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Preview paper
Pregledni rad

STEC in the beef chain – One Health Approach

Nastasijević Ivan[1], Mitrović Radmila[1], Janković Vesna[1]

A b s t r a c t: Since the early 1980s, E. coli O157 has emerged as one of the most significant pathogens of public health relevance not because of the incidence of the illness, which is much lower than that of other food borne pathogens such as Campylobacter or Salmonella, but because of the severity of the symptoms, the low infectious dose and potential sequelae. Shiga toxin – producing (STEC) Escherichia coli is a human pathogen that can cause hemorrhagic colitis (bloody diarrhea) and sometimes hemolytic uremic syndrome (HUS), a life-threatening disease that causes kidney damage. Cattle carry mixtures of O157 and non-O157 STEC in their intestines that are not necessarily pathogenic to humans. The O157 STEC serogroup was until recently responsible for the majority of disease outbreaks reported for STECs in North America, however now the non-O157 STECs account for almost 50% of the reported human disease outbreaks in North America and Europe. The STECs are shed at significant levels by healthy/asymptomatic cattle, e.g. cattle with jejunal hemorrhage syndrome. The shedding leads to contamination of the farm environment. This may lead to direct or indirect contamination of cattle hides, which, in turn, can serve as the main source of carcass contamination during slaughter and dressing of cattle at abattoirs or contamination of fresh beef and beef products. A science-based risk assessment is needed to assess the public health impact, the consumer exposure to the pathogen and for design of the most effective risk mitigation strategies regarding prevention and reduction of beef-borne O157 and non-O157 STECs.

Key words: *STEC, risk assessment, public health, risk mitigation.*

1. Introduction

Escherichia coli O157 is a potential food borne pathogen and a toxin-producing serogroup that-after ingestion-can cause severe damage to the intestinal mucosa and, in some cases, other internal organs of the human host. Since the early 1980s, *E. coli* O157 emerged as one of the most significant pathogens of public health relevance not because the incidence of the illness, which is much lower than that of other food borne pathogens e.g. *Campylobacter* or *Salmonella*, but because of the severity of the symptoms, the low infectious dose and potential sequelae. Shiga toxin-producing *Escherichia coli* (STEC) is a human pathogenic *E. coli* bacterium that is able to cause hemorrhagic colitis (HC; bloody diarrhea), which sometimes develops into hemolytic uremic syndrome (HUS). HUS is a life-threatening disease that causes kidney damage and is a severe complication of STEC infection (*Fairbrother and Nadeau*, 2006) (Figure 1). In most of cases, HUS is developed in children and immuno-suppressed individuals, while HC (Haemorrhagic Colitis) is usually associated with elder people. Faecally excreted by healthy, asymptomatic cattle, STEC can be spread to environment, water and/or foods directly or indirectly contaminated by the fecal material.

Cattle can carry different types of STEC in their intestines that are not necessarily pathogenic for humans. *E. coli* O157 is STEC serogroup that is responsible for the majority of outbreaks reported for shiga (verotoxin)-producing strains, although other non-O157 serotypes are also involved in human outbreaks (e.g. serogroups O26, O103, O111 and O145). Most available information relates to serotype O157:H7, since it is easily differentiated biochemically from other *E. coli* strains.

Cattle carry mixtures of STEC O157 and non-O157 in their intestines which are not necessarily pathogenic to humans and those healthy cattle may intermittently excrete VTEC seropathotypes, by fecal shedding, (*Nastasijevic,* 2011). The infectious dose of *E. coli* O157 is not known. In some cases of food borne human disease, only a few cells, perhaps lower than 100 CFU, may have been ingested (*Tilden et al.,* 1996). Therefore, the prevention of foodborne *E. coli* O157 infections requires not only growth suppression in foods, but also elimination of the pathogen from foods (*Buncic et al.,* 2004).

Advancement of the knowledge and development of efficient risk mitigation strategies for *E. coli*

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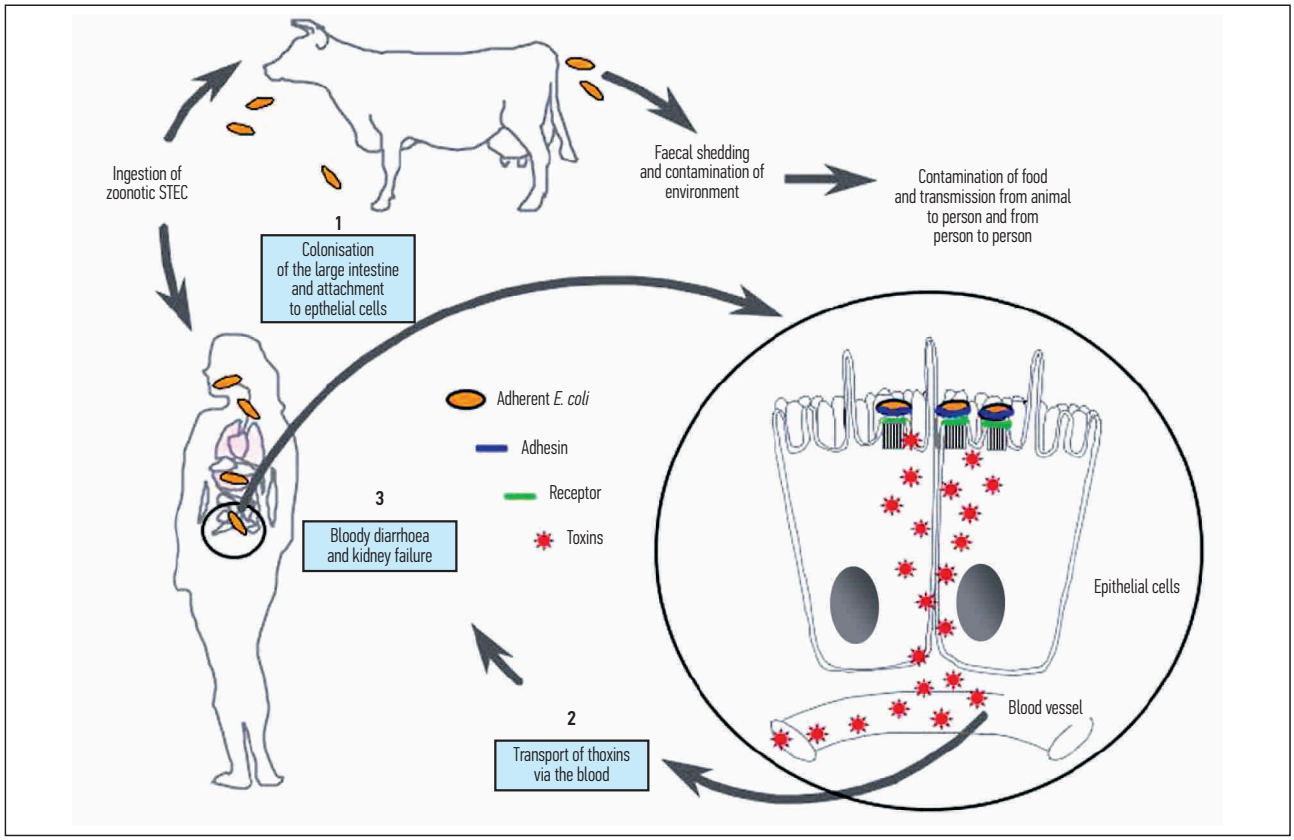

Figure 1. Bloody diarrhea and hemolytic uremic syndrome in humans caused by STEC O157 (Fairbrother and Nadeau, 2006)

Slika 1. Krvava dijareja i hemolitički uremički sindrom kod ljudi uzrokovan sa STEC O157 (Fairbrother i Nadeau, 2006)

O157 in the context of science-based risk assessment, longitudinal and integrated approach to meat safety assurance, are and will remain for the foreseeable future, one of the priorities for both – researchers and regulators in the area of meat safety (*Buncic,* 2006). Availability and quality of relevant data at different points of the meat chain is one of the key pre-requisites.

2. Risk assessment and risk management

Risk assessment. This is the science-based component of risk analysis (risk assessment, risk management and risk communication) consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization. In this process, the hazards are identified and the risk posed by that particular hazard (i.e. pathogen) is calculated (*Codex*, 1999). Therefore, the risk assessment has the aim to estimate prevalence/occurrence/numbers of *E. coli* O157 at different points along the beef chain. In this paper, only first three components of risk assessment will be addressed. This is due to the reason that risk characterization should be completed only after in-depth interpretation of national baseline data, originated from targeted research.

Risk management. This is the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (*Codex,* 2001). It aims to define the main and most effective control options of *E. coli* O157 along the beef chain, which are also technically and financially sustainable.

2.1. Hazard identification

E. coli pathogenic for humans can be faecally shed by humans and healthy animals; they can be divided into different groups including:

- Enteropathogenic *E. coli* (EPEC): associated with infantile diarrhoea;

- Enteroinvasive *E. coli* (EIEC): cause dysentery-like disease;
- Enterotoxigenic *E. coli* (ETEC): produce enterotoxins and diarrhoea;
- Enteroaggregative *E. coli* (EAEC): express aggregative adherence;
- Diffusely adherent *E. coli* (DAEC): adhere to the surface of epithelial cells;
- Enterohaemorrhagic *E. coli* (EHEC), including serotype O157, is the subset of STEC: produce Shiga-like toxin (Stx) or Verocytotoxin (Vtx) and can provoke hemorrhagic colitis (HC) in humans, with some cases developing to Hemolytic Uremic Syndrome (HUS).

The primary reservoir of EHEC are farm ruminant shedders i.e. cattle, sheep, goats. These farmed ruminants are healthy carriers of EHEC. The bacteria reside in the gut and can be intermittently fecally excreted. Therefore, STEC (EHEC) is a zoonotic pathogen that can be transmitted from animals to humans via direct contact with fecally contaminated animals, or more commonly through the food chain or through water. Person-to-person faecal-oral route of transmission can also occur.

2.1.1. Definitions associated with STEC/VTEC

VTEC. In Europe, most commonly, the cytotoxin produced by *E. coli* serotypes O157 or non-O157 has been called verotoxin (verocytotoxin) due to its lethal *in vitro* effects on Vero cells.

STEC. In US, *E. coli* serotypes who has the ability to produce cytotoxin are usually called shigatoxin-producing *E. coli*.

STEC O157. It usually denotes shiga(vero)toxin producing *E. coli* of O (somatic) serogroup O157, but with either "unspecified" or "undetermined" H (flagellar) serovar.

STEC O157:H7. It denotes shigatoxin producing *E. coli* of O157 serogroup and of H (flagellar) 7 serovar. It does not indicate whether the strains produce other virulence factors (apart from shigatoxin) necessary for causing food borne illness.

STEC non-O157. It denotes the number of serogroups, other than O157, which are associated with ability to produce shigatoxin, as well as other virulence factors and thus have significant impact on public health (e.g. O26, O45, O91, O103, O111, O145).

EHEC. Those STEC that cause enterohemorrhagic colitis (i.e. a subset of STEC) have been called enterohemorrhagic *E. coli* (EHEC; including O157).

HP-VTEC. Use of term Human pathogenic verotoxigenic *E. coli* (including *E. coli* O157) has been proposed in an attempt to cover both key aspects; ability to cause illness of "any" clinical manifestation in humans and the ability to produce shiga(vero)toxin (SCVMPH, 2003).

2.1.2. Overview of E. coli O157 infections

Reporting according to the new rules in Zoonoses Directive 2003/99/EC, instated as of 12 June 2004, started with data collected during 2005.

In 2012, the total number of confirmed VTEC cases in the EU was 5,671 based on data submitted by 22 member states (MSs). This represents a decrease of 40% compared with 2011 (9,487 reported cases), when a large outbreak of STEC O104:H4 occurred in Germany. The outbreak was associated with the consumption of contaminated raw sprouted seeds affecting more than 3,800 persons alone in Germany and linked cases in an additional 15 countries; the EU-incidence was 1.15 cases per population of 100,000 (*EFSA*, 2014). Overall, the highest notification rates were reported in Ireland, the Netherlands and Sweden (8.99, 6.27 and 4.98 cases per 100,000 population, respectively), while the lowest rates were reported in Bulgaria, Cyprus, the Czech Republic, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Romania and Spain (<0.1 cases per 100,000). The different sensitivities of the reporting systems of the MSs may have also influenced these figures. Consequently, comparison between countries should be done with caution. Comparison between years within a country is, in general, more valid (Table 1).

Data presented in Table 1. could lead to conclusion that number of reported and confirmed STEC cases in humans generally increased over time, within the three years period of time (2008-2011), e.g. 3,162 to 9,487, respectively. However, this increase may be attributed not only to ineffectiveness of current risk management strategies in place, but also to improvement of surveillance and reporting systems in respective EU Member States (e.g. Austria, Denmark, Ireland, Italy, Netherlands, Spain, Sweden), as well as the massive STEC O104:H4 outbreak in Germany, 2011.

In addition, more than half (53.0%) of EU reported confirmed human STEC infections in 2008 were associated with the O157 serogroup, while the rest belonged to the most frequent non-O157 serogroups, i.e. O26, O103, O145, O91, O111, O128, O146, O117, respectively (Table 2).

Table 1. Reported STEC cases in humans, 2008-2011 and notification rates for confirmed cases, 2012[1] (adapted from *EFSA*, 2014)

Tabela 1. Prijavljeni slučajevi STEC kod ljudi, 2008–2011. i stepen prijavljivanja za potvrđene slučajeve, 2012[1] (adaptirano iz *EFSA*, 2014)

Country	2012				2011	2010	2009	2008
	Report type[2]	Cases	Confirmed cases	Confirmed cases/ 100,000	Confirmed cases			
Austria	C	131	130	1.54	120	88	91	69
Belgium	C	105	105	0.95	100	84	96	103
Bulgaria	U	0	0	0	1	0	0	0
Cyprus	U	0	0	0	0	0	0	0
Czech Republic	C	9	9	0.09	7	-	-	-
Denmark	C	193	193	3.46	215	178	160	161
Estonia	C	3	3	0.22	4	5	4	3
Finland	C	30	30	0.56	27	21	29	8
France	C	208	208	0.32	221	103	93	85
Germany	C	1587	1573	1.93	5558	955	887	876
Greece	U	0	0	0	1	1	0	0
Hungary	C	3	3	0.03	11	7	1	0
Ireland	C	554	412	8.99	275	197	237	213
Italy	C	68	50	0.08	51	33	51	26
Latvia	U	0	0	0	0	0	0	0
Lithuania	C	2	2	0.07	0	1	0	0
Luxembourg	C	21	21	4.00	14	7	5	4
Malta	C	1	1	0.24	2	1	8	8
Netherlands	C	1049	1049	6.27	845	478	314	92
Poland	C	3	1	< 0.01	5	3	0	3
Portugal[3]	-	-	-	-	-	-	-	-
Romania	C	1	1	< 0.01	2	2	0	4
Slovakia	C	9	9	0.17	5	10	14	8
Slovenia	C	29	29	1.41	25	20	12	7
Spain	C	31	31	0.07	20	18	14	24
Sweden	C	472	472	4.98	477	334	228	304
United Kingdom	C	1339	1339	2.17	1501	1110	1339	1164
EU Total		**5848**	**5671**	**1.15**	**9487**	**3656**	**3583**	**3162**
Iceland	C	1	1	0.31	2	2	8	4
Liechtenstein	-	-		-				0
Norway	C	75	75	1.50	47	52	108	22
Switzerland	C	63	63	0.79	71	31	40	72

Legend/Legenda:

1. C: case-based data reported; –: no report; U: unspecified.
2. Mandatory notification of VTEC in 2008 and reported to ECDC from 2011.
3. No surveillance system.
4. Switzerland provided data directly to EFSA.

