi producers so as to eliminate the risk of contamination (*Daminabo et al.*, 2013).

A total of 50 Suya meats from various Suya spots in Abuja were investigated for microbial safety. *Staphylococcus aureus* (54%), *Escherichia coli* (4%), *Salmonella* (26%) and *Bacillus* (16%) were isolated. Total viable counts of bacteria ranged from 4.0×10^8 to 2.2×10^9 cfu/g. At 70°C, *Bacillus* thrived and was more resistant to heat. The authors concluded the presence of these pathogenic bacteria in the Suya calls for serious public concern, because such organisms could result in gastroenteritis and other infections associated with food poisoning (*Amaeze et al.*, 2016).

A microbiological analysis of Suya meats from four locations, Baze University, Kubwa, Lugbe and Maitama within FCT, Abuja, was carried out in 2016. The study revealed the presence of *Escherichia coli*, *Staphylococcus aureus*, *Providencia*, *Bacillus*, and *Pseudomonas aeruginosa* in the meats. Suya meats had significantly (1.5 to 5.9 times) higher total aerobic bacterial counts than did the raw meats. The authors opined that the higher level of contamination could be from the old newspapers used to wrap the Suya meat, contaminated spices, and exposure to airborne (cough, saliva), vector borne (flies) and vehicle borne (kitchen utensils) microbes (*Alonge et al.*, 2017).

Olaoye and co-workers assessed the effects of processing techniques (grilling and roasting techniques) and packaging materials (glass jar, aluminium foil, cling film and paper wrap) on the quality of Suya procured from spots in Tanke Oke-odo, Ilorin, Kwara State. The study revealed the crude protein (41.82%) and fat (9.92%) contents of roasted meats were significantly higher than those of the grilled meats, which were 39.92% and 8.36%, respectively. The authors concluded the roasting method and

storage of samples in glass jars or aluminium foil should be adopted (*Olaoye et al.*, 2016).

In a recent study, Folorunso and co-workers evaluated the microbiological quality of street-vended Suya sold in six major motor parks in Bida city, Niger State. The socio-demographic data revealed five of the vendors never washed their hands before touching raw meat, nine of them preserved their left-over Suya by spreading them in the open air, while 19 of them were not trained on how the product should be processed. High microbial loads were found in all Suya, due to poor hygiene practices by the vendors and as such, these products could constitute a food safety risk for consumers (*Folorunso et al.*, 2018).

A study by Ogbu and co-workers on 20 samples of beef Suya sold in Jos and environs, Plateau State revealed contamination with bacteria and fungi. Bacteria isolated were *Salmonella* (18.84%), *Escherichia coli* (13.04%), *Serratia* (11.59%), *Enterobacter* (10.14%), *Klebsiella* (8.70%), *Staphylococcus* (7.25%) and *Streptococcus* (5.80%). Fungi detected were *Candida albicans* (68.97%), *Aspergillus* (13.79%), *Absidia* (13.79%) and *Cunnighamella* (3.45%). They concluded that beef Suya sold in Jos and its environs were contaminated with bacteria and fungi that could constitute a public health problem (*Ogbu et al.*, 2016).

In another study, 240 Suya meats from four major market locations, low level, high level, Wadata and North Bank in Markurdi city, Benue State were investigated for microbial contaminants. The study revealed that although levels of the organisms (*E. coli*, *S. aureus*, *Salmonella*, *Klebsiella*, *Shigella*, *Penicillium*, *Rhizopus*, yeasts and protozoans) among the selected locations did not vary significantly before and after treatment, the level of indicator organisms harboured by Suya from these locations indicate potential threats to human health. The authors recommended the application of hazard analyses and critical control point (HACCP) programs or International Organization for Standardization (ISO) programs as essential programs (*Manyi et al.*, 2014).

Inyang and co-workers evaluated the bacteriological quality of Suya selected from four locations in Makurdi city. Mean total plate and coliform counts of Suya varied from 3.7×10^5 cfu/g to 2.4×10^6 cfu/g and 1.9×10^2 cfu/g to 1.0×10^3 cfu/g, respectively. Although, total plate count and coliform counts of most Suya were within recommended safe limits, faecal coliform bacteria were isolated from all Suya except that from Wurukum. The authors called for exigent enhancement on the hygienic handling of Suya by processors (*Iyang et al.*, 2005).

North-west Nigeria

The states in this zone are Kebbi, Sokoto, Zamfara, Katsina, Kano, Jigawa and Kaduna *(Akinlua et al.*, 2015*)*. In a recent study, microbiological analysis of 15 samples of Tsire meat from five spots in Wudil town, Kano State, was carried out. Prevalences of bacterial isolates from the meats were: *Staphylococcus aureus* (43.5%), *Shigella* (21.7%), *Salmonella* (21.7%) and *Escherichia coli* (13.0%), while fungal isolate prevalences were: *Aspergillus niger* (66.7%) and *Penicillium* (33.3%). The authors recommended that appropriate care should be taken during the preparation and handling of Tsire meat (*El-Hassan et al.*, 2018). Microbiological safety analyses of meat samples in Kaura Namoda, Northern Nigeria, were carried out. In the study, six samples were selected weekly over a period of one year from five locations (Sabon-gari area, Motor-park, Market, Academic area, Gulubi area). Fresh meat during the dry season had the highest level of microbial contamination, while samples of kilishi in the rainy season had the highest number of microscopic filamentous fungi. The authors concluded that meats sold in Kaura Namoda are very contaminated, mostly with fungi (*Olayinka and Sani*, 2014).

The lead contents of three commonly consumed Suya meats (beef, chevon and mutton) sold in two major streets in Sokoto city were investigated. Although, the results revealed the presence of lead, with concentrations under the legal limits, the authors recommended every Suya spot should have protective means against atmospheric particulates arising from vehicular traffic in urban regions (*Garba et al.*, 2017).

In another study, the bacterial quality of 216 local fried ground beef products (Dambun nama) sold in different retail outlets around Sokoto city was investigated by *Salihu et al.* (2010). Aerobic mesophiles (100%) were found in all meat samples, followed by faecal coliforms (49.5%) and *E. coli* (36.6%). The authors concluded that the products were not safe for human consumption because the levels of bacteria were above the acceptable limits.

The safety of 116 samples of traditional RTE (38 balangu, 39 kilishi and 39 tsire) meat products from retail outlets in Kebbi and Sokoto States was assessed by *Ribah et al. (2018)* using standard cultural microbiological procedures. From the study, 35/116 (30.17%) meats were contaminated with some of the studied pathogens, with the following prevalences: *Staphylococcus aureus*, 18 (15.51%), *Escherichia coli*, 12 (10.34%) and *Salmonella*, 5 (4.31%). The mean total bacterial count was 23.82×10^6 cfu/g.

Ribah and co-workers recommended that Kebbi and Sokoto State governments should conduct full-scale risk assessment studies on RTE meats.

A study of the incidence of extended spectrum β-lactamase producing bacteria and multi-drug resistant strains from 150 Suya meats procured from three spots in Samaru campus, Ahmadu Bello University, Zaria, was undertaken by Adenaike and co-workers. A total of 40 isolated *E. coli* was screened for extended spectrum **β-lactamase** (ESBL) production and confirmed using a double disk synergy test (DDST). The study revealed that 80% of the isolates had multi-drug resistance (MAR) index of 0.2 and above. The authors recommended the hygienic conditions in the preparation of Suya should be improved. Also, in order to enlighten people on proper antibiotic use, public campaign teams should be set up. Addition of antibiotics to animal feed as growth promoters was also recommended to be not used (*Adenaike et al.*, 2013).

In 2014, a study investigated the prevalence of *E. coli* O157 on 182 samples of raw meat, Suya (roasted meat), balangu (barbequed meat), kilishi (spiced sun dried meat) and dambu (shredded fried meat) in four major markets in Zaria city and from a local abattoir. Multiple drug resistance to antimicrobial agents was exhibited by all isolates. The raw meats had an overall *E. coli* O157 prevalence of 2.2%. The authors concluded the presence of this pathogen in meats suggested that consumers could purchase contaminated meat and meat products which would expose them to this foodborne hazard (*Tafida et al.*, 2014).

According to Belo and co-workers, in a study which assessed the level of beef carcass contamination with *Escherichia coli,* including serovar O157, before and after washing with water in North-west state abattoirs, increasing contamination of carcasses was observed during processing. The authors suggested that non-portable water used to wash carcasses might have contributed to contamination in all the abattoirs investigated. Thus, they recommended good hygiene practice and the use of potable water by abattoirs (*Bello et al.*, 2011).

North-east Nigeria

The six states in this geopolitical zone are Bauchi, Yobe, Borno, Gombe, Adamawa and Taraba (*Akinlua et al.*, 2015). Yusulf and co-workers carried out bacteriological analysis of 10 spiced and 10 unspiced 20 balangu (roasted meat) products from five retail outlets in Bauchi city. Prevalences of 14 species of bacteria of public health importance included *Bacillus cereus* (19.6%), *Staphylococcus aureus* (12.5%), *Escherichia coli* (10.7%), *Bacillus alvis* (7.1%)*, Proteus mirabilis* (7.1%) and *Streptococcus faecalis* (7.1%) among others. Average aerobic plate counts (cfu/g) of unspiced balangu were 2.25×10^6, 2.05×10^6, 2.47×10^6, 2.79×10^6 and 2.78×10^6 while those of spiced balangu were 2.66×10^6, 2.36×10^6, 2.69×10^6, 2.85×10^6 and 2.89×10^6 for the five retail outlets. The authors concluded the presence of isolates in meat products could pose gastrointestinal disorders, food poisoning and foodborne diseases (*Yusuf et al.*, 2012).

Microbiological analyses for the presence of methicillin resistant *Staphylococcus aureus* (MRSA) was carried out on 75 samples of processed meat, including 30 skewer meats (tsire), 30 roasted meats (balangu) and 15 dried meats (kilishi*)* from vendors in Gombe, Gombe State. From the study, 13.33% of the isolates were MRSA. The authors recommended that all meat producers and the general public should utilise good hygiene practices to avoid cross contamination of food products (*Shamsuddeen and Puma*, 2016).

Approximately 34 years ago, the microbiological status and moisture content of tsire type Suya retail products in Maiduguri were studied. The study revealed the presence of *Bacillus, Streptococcus, Staphylococcus, Escherichia, Proteus, Pseudomonas* and *Klebsiella.* Tsire products had levels of total bacteria and coliform counts higher than the acceptable limits due to handling at the retail level. At the time of the investigation, the authors opined that it could be difficult to establish and enforce microbiological guidelines for tsire (*Igene and Abulu*, 1984).

In 2012, microbiological and proximate analyses were carried out on Suya meats from five popular markets in Maiduguri city. The result revealed variations in proximate composition of crude protein, crude fat, ash and moisture content in various Suya meats. Microbial counts ranged from log 0.0 to log 8.08 cfu/g. The authors concluded that raw meat and beef Suya sold in Maiduguri were microbiologically unsafe, and improved hygiene was required (*Ogbonna*, 2012).

Health implications of contaminated RTE meat

Foodborne diseases result from intake of foods or drinks contaminated with pathogenic microorganisms (bacteria, fungi, yeasts and moulds) or chemicals (heavy metals and PAHs) *(Ogbu et al.*, 2016*)*. Whenever the level of contaminant surpasses its permitted limit, it becomes harmful to human health (*Kigigha et al.*, 2016). Categories of RTE meat contaminants are highlighted below.

Heavy metal contamination

Heavy metals are those with densities exceeding 5 g/cm^3 (*Inobeme et al.*, 2018). They tend to accumulate in RTE foods and may not undergo proper biodegradation following consumption (*Inobeme et al.*, 2018*; Okeke et al.*, 2018). Repeated ingestion of RTE contaminated with heavy metals from the environment, such as lead, cadmium, mercury and zinc, has various health effects and could result in metal accumulation in human organs, liver, kidney, lung and brain tissue, when the metals are not properly metabolised. This can lead to interaction with cell components, causing depletion of essential nutrients, DNA damage, cell cycle modulation, cancer, reduced immunological function and impaired psycho-social behaviours (*Ojebah and Ewhre 2015; Dibofori-Orji and ThankGod*, 2018). Although zinc is an essential element for human diet for normal growth and development, excess zinc can be hazardous to health, causing nausea and vomiting, epigastric pain, abdominal cramps and diarrhoea. Lead is a major source of heavy metal poisoning which can result in anaemia, calcium and zinc deficiency, encephalopathy seizures and mental retardation. Excessive intake of oxidising chromium can cause, skin inflammation, allergy, lung disorder and lung cancer (*Okeke et al.*, 2018). Iron from nutritional sources is essential for good health, as it serves as the source of haemoglobin iron and catalyst for enzymatic reactions.

PAH contamination

PAHs, which are condensed compounds of linked aromatic rings, are formed when organic material is inadequately incinerated. Sources of PAHs include wood, incense, diesel, tobacco, fuels, gas, coal, oil and biomass as a result of a series of complex chemical reactions (*Farhadian et al.*, 2011*; Ogbonna et al.*, 2012*; Okeke et al.*, 2018).

PAHs can build up during domestic and industrial food processing procedures, e.g. during smoking, barbecuing, drying, roasting, frying and grilling. Inhaled air can also be a source of PAHs (*Ogbuagu and Ayoade*, 2012*; Okeke et al.*, 2018). On the other hand, steaming and boiling introduce hardly any PAHs into processed food (*Ogbuagu and Ayoade*, 2012). PAHs have varying levels of toxicity. Although, some of them have no physiological function or benefits, they can be toxic even at trace amounts (*Inobeme et al.*, 2018). In most cases, lungs, breast, oropharynx, genitourinary and gastrointestinal tracts are organs that trap PAHs. Human and animal studies show exposure to PAHs results in poor foetal development and carcinogenesis due to PAHs binding to DNA and inducing mutations; they are also a cause of colon cancer (*Bastrom et al.*, 2002*; Olabemiro et al.*, 2011). Apart from water, air and soil, food, RTE meat is a notable means by which people intake PAHs (*Inobeme et al.*, 2018).

Microbial contamination

Indeed, pathogenic microorganisms have been shown to be associated with ill-prepared, packaged and preserved Suya, because protein, vitamin, fat and phosphorus contents in Suya facilitate their growth (*Onuorah et al.*, 2015*; Okoli et al.*, 2018). It is well documented that microbial loads in raw meat and Suya products tend to increase as long as growth conditions are favourable (*Okoli et al.*, 2018). Also, acidity, pH, temperature, water activity, gas atmosphere, available nutrients and competition with other microbes are factors which can influence microbial multiplication in RTE meat (*Egbebi and Muhammad*, 2016). Climatic conditions in the tropics also favour the persistence and proliferation of most pathogenic microorganisms (*Ekere et al.*, 2018).

After vending or hawking, leftover Suya products are often kept to be sold the following day, thereby providing the opportunity for rancidity and spoilage to occur in the products (*Onuorah et al.*, 2015). Consumption of such products, which may not be properly reheated, could result in foodborne diseases.

In most cases, S*almonella*, *Campylobacter, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Brucella*, *Vibrio, Yersinia enterocolitica, Streptococcus pyogenes* and *Shigella dysenteriae* among others, are pathogenic microorganisms associated with food poisoning when they are ingested via contaminated RTE meat (*Adeyeye*, 2016). Clearly, it is advisable for proper hygiene to be adopted by producers and consumers of RTE meats.

Signs and symptoms emerging from ingestion of contaminated food include nausea, vomiting, diarrhoea, abdominal cramp, pain, fever, and other clinical manifestations such as bloody diarrhoea, renal failure, sepsis, bacteremia and death (*Okoli et al.*, 2018*; Umar et al.*, 2018). The severity of these signs and symptoms is, however, dependent on the immune status of the individual and on the pathogenic potentials of the ingested organisms (*Okoli et al.*, 2018). Proper cleaning and disinfection should be carried out whenever vomiting occurs in a food handling area (*Umar et al.*, 2018).

In regards to a global concern for the safety of RTE meat products in Nigeria, numerous studies published in scientific journals have been conducted

to evaluate the microbiological status associated with purchase and consumption of RTE meats, including skewered Suya and dried meat.

Means of contamination of RTE meat

Abattoir employees and facilities

Insalubrious practices by some abattoir workers have been reported to cause microbial contamination even before the meat processing stage (*Bello et al.*, 2011). In most abattoirs, cattle carcasses are placed on bare floors or they are washed in water of unproven microbiological quality. Cross contamination of already slaughtered meat could also occur from adjacent raw meat via flies or unclean hands of the handlers (*Ologhobo et al.*, 2010).

Microbial contamination of meat also occurs from the use of contaminated equipment during the bleeding process, and during poor evisceration practices, when meat can be contaminated with gut contents (*Bello et al 2011; Acco*, 2003).