Table 2. Reported confirmed STEC cases in humans by serogroup (top 10), 2007-2008 (Adapted from *EFSA,* 2010)

Tabela 2. Prijavljeni potvrđeni slučajevi STEC kod ljudi prema serogrupama (prvih 10), 2007–2008. (adaptirano iz *EFSA*, 2010)

2008				2007			
Serogroup	**No. of cases**	**% Total**	**% Known**	**Serogroup**	**No. of cases**	**% Total**	**% Known**
O157	1,673	53.0	53.0	O157	1,571	54.1	54.1
NT	819	25.9	25.9	NT	842	29.0	29.0
O26	166	5.3	5.3	O26	136	4.7	4.7
O103	88	2.8	2.8	O103	77	2.7	2.7
O145	49	1.6	1.6	O91	43	1.5	1.5
O91	50	1.6	1.6	O145	31	1.1	1.1
O111	43	1.4	1.4	O111	23	0.8	0.8
O128	28	0.9	0.9	O128	21	0.7	0.7
O146	25	0.8	0.8	O113	16	0.6	0.6
O117	20	0.6	0.6	O146	14	0.5	0.5
Other	198	6.3	6.3	Other	130	4.5	4.5
Total: 3,159				**Total: 2,904**			

Legend/Legenda: Source: Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, Malta, the Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden and the United Kingdom
NT = non typed/untypeable

The large majority of the STEC infections had diarrhea as a clinical manifestation (i.e. non-HUS infections), whilst STEC infections with HUS manifestations were markedly less frequent. The largest proportion (34.2%) of reported STEC infections occurred in infants aged 0 to 4 years and the most frequent detected serogroup was O157 (Figure 2).

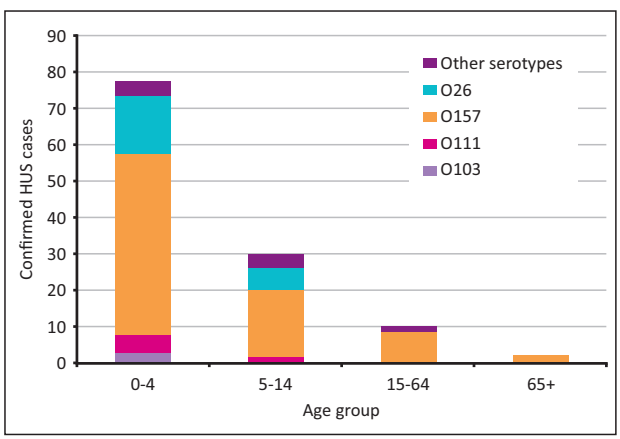

Figure 2. Hemolytic Uremic Syndrome (HUS) by age and serogroup in reporting MSs, 2008 (Adapted from *EFSA*, 2010)

Slika 2. Hemolitički uremički sindrom (HUS) po starosnoj kategoriji i serogrupama u zemljama članicama EU, 2008 (adaptirano iz *EFSA,* 2010)

However, in the massive outbreak recorded in Germany (May-July 2011), provoked by STEC O104:H4, it was reported that majority of HUS cases were adults (>87%), with a clear predominance of women (68%). Cases in children of school age are also reported. It was the unusual clinical manifestations having in mind that in the majority of previous STEC outbreaks HUS was developed mainly in children <5 years old. The number of people affected and the severity of disease (e.g. development of HUS) confirmed that it was the biggest ever recorded STEC outbreak in EU. By the end of the outbreak, the number of 3774 (750 HUS cases and 3024 non-HUS cases) of infected people was reported. The leading hypothesis was that seeds used for sprouting (distributed to local producers or retail outlets) contained a level of *E. coli* O104:H4 contamination, ultimately leading to contaminated sprouts destined for human consumption. The implicated food source was also attributed to consumption of faecally contaminated fresh produce/vegetables (cucumbers, tomatoes, etc.). However, the presence of the STEC O104:H4 in implicated foods was not precisely confirmed; the exact point of contamination in the food chain was not established (ECDC, 2011). The molecular characterization (e.g. PCR) of isolated STEC strain from the stools of infected people confirmed the unusual combination of virulence factors,

belonging both – to Enteroaggregative (EAEC) and Shigatoxin-producing (STEC) *E. coli*, as follows: Stx1-negative, Stx2-poisitive, Intimin (eae)-negative, enterohemolysin (hyl)-negative, EAggEC virulence plasmid-positive (aatA, aggR, aap) (*RKI*, 2011). This led to hypothesis that the acquisition of plasmid encoded gene for Stx2, possibly happened via direct horizontal transfer between STEC (animal/ruminant-host) and EAEC (human host). The combination of these factors – Stx2 and enteroaggregative characteristics of the isolated pathotype O104:H4, was the probable reason for the unusually high level of virulence of this epidemic strain. This biological indication that probably humans – and not animals – are the reservoir for this strain, backed up the finding of the epidemiology that the outbreak was not linked to meat or dairy products. Before this outbreak, there were some sporadic cases of O104 infection, reported in EU since 2008, e.g. Belgium (two cases in 2008), Denmark (one case in 2008), Norway (three cases in 2009), Austria (one case in 2010) and Sweden (one case in 2010).

In United States, the Center for Disease Control and Prevention (CDC) has estimated that 112,752 food borne illnesses annually are due to non-O157 STEC, which is nearly twice the number of illnesses attributed to *E. coli* O157:H7. According to United States Department for Agriculture (USDA), an estimated 36,700 illnesses annually due to non-O157 STEC could be attributed to beef products (*USDA FSIS,* 2011a). Therefore, USDA has declared six additional serogroups of Shiga toxin-producing *E. coli* (STEC) – O26, O103, O45, O111, O121 and O145 – adulterants in non-intact raw beef. The regular testing of these pathotypes, together with *E. coli* O157:H7, had begun in 2012 (*USDA FSIS,* 2011b). Such scheme had been introduced within the regular verification protocols related to cattle slaughter hygiene. To understand the prevalence of non-O157 STEC in beef, USDA Food Safety Inspection Service (FSIS) initiated a nationwide microbiological baseline survey on beef carcasses which ended in 2011. In the meantime, the FSIS advised establishments that manufacture raw, non-intact beef products or intact raw beef components of those products to evaluate whether non-O157 STEC are hazards reasonably likely to occur in their products (*USDA FSIS,* 2011a).

STEC – EU versus USA. It should be noted that certain differences exists between EU and USA, regarding the proposed methodology for monitoring of STEC in the beef chain. Namely, the detection and enumeration of STEC in EU is required to be carried out along the meat chain – at different, selected points (e.g. animals, food) in order to provide high quality data (*Nastasijevic,* 2014). Internationally recognized testing methodology should be also applied for obtaining the valid results. The EU regulations require that Member States should carry out regular monitoring/testing in three-year interval basis. The testing should primarily include VTEC O157, but also VTEC non-O157 (e.g. serogroups O26, O103, O111 and O145). On the other hand, the US Department of Agriculture, Food Safety and Inspection Service (*USDA FSIS*, 2011b) introduced verification procedures, including sampling and testing for raw ground/trimmed beef products, to ensure control of both *Escherichia coli* O157:H7 and six other serogroups of STECs (O26, O45, O103, O111, O121, and O145). These STECs are declared as adulterants of non-intact raw beef products within the meaning of the Federal Meat Inspection Act (FMIA). Therefore, it is evident that there is a certain difference regarding the scientific approach in prevention and control of STEC in the meat (beef) chain, between the EU and USA. For example, the recommended testing of STEC non-O157 in the EU doesn`t include serogroups O45 and O121, as it is the case in USA. It can be assumed that, in the light of ongoing negotiation between EU and USA within the framework of Transatlantic dialogue (Free Trade Agreement/FTA) and related food safety/public health issues, e.g. GMOs, pesticides, hormone-treated beef, growth promoters (*Hilary*, 2014) – the further harmonization regarding monitoring and testing protocols for STEC in the meat chain, might be also initiated.

2.1.3 Routes of infection

Person-to-person transmission. The faecal-oral route of infection of *E. coli* O157 appears to be commonly occurring in patients' homes, pre-schools, geriatric homes and hospitals (*CDCP,* 1993; *Bell et al.*, 1994; *CDCP, 1995*; *Chapman et al.,* 1997; *Paunio et al.,* 1999).

Contact with animals. *E. coli* O157 was found in a range of animal species including farm animals (primarily cattle, but also sheep and pigs), companion animals (horses, rabbits) and wild animals (gulls, rats, flies). Contact with faecally contaminated animals or animal-related environments can lead to the faecal/oral route of infection. For example, three children developed HC or HUS after animal contact on an open farm during a school visit in England (*Milne et al.,* 1999). Animal-to-person transmission was also confirmed by PFGE typing in Canada (*Louie et al.,* 1999). Hand-mouth contact and nail biting were significantly associated with disease (CDCP, 2001).

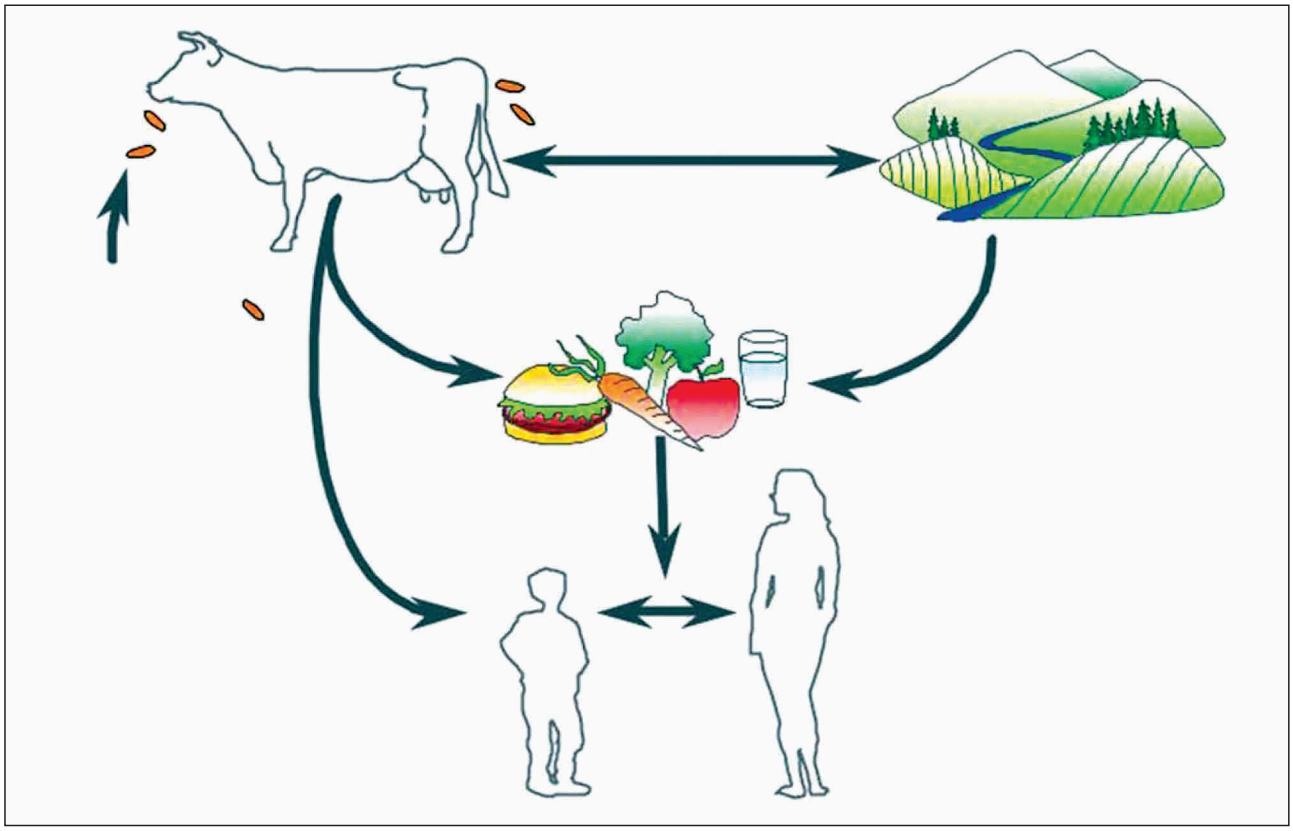

Figure 3. Exposure of humans to zoonotic Shiga toxin-producing *Escherichia coli* (STEC) (*Fairbrother and Nadeau*, 2006)

Slika 3. Izloženost ljudi ka zoonotskoj Šiga toksin-produkujućoj *Escherichia coli* (STEC) (*Fairbrother i Nadeau*, 2006)

Food borne. This is considered, overall, to be the main infection route, at least in the outbreaks (*Fairbrother and Nadeau*, 2006) (Figure 3). The implicated sources of food borne infections include:

- meats (e.g. meat patties, fermented sausages, deer jerky);
- milk/dairy (e.g. un-pasteurized milk, heat--treated milk, cheese from raw milk);
- produce (e.g. potato, alfalfa/radish sprouts);
- drinks (e.g. apple cider); and
- water (well water, reservoir water, mains' water supply).

Systemically presented information on the relative relevance of the food borne route *vs* other routes for *E. coli* O157 infections, is scarce. In the UK during 1995−2004 period, within O157 infections with identified infection routes, the food borne route was the most common and was responsible for 25% to 40% of the cases (*Smith,* 2004). In the US, over 20-year period, 52% of outbreaks were food borne, amongst which ground beef was implicated as a food vehicle in 41% of outbreaks (*Rangel et al.,* 2005). However, the situation with the routes of the food borne diseases can – and do – markedly vary between countries, and also both spatially and temporally within a given country.

2.2 Hazard characterization

2.2.1. Basic mechanism of E. coli O157 infection

Infection process in humans. After ingestion and incubation of around 4 (3 to 9) days, *E. coli* O157 is thought to be non-invasive, presumably colonizing the GI tract (large intestine, i.e. colon) by adhering to the external surface of gut epithelial cells (mediated by the Locus of Enterocyte Effacement-LEE). It seems that the exact location associated with A/E (attaching/effacing) lesions in the guts is not fully understood, possibly because human colon biopsy specimens are collected relatively late in the disease process, and lesions would only be visible during the early stages of infection (*Nataro and Kaper*, 1998). However, *Phillips et al.* (2000) found one *E. coli* O157 isolate did form microcolonies and attaching/effacing (A/E) lesions on human Payer's patches

in vitro, but not on proximal or distal small intestine nor on colon tissue. Once established in the GI tract, *E. coli* O157 cells do not move from the gut lumen, but produce one or more shiga(vero)toxins in the large intestine (*Griffin et al.,* 1990).

2.2.2. Manifestations of human infection

Hemorrhagic colitis (HC). HC or bloody diarrhea is usually accompanied by abdominal cramps causing severe pain. It may start with non-bloody diarrhea that progresses to bloody diarrhea within 2 to 3 days, and may be accompanied by vomiting and sometimes relatively mild fever (*Nataro and Kaper,* 1998).

Hemorrhagic uremic syndrome (HUS). HUS is characterized by acute kidney failure (Figure 1); it is the leading cause of kidney failures, overall, in children (*Park et al.,* 1999).

Thrombotic thrombocytopaenic purpura (TTP). In adults, *E. coli* O157 infection may result in thrombotic thrombocytopenic pupura (TTP). This disease is similar in course to HUS, but the central nervous system is involved in addition to the kidneys. Neurological complications occur in about 25% of HUS patients (*Mead and Griffin,* 1998).

Morbidity and mortality. Approximately 30% to 45% of the *E. coli* O157 infection cases are hospitalized. Long term complications are possible in patients that recovered from the infection, particularly in case of HUS (Hemolytic Uremic Syndrome). Fatalities are usually associated with HUS and the mortality rate is usually between 2 and 7% (*Mahon et al.,* 1997; *Bantavala et al.,* 2001; *Roberts and Upton,* 2001).

Asymptomatic carriers. Humans can be asymptomatic carriers of *E. coli* O157 (*Curnow*, 1999). Cattle carry *E. coli* O157 in their GIT (gastrointestinal tract) and they remain healthy. The organism may be a constituent of their naturally-occurring microflora, and longitudinal studies show most cattle occasionally carry *E. coli* O157 in their feces (*Hancock et al.,* 1997; *Lahti et al.,* 2003).