Processing

This is a major factor that predisposes RTE meat to microbial and chemical contamination. Although, it is common for raw meats to have no or low levels of microbial contamination (*Falegan et al.*, 2017), levels can increase with improper processing and handling (*Egbebi and Seidu 2011*). This occurs if the facility or instruments used, including water, raw meat, meat processing slabs, spices and utensils (*Ogbu et al.*, 2016*; Edema et al.*, 2008*; Egbebi and Seidu*, 2011) are of unproven microbiological quality (*Salihu et al.*, 2010). Inadequate municipal water supply can encourage producers to use alternative sources of water that are already contaminated. All these poor hygiene practices are the leading causes of microbial contamination of meat (*Okoli et al.*, 2018). Some meat processors do not subject their raw meat to proper heating because they assume that cooking beyond the stipulated time of about 30 minutes could cause loss of volatile nutrients. Also, some heat resistant toxins may not be completely eliminated during the heating period (*Okoli et al.*, 2018).

Packaging

Materials used to wrap processed RTE meats pose a significant means of contamination (*Eke et al.*, 2013). Most RTE meat vendors use inked paper, cement paper or old and abandoned newspapers which might be considered dirty, dusty and contaminated with insecticides. Importantly, use of packaging material of proven quality and stability would reduce product deterioration and extend the shelf-life of RTE meats (*Umar et al 2018*). Every good packaging material should retain proper thermal, mechanical, and optical properties for foods (*Chin et al.*, 2015).

Post-preparation, handling and vending processes

These processes can constitute public health risks. Occasionally, poor post-preparation handling of the meats can favour contamination of RTE meat with microorganisms from unwashed hands, due to poor personal hygienic practices of vendors (*Acco et al.*, 2003). *Staphylococcus* has been isolated from various body parts (hands, nose, hairs, skin and fingertips) of healthy individuals who purchase RTE meats (*Ogbu et al.*, 2016). RTE meat products can become highly contaminated during hawking processes or exposure to open air to attract potential buyers (*Uzeh et al.*, 2006). This latter exposure has posed an even greater means to contaminate RTE meats with environmental pollutants. Exposure of RTE meat to open air along streets, motor parks and highways could facilitate microbial and chemical contamination from vehicular traffic. Usually, wind currents and contaminated dust can also carry bacterial contaminants, which can be deposited on processed RTE meats (*Okoli et al.*, 2018). Studies have shown the high pH of meat encourages microbial growth, even under standard handling conditions (*Eke et al.*, 2013). Thus, improper storage of RTE meat until the following day by street vendors, which can involve ambient temperature and no proper reheating, could facilitate microbial proliferation (*Olaoye et al.*, 2016). Some vendors do not have the facilities required to reheat their leftover RTE meats (*Acco et al.*, 2003).

Measures to control contamination of RTE meat

With reference to the aforementioned literature, it is becoming increasingly clear that the health risks posed by microbial pathogens and potentially hazardous chemicals in the RTE meat sold in Nigeria cannot be overemphasised. This study has evaluated the safety status of RTE meat, especially Suya, sold in Nigeria. Categorically, there are three major RTE meat contaminants: microorganisms, heavy metals and PAHs. Most studies reviewed provided relevant recommendations to producers, regulatory bodies and consumers on how the quality of RTE meat sold in Nigeria could be improved, as highlighted below.

Advice to RTE meat producers

Suya vendors should be educated on good personal sanitation and hygiene practices in the course of processing and marketing of their products (*Yusuf et al.*, 2012*; Falegan et al.*, 2014*; Egbebi and Seidu*, 2011). Producers should ensure they properly wash their hands with soap and running potable tap water (*Fasoyiro 2012, Konne et al.*, 2018). They should consistently disinfect their facility and any equipment they use to process Suya meats (*Egbebi and Seidu 2011; Konne et al.*, 2018). *Hassan et al. (2014)* recommend Suya meat producers use aseptic techniques. Thus, healthy environment, proper handling, preservation and marketing would minimise microbial, heavy metal and PAH contamination. These steps would help minimise economic losses. Spices should be devoid of potential microbial, heavy metal and PAH contaminants. Processors should properly dispose of any solid and liquid waste acquired during processing to avoid cross contamination and environmental risks (*Fasoyiro*, 2012). *Folorunso et al. (2018)* recommend vendors store leftover Suya in freezers or cold rooms to prevent spoilage.

Adebiyi and co-workers recommend that Suya meat vendors should properly cover their products to minimise contamination from dust and vehicles, which are potential sources of heavy metals and airborne contaminants. They also suggest that Suya producers adopt indirect methods of heating Suya, such as microwave ovens (*Adebiyi*, 2008).

Studies in oil producing regions of the Niger Delta have revealed that various industrial activities in metallurgical, petrochemical, petroleum, oil and gas companies, and illegal refining of crude oil (bunkery) are major sources of heavy metals that can contaminate RTE meats (*Dibofori-Orji and Thank-God*, 2018). Thus, producers should take cognizance of these hazards.

In the area of packaging, Olaoye and co-workers recommend glass jars or aluminium foil is used for packaging RTE meats, because they yield better quality and hygiene than paper and other packaging materials, which are considered to be more affordable and economical (*Olaoye et al.*, 2016).

The roles of food safety regulatory organisations and agencies

This is a clarion call to the Federal and State Food Regulatory Authorities/Agencies to wake up to their constitutional tasks of ensuring that RTE meat products getting to consumers' tables are of better quality (*Ologhobo et al.*, 2010). Some of these agencies and organisations include the Federal Ministry of Health, the National Agency for Food and Drug Administration and Control (NAFDAC), the Standards Organization of Nigeria (SON), the National Codex Committee, the Federal Ministry of Agriculture, and state and local governments (*Omotayo and Denloye*, 2002*; Omojokun*, 2013).

There is a need for these agencies to set up local branches and laboratories at strategic locations nationwide to ensure proper monitoring of production, processing, packaging, distribution and vending sites and processes for RTE meats (*Yusuf et al.*, 2012). This can be achieved by periodic sampling and screening of such products for possible contamination (*Yusuf et al.*, 2012). Suya spots should be certified based on standards but not on a monetary basis (*Alonge et al.*, 2017). Suya producers and handlers having open wounds or skin infection should be banned from such services (*Konne et al.*, 2018). Also, sales of RTE meats should be restricted to specified safe locations, as this would aid the meats' sanitary condition (*Yusuf et al.*, 2012). A study carried out by *Orogu and Oshilim (2017)* revealed that hawked Suya meats were more contaminated with pathogenic bacteria than Suya meats sold via barbeque stands. In order to avoid excess contamination of raw meat and the spread of diseases, abattoir staff should ensure that sick animals are isolated from healthy animals for proper treatment (*Yusuf et al.*, 2012). Also, a control mechanism to improve the quality of spices by established, effective methods to decontaminate RTE meat spices is needed (*Odu and Best*, 2016).

Compulsory training and retraining workshops for certified RTE meat handlers should be established. This would aid in verifying the knowledge of processors and vendors. Topics that could be incorporated into such training curriculums include the importance of good hygiene and hand washing, relevance of sanitising work premises, sources, growing conditions, dangers and controls of microorganisms, foodborne diseases, allergies, cross contamination, storage temperature, packaging, fundamentals of HACCP, rapid methods of identifying microorganisms during processing and storage (*Fasoyiro 2012; Folorunso et al.*, 2018).

Possible dangers associated with contaminated RTE meat products should be communicated to producers (*Chin et al.*, 2015). Since RTE meats in oil-producing locations are susceptible to heavy metal contamination (*Dibofori-Orji and Thank-God*, 2018*),* statutory, regulatory laws banning cattle raising, abattoirs and Suya spots in these locations

should be put in place. Better still, such facilities should be sited very far from any environment capable of causing heavy metal discharge or emission.

Advice to consumers and the general public

Through various education programs, consumers should be instructed about the health implication of consuming contaminated RTE meat products (*Yusuf et al.*, 2012). Consumers of Suya and other RTE meats should properly reheat the meats to easily inactivate/kill microorganisms (*Yusuf et al.*, 2012). Suya meat is required to be reheated to about 75°C before consumption (*Konne et al.*, 2018). Consumers should also insist vendors use sterile foil paper or polyethylene to wrap Suya, but reject the use of newsprint, inked paper and cement paper (*Yusuf et al.*, 2012). Consumers should patronise only certified Suya vendors (*Alonge et al.*, 2017). Consumers could further heat process RTE meat products after purchase, which would aid in minimising microbial contamination (*Alonge et al.*, 2017). People in Niger Delta are advised not to consume the charred skin of roasted meat and fish, which can harbour chemical hazards, including PAHs and heavy metals, to a greater extent than the interior edible parts (*Amos-Tautua et al.*, 2015). Also, consumers should soak these charred products in hot water for a few minutes before consuming them, to aid in eliminating PAHs, heavy metals and other toxins (*Akpoghelie*, 2018).

Conclusion

To a large extent, this study has provided a review on hazards associated with RTE meat, particularly Suya meat, sold in Nigeria. The outrageous microbial, PAH and heavy metal contamination observed in RTE Suya meat in many studies was mainly attributed to poor hygiene, packaging, and vending processes. Thus, caution should be exercised by the populace who consume these RTE meat products. Implementation of the above recommendations would greatly minimise the number/spread of foodborne diseases and risks posed by substandard RTE meat in Nigeria.

Zdravstveni rizici povezani sa gotovim mesom za konzumiranje u Nigeriji: Razlog za zabrinutost javnosti i kritične intervencije

Earnest Erhirhie, Chuka Nwosu, Tedwins Emudainohwo, Chidimma Chukwunwejim, Peter Eze, Daniel Ajaghaku

A p s t r a k t: Naučna ispitivanja vrste gotovog mesa (RTE), popularno zvanog Suya, koje se prodaje u Nigeriji, izazivaju naučnu i javnu zabrinutost zbog mikrobioloških i hemijskih opasnosti povezanih sa proizvodima. U ovom pregledu ocenjen je bezbedonosni profil nigerijskog gotovog mesa RTE, sa posebnim naglaskom na Suyu mesu, kao potencijalnom izvoru mikrobiološke opasnosti u hrani, kao i na prisustvo teških metala i policikličnih aromatičnih ugljovodonika (PAH). Rezultati istraživačkih radova o bezbednosti RTE mesa objavljenih od 1984. do 2019. godine su procenjivani korišćenjem elektronskih baza podataka i pretraživanjem ključnih reči. Ishodi istraživanja svrstani su u šest delova koji predstavljaju šest geopolitičkih zona Nigerije (jug-jug, jugoistok, jugozapad, severoistok, severozapad i severno-centralni deo). Praktično svi nalazi istraživanja u različitim zonama otkrili su da su mikrobiološko prisustvo, kao i nivoi teških metala ili PAH u mesu RTE, viši od dozvoljenih granica i prihvatljivih standarda. Nehigijenske aktivnosti većine mesara (izvori sirovog mesa), prerađivača (koji pripremaju i pakuju RTE meso) i prodavaca (koji su uključeni u procese izlaganja) glavni su faktori koji doprinose mikrobiološkim i hemijskim rizicima. U tom cilju, predlažu se odgovarajuće bezbednosne i sanitarne mere, a nadležni organi i relevantne zainteresovane strane trebalo bi da primene druge osnovne stvari kako bi se osiguralo drastično smanjenje rizika od nehigijenskog RTE mesa.

Ključne reči: *RTE meso, bezbednost, mikrobiološka kontaminacija, hemijska kontaminacija, Nigerija.*

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Evaluation of sausages obtained from mechanically separated Nile tilapia (*Oreochromis niloticus*) meat and prepared using different homogenizing and refining processes

Angela Dulce Cavenaghi Altemio[1], Rosângela Cacho Ferreira[1], Gustavo Graciano Fonseca[2]*

A b s t r a c t: After filleting Nile tilapia (Oreochromis niloticus), unused parts can be processed to obtain the co-product, mechanically separated meat (MSM). The aims of this study were to use different processes for homogenizing and refining of Nile tilapia MSM sausages and to evaluate cooked sausages in terms of their microbiological, physical, chemical and sensory characteristics. Ingredients were processed according to three treatments: (T1) using a grinder and cutter, (T2) using a grinder and mixer, and (T3) using only the grinder. The protein content ranged from 15.08% (T3) to 15.91% (T1), lipids from 9.61% (T3) to 12.29% (T1), and ash from 1.83% (T1) to 2.73% (T3). The highest color lightness score was 57.28, for the sausage elaborated by the conventional method (T1). The obtained shear forces were 2.04 N (T1), 2.71 N (T2) and 1.77 N (T3). Only T2 sausages received an acceptability index higher than 70%. T2 sausages also were rated by 34% of panelists as "certainly would purchase" or "probably would purchase". In conclusion, it is feasible to produce sausages from Nile tilapia MSM by using a grinder and mixer for homogenizing and refining, which would be a good alternative method mainly for small producers.

Keyword: *fish, fish flesh, quality, sensory analysis, process methods.*

Introduction

The limited consumption of fish meat in some countries is not only due to economic and cultural factors, but it is also the consequence of a limited availability and diversity of species and products based on this type of meat (*Marques et al.*, 2020). Brazil has extremely favorable conditions for fish farming. In addition to the great market potential, the country has a favorable climate, good availability of land areas, extensive grain cropping to produce raw materials for animal feed, and good water potential (*Merengoni*, 2006). Nile tilapia (*Oreochromis niloticus*) is farmed worldwide due to its fast growth, easy handling, high yield and excellent quality meat (*Fonseca et al.*, 2013).

After filleting Nile tilapia, unused parts can be processed to obtain a co-product similar to mechanically separated red meat (MSM). A mechanized process separates the edible parts of the fish, generating skeletal meat particles free of viscera, bones and skin (*Cavenaghi-Altemio et al.*, 2018). Fish MSM is an intermediate product that serves as a raw material to produce surimi, fish burgers, fish fingers, nuggets, croquettes, pates, mortadella, sausages etc. (*Dallabona et al.*, 2013; *Palmeira et al.*, 2016; *Husein et al.*, 2020), so incorporating high-quality fish nutrients into these fish products (*Verdi et al*. 2020).

Sausages are the main products obtained from fish MSM. The sausages are made from an emulsion obtained by mixing water-soluble and fat-soluble ingredients in a cutter, preferably under vacuum and at low temperature. The resulting mixture, due to the extraction of soluble proteins, becomes viscous and the pieces of meat become adherent. The meat mixture is then filled into natural casings, bladders or other animal membranes or appropriate plastic casings. The sausages are made with meat or other edible animal parts, and can be dyed, skinned, cured, seasoned, cooked, smoked, and dried (*MAPA*, 2000).

[1]*University of Grande Dourados, Faculty of Engineering, Federal, Laboratory of Food Technology, Rua João Rosa Goes 1761, Vila Progresso, Dourados-MS, Brazil;*
[2]*Federal University of Grande Dourados, Faculty of Biological and Environmental Sciences, Laboratory of Bioengineering, Rua João Rosa Goes 1761, Vila Progresso, Dourados – MS, Brazil.*

***Corresponding author:** Gustavo Graciano Fonseca, *ggf@ufgd.edu.br*

The aim of this research was to evaluate different processes for homogenizing and refining sausage obtained from Nile tilapia (*Oreochromis niloticus*) MSM in terms of their microbiological, physical, chemical and sensory characteristics.

Materials and Methods

Mechanically separated Nile tilapia meat (MSM)

About 100 Nile tilapia (*Oreochromis niloticus*) carcasses were utilized in the research. The fishes were produced in a fish farming system and weighed, on average, 0.700 ± 0.025 kg. After filleting by a local fish processing plant, about 67% of the total weight remained in the carcasses, totaling approximately 47 Kg. These carcasses were transported for 40 min under refrigerated conditions to the Laboratory of Bioengineering at the Federal University of Grande Dourados, Dourados, MS, Brazil, and immediately utilized to produce MSM. The MSM was produced in 3 mm particle size using a meat-bone separator (HT 250, High Tech, Brazil), operating at an inlet temperature of 6°C and outlet temperature of 10°C (*Cavenaghi-Altemio et al.*, 2018). Approximately 35 Kg of MSM obtained was immediately utilized to produce sausages.

Sausages produced from Nile tilapia MSM using different homogenizing and refining processes

To prepare the Nile tilapia sausages, the same formulation for all treatments was used (%): MSM, 89.14; soybean protein, 4.00; cassava starch, 2.00; refined salt, 1.80; spices, 1.30; sodium polyphosphate, 0.50; sugar, 0.40; liquid smoke, 0.40, cochineal carmine, 0.40; ascorbic acid, 0.05; and sodium nitrite, 0.015. The additives and the condiments were supplied by Cavenaghi Eireli (Dourados, MS).

The treatments differed according to the type of process by which the sausage was elaborated: treatment 1 (T1) using the grinder and cutter (conventional production); treatment 2 (T2) using the grinder and the mixer; and treatment 3 (T3) using only the grinder. For T1, the MSM was thawed and weighed, milled using a 5 mm disc grinder (Weg, Jaraguá do Sul) and emulsified in a cutter (*Sire Filizola, São Paulo*) along with the other formulation ingredients, previously weighed. For T2, the MSM was thawed and weighed, milled using a 3 mm disc grinder, and mixed in a mixer (CAF Máquinas, São Paulo) together with the other formulation ingredients, previously weighed. For T3, the MSM was thawed and weighed, milled using a 5 mm disc grinder and mixed manually together with the other formulation ingredients, previously weighed. Then, this mass was milled a second time using a 3 mm disc grinder.

After that, the respective sausage stuffings were filled manually into cellulosic casing, caliber 26 (*Picelli, Rio Claro*). All sausages were cooked by maintaining them at 55ºC for 15 min, at 75°C, for 15 min, and at 85°C until the internal temperature of the product reached 74°C, before thermally shocking them at 0°C. The casings were removed, and the cooked sausages were left in a 5% solution of urucum dye for 20 min. They were then transferred to a phosphoric acid solution, pH 2.0 to 3.0, for 5 min. The sausages were refrigerated for 12 h, packed under vacuum, and kept under refrigeration prior the analyses. Each treatment was divided into three lots in order to evaluate the results of triplicates.

Chemical analysis

Moisture, crude protein, and crude ash contents of the sausages were determined in triplicate according to the methods described by *AOAC* (2012). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46), protein by the Kjeldahl method (method 928.08) and ash by the muffle oven technique (method 920.153). The lipid content was obtained in triplicate by the extraction method with cold organic solvent (*Bligh & Dyer*, 1959). The carbohydrate content was estimated by difference.

Physical analyses

Instrumental color

The color indices [CIE L*(lightness), a* (redness), b* (yellowness)] of the sausages elaborated from Nile tilapia MSM were determined using a colorimeter (Minolta Chroma Meter CR 410), with measurements standardized with respect to the white calibration plate (*Jiménez & Gutiérrez*, 2001). Six readings were made from the internal part of the sausages.

Shear force

Texture analysis of the sausages was carried out using a texture analyzer Model TAXTplus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Sausages were equilibrated at room temperature (28–30°C) before analysis. Samples of 15×15×20 cm were cut, placed in the texture analyzer and submitted to a cutting/shearing test (speed of 1.0 mm/s, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the

shear force (N), which indicated the firmness of the sample. A minimum of 10 replicates of each treatment were analyzed (*Kang & Chen*, 2014).

Microbiological analysis

Microbiological analyses of the sausages elaborated from Nile tilapia MSM were performed in triplicate for presence/absence of *Salmonella* spp., and counts of coagulase-positive staphylococci and thermo-tolerant coliforms at 45ºC, in accordance with the methodology described elsewhere (*USDA/FSIS*, 1998).

Sensory analysis

Sensory analyses of the sausages elaborated from Nile tilapia MSM were conducted by 50 non-trained panelists. A vertically structured nine-point hedonic scale of mixed category (9 = like extremely; 1 = dislike extremely) was used for evaluation of the attributes of color, taste, texture, odor, and overall acceptability. Samples (2 cm-long pieces) were prepared by steeping the sausages in boiling water for 3 min, draining the liquid, and holding the warmed sausage on a warming tray in covered plates for no longer than 30 min. Then, three slices of each treatment were presented to the panelists in monadic form, randomly coded with three digits. In the same sheet, the panelists recorded their purchase intention using a 5-point scale, where 5 = certainly would purchase, 4 = probably would purchase, 3 = perhaps would purchase / perhaps would not purchase, 2 = probably would not purchase and 1 = certainly would not purchase, which was expressed as the percentage of total score (*Cavenaghi-Altemio et al.*, 2018). The acceptability index (AI) was calculated according to the following equation: AI = (average of the attributed grades/maximum attributed grade) × 100. The sample was considered accepted if the AI was greater than 70% (*Stone and Sidel*, 2004).

Statistical analysis

Results were statistically evaluated using analysis of variance (ANOVA) and Tukey's test for comparison of means, at a level of 5% significance, using the software Statistica 7.0. The sensory attributes and the purchase intention were analyzed as percentages.

Results and Discussion

Microbiological analyses

Microbiological evaluations were conducted to ensure the safety of the raw materials and the efficiency of the sausage preparation processes. The microbiological results were within the limits established by Brazilian legislation (Table 1), which require the absence of *Salmonella* spp. in 25 g, and maximum counts of $3x10^3$ CFU g^{-1} for *Staphylococcus aureus* and $5x10^3$ CFU g^{-1} for coliforms at 45°C (*ANVISA*, 2001). These criteria are in accordance with the U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, which established a zero tolerance for *Salmonella* spp. and a limit of 10^4 CFU/g for *Staphylococcus aureus* in fish and fishery products (*FDA*, 2020). There was no clear relationship among the microbial microbiological results and the different treatments.

Chemical analyses

Proximate compositions obtained for sausages obtained from Nile tilapia MSM, elaborated using different homogenizing and refining processes are presented in Table 1. These values are very close

Table 1. Proximate composition and microbiological status of sausages prepared with mechanically separated Nile tilapia (*Oreochromis niloticus*) meat according to treatments T1, T2, and T3.

Treatment	Processing	Proximate composition (%)					Microbiological analyses (CFU/g)		
		Moisture	Protein	Lipids	Ash	Carbohydrates	TTC	CPS	*Salmonella* spp.
T1	grinder/ cutter	$60.66^a \pm 2.37$	$15.91^a \pm 1.70$	$12.29^a \pm 1.23$	$1.83^a \pm 0.17$	9.31	$<1\times10^2$	$<1\times10^3$	Absent in 25g
T2	grinder/ mixer	$68.73^a \pm 5.09$	$15.67^a \pm 0.09$	$11.35^a \pm 0.53$	$2.48^b \pm 0.49$	1.77	$<1\times10^2$	$<1\times10^3$	Absent in 25g
T3	Grinder	$60.64^a \pm 11.75$	$15.08^a \pm 0.22$	$9.61^a \pm 2.47$	$2.73^b \pm 0.13$	11.94	$4{,}5\times10^2$	$<1\times10^3$	Absent in 25g

Legend: Means with the same letter in the same column do not differ statistically at 5% (P>0.05). TTC: thermotolerant coliforms; PCS: coagulase-positive staphylococci bacteria; CFU: counting forming units

to those already reported for sausages from Nile tilapia MSM (*Uyhara et al.*, 2008; *Oliveira Filho et al.*, 2010; *Mélo et al.*, 2011).

Moisture, protein, and lipids did not differ between the three treatments ($P<0.05$). Ash was the unique parameter that differed significantly between the treatments ($P>0.05$).

The moisture content of the sausages (Table 1), on average, met the required level for identity and quality of sausages (maximum 65% moisture) (*MAPA*, 2000). Previously, an average moisture content of 70.75 % and range from 69.21 to 70.35% were reported in the literature for Nile tilapia MSM sausages, without significant differences in relation to the amount of MSM in the sausages (*Oliveira Filho et al.*, 2010), or to the nature of added colorant (*Uyhara et al.*, 2008), respectively.

The protein content ranged from 15.08% (T3) to 15.91% (T1) (Table 1). According to the Brazilian legislation, the minimum protein content in commercial meat products containing MSM is 12% (*MAPA*, 2000), and products containing lower protein levels can be considered as out of specification or even fraudulent. The prepared sausages contained superior protein levels and were considered satisfactory. Literature reported protein content in similar sausages ranged from 18.40 to 19.84% (*Uyhara et al.*, 2008), with average protein content of 13.02% (*Mélo et al.*, 2011), and 15.26% and 20.86% in Nile tilapia sausages containing 0% MSM and 100% MSM, respectively (*Oliveira Filho et al.*, 2010).

The average content of lipids found in the current study ranged from 9.61% (T3) to 12.29% (T1) (Table 1), which were also within the limits determined by Brazilian legislation (*MAPA*, 2000) that requires a maximum of 30% lipids. Lipid contents of 3.45% (*Uyhara et al.*, 2008) and 11.03% (*Mélo et al.*, 2011) were measured in sausage formulations containing Nile tilapia MSM, and 0% and 8.18% in Nile tilapia sausages containing 0% MSM and 100% MSM, respectively (*Oliveira Filho et al.*, 2010).

It was reported elsewhere that differences in composition could be related to differences in the raw materials and/or in the ingredients (*Cortez-Vega et al.*, 2013; *Cavenaghi-Altemio et al.*, 2018). This could explain the differences observed in relation to the results obtained by other authors. *Bordignon et al.* (2010) stated that MSM extracted from the abdominal muscle that is close to the cartilage of Nile tilapia has a high fat content. *Rebouças et al.* (2012) reported that the lipid content in the fish is very variable, depending on the species, age, body region, sexual cycle, and diet.

However, our present study utilized the same additives and condiments, and the Nile tilapia MSM was from the same batch; moreover, all ingredients were used at the same concentrations for the three treatments. On the other hand, the processing conditions might also affect the results (*Cortez-Vega et al.*, 2013), but statistical differences were not observed between the treatments in our study. This could affirm that utilization of grinder and cutter, grinder and mixer, or only grinder did not affect the sausage composition, so our particular types of sausage processing did not influence the characteristics of the MSM (*Mielnik et al.*, 2002), although our procedures could have altered the structure of biomolecules.

The average ash contents of the sausages were 1.83%, 2.48% and 2.73% for treatments T1, T2 and T3, respectively (Table 1). The ash content of T1 sausages differed from that of T2 and T3 sausages ($P > 0.05$). It is hard to explain how ash content could differ in the three treatments. However, the ash content found was more or less in accordance

Table 2. Instrumental color, shear force, and sensory analysis of sausages prepared with mechanically separated Nile tilapia (*Oreochromis niloticus*) meat according to treatments T1, T2, and T3.

Treatment	Instrumental color			Shear force (N)	Sensory analysis				
	L*	a*	b*		Color	Taste	Texture	Odor	OA
T1	$57.28^{a} \pm 0.98$	$8.75^{b} \pm 0.22$	$8.56^{a} \pm 0.26$	$2.04^{b} \pm 0.36$	$5.47^{a} \pm 0.37$ (60.8)	$5.56^{a} \pm 1.02$ (61.8)	$6.36^{a} \pm 0.45$ (70.7)	$6.06^{ab} \pm 0.75$ (67.3)	$5.60^{a} \pm 0.28$ (62.2)
T2	$45.54^{b} \pm 2.62$	$14.31^{a} \pm 1.43$	$5.30^{c} \pm 0.69$	$2.71^{a} \pm 0.47$	$5.84^{a} \pm 0.11$ (73)	$5.84^{a} \pm 1.30$ (73.0)	$5.82^{a} \pm 1.28$ (73.2)	$6.24^{a} \pm 1.58$ (78.0)	$5.84^{a} \pm 1.30$ (73.0)
T3	$46.28^{b} \pm 0.78$	$14.94^{a} \pm 1.00$	$6.26^{b} \pm 0.76$	$1.77^{b} \pm 0.17$	$5.30^{a} \pm 0.91$ (66.2)	$4.74^{b} \pm 1.23$ (59.2)	$4.80^{b} \pm 0.14$ (60.0)	$5.50^{b} \pm 1.06$ (68.7)	$4.80^{b} \pm 1.27$ (60.0)

Legend: T1, T2, and T3 treatments (see footnote to Table 1). L*: Lightness; a*: Redness, b*: Yellowness, OA: Overall acceptability. Means with the same letter in the same column do not differ statistically at 5% ($P>0.05$). Values in parenthesis are the acceptability index (%).

with the refined sodium mineral added in the form of NaCl (*Nowsad et al.*, 2000). Average ash contents of 3.40% and ranging from 3.2% to 3.7% were measured for Nile tilapia MSM sausages, without significant differences in relation to the inclusion of MSM (*Oliveira Filho et al.*, 2010) or the nature of added colorant (*Uyhara et al.*, 2008), respectively, and 1.08% in a Nile tilapia MSM emulsified-type sausage (*Mélo et al.*, 2011). These high ash levels in Nile tilapia sausages were due to the added curing salts that raised the mineral content (*Cavenaghi-Altemio et al.*, 2018; *Nascimento et al.*, 2017).

Physical analyses

Table 2 shows the results of instrumental color and shear force tests obtained for the Nile tilapia MSM sausages prepared using different homogenizing and refining processes.

Instrumental color

The values of L* (lightness), a* (redness), b* (yellowness) and W (whiteness) were significantly different (P>0.05) in all three treatments. When both grinder and cutter were utilized, sausages were lighter (P>0.05) but less red (P>0.05). Yellowness differed significantly (P>0.05) between the different treatments (Table 2).

The highest lightness value found was 57.28, for sausage elaborated by the conventional method (grinder and cutter). This value is comparable to that reported elsewhere (67.12) for sausage prepared from Nile tilapia MSM (*Lago et al.*, 2018). These differences in lightness could be related to different levels of incorporation of pigment from the remaining fins and skins present on the carcass frames during the passage of the residues by the meat and bone separator (*Uyhara et al.*, 2008). However, the significant difference (P>0.05) observed in the present study for all color parameters of T1 in relation to treatments T2 and T3 could be the result of some synergistic effect on meat rebinding during gelling, improving lightness and yellowness and reducing redness.

Shear force

The shear force differed among treatments T1, T2, and T3 (P>0.05) (Table 2). The obtained average shear forces were 2.04 N (T1), 2.71 N (T2) and 1.77 N (T3)). Similar results were obtained for Nile tilapia sausages without (2.02 N) and with 0.6% (2.95 N) of added transglutaminase (*Cavenaghi-Altemio et al.*, 2018).

Rupture force was reported to significantly correlate with the protein composition in cooked sausages, which suggests the increased gel rupture force is most likely due to the functional performance of the protein type rather than the protein content (*Wang and Xiong*, 1999). Despite the prepared sausages having the same composition, significant differences were obtained among the treatments (P>0.05), which could result from the different processes to which the fish meat (the main component) and the other ingredients were subjected. Therefore, we suggest the processing with different equipment could have differently altered the protein structure of the fish meat, by rupturing it at different levels. Moreover, the remaining protein structures have to be gelled to develop a proper sausage texture (*Jaczynski & Park*, 2003). The results indicate the combination of processing methods could have favored the disruption of the structures, and consequently the gelling during pasteurization, which could be beneficial to the firmness of the product. For example, despite the sausages having the same protein content, a greater exposure of functional groups in myofibrillar proteins would favor cross-linking interactions between –SH groups and the formation of S-S bonds (*Moosavi–Nasab et al.*, 2019).

Sensory analysis

The results of sensory analysis of the Nile tilapia MSM sausages are shown in Table 2. There was no significant difference in color scores between the three treatments (P<0.05). The mean color scores ranged from “like moderately” to “like very much”.

The odor scores did not differ between treatments T1 and T2, or between T1 and T3, but the odor score of T3 differed significantly (P>0.05) from that of T2.

The texture score did not differ between treatments T1 and T2, but that of T3 differed significantly (P>0.05) from those of T1 and T2. This indicates the grinder itself does not favor good homogenization, and consequently, a more uniform texture was achieved when the grinder was combined with the cutter (T1) or the mixer (T2). With the continuous mechanical action, the released compounds can react with each other, forming new structures, so the meat and fat particles, or their mixtures, adhere to each other due to the force of mixture.

The taste scores for T3 sausages differed significantly (P>0.05) from those for T1 and T2 sausages (Table 2). This could also be explained by the sole utilization of the grinder to prepare T3 sausages.

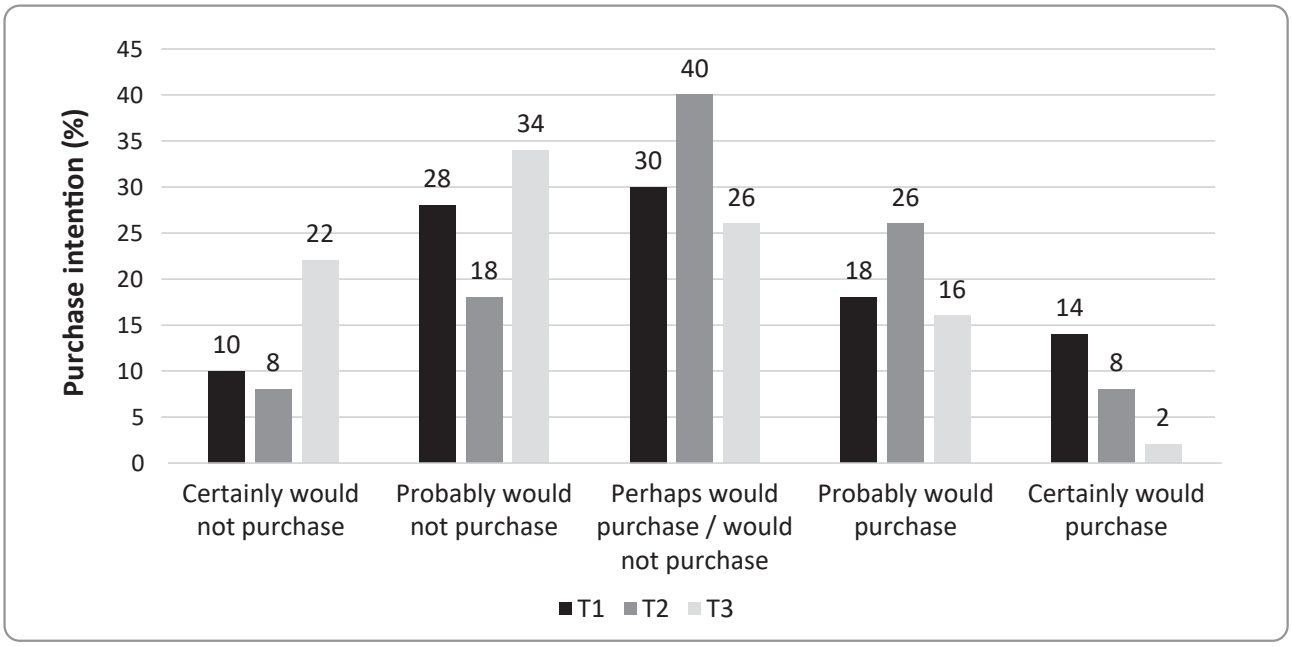

Figure 1. Panelists' purchase intentions for Nile tilapia MSM sausages prepared using different treatments (T1: grinder and cutter; T2: grinder and mixer; T3: grinder).