*DALY*s. In order to evaluate the public health impact regarding STEC-associated disease, a disability-adjusted life years model (DALY) is regularly used by public health agencies and international organizations (e.g. the World Health Organization) to assess a metric that combines the burden of mortality and morbidity (non-fatal health problems) into a single number. The DALY measure combines the years of life lost due to premature death (YLL) and the years lived with disability (YLD) for varying degrees of severity, making itself a valuable public health indicator. One DALY is a health measure, equating to 1 year of healthy life lost. (*WHO*, 2006). For instance, the mean disease burden of VTEC O157 in the Netherlands was 116 DALY per year (*Havellar et al,* 2003).

2.2.3. Beef meats associated with E. coli O157 infections

Around 52% of these cases were caused from ground beef (minces-burgers-patties), around 33% from other meats, around 12% from various fermented sausages, and around 0.5% from dried venison. These data outlined the dominant role of raw meats intended for cooking – particularly ground beef – followed by ready-to-eat sausages, i.e. fermented salamis (*Wells et al.,* 1983; *CDCP,* 2000; *CDCP,* 2003; *Pennington*, 2010).

2.2.4 Dose-response relationship

Assumption that exposure to a relatively low number of *E. coli* O157 cells can lead to the development of the illness is generally accepted (<100 CFU). If the infectious dose is very low, the consequence would be that infection may occur without pathogen's growth occurring in contaminated food (*Anon*. 1999).

2.2.5. Virulence factors affecting dose-response

Shigatoxins (Stx1, Stx2, Stx2c). Among the most important virulence characteristics of *E. coli* O157 is the ability to produce one or two Shiga toxins (verocytotoxins) (*Mead and Griffin*, 1998).

Enterohemolysin (hyl). Nearly all strains of *E. coli* O157 produce a hemolysin (termed enterohemolysin) that is encoded on the 60-MDa plasmid. Patients with HUS develop antibodies to enterohemolysin (*Schmidt et al.,* 1995), but it is still unclear whether/how it is involved in pathogenesis of disease.

Intimin (eae). Encoded by the *eae* gene, is an adherence factor that plays a role in intestinal colonization of *E. coli* O157 *in vivo* and in animal model (*Nataro and Kaper,* 1998).

pO157 plasmid. Encodes a catalase-peroxidase with unknown function. The plasmid is widely distributed among human EHEC isolates, but its role in the pathogenesis of disease is not yet determined and the results of *in vivo* and *in vitro* studies have been conflicting (*Nataro and Kaper,* 1998).

Iron transport. *E. coli* O157:H7 strains contain an iron transport system (a 69-kDA protein encoded by the *chu*A gene) allowing the use haeme/hemoglobin as an iron source (*Torres and Payne*, 1997), which possibly aids infection as it stimulates the growth of the pathogen.

EAST1. Many strains of *E. coli* O157:H7 possess the *ast*A gene encoding EAST1 (*Savarino et al*., 1996), the role of which in pathogenesis of disease is not known although it may be involved in non-bloody diarrhea.

Other intestinal adherence factors. Some adherence factors other than intimin have been reported for *E. coli* O157:H7 but they have not been well characterized or specifically demonstrated *in vivo*. For example, a 94-kDa OMP (distinct from intimin) mediated adherence adherence to Hep-2 epithelial cells (*Sherman et al.,* 1991) but it was not further characterized.

2.3. Exposure assessment

2.3.1. Introduction

Although this review reflects the STEC distribution along the beef chain, the majority of data are based on the published research on *E. coli* O157:H7. Therefore, it is hypothesized that different serotypes belonging to non-O157 STEC will most likely behave in a broadly similar fashion as *E. coli* O157:H7, in different ecological compartments – the gastrointestinal tract (GIT) and farm environment; this should be considered as an educated and informed assumption. It should be also considered that ecological distribution related to *E. coli* O157:H7 may not apply always to all STECs.

Presently, there is no single point along the meat (food) chain at which *E. coli* O157 can be reliably eliminated so to entirely prevent exposure of consumers to the pathogen, apart from sufficient heat treatment and reliable post-heating control of contamination. A longitudinally integrated approach to the meat (food) chain (i.e. LISA/Longitudinally Integrated Safety Assurance) including reduction of the pathogen at multiple points is necessary to reduce the risk of *E. coli* O157 infections occurring via meats (foods).

2.3.2. STEC in the beef chain

On-farm. Healthy cattle appear to shed STEC O157 and non-O157 serotypes sporadically, with high numbers being excreted in intermittent "bursts" in their faeces. The factors that contribute to a burst of shedding from a particular animal or herd are not fully defined yet (e.g. mixing of individual animals and/or herds, diet and the watering system). Published data have indicated that transmission via feedstuffs (*Davis et al.,* 2003), drinking/irrigation water (*Barham et al.,* 2002) and wilde-life (rats, flies, birds) may be involved. Recently, the study done by *Baines et al.* (2011) aimed to prove the link between mouldy feeds, mycotoxins, STEC colonization and development of Jejunal Hemorrhage Syndrome (JHS) in beef cattle. According to this study, until recently there have been no reports of STEC O157 disease in mature cattle (*Baines et al.,* 2008), but STECs do affect calf health from birth to weaning (*Hall et al.,* 1985; *Schoonderwoerd et al.,* 1988; *Sandhu and Gyles,* 2002). In some other studies (*Cray and Moon*, 1995; *Brown et al.,* 1997) it was confirmed that STEC infections cause high mortality in neonatal calves resulting from acute enteritis. Older calves may have transient watery diarrhea but are not seriously affected by STEC O157 infections. In addition, the similar A/E lesions, presented in hemorrhaged tissues in humans, were also found in the jejunum, ileum, cecum, colon, and rectum in neonatal calves, but not in older calves (*Cray et Moon,* 1995; *Brown et al.,* 1997; *Dean-Nystrom et al.,* 1997; *Dean-Nystrom et al.,* 1998). However, if STECs do cause disease in mature cattle, the most likely candidates are diseases with unclear etiologies such as JHS (*Puntenney et al.,* 2003). Current treatments for JHS include an aggressive medical and surgical therapy that can be effective, but the prognosis for long term survival relies upon early detection (*Peek et al.,* 2009).

While a number of factors have been identified and suggested as playing a role in the on-farm population dynamics of this pathogen, only season has been repeatedly and consistently shown to have an effect on shedding. Fecal shedding is typically low in the winter, increasing in the spring to peak levels during the summer months, then tapering off in the late autumn to very low winter levels (*Chapman et al.,* 1997; *Hancock et al.,* 1997; *Van Donkersgoed et al.,* 1999). The frequency of human outbreaks of *E. coli* O157:H7 occurring predominantly in the summer months, are complement with seasonal shedding patterns in cattle (*Besser et al.,* 1999; *Rangel et al.,* 2005).

Taking into consideration the intermittent/sporadic shedding of *E. coli* O157, the detected prevalence of *E. coli* O157 in the bovine faeces at farm was very low – 0.5% (*Buncic and Avery,* 1997), medium – 2.6% (*Nastasijevic et al.,* 2009a), 4.4% (*Conedera et al.,* 2001) and up to very high levels – 22.7% (*Smith et al.,* 2001). The detected level on the hides was 18% (*Barham et al.,* 2002), while higher levels, e.g. 24.6% were detected on farm surfaces (*Lahti et al.,* 2003). Commercial stock feeds sampled on-farm contained *E. coli*, but not *E. coli* O157 (*Lynn et al.,* 1998). It seems that proper silage fermentation reduces and, depending on initial levels, even can eliminate *E. coli* O157. This pathogen was

also found in slurry collected from cattle feedlots (*Cízek et al.,* 1999) and a dairy farm (*Porter et al.,* 1997). It is proved that *E. coli* O157 can survive up to 99 days in soil (*Bolton et al*., 1999) or even can proliferate in various soil types such as silt loam, sandy loam and clay loam (*Gagliardi and Karns*, 2000). Lastly, potential for "mechanical" spread of *E. coli* via vectors (e.g. rodents, birds, flies, vehicles, workers-visitors, feeds) exist on farms, in the same (well-known) way as with other food borne pathogens such as *Salmonella*.

Transport-market-lairage. Information on the fate of *E. coli* O157 during the transport and in livestock markets is limited. A recent study showed that the prevalence of marker organisms inoculated onto the hides of cattle entering the market process increased 2– to 5-fold on those animals post-market (*Collis et al.,* 2004). In some other studies, the prevalence of *E. coli* O157 dropped on cattle hides after transport and lairage (*Barham et al.,* 2002). In addition, *E. coli* O157 was also isolated from 7.3% of clean transport surfaces before cattle were loaded, indicating environmental spread of the pathogen could occur (*Barham et al.*, 2002). The detected prevalence of *E. coli* O157 in the bovine faeces post-transport, pre-lairage was 1.7% and 13% (*Minihan et al.,* 2003).

Lairage-to-dressing. A number of data accumulated in the past few years showed that lairage-to-dressing environment could play an important role in the spread of *E. coli* O157 in cattle at slaughterhouses through animal-animal and/or animal-environment-animal contacts (e.g. surfaces in lairage pens or stunning boxes). In addition to that, the occurrence of this pathogen in lairage varied from 7.8% (*Small et al.*, 2002) and even very high level– 50% (*Tutenel et al.,* 2003). It was confirmed that *E. coli* O157 survives very well on environmental surfaces; decimal reduction times (D values) on hide, concrete, metal or straw ranged between 3 and 15 days (*Small et al.,* 2003). An additional meat safety concern is that naturally-occurring *E. coli* O157 can persist on surfaces even after routine washing; not only in lairage areas (*Small et al.,* 2002), but also on surfaces on farm (*Lahti et al.,* 2003) and in transporters (*Barham et al.,* 2002). It should be taken into account that cattle lairage washing rarely includes treatments with detergents/sanitizers (*Small et al.,* 2003). Therefore, carry-over of *E. coli* O157 contamination on lairage surfaces from one day to subsequent days seems likely. One of the most important sites is the stunning box, the surfaces of which all animals contact in succession (*Small et al.,* 2002; *Avery et al.,* 2002). It means that through animal-environment-animal contacts the transfer and spread of persisting *E. coli* O157, amongst livestock slaughtered on different days, may occur and such cross-contamination could negate any successful control of the pathogen achieved on individual farms.

Slaughter-to-dressing. Visible cleanliness of hides and levels of microorganisms on carcasses may (*McEvoy et al*., 2000) or may not (*Kain et al.,* 2001) be correlated with quantitative total microbial viable counts. However, reported on-hide *E. coli* O157 prevalences on cattle at slaughter ranged from 4.5% (*Barham et al.,* 2002), 28.2% (*Nastasijevic et al.,* 2008a) to 56% (*Tutenel et al.,* 2003). This indicates the hides may be the very important sources of pathogen. This is a major concern, as in modern industrial slaughterhouses, carcass contamination is mainly due to hide-to-meat microbial cross-contamination, directly or via equipment, tools (*Tutenel et al.,* 2003) or airborne (*Rahkio and Korkeala,* 1997), rather than due to direct spills of digesta/faeces during evisceration. The skinning may be considered as a high-risk operation and *Elder et al.* (2000) found that most of the carcasses were contaminated with *E. coli* O157 in the pre-evisceration phase. The detected levels of carcass contamination were low – 2.8% (*Nastasijevic et al.,* 2009a), medium – 11.1% (*McEvoy et al.,* 2003) and high – 43.4% (*Elder et al.,* 2000).

Chilling-processing-retail. In a large 3-year survey in Belgium, 1.02% of the carcasses during chilling at the slaughterhouse were contaminated with *E. coli* O157 (*Tutenel et al.,* 2003), whilst other studies found higher prevalences – 5.5% (*McEvoy et al.,* 2003). *Carney et al.* (2006) found prevalence of pathogen in 2.4% samples of beef trimmings. Occurrences of *E. coli* O157 in minced beef (without ingredients) and completed sausage batter (i.e. intended for production of fermented sausages) were 6.2% and 2.1%, respectively (*Nastasijevic et al.,* 2009a). Other surveys of beef at retail level indicated a wide range of *E. coli* O157 occurrence, ranged from 0% (*Uhtil et al.,* 2001) and up to 36% (*Radu et al.,* 1998) with a median prevalence of around 6% in studies were pathogen was detected. Even if the contamination level on beef was low, that would still represent a serious public health risk because beef is often eaten undercooked (e.g. beef burgers) or even raw (e.g. beef tartar).

Catering-consumer level. Food safety problems associated with *E. coli* O157 in meats at catering and consumer levels relate to final preparation of food for consumption. The catering-level issues have been recently summarized in the form of brief guidelines (*Bolton and Maunsell*, 2004). At consumer level, epidemiological data from Europe (*Tirado and Schmidt*, 2000), North America, Australia, and

Table 3. Examples of confirmed *E. coli* O157 cases in meat borne catering outbreaks
Tabela 3. Primeri potvrđenih *E. coli* O157 slučajeva u alimentarnim epidemijama u vezi sa keteringom, nastalim nakon konzumacije mesa

Implicated meats (country)	**Cases (deaths)**	**Reference**
Beef tacos (USA)	13	*Conway* (1995)
Beef ("seeme rolle") (USA)	11	*Werber et al.* (2002)
Beef (roast) (USA)	65	*CDCP* (1990)
Cooked meat (Scotland)	496 (20)	*Pennington* (1998)
Cooked meat (UK)	30	*Rajpura et al*. (2003)
Genoa salami (Canada)	39	*Williams et al.* (2000)
Sausages (mortadella and teewurst) (Germany)	28 (3)	*Ammon et al.* (1999)

New Zealand indicate that substantial proportions of foodborne disease can be attributed to food preparation practices used in the domestic environment. The main risk factors include:

a) cross-contamination from raw to cooked foods via refrigerators, contaminated hands, cutting boards and kitchen towels;
b) inadequate refrigeration;
c) improper cooking; and
d) inadequate post-cooking handling including slow cooling and/or re-contamination.

In North and South America eating undercooked ground beef continues to pose a risk to the population (Table 3), but this has not been implicated as a food vehicle in the UK according to a study by *Parry et al*. (1998).

According to *Eblen* (2007) the prevalence of STEC O157 and some other non-O157 STEC strains in retail foods and retail meats can be very variable ranging from 0.1-12% in raw ground beef and up to 4.8% and 3.3% in fresh and dry sausages, respectively (Table 4).