Grinding uses mechanical energy to disorganize the tissue structure, leading to the formation of grains composed of more or less intact cells. However, the amounts of lipid and proteinaceous compounds obtained are scarce at this milling stage (*Cenci et al.*, 2018).

According to the hedonic sensory analysis test, T1 and T2 sausages received grades close to 6 (like slightly). The highest average AI was 72.2% for the T2 sausages. According to *Stone and Sidel* (2004), when the AI is equal to or greater than 70%, the product is considered accepted. Thus, only our T2 sausages were acceptable to the panelists.

When the panelists were asked about their intention to purchase sausages, for T2 sausages, 26.0% "probably would purchase", while 40.0% "perhaps would purchase, perhaps would not purchase", which were higher percentages than for T1 and T3. However, the highest score for "certainly would purchase" was obtained for T1 sausages (14.0%) (Figure 1). These results could be related to the greater consumption of red meat than fish derivative products in the region where the research was conducted.

Rejection rates ("certainly would not purchase") around 10% and 8% were obtained for T1 and T2, respectively. T3 sausages received a much higher rejection rate of 22% (Figure 1), which is consequence of this sausage receiving the lowest scores for the texture and taste (Table 2), as already discussed.

Conclusion

In conclusion, it is feasible to produce sausages from Nile tilapia MSM using the grinder and mixer for homogenizing and refining (treatment 2), which would be suitable production means for small manufacturers. Considering that this product would be accepted on the market if made commercially available, sausage production using this basic equipment is an opportunity that could be exploited by the fish industry to augment the consumption of lower-cost Nile tilapia meat products.

Ocena kobasica dobijenih od mehanički odvojenog mesa nilske tilapije (*Oreochromis niloticus*) i pripremljenih različitim postupcima homogenizacije i prerade

Angela Dulce Cavenaghi Altemio, Rosangela Cacho Ferreira, Gustavo Graciano Fonseca

A p s t r a k t : Posle filetiranja nilske tilapije (Oreochromis niloticus), neiskorišćeni delovi se mogu preraditi kako bi se dobio koprodukt, mehanički odvojeno meso (MSM). Ciljevi ovog istraživanja su bili korišćenje različitih postupaka za homogenizaciju i preradu kobasica od mehanički odvojenog mesa nilske tilapije i ocena kuvanih kobasica u pogledu njihovih mikrobioloških, fizičkih, hemijskih i senzornih karakteristika. Sastojci su obrađeni prema tri tretmana: (T1) korišćenjem mašine za mlevenje mesa i rezača/kutera, (T2) korišćenjem mašine za mlevenje mesa i mešalice/miksera i (T3) samo korišćenjem mašine za mlevenje mesa. Sadržaj proteina kretao se od 15,08% (T3) do 15,91% (T1), lipida od 9,61% (T3) do 12,29% (T1), a pepela od 1,83% (T1) do 2,73% (T3). Najviša ocena za boju bila je 57,28, za kobasicu izrađenu konvencionalnom metodom (T1). Dobijene sile presecanja bile su 2,04 N (T1), 2,71 N (T2) i 1,77 N (T3). Samo kobasice T2 su dobile indeks prihvatljivosti veći od 70%. Kobasice T2 su bile ocenjene od strane 34% učesnika u panel diskusiji kao proizvod koji bi „sigurno kupili" ili „verovatno bi kupili". Zaključno, moguće je proizvesti kobasice od mehanički odvojenog mesa nilske tilapije upotrebom mašine za mlevenje mesa i mešalice/miksera za homogenizaciju i preradu, što bi bila dobra alternativna metoda uglavnom za male proizvođače.

Ključne reči: *riba, meso ribe, kvalitet, senzorna analiza, metode prerade.*

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Original Scientific Paper

Computer Vision System: A better tool for assessing pork and beef colour than a standard colourimeter

Bojana Milovanović[1], Ilija Đekić[1], Bartosz Sołowiej[2], Saša Novaković[1], Vesna Đorđevic[3], Igor Tomašević[1]*

*A b s t r a c t: The aim of this paper was to evaluate the use of computer vision system (CVS) to calculate CIE colour coordinates of beef and pork, as compared to a traditional Minolta colourimeter. Statistical analysis revealed significant differences of the colour parameters (L*a*b*, hue angle and chroma) using these two different techniques for colour detection. The CVS methodology produced colours highly similar to the visual assessment tests, but the Minolta colourimeter did not. The CVS-obtained colours were similar to the colours of both pork and beef samples visualized by trained panellists on the monitor, but colourimeter-obtained colours differed. The frequency of similarity for CVS-obtained colours and the actual meat colours as seen by the trained panellists was 100%. These results indicate that the CVS could be a superior alternative over the conventional Minolta colourimeter by offering improved representativeness and accuracy. In addition to providing objective colour measurement, it offers other possibilities that can be of benefit in further quality control or research within the meat industry.*

Keywords: *colour, pork, beef, computer vision system, colourimeter.*

Introduction

These days, people usually select products based on colour (*Akcay et al.,* 2012), especially in the case of meat (*Mancini and Hunt,* 2005). In general, it is well known that colour is one of the main aspects in sensory acceptance (*Fernández-Vázquez et al.,* 2011). Consumers associate colour with freshness, ripeness, desirability and flavour. Regarding fresh meat, a bright red colour is related to freshness, whereas a brownish colour denotes undesirability and unacceptability. At the present time, all food goods need to be monitored, in order to guarantee a satisfactory level of quality and safety.

For reliable and objective colour detection, colour measuring devices are used. So far, two types of commercial colourimeters have been most commonly used: the Minolta chromameter and the Hunter Lab colourimeter. Currently, the Minolta colourimeter is frequently used for meat colour assessment (*Tapp et al.,* 2011). Both devices offer simple and fast food colour analysis, moreover, they are easy to handle and calibrate. However, each colourimetric instrument has various settings such as (1) colour system i.e. CIE, Hunter, tristimulus, (2) illuminants (A, C, D_{65}), (3) observers (0, 2, 10) and (4) aperture size (0.64–3.2 cm).

The colourimeters are the handheld instruments that provide simple, rapid and easy to apply routine analysis of meat colour. However, there are some limitations related to the colourimeters; the measurements could be subjective and hard to reproduce (*Larraín et al.,* 2008). Moreover, these devices only provide average values of a small portion of the entire surface area (only a few cm^2) and therefore, many sampling locations and the number of readings must be measured to obtain a representative colour data (*Mendoza et al.,* 2006). Additionally, the food should have a uniform surface and colour (*Goñi and Salvadori,* 201). As a main reason for deviations in measurements many researchers quoted light reflection (*Trinderup et al.,* 2014) especially in the case of meat (*Girolami et al.,* 2013).

To overcome some of the limitations of the colourimeter we suggest using a computer vision system (CVS). Unlike the traditional colourimeter, the CVS measures colour readings across the entire sample. CVS has the advantage of determining L*, a*, b* values for each pixel of a sample's images, providing rapidness, precision, objectiveness, efficiency and

[1]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of Serbia;*
[2]*University of Life Sciences in Lublin, Faculty of Food Sciences and Biotechnology, Department of Milk Technology and Hydrocolloids, Skromna 8, 20–704, Lublin, Poland;*
[3]*Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Republic of Serbia*

***Corresponding author:** Bojana Milovanović, *m.bojana@agrif.bg.ac.rs*

non-destruction. Furthermore, many studies used CVS to detect PSE or DFD pork meat (*Chmiel et al.,* 2012; *Chmiel and Slowinski*, 2016a*; Chmiel et al.,* 2016b), or to predict pork and beef colour and marbling (*Jackman et al.,* 2008; *Sun et al.,* 2016; *Sun et al.,* 2018).

Hence, the purpose of this study was to evaluate the performance of using CVS and its possible advantages over Minolta colourimeter. Comparisons between CVS and colourimeter for meat colour measurement have already been investigated (*Tomasevic et al.,* 2019a; *Tomasevic et al.,* 2019b; *Tomasevic et al.,* 2019c). Nevertheless, to the best of our knowledge, there are no studies evaluating the suitability of CVS for evaluating pork and beef colour parameters. Thus, the aim of this study was to apply CVS to pork and beef in order to investigate whether it could be a superior tool over a conventional colourimeter for colour assessment of these meats.

Materials and Methods

Sample preparation

The research was conducted on *m. longissimus dorsi* pork and beef (three of each species), which we purchased in a retail setting. The meats were individually placed on white polystyrene plates with a consistent colour and overwrapped with a transparent PVC film permeable to oxygen. The PVC film was removed before colour measurement. Measurements were taken at room temperature on a freshly cut surfaces of slices about 3 cm thick of loin and after 30 min bloom time at 4°C.

Colour assessment

Two different colourimetric instruments were used to assess pork and beef colour. Colour of pork and beef samples was estimated using following the methods as reported in our previous study (*Tomasevic et al.,* 2019a). Colour readings (L*a*b*) were read by a traditional Minolta colourimeter and a computer vision system (CVS). Seven replicate measurements on different parts of the freshly cut loin surfaces were taken for all six loins (3 pork and 3 beef) and results were expressed as means.

Minolta colourimeter

We used a Minolta CR-400 colourimeter (Konica Minolta, Osaka, Japan). Each of the meat samples was measured at seven circular sites, each with a diameter of 8 mm. The measurements were performed under D65 standard illumination and pulsed xenon lamp as a default light source, 2° standard observer. Before the colour assessment, the device was calibrated with its white reference tile supplied by the manufacturer (Y=88.6, x=0.3175 and y=0.3350). Furthermore, this device was equipped with a CR-A33a accessory in order to measure the colour of solid samples.

Computer Vision system (CVS)

A CVS was used in this work for image acquisition (*Tomasevic et al.,* 2019a). It basically consists of the following elements: a cubical box, an illumination source, a high-resolution digital camera and a PC with image processing software. The computer vision system is shown in Figure 1.

Figure 1. Computer vision system (*Tomasevic et al.*, 2019a)

Cubical (light) box: Black box (a=80 cm) with a removable top designed for the colour measurement was constructed from the wood. All internal walls were covered with matt black material in order to reduce any kind of light reflection. The entry for samples is located in the foreground of the box.

Light source: The samples were illuminated using 4 lamps (60-cm long), each a fluorescent tube (Master Graphica TL-D 90) with a colour temperature of 6500 K (D65; the standard light source widely used in food research) and a colour-rendering index (Ra) approaching 98%. Each lamp is located at a 45° angle and 50 cm above the samples in order to produce as uniform and diffuse illumination as possible.

Digital camera: A colour digital camera Sony Alpha DSLR-A200 was placed over the sample

holder inside the imaging-acquisition apparatus. The settings of the digital camera used in the colour measurements are summarised in Table 1. The high-resolution pictures were stored in RAW format. The digital camera was placed at distance of 30 cm from the samples. Before taking digital images, the camera was calibrated using a 24-tile patter colour sheets with different hues (X-Rite Colourchecker Passpord, Michigan, USA) represented by coloured quadrates (4×4 cm^2). This procedure was done by photographing the card and putting it into specific software (ColourChecker Passport 1.0.1, X-Rite Inc.). The calibrated card inside the CVS apparatus was photographed and analysed in order to obtain the L*c, a*c and b*c values for each colour sheet, which were then compared with the measured colours (L*m, a*m and b*m).

Table 1. Digital camera settings

Parameters	Values
Size of the image	3872×2592
Image file format	RAW
Iso velocity	100
Aperture Av	F/11.0
Exposure Tv	1/6s
Image sensor	CCD
Focal distance	30mm
Lens	DT-S18-70mm f 3.5-5.6
Flash	Off
Modes	Manual (M)

Software: The computer hardware and software, arranged to simulate the human brain, is another key component of the CVS. The hardware consists of a personal computer and monitor. The PC provides disk storage for images and specific application programs. A high-resolution colour monitor provides the visualisation of captured images and the effects of various image analyses. The external monitor with sRGB was previously separate hardware calibrated using X-rite i1 display pro device. Colour management includes creating ICC profile with i1Profiler 1.5.6. software by selecting settings of brightness (white point) adjusted at 6500 K (D65), luminance (140 cd/m^2) and contrast (gamma) at 2.2. Adobe Photoshop was used to scrutinise images, due to its many advantages such as low cost, availability and many image editing features (*Yam and Papadakis*, 2004). The colour parameters were measured with RAW image format using the special average colour sampler tool (31×31 pixels).

Quantification of colour

The colour parameters measured were L*a*b*, hue angle, chroma, ΔL, ΔH, ΔC and total colour difference.

The L* value defines the lightness and can vary from 0 (black) to 100 (white). The a* value (+/) signifies the redness (red to green), and the b* value (+/) characterises the yellowness (yellow to blue).

Hue angle (h°) refers to the degree of the dominant spectral component, such as red, green, and blue, and ranges from 0° to 360°. An angle of 0º or 360º represents red hue, while angles 90º, 180º and 270º define yellow, green and blue hues, respectively. Combining a* and b* provides a better indication than their individual values; it is calculated based on the formula (*Salueña et al.,* 2019):

$$h_{ab} = \arctan\left(\frac{b^*}{a^*}\right)$$

Chroma (C*) is defined according to the following mathematical function:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

and it defines the vividness or saturation of a colour (*Salueña et al.,* 2019).

The difference between chroma and lightness values was calculated using following equations:

$$\Delta C^* = C_c^* - C_M^*$$

$$\Delta L = L_c^* - L_M^*$$

Values for C_c^* and L_c^* were obtained from the meat samples using CVS and for and using the Minolta colourimeter.

Hue difference (H^*) was measured using the following formula according to (*Mokrzycki and Tatol,* 2011):

$$\Delta H^* = \sqrt{\Delta E^2 - \Delta L^2 - \Delta C^2}$$

Colour changes can be measured as total colour difference (ΔE). ΔE indicates the magnitude of colour difference between any two samples using the following equation:

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$$

$$\Delta L^* = L_1 - L_2$$

$$\Delta a^* = a_1 - a_2$$

$$\Delta b^* = b_1 - b_2$$

Values for a_1, b_1, L_1 were acquired using the CVS, whereas a_2, b_2, L_2 were acquired using the Minolta colourimeter.

Visual assessment

A trained panel of 12 assessors was recruited to carry out three sensory tests according to their normal colour vision. The Ishihara test (The Colblinder online Ishihara 38 plate) is used to diagnose possible colour blindness due to the fact that it is a valid screening test for colour vision deficiency (*Van Staden et al*., 2018). The minimum passing result was 18/21. Panellists' training was performed using Blendoku (blendoku.com) software. To access the ability of their eye colour perception, they completed the hue test (IQ colour test; X-Rite, Prato, Italy) with a maximum passing result of 20, which means almost perfect colour eyesight.

For all the sensory tests, panellists were kept a distance of approximately 60 cm from the calibrated monitor, equipped with a shade that reduces glare (Compushade Universal Monitor Hood, DulCO, USA), and from the meat samples presented inside the wooden light box. For the first test (test A), panellists were requested to compare a digital photograph on the monitor and a meat sample presented inside the light box. They assessed if there was similarity between them by answering "yes" or "no". If yes, the panelists recorded the level of similarity according to a 5-point scale from 1 – very low, 2 – low, 3 – moderate, 4 – high to 5 – very high. For test B, they were asked to estimate which of the two generated colours was more similar to the product colour visualised on the monitor. During the final test (test C), the panellists were asked to complete the triangle test. In this test, three colours were presented on the monitor, one of which was odd. Additionally, the assessors graded the extent of dissimilarity from 1 – very low level of dissimilarity to 5 – very high level of dissimilarity.

Statistical analysis

All statistical analyses were conducted with SPSS software (SPSS 23.0, Chicago, IL, USA). Instrumental colour measurement differences were measured with the t-test, whereas the data obtained from visual assessment tests (A, B) were analysed to determine based on the frequency of each response ($\chi 2$ one-sample test), where the expected frequency was 50%.

Results and Discussion

Pork colour assessment

Emphasize that this refers to meat part of pork muscle (the meat part of pork muscle), the colour traits measured with CVS and colourimeter were significantly different with the exception of b* (Table 3). Higher lightness (L*), lower redness (a*), and relatively higher yellowness (b*) indexes of pork meat were read by the colourimeter compared to the CVS. The magnitude of colour difference between the two methods used is best represented by the . For meat and fat parts of the muscle, was 16.7

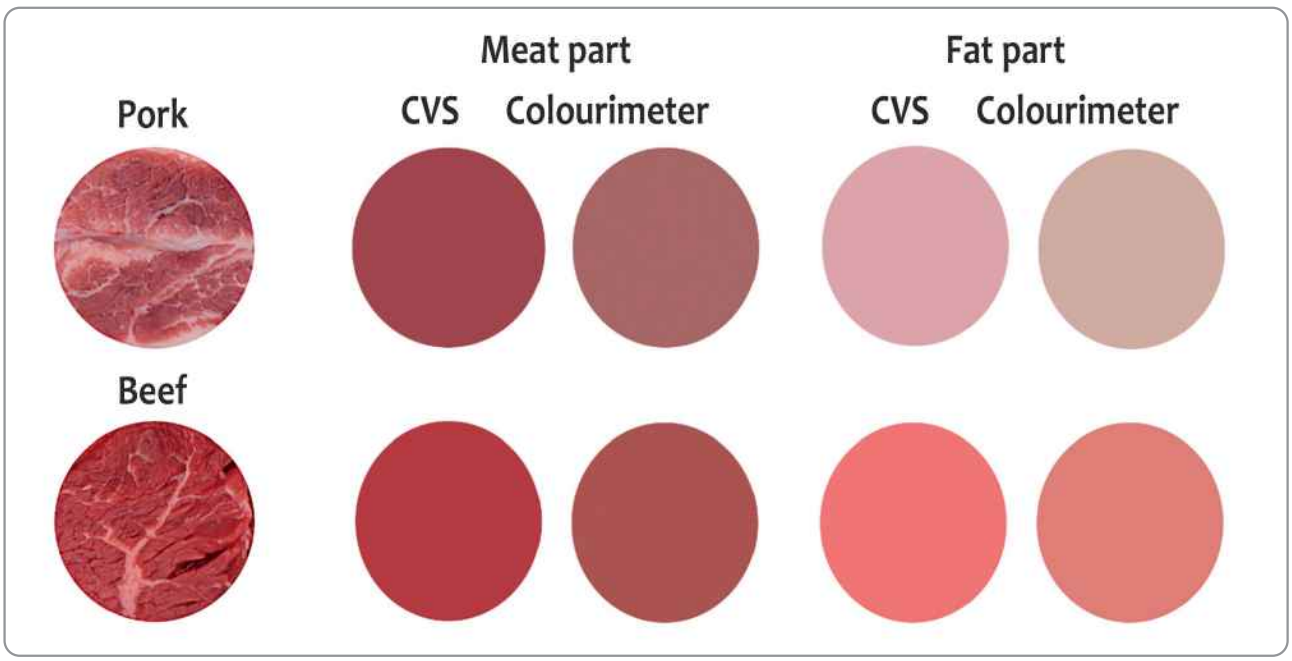

Figure 2. Colour of meat samples (pork and beef) measured by the computer vision system (CVS) and colourimeter

and 10.8, respectively, indicating that for meat parts, the colour difference between the two methods was even contrasting. Meat and fat parts were assessed as having darker colours when measured with the CVS than when measured with the colourimeter (Figure 2). The CVS-obtained colours of meat and fat parts were more saturated (positive values) than colourimeter values. All hue angle values of pork (both meat and fat parts) were significantly larger when measured with the colourimeter compared to CVS measurements. The CVS-generated colours of meat and fat parts were shifted in a clockwise direction from colourimeter-generated colours, representing, once again, a shift in the red direction.

The surface roughness and texture, the amount of surface gloss, the geometry of the measuring instrument and various other factors can affect colour analyses. In the case of fresh pork, as a bi-coloured meat that consists of meat and fat parts, its shininess can lead to specular reflectance, which results in chromatic components having smaller measurements. In addition, the colourimeter is dependent on both absorption and scattering properties of the test material. In our investigation, light employed in both instruments had the same colour conditions (6,500 K), but the light interface with the meat was obviously device dependent. Therefore, our results revealed that the colourimeter could not be suitable for the colour analysis of meat due to the fact that meat is a translucent and optically non-homogenous medium. This causes deviations in meat colour measurement resulting from the diffusion of light from illumination, making the colourimeter less accurate than the CVS. This study demonstrated the fact that CVS depicted more realistic meat colours than the colourimeter. Our observations are in good agreement with *Girolami et al.* (2013), who confirmed CVS was more precise and results were closer to the exact colour values than those of the colourimeter. This aspect was also reported by *Yagiz et al.* (2009), who stated that the reflectance properties of fresh meat can affect the colourimeter measurements and that diffuse illumination of the sample can be a way of overcoming this problem. In addition, *O'Sullivan*

Table 2. Colour values obtained using computer vision system (CVS) and colourimeter (mean±standard deviation; n=3)

Parameter	CVS	Colourimeter	Significance	CVS	Colourimeter	Significance
	Pork (meat part)			**Pork (fat part)**		
L*	39.3±2.3	49.8±2.8	***	73.3±4.5	73.9±2.2	
a*	33.1±1.6	20.4±2.4	***	15.0±4.1	7.9±1.6	**
b*	10.9±1.3	11.3±1.3		5.1±3.1	9.6±2.0	*
Chroma	34.9±1.4	23.4±2.4	***	15.9±4.9	12.5±2.4	
Hue angle	18.2±2.5	29.1±3.0	***	17.3±6.9	50.6±5.2	***
ΔE*	16.7±3.1		ΔE*	10.8±2.8		
ΔL*	−10.3±4.0		ΔL*	−0.6±5.1		
ΔC*	11.5±1.3		ΔC*	3.4±5.5		
ΔH*	5.5±2.0		ΔH*	7.8±1.7		
	Beef (meat part)			**Beef (fat part)**		
L*	39.3±2.6	44.1±2.1	*	60.2±2.2	59.6±3.4	
a*	42.6±1.4	29.4±2.8	***	42.2±1.1	30.4±2.6	***
b*	19.6±1.7	16.6±2.3	*	19.2±1.9	17.3±2.4	
Chroma	46.9±1.9	33.8±3.6	***	46.4±1.8	35.0±3.4	**
Hue angle	24.7±1.4	29.4±1.2	***	24.4±1.6	29.6±1.4	**
ΔE*	15.1±3.9		ΔE*	13.0±2.4		
ΔL*	−4.8±4.5		ΔL*	0.6±4.8		
ΔC*	13.1±4.3		ΔC*	11.4±3.3		
ΔH*	3.3±1.3		ΔH*	3.6±1.5		

Level of significance: * = P<0.05; ** = P<0.01; *** = P<0.001

et al. (2003) also postulated that CVS is more representative of real colour than the colourimeter, when pork colour was evaluated.

Beef colour assessment

Considering beef meat, the colour results returned by the two methods showed statistically significant differences. The values of the L*, a*, b*, hue angle and chroma obtained with the CVS and the colourimeter are shown in Table 2. Lightness (L*) for the meat part of beef muscle measured with the colourimeter was higher than that obtained using the CVS. In contrast, the other colour attributes of a*, b* and chroma values, gathered through the CVS, were always higher in both meat and fat parts than measurements obtained using the colourimeter. Hue angle values were higher with the colourimeter than with CVS, resulting in the non-real appearance of beef sample. We emphasized that total colour difference refers to meat part of beef muscle () for the meat part of beef muscle was 15.1, indicating the colours assessed by the two methods were opposite (*Brainard,* 2003). The colour obtained by the colourimeter has a non-real appearance, and that could be related to the penetration distance of the light into the samples. In beef samples*, Girolami et al.* (2013) assessed that the light from a colourimeter illuminates about 15–20 mm deep, but light from the CVS penetrates about 5 mm. Similarly, *Trinderup et al.* (2015) found that light penetrates about 20 mm from a colourimeter, and a few mm from the CVS. With regard to our results, they are in good agreement with findings from previous investigations (*Goni et al.,* 2016; *Girolami et al.,* 2013) that the colour predicted with the CVS is closer to the sample than the colour read by the colourimeter, making CVS more representative for beef colour evaluation.

Visual assessment tests

The results of the first visual test (test A) between the colour of the sample inside the light box and the CVS-produced colour on the display screen showed the panellists found the same colour of meats inside the box as the samples presented on the display. The frequency of similarity was 100.0% for both pork and beef meats (Table 3). This means that 12 out of 12 panelists found the sample colour was similar to the colour produced using CVS. The level of similarity recorded by the panellists ranged from moderate to high (Table 3).

The second test (test B) exposed that fact that CVS-observed colours were more similar to the actual meat sample when displayed on the PC, than were the colourimeter-observed colours, with panellists finding 100% similarity for pork and beef samples (Table 3).

The triangle test (test C) revealed there is a large difference between CVS and Minolta colour results, and this is a good agreement with our instrumental results (Table 3). The colour difference between these two methods ranged from 4.0 to 4.2 (high) for pork and beef, respectively.

According to the visual assessment tests, we found the CVS-produced colours more resembled the actual colours of the meat than did the colourimeter-produced colours. In conclusion, colours read by CVS are more realistic and representative of the true colours of both pork and beef muscle than those produced by the Minolta colourimeter.

Conclusion

Overall, our research on colour assessment proved that despite similar measurement conditions for the two studied methods, significant differences were observed. Our results show that employing a CVS is a valid alternative to the standard colourimeter. In fact, the CVS-obtained colours better represent the actual colour of meat samples as perceived by trained assessors (visual assessment tests) than the colourimeter-obtained colours. Taken together, our data clearly demonstrate the CVS methodology is more accurate and precise than the colourimeter for measuring colour of beef and pork. Although using a colourimeter for meat colour evaluation is

Table 3. Visual assessment tests (mean±standard deviation; n=3)

	Frequency of similarity (test A)	Level of similarity (test A)	CVS vs. Colourimeter (test B)	Level of difference test (test C)
Pork meat	100.0%	2.6±0.8[a]	CVS (100.0%)	4.2±0.7[a]
Beef meat	100.0%	4.1±0.5[b]	CVS (100.0%)	4.0±0.7[a]

CVS: computer vision system. Means in the same column with different small letters are significantly different ($p<0.05$). 5-point scale ranks from 1 – very low, 2 – low, 3 – moderate, 4 – high, 5 – very high.

regarded as reliable, it proved to be less accurate than CVS. Therefore, the CVS should be seriously taken into account as a more suitable alternative to the conventional method for measuring the colour of meat samples. Besides offering better objective measurement, it provides other possibilities that can be of benefit in quality control and research in meat science.

Kompjuterski vizuelni sistem kao alternativno sredstvo za procenu boje svinjskog i goveđeg mesa

Bojana Milovanović, Ilija Đekić, Bartosz Sołowiej, Saša Novaković, Vesna Đorđević, Igor Tomašević

*A p s t r a k t: Cilj ovog rada bio je da se proceni upotreba kompjuterskog vizuelnog sistema za izračunavanje CIE koordinata boje govedine i svinjetine u poređenju sa tradicionalnim Minolta kolorimetrom. Statistička analiza otkrila je značajne razlike u parametrima boje (L *, a *, b *, nijansa i hroma) koristeći ove dve različite tehnike za detekciju boje. CVS metod je bio vrlo sličan testovima vizuelne procene u poređenju sa Minolta kolorimetrom. Boja dobijena pomoću uređaja CVS bila je sličnija uzorcima svinjetine i govedine u odnosu na boju dobijenu kolorimetrom. Učestalost sličnosti bila je 100%. Ovi rezultati pokazuju da bi CVS mogao biti superiorna alternativa u odnosu na klasični Minolta kolorimetar nudeći poboljšanu reprezentativnost i tačnost. Osim što pruža objektivno merenje boje, nudi i druge mogućnosti koje mogu biti od koristi u daljoj kontroli kvaliteta ili istraživanju u industriji mesa.*

Ključne reči: *boja, svinjetina, govedina, kompjutre vision sistem, kolormetar.*

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Brief paper

Application of FMEA analysis in the short cheese supply chain

Biljana Aleksić[1], Ilija Đekić[2], Jelena Miočinović[3], Nurgin Memiši[4], Nada Šmigić[2]*

A b s t r a c t: The aim of this study was to apply Failure Mode and Effect Analysis (FMEA) methodology to determine the biological, chemical and physical failures that could occur during the farmhouse production of white brined cheese (short cheese supply chain) in Serbia. For that purpose, the values for occurrence (O), severity (S) and detection (D) of failures were determined. These estimated values were used to calculate risk priority number (RPN), for each potential failure. Very high RPNs were determined for biological failures in this short cheese supply chain. The highest RPNs were determined for the milking step, followed by the cheese ripening step and the transport of cheese by personal vehicle. The main chemical risks associated with raw milk were the presence of aflatoxins and antibiotic residues. Our results indicate the greatest risks in the short cheese supply chain can be attributed to biological and chemical failures, due to any failures being unlikely to be detected by cheese producers and having severe consequences. The proposed corrective measures include different pre-requisite programs. Even the application of these measures will not result in great risk reduction, as the severity and detection will remain the same. The lowest RPNs were obtained for physical failures, as they are visible and, therefore, easier to detect.

Keywords: *short cheese supply chain, farmhouse cheese production, FMEA analysis, risk.*

Introduction

Cheese is a fresh product or a product of different stages of maturity, which is obtained by separating whey after coagulation of milk (cow, sheep, goat, buffalo and/or their mixtures), cream, whey, or a combination of these raw materials or by using other technological solutions to achieve milk coagulation. Cheese can also be seen as a way to conserve raw milk, because the technological process of cheese production usually results in a reduction of pathogenic microorganisms **(***Serbia*, 2010a; *Zhao*, 2013**).**

According to the data published by the Serbian Bureau of Statistics, a total of 1,475,000,000 L of cow and sheep milk were produced in Serbia in 2018 (*Serbia*, 2019). Data published by authors from the Faculty of Agriculture in Novi Sad (*Vlahović et al.*, 2018) revealed that 54% of the total amount of raw milk produced was delivered to dairies, while about 18.6% was processed into cheese. Cheese production can be performed in households, i.e., small artisan processing, or in industrial conditions in which large quantities of milk are processed daily. In this paper, we use the term farmhouse cheese to denote a product produced according to traditional methods by cheese producers in households from milk derived from their own cows. This type of production, which implies a direct link between producers and consumers, is known as a short food supply chain (*Malak-Rawlikowska et al.*, 2019) and was considered in our study. In our case, this chain is further referred to as the "short cheese supply chain", since in Serbia, almost 15% of total cheese produced is made in households and is sold at green markets. Contrary to this, long food supply chains are very complex, with observed changes of food quality and safety throughout the entire supply chain, until food is finally consumed (*Yu et al*., *2013*).

Each year, according to the World Health Organization (*WHO*, 2019), about 600 million people become ill with food-borne and water-borne

[1]*Dairy Institute, Autoput za Zagreb 3, 11070, Belgrade, Republic of Serbia,*
[2]*Department of Food Safety and Quality Management, University of Belgrade – Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Republic of Serbia,*
[3]*Department of Technology of Animal Products, University of Belgrade – Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Republic of Serbia,*
[4]*AD Mlekara – Subotica, Tolminska 10, 24000 Subotica.*

***Corresponding author:** Nada Šmigić, *nadasmigic@agrif.bg.ac.rs*

diseases, resulting in about 420,000 deaths. More than 320,000 food-borne illnesses are registered in the EU each year. According to data from 2018, a total of 5,146 food- and water-borne outbreaks were registered caused by zoonotic agents, among which campylobacteriosis was the most common food-borne disease in the EU, followed by salmonellosis, from which most of the cases were caused by eggs and egg products, 2.2% cases were caused by cheeses, Shiga-toxin producing *E. coli*-induced diseases and yersiniosis (*EFSA*, 2019). It was also determined that 2,549 cases of listeriosis were detected in in the EU, of which 15.6% ended fatally, which is why listeriosis is considered to be one of the most severe food-borne diseases (*EFSA*, 2019). According to data reported by the Public Health Institute of Serbia, the most commonly diagnosed food-borne diseases in Serbia were salmonellosis, campylobacteriosis, stomach flu (norovirus) and staphylococcal poisoning (*Serbia*, 2018). There are no published and available data of food-borne diseases caused by farmhouse cheeses sold at the green markets in the Serbia.