Table 4. The prevalence of STEC O157 and non-O157 STEC strains in retail foods and retail meats
Tabela 4. Prevalenca STEC O157 i non-O157 STEC sojeva u hrani iz maloprodaje i mesa iz maloprodaje

Country	Organism	Reported prevalence	**Reference**
Argentina	*E. coli* O157:H7	4.8% of fresh sausages; 3.8% of raw ground beef; 3.3% of dry sausages	*Chinen et al.,* 2001
Belgium	All STEC	4.6% of raw meat samples (beef, mutton and venison)	*Pierard et al*., 1999
Botswana	*E. coli* O157:H7	5.2% of meat cubes; 3.8% of raw ground beef; 2.3% of fresh sausages	*Magwira et al*., 2005
England	*E. coli* O157:H7	2.9% of lamb products, 1.1% of beef products	*Chapman et al*., 2000
France	All STEC	11% of beef	*Pradel et al*., 2000
France	*E. coli* O157:H7	0.1% of raw ground beef	*Vernozy-Rozand et al*., 2002
Italy	*E. coli* O157:H7	0.4% of raw ground beef	*Conedera et al*., 2004
New Zealand	All STEC	12% of beef, 17% of lamb; 4% of pork	*Brooks et al*., 2005
Sweden	*E. coli* O157:H7 All STEC	0.06-0.5% of raw ground beef 4% of raw ground beef	*Lindqvist et al*., 1998

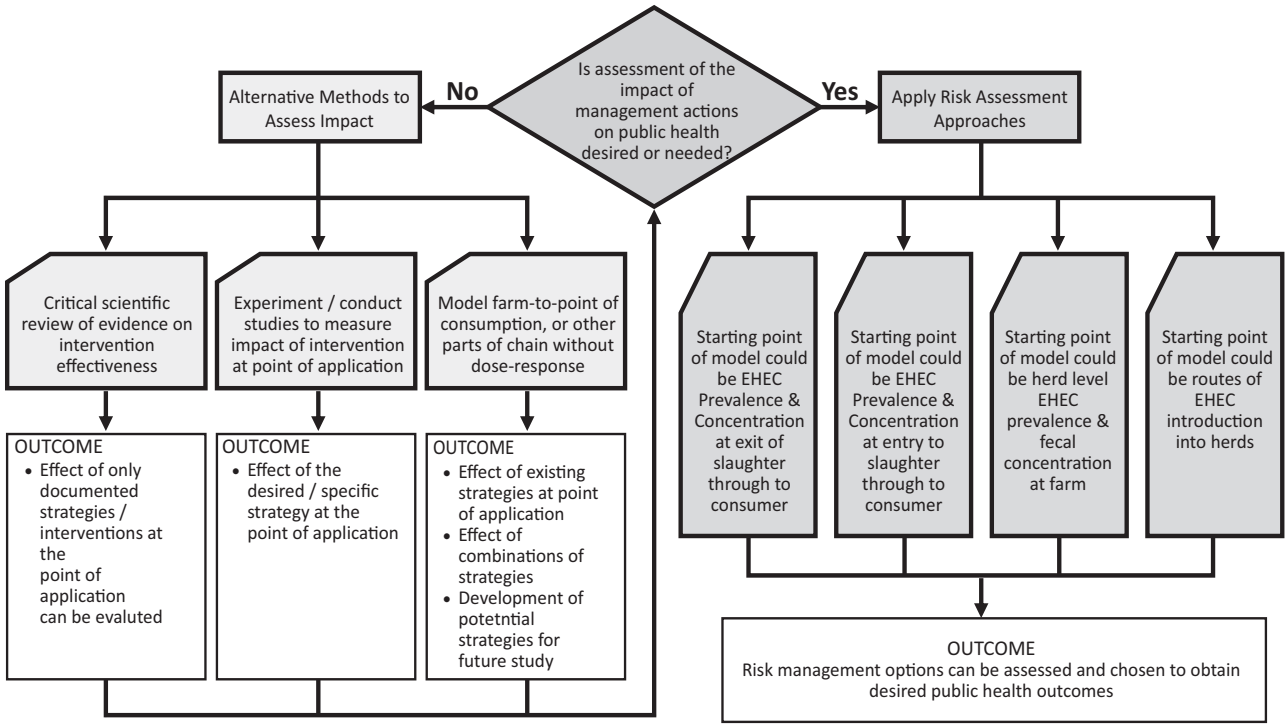

Figure 4. Roadmap for the application of risk assessment approaches in managing the public health impact of EHEC (*WHO*, 2011)

Slika 4. Mapa puta za primenu pristupa u oceni rizika za upravljanje uticaja EHEC na javno zdravlje (*WHO*, 2011)

Lastly, for successful science-based risk assessment, risk assessors need to have good communications with risk managers to provide guidance on what risk assessment can and cannot answer, i.e. to condition the expectations of the risk manager (e.g. depending on the risk question it may not be necessary to consider the full chain (e.g. if only interested in interventions at the consumer level, then no model, or a less detailed model, would be needed at the pre-harvest and harvest levels). Examples of the types of risk assessments that could be undertaken to assist risk managers in controlling STEC/EHEC in beef and beef products and their potential areas of application are as follows (*WHO,* 2011) (Figure 4):

- Prevalence and concentration of STEC/EHEC in beef products exiting processing plant
- Prevalence of contaminated cattle and contamination level of STEC/EHEC on animals entering the slaughter and processing plant
- Herd prevalence and fecal contamination levels at the farm
- Routes of introduction and spread of STEC/EHEC into and within herds

3. Risk management

In considering the risk management issues associated with STEC it has to be noted that to date most of these have been addressed in the absence of quantitative microbial risk assessments. In some countries a risk assessment has been carried out, irrespective of whether or not the risk assessment contributed to the risk management decision. For the most effective risk management decisions, it is suggested that it will be useful to look at one country where no risk assessment has been undertaken, as such an example may also reflect the situation in many other countries, especially with respect to the adoption of HACCP and GHP in slaughterhouses (WHO, 2011). In the process of risk management, the main and the most effective control options of *E. coli* O157 along the beef chain, which are also technically and financially sustainable, have to be selected. Risk management strategies should be implemented by the Competent Authorities responsible for the enforcement of official controls in the food (beef) chain and should be based on holistic and integrated approach to the food safety – "farm to fork". These strategies may include: managing farms to reduce fecal shedding in cattle and spread of pathogen

in environment; transport-market-lairaging procedures to control cross-contamination; GHPs and HACCP procedures in slaughter operations and beef processing; proper handling during transport and by retail outlets; proper handling and cooking by consumers. Over-reliance on any one sector may result in a false sense of security of the final product.

3.1. Pre-harvest control options

Currently, there are no on-farm measures that can be relied upon to guarantee complete absence of *E. coli* O157 carriage in individual cattle or herds. This is very difficult even if herds are subjected to an on-farm testing regime, because the intermittent nature of the shedding. Due to multi-factorial nature of the *E. coli* problem on-farm, the efficacies of individual control measures or their combinations at pre-harvest level are difficult to quantify and carry many uncertainties. Therefore, when cattle destined to enter the human meat chain are transported from farms for slaughter, their *E. coli* O157-carriage status is largely unknown. Presently, on-farm control measures could only reduce *E. coli* O157 incidences/prevalences, and only to uncertain extents. The main considerations related to on-farm controls include:

3.1.1 Prevention of the pathogen's recycling

Slurry used as a fertilizer. E. coli O157 was found in slurry collected from cattle feedlots (*Cízek* et al., 1999) and a dairy farm (*Porter et al.,* 1997). Additionally, *E. coli* O157 occurring naturally in manure originated from bovine shedders can survive for very extensive times – ranging from a few weeks to 21 months (*Kudva et al*., 1998; *Bolton et al.,* 1999).

Soil. *E. coli* O157 survived up to 99 days in soil (*Bolton et al.,* 1999) or proliferated in various soil types including silt loam, sandy loam and clay loam (*Gagliardi and Karns*, 2000). Therefore, farmers have a responsibility to manage land and organic agricultural materials, i.e. slurry and manure, in ways that prevent contamination of ready-to-eat food crops, water supplies, feed and grassland and also to apply other necessary biosecurity measures such as deratization, disinfection of farm and surrounding environment, control of wildlife, movement of workers and mechanical vectors. Farmers are also obliged to present animals for slaughter with the minimum amount of soil and faecal contamination on their hides, so that cross-contamination of carcasses/meat during slaughter and dressing will be minimized.

3.1.2. Prevention of ingestion of the pathogen

Feed. STEC *E. coli* was detected in 6.3% of fresh grass samples, indicating that pastures have the potential to act as sources of transmission of the pathogen for grazing livestock (*Hutchison et al.,* 2006). On the other side, feeding hay, grass, or silage high in propionic or acetic acids may reduce the likelihood of STEC shedding by cattle (*Lynn et al.,* 1998). Due to changes in farm husbandry practices, cattle nowadays are fed more grain and concentrates. It has been suggested that these practices may promote the growth of *E. coli* populations. However, further studies are required before definitive advice can be formulated in relation to the effect of feeds on the incidence of STEC (*FSAI,* 2010).

Water. Studies have shown that *E. coli* O157:H7 can survive in water for up to 109 days (*Scott et al.,* 2006). Water supplies contaminated with livestock effluent have been implicated in a number of outbreaks (*Locking et al.,* 2006). STEC survives in water trough sediments for at least four months and appears to multiply there, especially in warm weather. Farmers should clean water troughs frequently to prevent the accumulation of sediments. Water troughs should be positioned away from feed troughs/feed passageways, as contamination of water with feed can providing a nutrient-rich substrate for bacterial growth and survival at the bottom of the trough (*Lejeune et al.,* 2001). Specific water treatments (i.e. disinfection) may be also needed.

Animal interactions (suckling, licking, etc). The design of the farm holding should allow logistic organization of animal feeding and breeding so to avoid unnecessary contacts between them. This is because by suckling and licking of fecally contaminated hides, pathogen can be easily transmitted, by fecal-oral route (*Pearce et al.* 2004).

3.1.3. Suppression of the ingested pathogen

Dietary manipulation. It has been shown that zoonotic STEC O157 and non-O157 survive in acid conditions and persist in rumen contents (*Boukhors et al.* 2002), which supports the proposal that a grain-rich diet may induce acid resistance of STEC in the rumen and permit the bacteria to survive in the abomasum, leading to increased fecal shedding. However, numerous field studies have demonstrated the opposite effect: hay-fed sheep (*Kudva et al.,* 1997) and cattle (*Hovde et al.,* 1999) shed STEC O157 for shorter periods than grain-fed animals of the same species. In another study, (*Grauke et al.,* 2003) no difference in fecal shedding of STEC O157

was observed between hay-fed and grain-fed cattle. Further investigation is needed in order to give proper risk management recommendations regarding dietary regimes/manipulations which could minimize shedding of STEC O157.

Probiotics, prebiotics, competitive exclusion. Treatment with different probiotic strains has had variable effects on fecal shedding of STEC in cattle. Encouragingly, daily treatment of finisher beef cattle with direct-fed microbials, such as certain strains of *Lactobacillus acidophilus* (*Younts-Dahl et al.,* 2005), reduced fecal shedding of STEC O157 by over 50%. Treatment with a competitive exclusion probiotic containing *E. coli* strains reduced fecal shedding of both O157 and O111, but not O26 zoonotic STEC in weaned calves (*Tkalcic et al.,* 2003). Hence, these results suggest that a judicious choice of probiotic bacterial strains for the treatment of cattle could eventually permit a reduction in fecal shedding of not only STEC O157 but also a variety of zoonotic STEC non-O157 serotypes. Lastly, the study performed by *Baines et al.* (2011) confirmed that mycotoxins and STEC are part of the disease complex for JHS (Jejunal Hemorhagic Syndrome) in beef cattle. A prebiotic treatment alleviated the development of disease in symptomatic beef calves. Future studies should examine the role of STECs and mycotoxins in the infection process that leads to JHS and the mode of action of prebiotics.

Phage therapy. Antibacterial viruses, known as bacteriophages, that specifically target STEC O157 appear to be able to control the growth of these bacteria under laboratory conditions and have shown promising results in sheep; however, further work is necessary before the viruses can be considered a feasible approach for the control of STEC in cattle (*Callaway et al.,* 2004; *Niu et al.,* 2012).

3.1.4. Modification of the animal's response

Vaccination. Vaccination performed with type III secreted proteins, resulted in significant decrease of the number of animals shedding faecally the organism and the number of challenge organisms shed per animal. These studies were done in experimentally infected cattle and in clinical trials in feedlot cattle, demonstrating the potential benefits of such an approach (*Potter et al.,* 2004; *Allen et al.,* 2011). Nevertheless, this approach still requires some optimization as faecal shedding was not reduced after administration of the same vaccine to feedlot cattle in commercial operations (*Van Donkersgoed et al.,* 2005). Furthermore, in the recent study carried out by *Cernicchiaro et al.* (2014) it was revealed that the *E. coli* O157:H7 vaccine, which reduced STEC O157 fecal shedding, didn`t significantly affected fecal shedding of non-O157 STEC serogroups, despite the fact that the most prevalent non-O157 STEC serogroups tended to occur concurrently with O157 STEC strains within fecal samples; O157, O26 and O103 were the most prevalent STEC O serogroups that have been fecally shed by feedlot cattle.

3.2. Transport-market-lairage

3.2.1. Transport

Significant spread of the pathogen contaminating animal coats during transport can occur. This occurs through the same mechanisms of animal-to-animal and/or animal-surfaces-animal cross-contamination taking place during lairaging (*Childs et al.,* 2006). Therefore, transportation of cattle intended to slaughter should be always performed in properly sanitized vehicles and with minimal duration because stress that may happen can increase shedding of STEC *E. coli* O157 and even increase subsequent cross-contamination between animals and/or animal-surface-animal.

3.2.2. Livestock market

A recent study showed that the prevalence of marker organisms (including generic *E. coli*) inoculated onto the hides of cattle entering the market process increased 2– to 5-fold on those animals post-market (*Collis et al.,* 2004). It is recommended that livestock markets should be avoided, if possible. That is because the mixing of animals from different farms (e.g. pathogen-free farms and others) can increase the cross-contamination of non contaminated cattle either through direct contact and/or through contamination of environment.

3.3. Lairage-to-dressing

3.3.1. Lairaging

The lairage-to-dressing environment plays an important role in the spread of *E. coli* O157 in cattle at slaughterhouses through animal-animal and/or animal-environment-animal contacts (*Avery and Buncic*, 2005). Cattle should be kept in sanitized pens and with minimal duration, because carry-over of pathogens on surfaces from one day to another may increase the probability for cross-contamination of subsequent animal batches coming from different farms (e.g. animals lying on contaminated floor).

3.3.2. Sanitation of stun boxes

During the process of stunning, the first slaughter operation, animals can contaminate the surfaces of stun box via contact of their fecally contaminated hides and surfaces-mediated cross-contamination of consecutively stunned animals may occur (*Small et al.,* 2006). Therefore, the proper sanitation of stun box between different slaughter batches is highly recommended.

3.4. Slaughter-to-dressing (Harvest)

3.4.1. Efficient cleaning-sanitation of the slaughter-hall environment

Effective cleaning-sanitation of the slaughter-hall environment is necessary and beneficial for microbial safety of the meat (*Nørrung and Buncic*, 2008), because the potential for transfer of pathogen via cross-contamination between carcasses and slaughter equipment, floor and walls, is minimized.

3.4.2. Minimizing microbial contamination through application of GHP/GMP and HACCP principles

A range of standard operational procedures are used at slaughter line to prevent/minimize microbial cross-contamination during slaughter. According to the best GHPs, it is advisable to slaughter only visually clean animals and to reject dirty ones; to perform mechanical skinning, bagging of anus and tying ("rodding") of esophagus before evisceration; and to apply procedures, such as regular hot water/steam "sterilization" of all tools and equipment coming in direct contact with meat, so to avoid and/or minimize the possibility of cross-contamination (*Nastasijevic et al.,* 2008a; *Nastasijevic et al*., 2008b). The HACCP principles should ensure science-based hazard analysis and risk categorization of all steps along the beef slaughter line. The potential Critical Control Points (CCPs) may be visual assessment of cattle hides` contamination, skinning, evisceration and rapid chilling (<4ºC/24h). Lastly, the use of indicator organisms may be helpful to assess the probability of the presence of *E. coli* O157 on bovine carcasses (e.g. APC, Generic *E. coli*, *Enterobacteriaceae*), through continuous monitoring of microbial process hygiene (USDA FSIS, 2002).

3.4.3. Decontamination treatments

The higher level of safety assurance against STEC, regarding prevention and minimization of hide-mediated cross-contamination of carcasses, can be achieved by "pro-active" decontamination of hides before skinning and "reactive" decontamination of carcasses (Table 5). Decontamination treatments of hides (*Castillo et al.*, 1998a; 1998b; *McEvoy et al.,* 2001; *Bosilevac et al.,* 2005) and dressed carcasses (e.g. steam vacuuming, steam pasteurization, hot water washes, organic acid washes, etc.) (*Dickson et al.,* 1994; *Carneiro et al.,* 1998; *Uyttendaele et al.*, 2001) and their combinations can be used. Carcass decontamination is a mandatory CCP (Critical Control Point in HACCP-based food safety management system) at abattoirs in USA, whilst it is still not widely used in the EU. Namely, *EC Regulation 853/2004* (article 3 and 12) allows decontamination treatments to be considered as a supplement to good hygiene practices. In the EU, risk

Table 5. Example of the effects of decontamination treatments on *E. coli* O157 on hide or meat

Tabela 5. Primer efekata dekontaminacionih tretmana u odnosu na *E. coli* O157 na koži ili mesu

Treatment	Anti-E. coli O157 effects achieved (approx.)	Reference
Hide decontamination		
Sodium sulphide-hydrogen peroxide combination (chemical dehairing)	5 log reduction	*Castillo et al*. 1998a
Steam (condensing at 80ºC; sub-atmospheric pressures)	4 to 6 log reduction	*McEvoy et al*. 2001
Sodium hydroxide wash plus chlorinated (1 ppm) water rinse	Prevalence reduced from 44% to 17%	*Bosilevac et al*. 2005
Meat decontamination		
Hot water (74-80ºC)	3.7 log reduction	*Castillo et al.* 1998b
Steam pasteurization (above atmospheric pressure)	3.7 to 4.4 reduction	*Phebus et al.* 1997

assessors and risk managers need to ensure any such substance is first shown to be safe and effective at significantly reducing microbial contamination before it can be approved. In addition, EU authorities (e.g. EFSA, EU Commission) are also seriously considering a possibility of microorganisms developing resistance to substances used for decontamination of carcasses/meat – as a result of their use. Currently, only lactic acid is approved to reduce microbiological surface contamination on bovine carcasses, and no other substances are authorized for this purpose within the EU (*EC Regulation 101/2013*).

3.4.4. Novel approaches

Microbial immobilization treatments. Recently, the novel approaches regarding microbial immobilization treatment of hides are considered. It is proved that bacterial on-hide immobilization, rather than decontamination of hide, could be very effective in reducing transmission of bacteria (including *E. coli* O157) from cattle hide onto the meat. This novel approach is more proactive and preventative because it aims to prevent pathogen transfer from their main source – surface of hide, to the carcass. This is achieved by immobilization (fixation) of pathogens on the hair. With this approach the killing of the entire target hide microbiota may not be necessary. Rather, the hide could be treated with some compound(s) "glueing" the microorganisms to the hair so as to prevent their detachment from the hair and transmission onto the carcass during the skinning operation at slaughter line. This approach can be even more effective from decontamination treatments, by reducing swab-recoveries of TVC (Total Viable Counts) by 6.6 logs, fecal indicators (GEC-Generic *E. coli* and EC-Enterobacteriaceae) by 2.9 and 4.8 logs, respectively and *E. coli* O157 by 2.1 logs (*Antic et al.*, 2010).

Revision of post-mortem protocol in the cattle slaughter line. Current abattoir protocols do not include examination of the jejunum for lesions suggestive of hemorrhagic disease (JHS) and as such, it provides a novel approach to identifying suspect animals and removing them from the food chain (*Baines at al.,* 2011).

3.5. Chilling-processing-retail (Post-harvest)

The cold chain during all stages after slaughter should be maintained. Effective cleaning and sanitation in related premises should be performed in order to prevent cross-contamination during cutting, de-boning and further processing. Bactericidal step (e.g. heating/cooking) should be included in the process, e.g. >71°C/1min, throughout the product (*USDA,* 2003); recontamination of the heated products during further handling (e.g. slicing, packaging) should be prevented; For non-heated products, e.g. fermented sausages, the "hurdle" concept should be applied, e.g. validated 5D inactivation treatment (*Reed,* 1995); cross-contamination of ready-to-eat products from raw meats (and other raw ingredients) during food preparation should be prevented.

3.6. Catering-consumer level

To avoid/prevent the cross-contamination of foods with *E. coli* O157, as well as other food borne pathogens, general hygiene principles of WHO Five Keys for Safer Food (WHO, 2006) should be applied. The core messages of the document are: (1) keep clean; (2) separate raw and cooked; (3) cook thoroughly; (4) keep food at safe temperatures; and (5) use safe water and raw materials. It is noteworthy to emphasize that adequate cooking is currently the only bactericidal step in the meat chain by which any level of *E. coli* O157 can be reliably and completely eliminated (*Duffy et al.,* 1999).

4. One health approach to diagnosis, treatment and prevention of STEC

Earlier and timely diagnosis and effective responses to infection provoked by VTEC may be achieved if current recommendations for EHEC diagnosis in humans, issued by clinical laboratories, are followed (*Gould et al.*, 2009). The efficient protocol for detection/diagnosis of VTEC/EHEC O157 and non-O157 pathotypes should encompass culturing on selective and differential agar (e.g. CTSMAC, ChromAgar) and simultaneous molecular methods that can detect shiga-toxins or genes that encode them (e.g. multiplex PCR). No specific treatments are available for HUS in humans. Supportive therapy includes intravenous fluids and volume expansion *(Ake et al,* 2005). Antibiotic treatments are contraindicated in suspected or confirmed cases of O157:H7 infection or infection provoked by other non-O157 pathotypes, due to the possibility of increased risk of HUS by lysis of pathogen` cells and induction of Stx-encoding bacteriophages, which may subsequently lead to increased release of shiga-toxin into blood stream (*Ahn et al.,* 2009; *Zhang et al.*, 2000). Therefore, the recommended intervention strategies in humans consist of vaccines (Gb3 receptor analogues), and monoclonal antibodies against Stx (*Bitzan*, 2009; *Orth et al.*, 2008). Prevention of STEC/EHEC O157 and non-O157 infection is the

best approach to avoid HUS. Hand washing is the most important step for reducing the risk of EHEC O157 and non-O157 transmission (*NASPHV*, 2009).

The overall success of risk mitigation strategies regarding STEC occurrence in the beef chain and protection of public health, is inevitably associated with the implementation of targeted and synergistic control measures within the "One Health" concept which encompass environment/wildlife-animal-human interface. Such integrated approach should enable active monitoring and control of pathogen entering routes along the beef chain (*EC Regulation* 99/2003; *Nastasijevic,* 2009b; *Nastasijevic,* 2014; *Buncic et al.*, 2014), e.g. environment (soil, water, on-farm surfaces, wildlife/pests, birds, workers, vehicles movement), animal (hygienic conditions on-farm, vaccination, dietary manipulations – prebiotics/probiotics, monitoring of fecal shedders, introduction of new animals, etc.) and human (public health education, prevention strategies, early diagnosis, prevention and effective disease management). The "One Health" concept is widely accepted approach in mitigating the public health risks of zoonotic origin and also advocates active and structured inter-sectoral cooperation between the key stakeholders involved in the public health – environmental, veterinary, food and health authorities (Figure 5).

5. Conclusion

Many beef-borne STEC O157 and non-O157 serotypes (O26, O45, O103, O91, O111, O121, O145) are intermittently fecally shed by healthy/asymptomatic cattle and may represent a significant threat to human consumers and public health. STECs colonization and growth in small and large intestine (jejunum, ileum, caecum, colon and rectum) may play important role in that. Calves may develop life-threatening STEC infections in the first months of their life (JHS) and this may also contribute to long term carriage or shedding. The seasonal variations of shedding are reported, with increase in the spring – to peak levels during the summer months, then tapering off in the late autumn to very low winter levels. The shedding leads to contamination of farm environment (slurry, water, soil) which may lead to direct or indirect contamination of hides, which, in turn, serves as the main source of

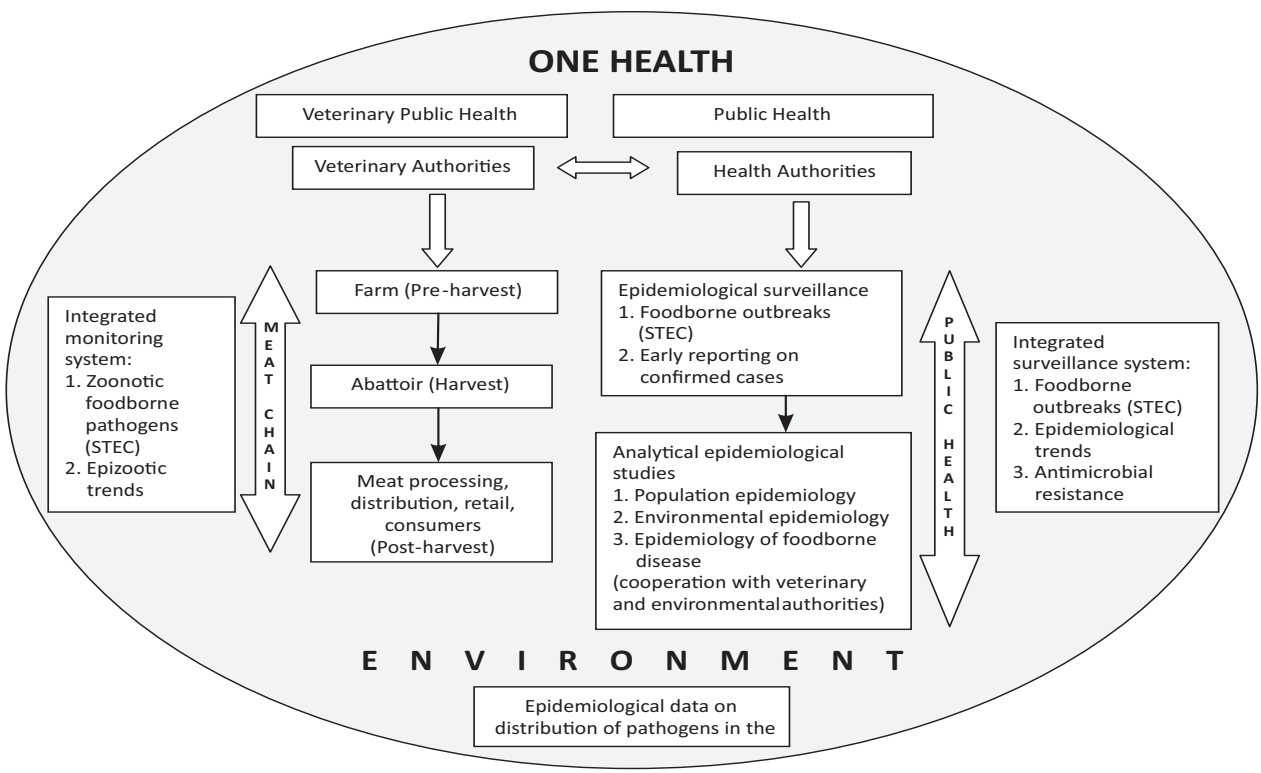

Figure 5. Inter-sectoral collaboration between environmental, veterinary and health authorities in mitigating the public health risks originated from foodborne hazards (adapted from Nastasijevic, 2009b)

Figure 5. Inter-sektoralna saradnja između agencija nadležnih za životnu sredinu, veterinarstvo i zdravstvo u suzbijanju rizika po javno zdravlje poreklom od hazarda koji se prenose hranom (adaptirano iz Nastasijević, 2009b)

carcass contamination during slaughter and dressing of cattle at abattoirs, and further spread to fresh beef and products thereof.

Therefore, the main control options to reduce the beef-borne risk of *E. coli* O157 include: 1) on-farm interventions to reduce its shedding in cattle and prevent contamination of environment and hides – risk management strategies should correspond with peak shedding times during the year (pre-harvest control strategies); and, 2) at-abattoir interventions to reduce hide-to-carcass cross-contamination (harvest control strategies).

It is known that pre-harvest controls in cattle hold great potential to reduce STEC dissemination on farms, in the environment, and entering the food chain. However, none of the on farm management-based controls can completely eliminate STEC from cattle and will certainly not eliminate the need for proper procedures in the processing plant. Therefore, risk-based and well designed meat safety management system (GHP/HACCP), which should include decontamination treatments of hides and carcasses and/or microbial immobilization treatment of hides, regular monitoring of microbial process hygiene (APC – general hygiene indicator, EC/Generic *E. coli* – indicators of fecal contamination) and pathogen occurrence (STEC) on carcasses/meat, as well as targeted post-mortem examination of jejunum, will present the novel approach (harvest controls). The integrated meat safety approach should be also continuously applied in further processing (meat boning, meat processing, distribution, retail). These aforementioned in-plant interventions should maximize the reduction in pathogen entry to the food supply.

At the catering-consumer level, the basic hygienic principles should be applied in meat handling and preparation (e.g. WHO guideline "Five Keys for Safer Food"; WHO, 2006).

Lastly, it should be considered that relatively limited *E. coli* O157 reductions are achieved by applying only one control measure (e.g. decontamination treatments of hides and carcasses or adequate cooking). Therefore, a longitudinally integrated approach to the beef chain, within farm-to-fork continuum, with coordinated and targeted control measures at multiple points is necessary to manage the risk of beef-borne STEC infections. Such approach should be based upon: on-farm controls; transport-livestock market-lairage controls; slaughter-dressing controls; chilling-processing-retail controls; and controls at the catering-consumer level. The competent authorities (veterinary, health, food, environmental) should be encouraged to strengthen the inter-sectoral cooperation within `One Health` concept and to support further, deeper research regarding occurrence and distribution of STEC in the meat chain, so that valid and top quality data needed for the science-based risk assessment and design of effective control measures are generated.

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STEC u lancu goveđeg mesa – Koncept „Jedno zdravlje“

R e z i m e: Još od ranih 80-ih godina prošlog veka E. coli O157 se pojavila kao jedan od najznačajnijih patogena relevantnih za javno zdravlje, ne zbog incidence oboljenja, koja je mnogo manja u odnosu na druge patogene koji se prenose hranom poput Campylobacter ili Salmonella, već zbog težine simptoma, niske infektivne doze i potencijalnih hroničnih posledica. Šiga toksin-produkujuća Escherichia coli (STEC) je pathogena za ljude i može da izazove hemoragični colitis (krvava dijareja) i ponekad hemolitički uremički sindrom (HUS), bolest koja je opasna po život jer dovodi do oštećenja bubrežne funkcije. U svom intestinalnom traktu, goveda nose mešavinu O157 i non-O157 sojeva koji nisu uvek patogeni za ljude. Donedavno se smatralo se da je O157 STEC serogrupa odgovorna za većinu alimentarnih epidemija u vezi sa STEC u Severnoj Americi, ali je nedavno potvrđeno da su non-O157 STEC serogrupe odgovorne za skoro 50% alimentarnih epidemija, odnosno oboljenja ljudi, u Severnoj Americi i Evropi. STEC sojevi se najčešće fekalno izlučuju u značajnim nivoima od strane zdravih/asimptomatskih goveda, npr. goveda sa jejunalnim hemoragičnim sindromom. Takvo izlučivanje dovodi do kontaminacije farmskog okruženja. To može da uzrokuje direktnu ili indirektnu kontaminaciju koža goveda, koja, sa druge strane, može da posluži kao glavni izvor kontaminacije trupa u toku klanja i obrade goveda u klanicama ili kontaminacije sirovog goveđeg mesa, odnosno proizvoda od mesa. Naučno bazirana ocena rizika je potrebna da bi se utvrdio uticaj na javno zdravlje, izloženost potrošača patogenu i za dizajn najefektivnijih strategija za redukciju rizika, odnosno za prevenciju i redukciju alimentarnih O157 i non-O157 STEC sojeva poreklom od goveda.

Ključne reči: *STEC, ocena rizika, javno zdravlje, redukcija rizika.*

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Prevalence of *Listeria monocytogenes* in ready – to – eat food of animal origin

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A b s t r a c t: In this study, the presence of Listeria monocytogenes in ready– to– eat meat, milk and fish products has been investigated. In addition, the presence of L. monocytogenes on food – contact surfaces, as a potential source of food contamination, has been investigated as well. Samples were analyzed by fluorescent immunoenzyme assay on miniVidas® device and by standard microbiological SRPS EN ISO 11290-1: 2010 and SRPS EN ISO 11290-2: 2010 methods. Out of 881 food samples tested, 12,25% were Listeria spp. positive, out of which 8,4% were positive for L. monocytogenes. L. monocytogenes was most commonly found in smoked salmon, which confirmed fact that smoked fish is a high risk food for the L. monocytogenes growth and survival. Out of 512 samples from the food – contact surfaces, L. monocytogenes was found in 8,78% of swab samples. This paper highlightes the importance of implementing appropriate prevention and control measures, verification procedures, and monitoring and maintenance programs that will help to prevent L. monocytogenes food contamination.