The term "hazard" means a biological, chemical or physical agent or a food condition that has a potential to cause an adverse health effect, while the term "risk" is the likelihood the hazard will occur, as well as the seriousness of any possible health consequences. Food-related hazards can be divided into three main groups, microbiological, chemical and physical (*Shirani et al.*, 2015). In the food supply chain, different hazards can be identified at each stage from primary production to the consumer (*Shirani et al.*, 2015; *Motarjemi and Lelieveld*, 2013). Risk can be assessed using different methods and tools, including Hazard Analysis and Critical Control Points (HACCP), Failure Mode and Effect Analysis (FMEA), Preliminary Hazard Analysis (PHA), Risk Ranking, etc. (*ICH*, *2005*).

FMEA is a systematic approach that improves production lines and is used to define, identify, eliminate or reduce potential failures in each step of the process, before they enter the next stage (*Scipioni et al.*, 2002; *Kurt and Özilgen*, 2013). It is an engineering tool (*Djekic at al.*, 2018), which consists of several successive steps, organised by dividing the manufacturing process into phases and calculating the risk priority number (RPN) for each potential failure at all stages of production (*Scipioni et al.*, 2002; *Özilgen at al.*, 2013). This methodology was developed in 1949 by the United States Army, and nowadays, it has been implemented in different areas including the food industry (*Scipioni et al.*, 2002). FMEA is a "bottom up" quantitative evaluation of the risks, by which the risk is assessed as the product of multiplication of values determined for severity (S), detection (D) and frequency of occurrence (O) of possible failures. Establishing a critical value for the RPN determines the need for appropriate corrective action. After corrective measures have been applied, the values for O and D have to be re-evaluated, while the value of S remains unchanged (*Djekic at al.*, 2018; *Arvanitoyanis et al.*, 2007).The assessment of the severity (S) of a failure is a measure of the impact that a failure can have on the health of the consumer, the required quality of the product and/or legislation. Assessment of occurrence (O) determines the frequency of occurrence of a given failure, while detection (D) is a measure of the possibility of easier or more difficult detection of a given failure (*Kurt and Özilgen*, 2013). In this paper, FMEA methodology was used to determine the biological, chemical and physical failures that may occur during the farmhouse production of white brined cheese (short cheese supply chain) in Serbia.

Materials and methods

Cheese supply chain

To apply FMEA methodology, a flow diagram was made for the production of white brined cheese production in farmhouse conditions and green market sale (short cheese supply chain, Figure 1). This was done according to *Popovic-Vranjes* (*2015*) and the authors' personal experience.

FMEA analysis

After the flow diagram was prepared, the potential failure modes were identified for each step, and the possible effects and causes of each failure mode were also identified. Afterwards, the risk level of each failure mode was assessed, and corrective actions to reduce and eliminate the potential failures were suggested. Finally, the risk level of the corrected design was recalculated.

To assess biological, chemical and physical failures, the values for occurrence (O), severity (S) and detection (D) in the manner shown in Table 1 were determined, according to *Djekic et al*. (2018). For each potential failure, the RPN was calculated using estimated values for O, S and D. A numerical ranking for values of O, S and D was established taking into consideration available literature data, epidemiological studies and our own expertise and

knowledge. All co-authors of this study, as experts in the field, participated in ranking the risks, using the Delphi method to stimulate and synthesize the opinions of experts and achieve consensus (*Heiko*, 2012). There were no holdouts and consensus for each type of risk analysed was achieved. Also, all participants confirmed that all important food safety hazards had been included in each activity within cheese supply chains. Depending on the likelihood of occurrence, each failure was assigned with an O value in the range from 1 to 5. The highest O value represented the greatest probability the failure would occur. The possibility to detect the failure before it occurs and the seriousness of the failure were also ranked from 1 to 5, where the values increase as it becomes more difficult to detect the failure before occurring, or as the potential damage caused by the failure increases.

Table 1. Severity (S), occurrence (O) and detection (D) rating scale used for FEMA analysis (according to *Djekic et al.*, 2019)

SEVERITY (S)	
Estimation	**Consequence**
1	no consequences
2	minimal consequences
3	Low
4	High
5	Severe
OCCURRENCE (O)	
Estimation	**Probability**
1	very unlikely
2	Unlikely
3	Possible
4	great probability
5	Certainly
DETECTION (D)	
Estimation	**Control potential**
1	easy to detect
2	a great opportunity for detecting
3	little chance of detecting
4	difficult to detect
5	very difficult to detect

The calculation of RPN was based on the work of *Kurt et al.* (*2013*), and it included multiplication of the values of O, S and D. The maximum value for RPN that could be obtained was set at 125. A RNP value of 6 has been accepted as the critical limit, above which corrective measures need to be taken (5% of the maximum value with a statistical significance of 95%). The RPNs after the corrective actions were also recalculated.

Results and Discussion

White brined cheese refers to a large group of cheeses characterised by the name of the geographic area where they are produced. Although this group accounts for a large number of cheeses and, hence, great variety in their specific properties, their common characteristic is that they are matured in brine under anaerobic conditions. The most popular cheeses that belong to this group in Serbia include Sjenica Cheese, Zlatarski Cheese, Svrljiški Cheese, Serbian white cheese etc., while Greek Feta Cheese, Cypriot Haloumi, Egyptian Domiati, etc. are white cheeses known worldwide. Traditionally, these cheeses are made from cow, sheep or goat milk, or their mixtures. In the farmhouse production of white cheeses, raw milk is often used, giving the product specific sensory characteristics, due to the presence of indigenous microbiota (*Popovic-Vranjes*, 2015; *Radulovic et al.*, 2011; *Terzic-Vidojevic et al.*, 2006).

In this paper, Serbian white brined cheese, traditionally produced in Pomoravlje, is described as an example of farmhouse cheese and a general flow diagram showing its production steps is presented in Figure 1. This cheese is usually made from raw cow milk, which is heated to the required temperature (in summer 18–20°C, in winter 25–30°C), and at this temperature, rennet is added to the milk. After coagulation, which lasts for 4–6 h, the cheese curd is left in a strainer and hung to drain; this is followed by pressing the cheese curd with a board and a stone (the pressure should be 1–2 kg/1 kg of cheese curd). Drainage and pressing usually takes up to 24 hours. The drained cheese curd is cut into slices, sprinkled with dry salt and arranged in wooden buckets or plastic cans. Ripening is done under load and usually lasts for 2–3 weeks (*Popovic-Vranjes*, 2015). The characteristics of farmhouse white brined cheeses depend on the microbiological composition of raw milk, as the types of indigenous non-starter lactic acid bacteria (LAB) present in milk depend on the ecological characteristics of the

climate in which the cheese is produced (*Terzic-Vidojevic et al.*, 2007; *Radulovic et al.*, 2011; *Popovic-Vranjes*, 2015).

FMEA analysis in the short cheese supply chain

The identified failures in cheese production and distribution chains, the calculated RPN values, corrective measures that can be applied, as well as the recalculated values for RPNs are presented in Table 2.

Assessment of biological failures in the short cheese supply chain

Very high values for RPN were determined for biological failures in the short cheese supply chain (Table 2). The highest values were determined for the milking step (total RPN for different biological failures=175), followed by cheese ripening step (RPN=132) and transportation of cheese by personal vehicle (RPN=110). The presence of pathogenic microorganisms in raw milk might indicate the animals' impaired health condition, but also inadequate hygienic milking conditions. Dairy products play a significant role in the occurrence of food-borne disease outbreaks, because raw milk can contain pathogens that might remain in the dairy products and consequently lead to food-borne diseases. Due to its composition (water content, neutral pH, nutrient content), milk is a suitable medium for microbial multiplication. The most commonly isolated pathogens from raw milk include *Staphylococcus aureus, Streptococcus* spp*., Listeria monocytogenes, Campylobacter jejuni, Escherichia coli,* etc. Special attention towards the legislation was given to the infectious diseases, brucellosis and tuberculosis (*Oliver et al.*, 2005*, Kurt et al.*, 2013*, Le et al.*, 2014*, Serbia*, 2011). Some of these microbiological hazards can be controlled at the primary production level (e.g. tuberculosis, brucellosis), while *Listeria monocytogenes* is much more widespread in the environment and the introduction of this bacterium into dairies can result in product contamination, biofilm formation, etc. (*Oliver et al.,2005*; *Kurt et al.*, 2013; *Le et al.*, 2014; *Shirani et al.*, 2015). Legislation foresees the conditions that raw milk should meet in terms of total number of microorganisms and somatic cell counts (*Serbia*, 2010a; *Serbia*, 2011; *Serbia*, 2017b; *Serbia*, 2017c; *Serbia*, 2019). Application of good breeding practice and good veterinary practice, utilisation of animal feed from verified suppliers, education of owners and breeders of animals, who are often also the cheese producers in

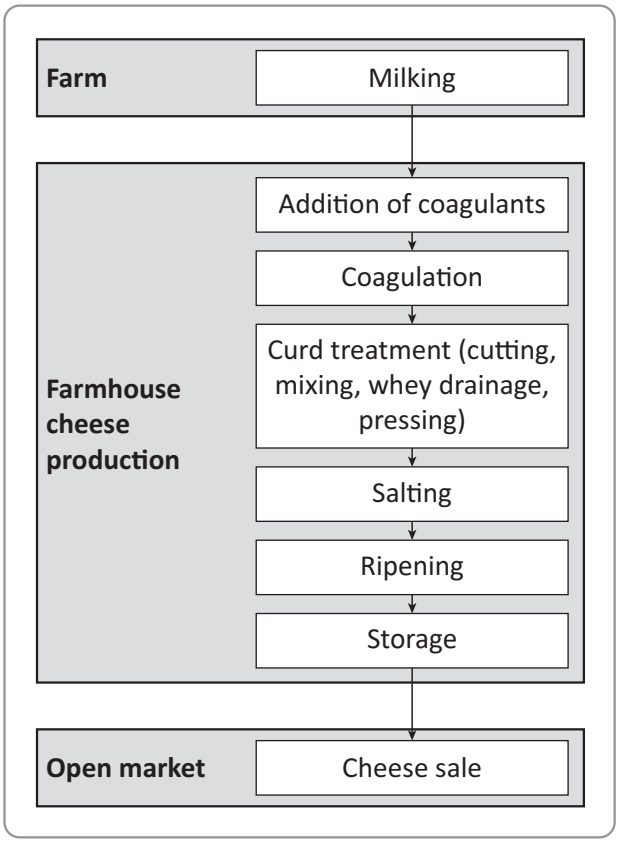

Figure 1. Short supply chain for farmhouse white brined cheese (according to *Dozet et al.*, 2004; *Popovic – Vranjes*, 2015)

farmhouse production conditions, and application of legally determined norms could be used as corrective actions to control some biological failures in this cheese chain. By applying the proposed corrective measures, the recalculated RPNs values were reduced (Table 2).

Farmhouse cheese production, from the aspect of using raw milk that has not undergone any thermal treatment (Figure 1), carries the risk of causing food-borne diseases (*Mauropoulos et al.*, 1999; *Oliver et al.*, 2005). However, the controlled processes of coagulation and ripening of cheeses, due to the action of naturally occurring LAB, can still result in a product that is recognized as safe. Namely, LAB activity leads to a decrease in pH, and in conjunction with other factors such as storage temperature, salt concentration and water activity (a_w), might be limiting to the survival and multiplication of pathogenic microorganisms. In addition to this, LAB also produce antimicrobial substances such as bacteriocins, hydrogen peroxide, fatty acids, diacetyl, bacteriocin-like molecules, etc. (*Mauropoulos et al.*, 1999; *Bintsis et al.*, 2002; *Terzic-Vidojevic et al.*, 2006; *Veskovic-Moracanin et al.*, 2007; *Bulajic et al.*, 2017).

Table 2. FMEA analysis for farmhouse white brined cheese (short supply chain)

Production, processing and marketing phase	Failure /cause	O[1]	S[2]	D[3]	RPN[4]	Corrective actions	O	S	D	RPN
Register of identification of potential biological failures and proposed corrective actions										
Milking	Contamination with pathogenic microorganisms *(E. coli* O157:H7, S*almonell*a spp., *Mb. tuberculosis*, *Listeria monocytogenes*, *Staphylococcus aureus,* etc.) due to milking	3	5	5	75	Cheese production must be done in registered households Cheese production in the household can only be done from milk produced in that household (*Serbia*, 2017c) Milking animals must be covered by the program of measures and that the conditions for the production of raw milk and milk should meet the legal requirements (*Serbia*, 2011a)	2	5	5	50
	Contamination with pathogenic microorganisms in milk equipment due to: ▪ usage of contaminated water for washing dishes (i.e. buckets) ▪ faulty washing / milk trapping ▪ poor storage conditions of washed equipment	2	5	5	50	Water quality should be periodically controlled Disinfection of containers must be done Washing the equipment must be complete The storage room for milk-contacted equipment must be kept clean and protected from pests	1	5	5	25
	Contamination with pathogenic microorganisms due to impaired health of the person handling raw milk	2	3	5	30	Intermittent sanitary inspections must be done Education of farmers / persons handling raw milk is recommended Maintaining personal hygiene is essential People with signs of illness are not allowed to work with food	1	3	5	15
	Psychrotrophic microorganisms present in raw milk (spoilage microorganisms) and their possible outgrowth during cooling	2	2	5	20	Adequate temperature	1	2	5	10
Addition of coagulants	Contamination with pathogenic microorganisms due to impaired health of the food handler / cheese producer	2	3	5	30	Intermittent sanitary inspections must be done Education of cheese producers is recommended Maintaining personal hygiene is essential People with signs of illness are not allowed to work with food	1	3	5	15
Coagulation	Coagulation vats - inadequate washing / residues of milk and gel from previous productions	2	3	5	30	Utensils have to be washed and rinsed after each usage Using of potable water is recommended	1	3	5	15
Curd treatment	Microbiological contamination of cutting equipment (knife, cutting wires) Microbiological contamination due to manual manipulation	2	3	5	30	Regular cleaning of cutting equipment must be done Intermittent sanitary inspections must be done Education of cheese producers is recommended Maintaining personal hygiene is essential People with signs of illness are not allowed to work with food	1	3	5	15
	Microbiological contamination due to unhygienic utensils used (strainer, spoon and cloths)	2	3	5	30	Adequate cleaning and disinfection of strainer and spoon A new cheese cloth is recommended to be used each time	1	3	5	15
	Mold development due to: ▪ inadequate hygiene of the pressing table ▪ wooden pressing circles ▪ load stone	3	2	4	24	Regular cleaning and disinfection must be done Hygiene of wooden circles and stone using other materials instead of wood must be done	1	2	4	12

Production, processing and marketing phase	Failure /cause	O[1]	S[2]	D[3]	RPN[4]	Corrective actions	O	S	D	RPN
Ripening	Mold development	4	2	4	32	Cheese must be cleaned during ripening Stone and circle must be cleaned with brushes and potable water Mold growth inhibitors may be used	2	2	4	16
	Microbial contamination of water for making brine solution	2	5	5	50	Visual inspection of brine must be done Potable water is recommended to be used	1	5	5	25
	Growth of pathogens and spoilage microorganisms due to inadequate pre-requisite for cheese ripening room (temperature, room hygiene, room humidity, air flow)	2	5	5	50	The ripening of cheese must take place under controlled conditions The ripening room must be tide and clean The temperature in the maturation room should be below 18°C	1	5	5	25
Storage	Growth of pathogens and spoilage microorganisms during ripening (depends on room temperature, ripening time and hygienic conditions)	2	5	3	30	For cheeses with a long ripening period 10–15°C For cheeses with a shorter ripening period 0–4°C	1	5	3	15
Transportation	Microbial contamination due to poor vehicle hygiene	2	2	5	20	Regular maintenance of vehicle hygiene must be done	1	2	5	10
	Microbial contamination due to inappropriate temperature in the vehicle and / or inappropriate transport time	2	4	5	40	Cold chain must be maintained Transport / handheld refrigerator / thermo bag may be used Transport time should be maximal 2 hours (*Serbia*, 2017c)	1	4	5	20
	Microbial contamination due to the simultaneous transportation of different types of food	2	5	5	50	Physical separation of different types of food must be done	1	5	5	25
Direct sale (open market)	Microbial contamination due to the usage of water which is not adequate quality (uncontrolled wells, spring water)	2	5	5	50	Using of potable water is recommended	1	5	5	25
	Microbial contamination due to cheese seller (wounds on the hands, diseases)	2	3	5	30	Intermittent sanitary inspections must be done Education of cheese producers is recommended Maintaining personal hygiene is essential People with signs of illness are not allowed to work with food	1	3	5	15
	Microbial contamination due to: ▪ poor hygiene maintenance of refrigerator units ▪ exposure of the product in open containers ▪ customers touching cheese products to taste ▪ simultaneously exposal of several different types of products (meat, eggs, poultry) ▪ returning unsold cheese from the market ▪ poor hygiene of utensils (knives, spoons, dishes, cloths)	2	4	5	40	The refrigerator units must be regularly cleaned Using of containers with lids, using of foil or cloths is recommended Disposable accessories for food tasting (toothpicks, plastic clip, cardboard coasters) may be used Exposure to different types of food is allowed, but care must be taken to avoid cross-contamination Cold chain must be maintained Using of protective clothes / foils is recommended Washing unclean dishes, replacing of worn-out dishes must be done	1	4	5	20

Production, processing and marketing phase	Failure /cause	O[1]	S[2]	D[3]	RPN[4]	Corrective actions	O	S	D	RPN
Register of identification of potential chemical failures and proposed corrective actions										
Milking	The presence of residues of veterinary medicine drugs (antibiotics, hormones, growth stimulants) due to non-compliance with the prescribed withdrawal period	3	3	5	45	Veterinary medicines should be given only according to the instructions of the veterinarian	1	3	5	15
	The presence of aflatoxins in raw milk due to poor agricultural practices (animal feed)	3	3	5	45	Good agricultural practice must be performed Animal nutrition should be done by commercial feed from verified suppliers (authorized animal feed sales)	2	3	5	30
	The presence of chemical contaminants (pesticides, dioxins, organophosphates, etc.) in raw milk due to poor agricultural practices (animal feed)	2	3	5	30	Good agricultural practice must be performed Animal nutrition should be done by commercial feed from verified suppliers (authorized animal feed sales)	1	3	5	15
	The presence of detergents and disinfectants in raw milk collection equipment due to improper washing and rinsing	2	2	5	20	Using of approved agents for washing and disinfection according to the manufacturer's instructions, good rinsing must be done	1	2	5	10
Coagulation	Chemical contamination due to storage of cleaning and disinfecting agents	2	4	5	40	Regular cleaning and disinfection must be done Hygiene products should be stored separately from food	1	4	5	20
	Residues of cleaning and disinfecting agents on the utensils used for milk coagulation	2	2	5	20	Utensils must be washed and rinsed after each production Detergent must be allowed for the use in the food industry Usage of detergents and disinfectants according to the manufacturer	1	2	5	10
Curd treatment	The presence of mycotoxins (following mold development) due to: ▪ inadequate hygiene of the pressing table ▪ wooden pressing circles ▪ load stone	2	3	5	30	Regular cleaning and disinfection of the room must be done Wood and stone hygiene The usage of other materials instead of wood or stone is recommended	1	3	5	15
Salting	The presence of heavy metals (Hg, Pb, As, Cd) in salt	2	3	5	30	Salt must be obtained from the reliable suppliers	1	3	5	15
Ripening	The presence of mycotoxins due to mold development during ripening	2	3	5	30	Cheese must be cleaned during ripening Stone and circle cleaning must be done Mold growth inhibitors may be used	1	3	5	15
	Chemical contamination of the water used to make brine with heavy metals and / or residues of the chlorine	2	3	5	30	Water quality testing must be done regularly Potable water is recommended to be used	1	3	5	15
Direct sale (open market)	Residues of detergents for dishwashing	2	3	5	30	Approved detergents and disinfectants must be used Good rinsing after washing must be done Disinfectants must be used according to the manufacturer's instruction	1	3	5	15
	Chemical contamination due to utensils (dishes, knives, etc.)	2	2	5	20	Materials that are allowed in the food industry must be used Equipment that can be easily maintained and where necessary disinfected (e.g. stainless steel) must be used	1	2	5	10
	Chemical contamination due to inadequate material used to cover the bowl (wood, rusted lids, newspapers, rags)	2	2	5	20	Plastic lids or / and foil may be used	1	2	5	10

Production, processing and marketing phase	Failure /cause	O[1]	S[2]	D[3]	RPN[4]	Corrective actions	O	S	D	RPN
Register of identification of potential physical failures and proposed corrective actions										
Milking	The presence of foreign bodies originating from damaged equipment (pieces of metal, strand wire, cloth parts, etc.)	2	4	2	16	Equipment made of adequate materials must be used Damaged utensils must be replaced Raw milk filtration must be done	1	4	2	8
	The presence of straw / litter, hairs, mud, insects, rodent faces, etc. due to poor hygiene and breeding practices and / or machine milk due to the fall of suction cups on the ground	2	3	2	12	Good hygiene and breeding practices must be applied Protection of raw milk from pest must be done Filtration of raw milk must be done	1	3	2	6
	The presence of foreign bodies originating from a person handling raw milk (buttons, jewelry, etc.)	2	4	1	8	Good hygiene and manufacturing practice Education of farmers / persons handling raw milk is recommended	1	4	1	4
Coagulation	The presence of foreign bodies such as insects, larvae, glass, metal parts, hair) in utensils	2	3	2	12	Manipulation of glass materials must be avoided Protection of utensil from insects and other pests must be done Personal hygiene is required	1	3	2	6
Curd treatment	The presence of foreign bodies originating from the person who cuts curd into slices (hair, buttons, jewelry)	2	4	1	8	Personal hygiene must be regular	1	4	1	4
Salting	The presence of foreign bodies in salt	2	3	1	6	Salt must be obtained from registered stores	1	3	1	3
Transport	The presence of foreign bodies (glass, metal parts, hair, dust, insects, insect larvae, etc.) due to poor vehicle hygiene	2	3	2	12	Regular maintenance of vehicle hygiene must be done Transport in closed containers is recommended Regular cleaning and disinfection must be done	1	3	2	6
Direct sale (open market)	Physical contamination due to cheese exposure in open dishes	2	3	1	6	It is recommended that the cheese is sold at open markets in sealed containers, as well as during the sale remains covered with plastic foil	1	3	1	3
	Contamination due to the presence of metal particles originating from damaged dishes or other equipment (e.g. knives)	2	5	2	20	Cheese in which the presence of metallic particles has been determined must be removed and destroyed Damaged and broken equipment must be replaced	1	5	2	10
	Physical contamination due to touching the product by the customers or sellers (hair)	2	2	2	8	Disposable accessories should be used (toothpicks, plastic clip, cardboard coasters)	1	2	2	4
	Physical contamination due to accidental breakage of glass bottles or glasses	2	4	1	8	Cheese in which the presence of glass has been established should be destroyed and removed Handling with glass near food should be avoided	1	4	1	4

[1]O – occurrence, [2]S – severity, [3]D – detection, [4]RPN – risk priority number

During coagulation and whey drainage, pH should decrease to a value of 4.6 or lower. Syneresis and salt addition decreases the a_w, which slows the growth of pathogenic microorganisms, and affects the cheese structure, enzymatic activity, etc. (*Pacheco et al.*, 2010; *Bulajic et al.*, 2017). In household production, the key moment is the visual inspection of whey, when the appearance of clear, light green whey with a pleasant taste and smell indicates the milk has coagulated well (*Popovic-Vranjes*, 2015). If changes in whey colour, a sour taste of curd or unpleasant smell occur, the production must be stopped and all

obtained curd and whey must be discarded (*Serbia*, 2019; *Olofson*, 2010).

In farmhouse cheese production, most of the steps involve manual manipulations, such as milking, rennet addition, gel cutting, salting and stacking in buckets (Figure 1). In addition, during cheese sale at the green markets, manual manipulation is inevitable. Therefore, it is clear that the cheese producers' personal hygiene is very important. Legislation in Serbia determines the conditions that persons involved in food production must meet in terms of maintaining personal hygiene and wearing of appropriate and clean protective clothing. Food handlers must not suffer from food-borne diseases, must not have infected wounds, skin infections and injuries or diarrhoea, and food handlers are obligated to report illnesses or symptoms. For persons who perform activities involving direct contact with food, the legislator mandates health examinations twice a year. Hand hygiene of persons handling food must be maintained, which is achieved by installing a sufficient number of hand washing stations with hot and cold water, hand washing agents and suitable disinfectants (*Serbia*, 2010a; *Serbia*, 2011; *Kurt et al.*, 2013; *Serbia*, 2017).

In farmhouse cheese production, it is essential the production steps of coagulation, gel cutting, whey drainage, salting and stacking in buckets take place in clean environments. Identical hygienic conditions must be met in both industrial production and distribution in retail chains. In farmhouse cheese production, the washing step is usually performed manually, while in industrial facilities, automatic cleaning in place (CIP) technology is applied. The utensils should be washed as soon as possible after the production of cheese, but the washing step must not be performed during the production process. In order to preserve the desirable microbiota in farmhouse conditions, mechanical cleaning and washing of surfaces is recommended, but not the use of disinfectants. In such conditions, special attention should be paid to the mechanical cleaning of cheese making equipment, the appropriate water temperature and detergent concentration, as well as the washing time (*Olofson*, 2010).

Untimely and inadequate washing of equipment used in household cheese production can result in microbiological contamination, due to retention of milk and gel that remain from previous production. We rated this failure as RPN=30. This failure could also be seen through the possibility of biofilm formation. Namely, the occurrence of biofilms on materials used for the food production (stainless steel, plastic, glass, etc.) and from which kitchen equipment used in households is also made, is described in the literature (*Olofson*, 2010; *Giaouris et al.*, 2012; *Moretro et al.*, 2004; *Katic*, 1995). The possibility of this phenomenon in farmhouse cheese production should not be neglected. Although special attention is paid to pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus*, the occurrence of LAB biofilms has also been described, in which pathogens have been identified.

According to Serbian legislation (*Serbia*, 2017), the mandatory data the small producer must state on the food label are name and address of the manufacturer, production date, product name, shelf life, storage conditions and household registration number. The shelf life of farmhouse white cheeses, and therefore their quality and safety parameters, is dependent on many different factors, including composition, degree of maturity, storage conditions etc. According to *Popovic-Vranjes* (*2015*), white brined cheeses matured for 2–3 weeks might be stored for months. However, when the maturation period is shorter in order to produce fresh cheeses, the subsequent shelf life is also shorter. The production of these cheeses is based on the tradition and experience that generations of housewives have gained. Passing the experience from generation to generation has contributed to the preservation of this traditional production. According to the Serbian legislation (*Serbia*, 2010b), cheeses can be divided into the following categories: extra hard cheeses with a ripening period that must not be shorter than 6 months, hard cheeses that must ripen for at least 5 weeks, semi-hard cheeses that ripen for at least 2 weeks and soft cheeses that ripen for at least 7 days. Depending on the length of the ripening period, the shelf life of these cheeses also changes, as a longer ripening period is associated with a longer shelf life due to microbiological and biochemical changes that characterise the ripening period and by which the desired sensory and textural characteristics are achieved. Fresh cheeses are characterised by low dry matter content with a consequently high a_w, and low fat and protein contents but a high lactose content, which makes them perishable foods (they can remain unspoiled for only a few days). On the contrary, in ripened cheeses, dry matter, fat and protein contents are higher, but lactose and water contents are lower, which prolong their shelf life (*Pacheco et al.*, 2010). Questions remain as to how long the shelf life is of farmhouse-produced cheeses that are sold at green markets in Serbia, and how should the small producers determine the shelf life of their cheeses.

Assessment of chemical failures in the short cheese supply chain

Chemical failures can be identified at all stages of farmhouse cheese production and distribution. The main chemical risks we associated with raw milk were the presence of aflatoxins and antibiotic residues, as a consequence of poor breeding practices and veterinary malpractice, respectively. The highest RPNs were determined for the presence of antibiotic residues and aflatoxin contamination in raw milk. In farmhouse conditions, RPN values were rated as 45, 45 and 30 for antibiotic residue, aflatoxin and chemical contamination, respectively. The occurrence of pharmacologically active substances in food of animal origin is most often caused by the use of veterinary drugs used in the treatment of dairy animals. Residues of veterinary medicinal products are classified in group B of chemicals that can be found in food (according to Annex I of Council Directive 96/23/EC), where environmental contaminants are also present. Here, the greatest importance is given to antibiotics, which are relatively stable at pasteurisation temperatures, but also at low temperatures. The influence of antibiotics in cheese production can be divided into two areas: 1) influence on consumer health (hypersensitivity reactions, development of antibiotic resistance in pathogenic microorganisms, changes in digestive tract microbiota) and; 2) influence on cheese production (inhibition of LAB, delay in achievement of the appropriate pH, as well as altered sensory characteristics and possible growth of pathogenic microorganisms due to inadequate LAB activity) (*Marth et al.*, 1959; *Albright et al.*, 1961; *Kurt et al.*, 2013*, Katic & Bulajic*, 2018). Farmhouse cheeses are made of milk that is produced in that household (*Serbia*, 2017). The presence of antibiotics in milk can be prevented by applying good breeding practices, regular health checks of dairy animals according to legislation (*Serbia*, 2005) and by educating the breeder, who is also the cheese producer, to respect the prescribed withdrawal period.

Mycotoxins are secondary metabolites of fungi that reach the milk via contaminated animal feed. In dairy farming, the highest importance is attached to aflatoxin M1. Aflatoxin M1 is excreted by cows into raw milk, is resistant to heat treatment and due to its binding to casein micelles in cheese, it occurs in higher concentrations in cheese than in the milk from which the cheese is produced. Usually the concentration increase (from milk to cheese) ranges from 3-fold in soft cheeses to 5-fold in hard cheeses. Cheese is considered to be the most important source of aflatoxin M1 among all dairy products (*Ardic et al.*, 2009; *Skrbic et al.*, 2014; *Kos et al.*, 2014; *Polovinski-Horvatovic et al.*, 2009; *Tomasevic et al.*, 2015; *Miocinovic et al.*, 2016). At this point, the control of aflatoxin M1 in farmhouse cheese production is questionable, because no controls of aflatoxin in raw milk are performed at the household level. Aflatoxin M1 in milk originates from animal feed. Therefore, application of good agricultural practice, with special attention to the conditions of storage of animal feed, and good breeding practices are considered as suitable corrective measures.

Chemical contamination resulting from non-compliance with hygiene standards (presence of residues of detergents and disinfectants, inappropriate materials from which equipment is made, etc.) can be prevented by educating individual producers, by applying good manufacturing practices and using permitted means of hygiene and disinfection at all stages of production and trade (Table 2). Also, water used on food production farms must meet the requirements for potable water, regardless of whether it is used from the public supply system of consumers or from their own wells (*Serbia*, 2010a; *Serbia*, 2017).

Assessment of physical failures in the short cheese supply chain

An artefact is defined as any unwanted object in food that originates from the food itself (intrinsic such as bones in meat products, fruit seeds, etc.) or originates from other sources (extrinsic such as glass, plastic, metal parts). The presence of artefacts in food can cause serious consequences (oral cavity injuries, suffocation, damage to the digestive tract, internal bleeding and even death). The most common injuries recorded as a result of ingestion of artefacts were caused by sharp metal objects (parts of equipment, wires, etc.), but such dangers can be caused by other types of artefacts such as jewellery, artificial nails, pieces of wood, etc. (*Trafialek et al.*, 2016). Metal artefacts can be present in cheese due to contamination of raw milk because of poor milking hygiene or can occur during the different production steps as presented in Table 2.

The RPN value calculated for the identified physical failures (116) was significantly lower than those obtained for chemical and biological failures (390 and 741, respectively). The short cheese supply chain consists of a series of manual processes from production to sale, which can result in the occurrence of artefacts in the product (from hair that

can lead to consumers' nausea, to metal parts that can lead to injuries). The application of appropriate hygienic conditions during the production, transport and trade of cheese at the markets as well as the education of individual producers are considered as appropriate corrective measures. The highest value for physical defects was determined for the presence of metal foreign bodies originating from damaged equipment during sale at the green markets (RPN=20). Destroying suspect products and replacing damaged equipment are considered good corrective measures to reduce the RPN to 10.

Conclusion

In this paper, FMEA methodology was used to quantitatively determine the risks that can be observed in different phases of white brined cheese production and trade in a short supply chain (farmhouse-produced cheese which is sold at green markets). Our results indicate the greatest risks in the short cheese supply chain can be attributed to biological and chemical failures, due to any failures being unlikely to be detected by cheese producers and having severe consequences. The proposed corrective measures include different pre-requisite programs. Even the application of these measures will not result in great risk reduction, as the severity and detection will remain the same. Small cheese producers on their own initiative rarely send cheese for the external analysis, as this is most often done by competent authority during regular controls. Therefore, the biological and chemical failures are usually not detected at all in farmhouse cheese production. To increase detection and consequently to decrease the risk, some rapid hygiene monitoring techniques such as ATP (detection of adenosine triphosphate by bioluminescence) and protein kits might be used. They are designed to provide rapid results and to be used by unskilled personnel, such as cheese producers, to assess the effectiveness of their cleaning procedures. This might be supported and organised by government institutions. At the same time, this conclusion cannot be applied to physical defects because they are visible and, therefore, easier to detect, which is indicated by the relatively low RPN values for these faults calculated in this study.