Key words: *Listeria monocytogenes, ready– to– eat food, prevalence, control.*

Introduction

Listeria monocytogenes is a Gram – positive, non – spore – forming, facultative intracellular microorganism, ubiquitous in natural environment. It is a significant foodborne pathogen that causes listeriosis in both, humans and animals. Listeriosis in humans occurs infrequently, but it has severity of serious manifestations (including septicaemia, meningitis and fetal death), with a case fatality rate between 20% and 50% (*Vazquez-Boland et al*., 2001). Although listeriosis can occur in apparently healthy individuals, it is primarily pregnant women and their neonates, elderly people, and immunocompromised individuals who are considered to be at the highest risk (*Slutsker and Schuchat*, 1999). *Listeria monocytogenes* is widely disseminated throughout the natural environment (*Fenlon et al.,* 1996) and consequently, it is present in many animal and plant food products. The primary mode of transmission of *L. monocytogenes* to humans is the consumption of contaminated minimally processed food (*Lakićević et al*., 2011; *Kathariou,* 2002; *Shen et al*., 2006; *Schlech*, 2000) and contaminated ready– to-eat foods (RTE) (*Gombas et al*., 2003), as well. Its extended distribution in the environment, combined with the specific growth conditions of the pathogen, appear to be the main cause of its high prevalence in different kinds of food products. Studies conducted by several authors (*Pan et al*., 2006; *Kathariou*, 2002; *Tompkin*, 2002) have indicated that certain strains of *L. monocytogenes* survive well within the food – processing environment and the persistence of such strains is of concern as they have the potential to act as a continual source of contamination (*Lakićević et al*., 2010; *Pan et al.*, 2006). In addition, physical and chemical characteristics of the product and their storage allow us to classify foodstuffs as high and low risk foods (*Vitas et al.*, 2004) for *L. monocytogenes* occurrence/ growth. Various RTE food such as dairy products, meat products, fish products, vegetables and complex food were associated with transmission of listeriosis. This confirms the fact that *L. monocytogenes* is a highly resistant organism with the ability to grow under harsh environmental conditions

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such as extreme temperature (−0.1 to 45°C), pH (3.0 – 9.5) and salt (up to 10%) ranges (*Wagner and McLauchin,* 2008). Some products that support the growth of *L. monocytogenes* include: prepacked sliced meat products, fermented sausages, pâté, cured meat products, cooked sausages, smoked meats, prepared roast meat and grill meat, meat salads and related products, pasteurized cans, cold smoked packaged meat products and marinated meat products, soft cheese and cream. Products that do not support the growth of *L. monocytogenes* include: products with a shelf life of less than 5 days, ice cream, fermented milk products, dairy desserts, dairy spreads, semi-hard and hard cheese and products with the pH value less than 4.4. *Vitas et al*. (2004) have reported that 19.4% of 3685 fresh and processed food analyzed during 4 years were *Listeria* positive, of which 8.3% were *L. monocytogenes* positive. In study conducted by *Gusman et al*. (2014), the prevalence of *L. monocytogenes* in examined samples of RTE foods was 1.97%, and the count of *L. monocytogenes* in all positive samples exceeded the limit of 100 colony forming units (CFUs) per gram. According to the data reported by the *European Food Safety Authority* (2009), prevalence rate of *L. monocytogenes* in RTE foods was 4.4%.

In Serbia, there are still not enough data on the presence of *L. monocytogenes* in RTE products.

The aim of this study was to determine prevalence of *L. monocytogenes* in RTE food of animal origin, before the food has left the immediate control of the food business operator who has produced it. In addition, environmental samples were examined for *L. monocytogenes* presence, in order to perceive the risk of additional food contamination.

Material and methods

Samples

A total of 1393 samples were analyzed over 1– year period (2013). The samples included RTE meat, fish and seafood products (cooked and cured meats, smoked fish, seafood salads), RTE milk products (yogurt, sour cream, butter, cheeses, cheese spreads, dairy desserts) and environmental samples (swabs from the surfaces in food processing facilities and retail establishments).

Environmental, equipment surfaces and working surfaces were sampled according to reference *SRPS ISO 18593:2010* method.

The samples were kept refrigerated and analyzed within 2h.

Microbiological and immunoassay analysis

Comparative analysis of *L. monocytogenes* presence in food and environmental samples was performed by standard microbiological *SRPS EN ISO 11290-1:2010* method, as well as by enzyme-linked fluorescent immunoassay. Also, enumeration of *L. monocytogenes* in food samples was performed according to standard *SRPS EN ISO 11290-2:2010* method. Immunoassay was performed by fully automated miniVIDAS® system (*bioMérieux, France*) using VIDAS® LMX test kit (REF. 30123, *bioMérieux, France*) for detection of *L. monocytogenes* antigens in food. According to manufacturers' protocol, 25 g/mL of food sample or 1:10 dilution of environmental sample was enriched with 225 mL of LMX broth (REF. 42647, *bioMérieux*, *France*) and subsequently incubated during 26−30 h at 37°C ± 1°C. After incubation, 1−2 ml of enrichment was heated 5±1 min at 95°C, than the tube was cooled down and 250 µL of the enriched sample was taken to test and analyzed according to manufacturers' instruction.

Results and discussion

The overview of analyzed samples and prevalence of *L. monocytogenes* in RTE food of animal origin are presented in Table 1.

Results obtained by immunoassay, as well as from the standard microbiological method used in this study, were in compliance. From the total of 881 food samples examined, 74 samples (8.40%) were positive for *L. monocytogenes*, nevertheless count of *L. monocytogenes* in all positive samples was lower than 100 CFUs/g (data not shown).

In our study, the highest prevalence of *L. monocytogenes* was found in smoked fish, especially smoked salmon, where 29.54% of analyzed fish samples were positive. This could be the reason for higher prevalence of *L. monocytogenes* obtained in our study, with regard to the study *by Gusman et al.* (2014) who has not examined this type of food. *Garrido et al*. (2009) have reported that from 783 different food samples being analyzed, RTE smoked fish was the most frequently contaminated food category (25% positive). The similar prevalence of *L. monocytogenes* in smoked fish was reported by *Beaufort et al.* (2007) and *Lončarević et al.* (1996). In addition to smoked fish, a relatively high prevalence was observed in seafood salads samples. Out of 86 seafood salads examined, 13.95% were positive for *L. monocytogenes*.

Table 1. Overview of analyzed samples and prevalence of *Listeria monocytogenes* in RTE foods of animal origin

Tabela 1. Pregled ispitanih uzoraka i prevalenca *L. monocytogenes* u hrani animalnog porekla spremnoj za konzumiranje

Food group/*Grupa proizvoda*	**Type of product/ *Tip proizvoda***	**Samples analyzed/ *Analizirani uzorci***	***L. monocytogenes* positive samples/ *L. monocytogenes pozitivni uzorci***	***Listeria* spp. positive samples/ *Listeria spp. pozitivni uzorci***
RTE meat products/ *Proizvodi od mesa spremni za konzumiranje*	Cooked meat products/ Kuvani proizvodi od mesa	311	3(0.96%)	5(1.61%)
	Cured meat products/ Suhomesnati proizvodi	111	5(4.50%)	10(9.01%)
RTE fish products and seafood products/*Proizvodi od ribe i plodova mora spremni za konzumiranje*	Smoked fish/ Dimljena riba	176	52(29.54%)	72(40.91%)
	Seafood salads/ Salate od morskih plodova	86	12(13.95%)	10(11.63%)
RTE milk products/ *Proizvodi od mleka spremni za konzumiranje*	Cheese spreads/ Sirni namazi	68	0	0
	Fermented milk products/ Fermentisani proizvodi od mleka	25	0	0
	Cheeses/ Sirevi	91	2(2.20%)	8(8.79%)
	Dairy desserts/ Mlečni dezerti	13	0	0
Total/*Ukupno*		881	74 (8.40%)	108 (12.25%)

Among RTE meat products, *L. monocytogenes* was detected in 0,96% of cooked meat products and 4,50% of cured meat products. Out of 311 cooked meat products examined, *L. monocytogenes* was detected in three frankfurter samples. *Čaklovica et al.* (2011) examined the survival of *L. monocytogenes* in frankfurters cooked at 65 and 72°C, and stored at 0.5°C for 45 days. They concluded that *L. monocytogenes*, compared to other foodborne pathogens, is highly resistant to different heat treatments (65 and 72°C), and that storage temperature of 0,5°C does not inhibit the growth of *L. monocytogenes* in frankfurters, which may explain our findings. Understanding the factors that impact positively and negatively on the ability of *L. monocytogenes* to survive and proliferate in food and in the food processing environment is essential to the development and management of effective *L. monocytogenes* control measures (*Lakićević et al.*, 2014).

The smoking, cooking and drying processes can be considered as antimicrobial processes. Nevertheless, we have detected *L. monocytogenes* in five samples of cured meats (two fermented sausages, one smoked ham and two smoked bacons). The principal factors that influence the survival and growth of *L. monocytogenes* in food are temperature, pH and water activity. As similar to other bacteria, the tolerance of *L. monocytogenes* to particular environmental constraints (processing and/or storage conditions) is greatest when all other conditions are optimal for growth (*Lakićević et al*., 2014). The growth of *L. monocytogenes* in cured meats should be limited by a_w and pH value, and water phase-salt content. Besides that, contamination level of raw meat, process hygiene, as well as storage conditions can significantly affect the growth of *L. monocytogenes* in these products. *Ingham et al.* (2004) evaluated survival of *L. monocytogenes* on 15 ready – to – eat meat products made using drying,

Table 2. Prevalence of *Listeria monocytogenes* on surfaces and equipment in food processing establishments and retail stores

Tabela 2. Prevalenca *L. monocytogenes* na površini opreme u industrijskim pogonima i maloprodajnim objektima

Sample/*Uzorak*	**Samples analyzed/ *Analizirani uzorci***	***L. monocytogenes* positive samples/ *L. monocytogenes pozitivni uzorci***
Knives/*Noževi*	47	4 (8.51%)
Slicing machines/*Mašine za narezivanje*	35	3 (8.57%)
Worktables/*Radni stolovi*	19	4 (21.05%)
Floor/*Podovi*	7	3 (42.86%)
Dishes/*Posuđe*	10	6 (60.0%)
Other surfaces/*Ostale površine*	394	25 (6.34%)
Total/*Ukupn*o	512	45 (8.78%)

fermentation and/or smoking. They found that numbers of *L. monocytogenes* decreased for all products during storage ranging from a decrease of 0.8 log CFU on smoked cured beef slices during 11 weeks under vacuum at 5 ºC to a decrease of 3.3 log CFU on a pork rind product stored 5 weeks under air at 21ºC.

Authors suggested that 1– week post– packaging room-temperature storage prior to shipment could act as an effective post-lethality treatment for *L. monocytogenes* occurrence in meat products.

Among milk products examined, *L. monocytogenes* was detected in two soft cheeses, while in cheese spreads and fermented milk products this pathogen has not been detected. In the dairy industry, many problems associated with *L. monocytogenes* contamination are related to post-pasteurization contamination.

The second part of our study was related to the detection of *L. monocytogenes* on food contact surfaces in production facilities and retail stores, as significant sources of food contamination. Several studies have focused on the sources and contamination routes of *L. monocytogenes* in food-processing environments. These studies concluded that raw materials were not a major source of contamination, but that contamination occurred during processing and that the food-processing equipment can act as a reservoir of *L. monocytogenes* (*Möretrö and Langsrud,* 2004). It is well known that some serotypes of *L. monocytogenes* have the ability to form biofilms on food contact surfaces, and thus represent a continuous source of contamination. *L. monocytogenes* can survive for long period at low temperatures on process equipment, and the ability of bacteria to survive on the equipment used in production is often cause of the outbreaks described in the literature (*Conly and Johnston*, 2008).

Out of 512 environmental samples (swabs) analyzed, 45 samples (8,78%) were positive for *L. monocytogenes*. These results reflect the need to improve hygiene and disinfection programs by addressing more accurate cleaning practices and continuous education of food workers in order to obtain microbiologically safe environment.

Listeria monocytogenes should be considered a serious hazard in retail and food processing establishments. To protect customers and to protect the business, operators should implement a program to control *L. monocytogenes*. Understanding the sources of the pathogen and factors that contribute to the risk of contamination, growth and spread of the pathogen are important building blocks to an effective control program.

Conclusions

Relatively low prevalence of *L. monocytogenes* was found in RTE meat and milk products, and the count of the pathogen in positive samples was below the acceptable limit of 100 CFU/g or mL. The obtained data highlighted the importance of good manufacturing and hygiene practices to improve the microbiological safety of the product. Extension of the storage period, temperature variations during storage and handling, and poor hygiene during handling of RTE products, increase the risk of creating favorable conditions for the growth of *L. monocytogenes* and consequently increase the risk for consumers' health. Out of all tested RTE foods, the highest prevalence of *L. monocytogenes* was found in smoked

fish and seafood salads. Results of our study suggest that this food category carries a high risk for *L. monocytogenes* contamination. We have also found *L. monocytogenes* in heat-treated meat products, as well as in cured meats, which confirms the fact that this pathogen is highly resistant and adaptable to different environmental conditions. Findings of *L. monocytogenes* in environmental samples indicate poor hygiene and indices equipment as possible source of contamination of the final product. An effective control program is the best defense against this pathogen.

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Učestalost nalaza *Listeria monocitogenes* u hrani animalnog porekla spremnoj za konzumiranje

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R e z i m e: U okviru ovog istraživanja ispitivano je prisustvo L. monocitogenes u proizvodima od mesa, mleka i ribe, spremnim za konzumiranje. Pored toga, ispitivano je i prisustvo L. monocytogenes na površinama koje dolaze u kontakt sa hranom, kao mogućeg izvora kontaminacije hrane. Uzorci su ispitivani imunoenzimskom metodom na miniVidas® uređaju i standardnim mikrobiološkim metodama SRPS EN ISO 11290-1: 2010 i SRPS EN ISO 11290-2: 2010. Ispitan je 881 uzorak hrane, od čega je 12,25% bilo Listeria spp. pozitivno, a 8,4% pozitivno na L. monocitogenes. Najčešći nalaz L. monocytogenes utvrđen je kod uzoraka dimljenog lososa, što je potvrdilo činjenicu da je dimljena riba hrana sa visokim rizikom za rast i preživljavanje L. monocitogenes. Od 512 ispitanih uzoraka sa površina koje dolaze u kontakt sa hranom, L. monocytogenes je utvrđena kod 8,78% uzoraka briseva. Ovim radom istaknut je značaj sprovođenja odgovarajućih mera prevencije i kontrole, procedura verifikacije i praćenja, i programa održavanja koji će pomoći da se spreči kontaminacija hrane L. monocitogenes.

***Ključne reči**: Listeria monocitogenes, hrana spremna za konzumiranje, prevalenca, kontrola.*

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Microbial ecosystem of processing units during production process of *Petrovská klobása*

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A b s t r a c t: Production of traditional dry-fermented sausages is associated with natural contamination by environmental flora. This microbiota is usually referred to as "house flora". This contamination occurs during slaughtering and increases during manufacturing. The diversity of microbiota in small-scale processing units, during production process of the traditional fermented dry sausages – Petrovská klobása, is reviewed in the present paper. Test samples were collected in two village households in Bački Petrovac, where the preparation of Petrovská klobása samples was performed in a traditional manner. The total number of 43 samples was subjected to laboratory examination. Generally, before stuffing, Listeria monocytogenes and Staphylococcus aureus were detected in 6.97 and 9.30%, respectively. Escherichia coli was found in 18.60%. The tested samples of end product at the end of the storage period (270th day) were safe with presence of bacteria populations from the working environment, such as: aerobic bacteria, Micrococcaceae, Lactic acid bacteria and Enterococcus spp. Examination of the hygienic status of the food processing environment, equipment, raw materials and final product provides an overview of growth trends and the disappearance of bacterial populations.