Primena FMEA analize u ocenjivanju kratkog lanca snabdevanja sirom

Biljana Aleksić, Ilija Đekić, Jelena Miočinović, Nurgin Memiši, Nada Šmigić

A p s t r a k t: Cilj ovog rada bio je da se primeni kvantitativna FMEA metodologija (engl. Failure Mode Effect Analysis) u ocenjivanju potencijalnih bioloških, hemijskih i fizičkih nedostataka koji se javljaju tokom proizvodnje i distribucije belog sira u salamuri proizvedenog na gazdinstvima (kratak lanac snabdevanja) u Republici Srbiji. U tu svrhu utvrđene su vrednosti za učestalost pojavljivanja potencijalnih nedostataka (O), ozbiljnost posledica koje izazivaju (S) i mogućnost detekcije (D). Množenjem utvrđenih vrednosti izračunate su RPN (engl. risk priority numbers) vrednosti za svaki potencijalni nedostatak. U kratkom lancu snabdevanja utvrđene su visoke vrednosti za RPN za biološke nedostatke. Najviše vrednosti izračunate su za fazu muže muznih životinja, dok su nešto niže vrednosti izračunate za fazu zrenja sireva i transport ličnim vozilom do pijaca. Hemijski nedostaci za koje je izračunata najvša RPN vrednost odnose se na kontaminaciju sirovog mleka aflatoksinom i reziduama veterinarskih lekova. Naši rezultati ukazuju da biološke i hemijske opasnosti predstavljaju najznačajnije rizike u kratkom lancu iz razloga što je za njihovu detekciju neophodno izvršiti analize, a posledice koje izazivaju po zdravlje potrošača mogu da budu veoma ozbiljne. Predložene korektivne mere zasnivaju se na primeni odgovarajućih preduslovnih programa. Ipak i primenom predloženih korektivnih mera ne može se postići značajnije smanjenje rizika za pojavljivanje odgovarajućih opasnosti, iz razloga što je za detekciju i dalje neophodno primeniti iste postu pke, ozbiljnost posledica koje predstavljaju po zdravlje potrošača ostaje ista, pa su vrednosti za D i O nepromenjene. Kako su fizičke opasnosti lako vidljive i samim tim lakše za otkrivanje, najniže RPN vrednosti utvrđene su za fizičke nedostatke.

Ključne reči: *kratak lanac snabdevanja sirom, proizvodnja sira na gazdinstvima, FMEA analiza, rizik*

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Evaluation of n-3 polyunsaturated fatty acid content in various foods: health impact assessment

Dejana Trbović[1], Mirjana Lukić[1], Radivoj Petronijević[1], Brankica Lakićević[1], Mladen Rašeta[1], Ivana Branković Lazić[1], Nenad Parunović[1]*

A b s t r a c t: The objectives of this study were to verify the on-label claims of foods labelled as rich in n-3 polyunsaturated fatty acids (FA) and to assess their potential effects on human health in relation to European legislation. All the foods tested, i.e., chicken meat, anchovy fish oil, linseed oil, shellfish, capsule oil concentrate, egg, cold-smoked mackerel, frozen seafood, squid, hake, salmon and sardine, were evaluated for their contribution to the amount of n-3 polyunsaturated FA (n-3 PUFA) and the ratio of n-6/n-3 PUFA in relation to European dietary regulations. Lipids were extracted from the samples and then detected using capillary gas chromatography with flame ionization. An intake of 250 mg eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) per day, which is sufficient for primary prevention of chronic diseases in healthy volunteers, was found for 100 g of the edible part of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel and sardine. The European regulation defines high n-3 PUFA food as food with a content of at least 0.6 g 100 g^{-1} α-linolenic acid or at least 80 mg 100 g^{-1} EPA+DHA. This means that linseed oil and anchovy fish oil were the foods best suited to fulfil the first recommendation (>0.6 g 100 g^{-1} α-linolenic acid). The edible part of shellfish, frozen seafood, squid, hake, salmon, sardine, cold-smoked mackerel, capsule oil concentrate and anchovy fish oil met the second recommendation (>80 mg 100 g^{-1} EPA+DHA). With regard to the nutrition recommendations, the least favourable foods in terms of EPA+DHA content were eggs and chicken meat. An n-6/n-3 PUFA ratio closer to 4:1 is necessary for the prevention and treatment of chronic diseases. The results obtained in this study should be relevant for the establishment of Serbian tables of nutritional values of products.

Key words: *total fat content, n-3 PUFA, n-6 PUFA, ratio n-6/n-3 PUFA.*

Introduction

Fat in food consists mainly of fatty acids (FA), which are chemically coupled to glycerol. FA can be saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) (*Simopoulos,* 2008; *Gibson et al.,* 2013). Whereas PUFA have historically contained about one n-3 FA (omega in popular literature) for every four n-6 FA (1:4), modern diets can contain up to fifty to a hundred times more n-6 FA than n-3 FA (50:1) (*Simopoulos et al.*, 2013). The evidence that this imbalance contributes to disease is now strong, and governments should formulate agricultural and food policies to influence costs (*Simopoulos et al*., 2013, *Simopoulos*, 2008). The n-6/n-3 PUFA ratio could again approach that to which we are genetically adapted, i.e. four to one (4:1) (*Simopoulos*, 2004; *Simopoulos and Cleland*, 2003). A high n-6/n-3 PUFA ratio is typical of Western and, increasingly, global diets and is associated with an increased risk of cardiovascular disease, obesity, type 2 diabetes and breast and prostate cancer, especially in people with genetic predispositions. Of concern, animal studies show that low intake of docosahexaenoic acid (DHA, C22:6n-3), an n-3 PUFA, combined with high intake of fructose leads to a metabolic syndrome in the brain (*Agrawal and Gomez-Pinilla*, 2012).

n-3 PUFA have numerous functions in the human body. They play an important role in the structure and function of biological membranes. Any increase in n-3 PUFA could cause changes in membrane fluids that can affect enzymatic activity, receptor-ligand interaction, cell interaction and nutrient transport through the membranes (*Horrobin*, 1995; *von Schacky et al.*, 1985). Studies have shown that n-3 PUFA are essential for infant growth and development and for the prevention of various clinical conditions such as arthritis, diabetes, cancer and skin diseases.

Most diets, although with regional differences, are deficient in n-3 PUFA and too high in n-6 PUFA. A concerted effort is needed to narrow the n-6/n-3

[1]*Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia.*

***Corresponding author:** Dejana Trbović, *dejana.trbovic@inmes.rs*

PUFA ratio in the diet. Consumers should be encouraged, through education and, if necessary, through government intervention to switch from oils with high n-6 PUFA content such as corn, safflower, and sunflower oil, to those with high n-3 PUFA content such as rapeseed and linseed oils and oils with high MUFA content such as olive oil or hazelnut oil in combination with rapeseed oil. The increased consumption of fish should also be emphasized. Scientists should work with the fishing industry to achieve this goal. Aquatic organisms and fish from aquaculture are the main source of the essential FA (*Arts et al*., 2001; *Hunter and Roberts*, 2000). The nutritional and health benefits of consuming fish and fish products are the reason for increased consumer demand for fish (*Hunter and Roberts*, 2000). Specifically, a 4:1 ratio of n-6/n-3 PUFA in the diet should be the goal (*Simopulos,* 2008, *Simopoulos and Cleland,* 2003). The aims of the present study were to verify the on-label claims of foods declared to be rich in n-3 PUFA and to assess their potential effects on human health.

Materials and Methods

Food samples

All foods tested were labelled as rich in n-3 PUFA: three chicken meat samples with skin, three anchovy fish oil samples, three linseed oil samples, eighteen edible shellfish samples, two capsule oil concentrate samples present on the Serbian market, six whole egg samples, three cold-smoked mackerel samples, three frozen seafood samples, three frozen squid samples, three frozen hake samples, three frozen salmon samples and eighteen edible part of sardine samples.

FA analysis by capillary gas chromatography

Total lipids for FA determination were extracted from products by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) using a 33 ml stainless steel cell according to the method of *Spiric et al.* (2010). Fatty acid methyl esters (FAMEs) in the extracted lipids were transesterificated using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol (EN ISO 5509:2000). FAMEs were determined by gas-liquid chromatography (GLC, Shimadzu 2010, Japan) equipped 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 temperature were set at 250ºC and 280ºC, respectively. Nitrogen was used as the carrier gas at flow rate of 1.33 mL min^{-1}. The injector split ratio was set at 1:50 and programmed column oven temperature started at 125ºC and ended at 230ºC. Total analysis time was 50.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

Results and Discussion

The average total fat, the total n-3 PUFA, α-linolenic acid *(*ALA), eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) and n-6/n-3 ratio of the 69 samples are presented in Table 1.

This study included twelve food types and provided total n-3 PUFA in g 100 g^{-1} of samples, along with ALA, EPA and DHA contents. An intake of 250 mg per day of EPA+DHA is sufficient for primary prevention in healthy volunteers (*EFSA,* 2010). This recommendation would be fulfilled when at least 100 g of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel or sardine are consumed (Table 1). The American Heart Association (AHA) recommends at least two portions of fish per week for general health; cardiovascular patients are advised to consume 1 g EPA+DHA per day and patients with hypertriglyceraemia, 2 to 4 g EPA+DHA per day (*Lichtenstein* et al., 2006). As shown in Table 1, foods, if consumed in 100 g amounts, that fulfil the minimal AHA recommendation for EPA+DHA intake (1 g per day) were anchovy fish oil, sardine, capsule oil concentrate and cold-smoked mackerel.

ALA cannot be synthesized by the body, but it is necessary to maintain "metabolic integrity" and is, therefore, considered an essential FA. However, there is not enough scientific data to derive an average requirement or a population reference intake (*EFSA*, 2010). The foods that were relatively high in ALA were linseed oil, anchovy fish oil, frozen salmon, chicken meat and cold-smoked mackerel. However, the Annex of Regulation EC No 1924/2006 defines a high n-3 PUFA food as a foodstuff containing at least 0.6 g 100 g^{-1} ALA or at least 80 mg 100 g^{-1} EPA+DHA. For fulfilling the first recommendation (0.6 g 100 g^{-1} ALA), linseed oil and anchovy fish oil were the most suitable foods. Shellfish, frozen seafood, squid, hake, salmon, sardine, cold-smoked mackerel, capsule oil concentrate and anchovy fish oil complied with the second recommendation (80 mg 100 g^{-1} EPA+DHA). Samples of eggs and chicken meat were the most unfavourable

Table 1. The content of total fat, n-3 PUFA, ALA, EPA+DHA and the n-6/n-3 FA ratio in foods

Food sample	Total fat (g 100 g^{-1})	n-3 FA (g 100 g^{-1} of sample)	ALA (g 100 g^{-1} of sample)	EPA+DHA, (g 100 g^{-1} of sample)	n-6/n-3 FA ratio
Eggs whole (n = 6)	9.85	0.07	0.04	0.03	12.01
Chicken meat with skin (n = 3)	8.34	0.46	0.45	0.007	5.62
Anchovy fish oil (n = 3)	100	10.48	5.80	4.68	0.87
Linseed oil (n = 3)	100	58.14	58.14	0	0.19
Shellfish (n = 18)	1.72	0.34	0.05	0.29	0.26
Capsule oil concentrate (n = 2)	3.33	1.27	0.04	1.23	0.73
Mackerel cold-smoked (n = 3)	20.12	0.50	0.24	2.96	0.15
Seafood frozen (n = 3)	3.92	0.84	0.15	0.69	0.48
Squid frozen (n = 3)	2.48	0.48	0.03	0.45	0.11
Hake frozen (n = 3)	1.66	0.20	0.01	0.19	0.10
Salmon frozen (n = 3)	11.69	1.10	0.54	0.56	0.53
Sardine (n = 18)	10.0	2.53	0.11	2.42	0.26

Legend: n – Number of samples examined

foods examined in terms of EPA+DHA content and n-6/n-3 ratio. The n-6/n-3 PUFA ratios in eggs were above the recommended levels of 4:1 (*Simopoulos*, 2002) and averaged 12.01 (egg samples examined), which was consistent with the previously published data for eggs from Hy-line hens housed in a cage system (*Pavlovski et al.*, 2011). The n-6/n-3 PUFA ratios were higher in our study than in similar studies with Hy-line free range and Naked neck free range eggs (*Pavlovski et al*., 2011). The n-6/n-3 PUFA ratio of 5.62 in our chicken samples was lower than in the studies of Živković et al. (2017) and *Milićević et al*. (2014). With dietary manipulation, chicken meat enriched with n-3 PUFA with n-6/n-3 <5 can be produced (*Penko et al.,* 2015).

The n-6/n-3 PUFA ratio in frozen fish ranged from 0.10 (frozen hake) to 0.48 (frozen seafood), which were similar ratios to those of freshwater fish such as silver carp, Wells catfish and zander, namely from 0.33 to 0.93 (Ćirk*ović et al.,* 2011). The n-6/n-3 PUFA ratio of cold-smoked mackerel was 0.15, similar to that of smoked salmon (*Djordjević et al.,* 2016). Fish generally has high EPA+DHA ratios with low n-6/n-3 PUFA ratios, as was shown for rainbow trout with an n-6/n-3 PUFA ratio of 0.62–0.72 (*Trbović et al.,* 2012; *Lušnic Polak et al.,* 2017) and carp reared with extruded or pelleted feed, with an n-6/n-3 PUFA ratio of 3.79 (Ćirković et al., 2011). Certainly, even more favourable n-6/n-3 ratios in fish can be achieved by animal dietary measures.

Conclusion

Whereas the PUFA content of food declared as rich in n-3 PUFA have historically contained an n-6/n-3 PUFA ratio of about 1:4, modern diets can contain as much as 50:1. A concerted effort is needed to decrease the ratio of n-6/n-3 PUFA in modern human diets. The aim of the present study was to verify food samples labelled as rich in n-3 PUFA. The foods tested were chicken meat, fish and linseed oil, shellfish, capsule oil concentrate, egg, cold-smoked mackerel, frozen seafood, squid, hake, salmon and sardine. Sufficient intake for primary prevention in healthy subjects is 250 mg EPA+DHA per day. Consumption of 100 g of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel or sardine meets this recommendation. The AHA recommends for general health at least two portions of fish per week, while cardiovascular patients are advised to consume 1 g of EPA+DHA per day and patients with hypertriglyceraemia to take 2 to 4 g of EPA+DHA per day. Foods meeting the AHA recommendation for EPA+DHA content were anchovy fish oil, sardine, capsule oil concentrate and cold-smoked mackerel. Eggs and chicken meat contained the least favourable EPA+DHA ratios. The results obtained in this study should be relevant for the establishment of Serbian food composition tables in the field of meat and meat products.

Procena sadržaja n-3 polinezasićenih masnih kiselina u različitim namirnicama: procena uticaja na zdravlje

Dejana Trbović, Mirjana Lukić, Radivoj Petronijević, Brankica Lakićević, Mladen Rašeta, Ivana Branković Lazić, Nenad Parunović

A p s t r a k t: Cilj ove studije su verifikacija uzoraka hrane označeni kao bogata n-3 polinezasićenim masnim kiselinama (PUFA) i procena njihovog uticaja na zdravlje ljudi u odnosu na evropsko zakonodavstvo. Svi ispitivani uzorci, poput pilećeg mesa, ribljeg i lanenog ulja, školjki, koncentrata ulja u kapsuli, jaja, hladno dimljene skuše, smrznute morske hrane, lignji, oslića i lososa, kao i sardine, ocenjeni su zbog njihovog doprinosa količini n-3 PUFA i odnos n-6 / n-3 PUFA u odnosu na evropske propise. Izvršena je ekstrakcija lipida iz uzoraka i ispitano je kapilarnom gasnom hromatografijom sa detekcijom plamenske jonizacije. Unos 250 mg eikosapentaenske kiseline plus dokozaheksanske kiseline (EPA + DHA) dnevno, koji je dovoljan za primarnu prevenciju kod zdravog subjekta, za 100 g jestivog dela školjki, smrznutih morskih plodova, lignji i lososa, ribljeg ulja, koncentrata kapsula ulja, hladno dimljena skuša i sardina. Evropska uredba definiše visoki nivo n-3 PUFA kao hranu sa sadržajem od najmanje 0,6 g / 100 g a-linolenske kiseline ili najmanje 80 mg / 100 g EPA + DHA. To znači da su laneno i riblje ulje najprikladnije za prvu preporuku. Hrana, poput jestivog dijela školjkaša, smrznute morske hrane, lignje, oslić i losos, sardina, hladno dimljeni skuša, koncentrat ulja u kapsuli i riblje ulje ispunjava drugu preporuku. U pogledu ishrane, najmanje povoljni uzorci su jaja i pileće meso. Bliži odnos PUFA n-6 / n-3 neophodan je za prevenciju i lečenje hroničnih bolesti. Rezultati dobijeni ovom studijom trebalo bi da budu relevantni za formiranje tabela hranjivih vrednosti proizvoda.

Ključne reči: *određivanje ukupne masti, n-3 PUFA, n-6 PUFA, odnos n-6/n-3 PUFA*

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