Key words: *Petrovská klobása, house flora, processing environment, growth trends.*

Introduction

In many European countries, the demand for traditional food products has increased. Moreover, food and gastronomy form an inherent link with tourism in Europe, with a renewed interest of consumers in typical and regional food. *Petrovská klobása*, traditional and autochthonous dry – cured sausage, presents a part of gastronomic heritage of Slovaks in Vojvodina. Nowadays, they are manufacturing the product in a traditional way according to the original recipe of their ancestors, without the use of nitrate/nitrite, glucono delta-lactone (GDL) and microbial starters. In rural households, in the Municipality of Bački Petrovac, this sausage is made by the end of November and during December. *Petrovská klobása* is made by mixing partly cooled (cca 4 h p.m) or cold (cca 24 h p.m) medium chopped lean pork and fat (up to10 mm) with addition of powdered red hot spicy paprika, salt, crushed garlic, caraway and sugar. A well-mixed filling, which is prepared within 15–30 minutes by using a unique technique of manual mixing with kneading and overturning, is stuffed into natural casings consisting of the rear part of pig intestines (colon), forming units 35–45 cm long and 4.5–5.0 cm in diameter. After stuffing, the sausages are left to drain for a while and then subjected to cold smoking for about 10-15 days, using specific kinds of wood (cherry wood in particular). When the smoking process is finished, the sausage is kept in a dry and well ventilated place to dry and ripen, until it achieves an optimum quality, which takes about four months (Tasić, 2012; Janković, et al., 2013; Šojić et al., 2014). *Petrovská klobása* is a product of protected geographical origin, under number 44, based on the order issued by the Republic Bureau for Intellectual Property, number 9652/06 G-03/06, on 21/05/2007. In order to achieve a recognizable product of standardized supreme quality which will be continually produced in the controlled conditions, the aim of this study is to determine the parameters of typical house flora during the production

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process of *Petrovská klobása*, which is crucial because of the safety of the final product.

Materials and methods

1. Samples

Test samples were collected from two village households (A and B) in Bački Petrovac, where the preparation of *Petrovská klobása* samples was performed in a traditional manner. Testing included examination of 43 samples, such as: swabs – workers' hands (n = 11), working surfaces (n = 1), equipment before beginning the operation (n = 9), equipment after the operation (n = 9), and other swabs from the working area – wall, drains etc. (n = 7) and samples of fillings (n = 2) and sausages after the drying process (n = 4). The sample area was swabbed using a maximum recovery diluent (MRD, Oxoid) moistened cotton swab. The samples were stored at 0-4°C (coolbox) and then transported to the laboratory under chilled conditions 0−4°C. The samples were examined within 24 hours.

Each sample was tested on the presence of the set of the following bacteria: (1) Total Viable Count/ TVC (SRPS ISO 4833-1; Plate Count Agar -PCA, Oxoid); incubated at 30°C for 72h; (2) Total bacterial count of the *Micrococaceae* family (Manitol salt phenol – red agar, Oxoid), incubated at 30°C for 72 h; (3) Total *Enterobacteriaceae* count (SRPS ISO 21528-2; Violet Red Bile agar with glucose − VRBG, Oxoid), incubated at 30°C for 72 h; (4) Total count of β – glucuronidase positive *E. coli* (SRPS ISO 16649-2; Tripton Bile x Glucuronide agar/TBX, Oxoid), incubated at 44°C for 24 h; (5) *Enterococcus* spp. counts (Bile esculin azide agar, Biokar diagnostics), incubated at 37°C for 48 h; (6) Total count of coagulase positive staphylococci (SRPS ISO 6888 – 1, Baird Parker, Oxoid), incubated at 37°C for 24 h; (7) *Pseudomonas* spp. counts, (Pseudomonas Selective Agar – Cetrimide Agar, Merck), incubated at 35°C for 48 h; (8) Total count of sulphate-reducing bacteria, which grow in anaerobic conditions (SRPS ISO 15213; Iron Sulfite Agar, Oxoid), incubated at 37°C for 48 h; (9) Total count of *Clostridium perfringens* (SRPS ISO 7937; Sulfite cycloserine Agar, Oxoid), incubated at 37°C for 20 h; (10) *Salmonella* spp. (SRPS ISO 6579; modified Rappaport Vasilidis Soft Agar), incubated at 42°C for 24 h; (Rambach, Merck), incubated at 37°C for 24 h; (11) Lactic acid bacteria presence in samples of chunk meat and filling (ISO 15241; Man-Ragosa Sharpe/MRS, Merck, Darmstad, Germany), incubated at 30°C for 48−72; (12) *Listeria monocytogenes* presence and total count (SRPS ISO 11290−1, 2; ALOA, Merck).

2. Immunoenzymatic assay

For detection of *Listeria monocytogenes,* a Vidas − *L. monocytogenes* Xpress (LMX, BioMérieux) was used. In case of food samples, 25 g of sample (analytical unit) was aseptically added to 225 mL of LMX broth in a stomacher bag (Seward). In case of environmental samples, for each swab, 10 mL of LMX broth was aseptically added for each swab. Incubation period was 30 ± 1°C for 22 − 24 h for food samples or 24 − 26 h for environmental samples. After a specific period of incubation, 1− 2 ml broth was removed into a sterile test-tube (Sigma Aldrich), which was heated at 95 to 100°C for 5 ± 1 min. The tube was cooled down and 250 µl of the enriched sample was taken to test. All positive results obtained were confirmed by the reference SRPS ISO 11290-1 method or by using the ALOA chromogenic agar.

3. Statistical analysis

Statistical analysis was carried out using STATISTICA 9.1 (StatSoft, Inc., Tulsa, OK, USA). All data were presented as a mean value with the standard deviation indicated (mean ± SD).

Results

Results of testing are presented in Tables 1, 2, 3, 4 and 5. The environment of processing units was colonized at variable levels by resident spoilage and technological microbiota, with sporadic contamination by pathogenic microbiota. In the households A and B (Tables 1, 2, 3 and 4), the presence of aerobic bacteria, *E. coli*, enterococci, *Staphylococcus aureus*, *Enterobacteriaceae* and *Listeria* spp. was detected. In household A (Table 1), the aerobic bacteria counts ranged from 1.26 ± 0.17 $\log_{10}$cfu/cm^2 (knife) up to 8.04 ± 0.91 $\log_{10}$cfu/cm^2 (saw after cutting). *E. coli* was present in two samples (saw after cutting and table), while enterococci were found in all experimental samples, with a range between 2±0 $\log_{10}$cfu/cm^2 (workers hands) and 5.67 ± 0.06 $\log_{10}$cfu/cm^2. (workers' hands after slaughtering**).** *Staphylococcus aureus* was found in only one sample (workers' hands). *Enterobacteriaceae* had total counts that ranged between 2.67 ± 0.31 $\log_{10}$cfu/cm^2 (table) and saw after cutting (5.04 ± 0.4 $\log_{10}$cfu/cm^2). Other groups of bacteria were not detected. Household

Table 1. Microbiological contamination of processing environment, food contact surfaces, equipment and workers' hands in the household A during the meat production process (X ± SD, log_{10}cfu/cm^2).

Tabela 1. Mikrobiološka kontaminacija radne sredine, radnih površina, pribora i ruku radnika u okviru domaćinstva A tokom proizvodnje mesa (X ± SD, log_{10}cfu/cm^2)

Microbiological contamination/ Mikrobiološka kontaminacija	Workers' hands/ Ruke radnika	Workers' hands after slaughtering/ Ruke radnika posle klanja	Saw/ Testera	Saw after cutting/ Testera posle klanja	Knife/Nož	Knife after cutting/ Nož posle klanja	Table/Radna površina	Wall/ Zid
Total viable count/ Ukupan broj bakterija	4.13±0.16	6.09±0.52	7±0	8.04±0.91	1.26±0.17	1.33±0.17	6.83±0.02	7±0
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	3.11±0.16	ND	ND	1.98±003	ND
Enterococcus spp.	2±0	5.67±0.06	3.04±0.07	3.35±0.31	2.13±0.08	4.74±0.04	5.04±0.04	3.94±0.03
S. aureus	2.24±0.21	ND	ND	ND	ND	ND	ND	ND
Pseudomonas spp.	ND	ND	ND	ND	ND	ND	ND	ND
Sulphite-reducing bacteria	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriace	ND	ND	ND	5.04±0.4	ND	3.8±0.29	2.67±0.31	ND
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND

Legend/Legenda: ND – not detected/nije otkriven

Table 2. Microbiological contamination of processing environment, food contact surfaces, equipment and workers' hands in the household B during the meat production process (MS ± Sd, log_{10}CFU/cm^2).

Tabela 2. Mikrobiološka kontaminacija radne sredine, radnih površina, opreme i ruku radnika u okviru domaćinstva B tokom proizvodnje mesa (MS ± Sd, log_{10}cfu/cm^2)

Microbiological contamination/ Mikrobiološka kontaminacija	Workers' hands/ Ruke radnika	Workers' hands/ Ruke radnika	Workers' hands/ Ruke radnika	Saw/ Testera	Saw after cutting/ Testera posle klanja	Knife/ Nož	Knife after cutting/ Nož posle klanja	Chopper/ Mašina za sečenje	Chopper after Cutting/ Mašina za sečenje posle sečenja	Apron/ Kecelja	Drain/ odvod
Total viable count/ Ukupno bakterija	3.6±0.53	4.05±1.69	3.72±0.09	5.83±0.56	6.29±0.03	5.58±0.17	6.1±0.35	2.57±0.24	6.44±0.17	6.46±0.19	7.19±0.15
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	3.41±0.36	ND	1.87±0	ND	3.41±0.23	ND	3±0
Enterococcus spp.	ND	ND	ND	4.28±0.25	4.45±0.08	ND	3.66±0.16	ND	4.39±0.05	ND	3.33±0.28
S. aureus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulphite-reducing bacteria	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	1	ND	ND	3.52±0.17	5.07±0.5	ND	5.73±0.2	ND	4.64±0.06	ND	2.37±0
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L.monocytogenes.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.28±0.02

Legend/Legenda: ND – not detected/nije otkriven

Table 3. Microbiological contamination of processing environment, food contact surfaces, equipment and workers' hands in the household A during preparation of the filling (MS ± Sd, $log_{10}CFU/cm^2$)

Tabela 3. Mikrobiološka kontaminacija radne sredine, radnih površina, opreme i ruku radnika u okviru domaćinstva A tokom pripreme nadeva (MS ± Sd, $log_{10}cfu/cm^2$)

Microbiological contamination/ Mikrobiološka kontaminacija	Mincing machine (beginning)/ Mašina za mlevenje (početak)	Mincing machine (operation)/ Mašina za mlevenje (rad)	Stuffing machine (beginning)/ Mašina za punjenje (početak)	Stuffing machine (operation)/ Mašina za punjenje (rad)	Casing/ Crevo	Casing with the filling/ Crevo sa punjenjem	Workers' hands during grinding/ Ruke radnika tokom mlevenja	Workers' hands with spices/Ruke radnika sa začinima	Drain/ Odvod
Total viable count/ Ukupno bakterija	4.83±0.26	6.64±0.05	3.23±0.06	6.77±0.07	6.75±0.13	6.75±0.13	6.69±0.05	6.94±0.1	6.84±0.06
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	1.97±0.03	ND	ND	ND	2±0	ND
Enterococcus spp.	ND	5.27±0.25	ND	4.52±0.06	4.03±0.05	4.89±0.06	4.72±0.05	5±0	3.28±0.12
Staphylococcus aureus	ND	2±0	ND	ND	ND	ND	3.31±0.21	3±0	ND
Pseudomonas spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulphite-reducing bacteria	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	ND	ND	ND	1.32±0.04	ND	ND	ND	ND	2.33±0.05
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	2. 03±0	ND	ND	ND	ND	ND

Legend/Legenda: ND – not detected/nije otkriven

Table 4. Microbiological contamination of processing environment, food contact surfaces, equipment and workers' hands in the household B during preparation of the filling (MS ± Sd, $log_{10}CFU/cm^2$)

Tabela 4. Mikrobiološka kontaminacija radne sredine, radnih površina, opreme i ruku radnika u okviru domaćinstva B tokom pripreme nadeva (MS ± Sd, $log_{10}cfu/cm^2$)

Microbiological contamination/ Mikrobiološka kontaminacija	Mincing machine (beginning)/ Mašina za mlevenje (početak)	Mincing machine (operation)/ Mašina za mlevenje (rad)	Stuffing machine (beginning)/ Mašina za punjenje (početak)	Stuffing machine (operation)/ Mašina za punjenje (rad)	Workers' hands after cutting the meat/ Ruke radnika posle sečenja mesa	Workers' hands after cutting the meat/ Ruke radnika posle sečenja mesa	Workers' hands after cutting the meat/ Ruke radnika posle sečenja mesa	Workers' hands after mixing the filling/ Ruke radnika posle punjenja	Drain/ Odvod
Total viable count/ Ukupno bakterija	3.18±0.14	6.56±0.08	2.21±0.02	4.21±0.26	6.21±0.62	6.1±0.19	5.2±0.17	6.75±0.12	7.19±0.15
Micrococcaceae	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	ND	ND	3±0
Enterococcus spp.	ND	3.33±0.24	ND	3.57±0.27	ND	4.3±0.11	4.14±0.36	4.3±0.3	3.33±0.28
Staphylococcus aureus	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulphite-reducing bacteria	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	ND	2.33±0.58	ND	ND	2.33±0	2.33±0	ND	2.11±0.58	2.37±0
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	2.02±0.46	ND	ND	ND	ND	ND	ND	ND

Legend/Legenda: ND – not detected/nije otkriven

Table 5. Microbiological contamination of sausage batter and final product after a drying process (X ± SD, $log_{10}CFU/cm^2$)

Tabela 5. Mikrobiološka kontaminacija nadeva i kobasica nakon procesa sušenja (X ± SD, $log_{10}CFU/cm^2$)

Sample/ Uzorak	Total viable count/ Ukupno bakterija	Micrococcaceae	Enterococcus spp.	Lactic Acid Bacteria	*Enterobacteriaceae*	*L. monocytogenes*
Batter A/Masa A	7.03±0.05	4.23±0.42	3.24±0.24	ND	3.89±0.02	ND
Batter B/Masa B	7.05±0.07	4.51±0.45	3.19±0.17	ND	4.19±0.12	ND
Sausage A1/ Kobasica A1	4.19±0.22	2.75±0.35	<2	5.3±0.15	ND	ND
Sausage A2/ Kobasica A2	4.28±0.23	3.4±0.22	<2	6.4±0.22	ND	ND
Sausage B1/ Kobasica B1	4.5±0.05	2.64±0.1	<2	5.7±0.15	ND	ND
Sausage B2/ Kobasica B2	4.31±0.02	2.62±019	<2	6.2±0.30	ND	ND

Legend/Legenda: ND – not detected/nije otkriven

B (Table 2) showed similar situation with regard to the presence of microorganisms (aerobic bacteria, *E. coli*, enterococci and *Enterobacteriaceae*). The working surfaces, machines, tools and worker's hands had total aerobic counts between (chopper) and 7.19 ± 0.15 $log_{10}cfu/cm^2$. For *E. coli*, contamination level was 1.87 ± 00 $log_{10}cfu/cm^2$, 3.41 ± 0.23 $log_{10}cfu/cm^2$, 3 ± 0 $log_{10}cfu/cm^2$, respectively. *Enterobacteriaceae* were found in six samples, with maximum of 5.73 ± 0.2 $log_{10}cfu/cm^2$ (knife after cutting). The presence of *L. monocytogenes* was detected in swabs from the drain 2.28 ± 0.2 $log_{10}cfu/cm^2$ (Table 2). In households A and B, during preparation of the filling (Tables 3 and 4), the presence of *L. monocytogenes* was detected in swabs from the stuffing (2.03 ± 0 $log_{10}cfu/cm^2$) and mincing machine (2.02 ± 0.46 $log_{10}cfu/cm^2$). Also, *L. monocytogenes* was detected in a sausage batter A (2.07 ± 1270.07 $log_{10}cfu/cm^2$) and sausage batter B (2.08 ± 0.08 $log_{10}cfu/cm^2$) (Table 5).

In regard to the final product – sausage after drying process (Table 5), the presence of aerobic bacteria, micrococci, enterococci and Lactic Acid Bacteria (LAB) was detected while other groups of bacteria were not detected.

Discussion

Many authors support the belief that the microorganisms present in traditional sausages are derived from the raw materials or from the manufacturing (*Talon et al.,* 2007). This microbiota is usually referred to as "house flora". While the microbiota isolated from traditional sausages is well described, the resident microbiota in the environment of the processing unit is still poorly known. The presence of aerobic bacteria, enterobacteriaceae, enterococci and *L. monocytogenes* in A and B fillings, most probably resulted from the cross contamination of the sausage batter either with working surfaces or after the meat mincing and addition of spices; that is a consequence of the specific filling preparation technique by manual mixing on the wooden table for ca. 15-30 min (*Ikonić et al.,* 2010). Generally, *L. monocytogenes* and *S. aureus* were detected in 6.97 and 9.30%, respectively, while *E. coli* was found in 18.60%. Sausage samples at the end of the production cycle (270th day) were safe in regard to the presence of bacteria populations from the working environment, such as: aerobic bacteria, *Micrococcaceae, Lactic acid bacteria* and *Enterococcus*. The results are in accordance with the results obtained by *Lebert et al.* (2007), *Janković et al.* (2013), *Lakićević et al.* (2014). Several critical points were identified such as the drain, saws, workers' hands, mincing and stuffing machines. The current study revealed that the majority of the sampling sites (control point) tested were (2 to 6 log cfu/cm^2) contaminated by spoilage flora (*Enterobacteriaceae*) with knives and saws, mincing machines (*Listeria monocytogenes*), workers' hands (*Staph. aureus, E. coli*), which surely indicates an inappropriate slaughtering process, and to a low level of personal hygiene. Detection of *Listeria monocytogenes* can be considered as a useful indicator of a deterioration in hygiene or process conditions during food production. Unclean, insufficiently or inadequately cleaned pieces of equipment

have often been identified as a source of pathogens. The results are unique and crucial for the improvement of hygiene control systems in traditional meat processing units (*Talon et al.,* 2007).

Conclusion

Traditional dry sausages rely on natural contamination by environmental flora. This contamination occurs during slaughtering and increases during manufacturing. The results, during the production of the *Petrovská klobása* in the traditional manner, showed that processing units were colonised at various levels by spoilage and technological microflora with excessive contamination levels. Sporadic contamination by pathogenic microflora was recorded. *L. monocytogenes* and *S. aureus* were detected in 6.97 and 9.30% of the samples, respectively, while *E. coli* was enumerated in 18.60% of the samples. The variability of the contamination emphasized the different cleaning, disinfecting and manufacturing practices routinely followed by these (A and B households) small-scale processing units. The technological flora (coagulase negative staphylococci and lactic acid bacteria) were both in the environment and in products. Enterococci were present all along the manufacturing period. Examination of the hygienic status of the processing environment, equipment, raw materials and food safety criteria of the final product provides an overview of growth trends and the disappearance of bacterial populations.

Further studies will be carried out to detail phenotypic, genotypic and physiological characterization of the isolated strains of staphylococci (CNS) and LAB from the *Petrovská klobása* with additional purpose of creating Serbian bank of autochthonous functional starter cultures specific for industrial production of the *Petrovská klobása* . In this way, the product will meet all regulations, as needed on a broad market, and it will remain autochthonous, safe and recognizable.

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Mikrobiološki ekosistem malih proizvodnih jedinica tokom proizvodnog ciklusa *Petrovačke kobasice*

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R e z i m e: Proizvodnja tradicionalnih suvih-fermentisanih kobasica je u direktnoj vezi sa mikrobiološkim ekosistemom radne sredine (house flora), čije definisanje ima značajnu ulogu u mikrobiološkoj stabilnosti i bezbednosti gotovog proizvoda. U okviru rada, prezentovani su rezultati koji daju uvid u diverzitet microbiota u malim proizvodnim jedinicama, tokom proizvodnog ciklusa Petrovačke kobasice. Test uzorci su sakupljeni u dva seoska domaćinstva u Bačkom Petrovcu, gde je izvršena priprema Petrovačke kobasice na tradicionalan način. Ispitivanjem su obuhvaćena ukupno 43 uzorka. Generalno, Listeria monocytogenes i Staphylococcus aureus su detektovani u 6,97 i 9,30%, respektivno, dok je Escherichia coli detektovana u 18,60% uzoraka. Uzorci kobasica su na kraju perioda skladištenja (270. dan) bili bezbedni uz prisustvo sledećih grupa bakterija: aerobne bakterije, Micrococcaceae, bakterije mlečne kiseline i Enterococcus spp.

Key words: *Petrovská klobása, house flora, trend rasta, radna sredina.*

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Cholesterol and total lipid content in raw and heat processed commercially produced meat from two farms

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A b s t r a c t: The present study was conducted to present information about the cholesterol and total lipid content in fresh and roasted chicken meat with skin (breast and drumstick meat), most commonly consumed in Serbia. In addition, to assess the possible effects of carcass weight and nutrition on total lipid and cholesterol content in examined meat cuts. A total number of 48 samples of breast and drumstick muscle of broilers from two farms (Farm I and II), fed ad libitum by commercial diets for growing broilers,were taken in summer, 2012 and autumn, 2013. Total lipid and cholesterol content were determined in raw and heat-processed breast and drumstick muscle with skin. Total lipid content was determined by extraction of fat by petrol ether (Soxhlet) after acid hydrolysis of samples (SRPS ISO 1443/1992).Cholesterol determination was performed after direct saponification (without prior lipid extraction) by using HPLC/PDA system.

Generally, all parameters measured were influenced by interaction of care and management and broiler performance at the 5% level or less. The total lipid content in samples of raw breast muscle of chicken from Farm I were the highest in summer (5.53%), (4.2%, in autumn), compared to samples of chickens from Farm II (3.05% in summer and 2.61% in autumn). The total lipid content in samples of raw drumstick were significantly differ ($p < 0.001$) between Farm I (9.63%) and Farm II (5.19%), only in summer. Cholesterol content (mg/100 g) in the raw breast muscle from Farm I was 53.9 (autumn) and 62.1 (summer), while in samples from Farm II was 46.97 (autumn) and 49.53 (summer). There was significant difference ($p < 0.001$) in cholesterol content in raw breast muscle of chickens in summer from two farms. In raw drumstick from Farm I the average cholesterol content (mg/100 g) was 70.24 (autumn) and 83.95 (summer), while in samples of chickens from Farm II was 65.05 (autumn) and 60.92 (summer). These differences were significant for cholesterol levels in drumstic between farms in summer ($p < 0.001$).

In heat-processed meat belonging to chickens from Farm I, breast and drumstick contained higher quantities of total lipids compared to samples from Farm II. These differences was significant ($p < 0.01$) in drumstick between Farm I and II (autumn) and were 13.37%, and 11.10%, respectively. The average cholesterol content (mg/100 g) in samples of heat-processed meat of chicken from Farm I varied between 70.32 (autumn) and 87.37 (summer) and from 75.23 (autumn) to 78.92 (summer) in drumstick, versus 64.33 (summer) and 66.24 (autumn) in breast muscle and from 81.31(summer) to 91.6 (autumn) in drumstick samples of chickens from Farm II.

We conclude that factors, such as feed composition, genotype (breed) and gender, influence the total lipid and cholesterol content in the meat. There were no obviously effects of slaughter traits on cholesterol and total lipid content. The results presented here, also shows that further investigations have to be conducted on greater number of samples.

Key words: *chicken meat, cholesterol, total lipid.*

Intoduction

Poultry has a leading position among all types of meat in the most developed countries in the world because of many reasons: short breeding time, a greater ammount of live weight of poultry in the poultry house, the great reproductive power of breeding flocks, excellent feed conversion, relatively low selling price and the suitability through so-called "fast food" (*Jahić et al.,* 2012). The consumption of poultry meat has become very popular due to their nutritional, but also sensory and aeshtetic characteristics (*Bogosavljević-Bosković et al.,* 2010). Consumer needs not only lean, but tasty meat, characterized by good culinary, technological and biological properties (*Jukna et al*., 2005). In fact, skinless chicken meat provides high protein (around 20 g/100 g) and low fat intakes (around 5

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g/100 g). Moreover, chicken lipids are characterized by relatively high levels of unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA), which are regarded as a positive healthy aspect by consumers (*Bonoli et al.,* 2007). For most of many societies, meat and animal products represent a source of high quality protein, although high intakes of some animal products can lead to excessive fat intakes. The chemical composition of muscle tissue from major primal cuts is an important element of broiler meat quality (*Holcman et al.,* 2003; *Suchy et al*., 2002) depending on a number of biological factors (genotype, sex and age), (*Hellmeister et al*., 2003) and also numerous non-genetic factors (e.g. broiler rearing system), (*Ristic,* 2003; *Dou et al*., 2009). But despite its nutritional richness, meat has been considered a disease-promoting food.

In Western societies, coronary heart disease and arteriosclerosis are strongly related to the dietary intake of cholesterol and saturated fatty acids and are among the most important causes of human mortality (*Sacks, 2002*). Increases the amount and proportion of animal fat in human diets are associated with the occurrence of cardiovascular diseases (*Lichtenstein*, 1999; *Katan*, 2000). In addition, a strong relationship has been demonstrated between cellular cholesterol concentration and Alzheimer's disease (*Michikawa*, 2003). Relationships appear to exist, also, between a high-fat intake, especially saturated fat and an increased risk of some cancers, such as cancers of the colon, breast and prostate (*Reddy,* 1995). Among these factors, genetics and antioxidant dietary intakes appear to be very important (*Chizzolini et al*., 1999). It is widely acknowledged that there is an urgent need to return to a balanced fatty acid diet by decreasing intake of cholesterol and saturated fats (*Evans et al*., 2002). According to the *American Heart Association* (2004), the daily ingestion of lipids for individuals with normal blood cholesterol levels, should not be more than 30% of the total calorie intake; saturated fat should not exceed 10% of the total calorie intake and cholesterol intake should be below 300 mg/day. Therefore, the knowledge about the cholesterol content in food is important, especially in poultry and fish meat, because the consumption of these foods in nowadays increases in relation to recommendations of healthy nutrition. It is known that the cholesterol content in animal tissues can be influenced by dietary treatment such as feed rations (*Konjufca et al.,* 1997), despite the regulatory mechanisms at the level of synthesis and absorption, which supposedly maintain cholesterol concentration in these tissues (*Harris et al.,* 1993).

Meat composition, as well as its physicochemical properties, undergoes significant changes during heat treatment. It is well known that meat composition, especially its fat content, combined with a specific cooking methodology is among the factors that mostly affect the final quality of meat products (*Serrano et al*., 2007). Several authors pointed out that the cooking process can affect the lipid composition of meat, especially the fatty acid content, by changing the nutritional value of cooked products in relation to raw samples (*Badiani et al*., 2002). Moreover, it was reported that heat treatment can lead to undesirable changes, such as loss of essential fatty acids (FA), reducing the nutritional value of meat, mainly due to lipid oxidation (*Rodriguez-Estrada et al.,* 1997). Some lipid oxidation products (e.g. cholesterol oxidation products) can be involved in lipid metabolism, various chronic and degenerative diseases (such as cancer, aging and human atherosclerosis) and disturbance of cell functionality (*Schroepfer*, 2000; *Osada*, 2002). It is also reported (*Ono et al.,* 1985) that there is an increase in polyunsaturated/saturated ratio, probably because polyunsaturated fatty acids are part of the cell membrane and thus have less contact with heat. *Gerber et al.,* (2009) have shown considerable fat losses in several meat cuts submitted to grilling, broiling or pan-frying without addition of fat. Taking into account controversies around meat consumption and disease risk some studies distinguished the influence of meat cuts from processed meat products in cardiovascular disease pointing out the need to consider processing technique and cooking as important variables which could influence the final result and contribute to the bad image of meat *(Misha et al.,* 2010).

The present study was conducted to present information about the cholesterol content and total lipid content in fresh and roasted chicken meat; white meat (breast meat) and red meat (drumstick meat), most commonly consumed in Serbia. An assessment of possible effects of carcass weight and nutrition on total lipid and cholesterol content in the tissues reached at the slaughter age of the animals, was an integral part of the experiment.

Materials and methods

Bird management and dietary treatments

Two homogeneous groups of male and female (50:50%) Ross 508 and Hubbard broilers were housed under standard conditions of temperature, humidity and ventilation in two farms from different locations of the north province of Serbia – Vojvodina (Farm I and

Farm II). The feeding programme consisted of starter (0–15 d), grower (16–32 d), and finisher (33–38 d) basal diets that were formulated to meet the bird's dietary nutrient requirements (Table 1). Feed and fresh water were offered *ad libitum* throughout the 38-day rearing period. Feed:gain and body weight (BW) were recorded on a cage, based at weekly intervals. Feed conversion ratio, corrected for mortality was calculated.

Slaughter procedure and sampling

The birds (n = 12) aged 39 days selected on the basis of live weight within wider possible range were slaughtered in the approved abattoir. After slaughtering and dressing, hot carcasses were chilled for two hours at 4°C. The carcasses were weighted and refrigerated for 24 h. The breast meat (*Mm. pectoralis major et minor*) with skin and drumstick meat with skin (muscles of *regio tibio-femoralis*) were cut up, separated and weighted. The total of 48 breast and drumstick meat samples were taken from chilled broiler carcasses, collected in the summer, 2012 and the autumn, 2013. Samples of both meats were roasted in an electric oven in the open aluminum pan at 220°C for 30 min.

Determination of total lipid and cholesterol content

Total lipid and cholesterol content were determined in raw and roasted breast and drumstick meat with skin. Total lipid content was determined by extraction of fat by petrol ether (Soxhlet) after acid hydrolysis of samples (*SRPS ISO* 1443/1992). Cholesterol determination was performed after direct saponification (without prior lipid extraction) by using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, USA), according to the method described by *Maraschiello et al*. (1996). *Empower Pro* software was used to control the HPLC system as well as for data acquisition and data processing.

Statistical Analysis

Statistical analysis was performed by the MINITAB software package, version 16.0. Data obtained from the experiment were analyzed by descriptive statistics (mean, standard deviation, range). The One Way ANOVA and the post-hoc HSD Tukey test were used to examine statistical differences betewen examined parameters within the same and accros the farms. The differences were considered statistically significant when the p value was less than 0.05. The significance of correlations (Pearson′s correlation coefficient – r) were calculated using the correlation procedures.

Results and discussion

Table 1 shows ingredients (%) and nutrition composition (%) of commercial broiler feed mixtures which were used for chicks feeding in Farm I and Farm II, in the summer (a) and the autumn (b).

Growth performances of the broilers

Growth performances and slaughter characteristics of broilers are reported in Table 2. Average live weight, daily gain, carcass, breast and drumstick meat weights (2240g, 57g, 1764.87 ± 227.83g, 647.95 ± 78.70g and 536.40 ± 88.41g, respectively) were the highest and the least mortality rate (3.19%) were determined for chicken at farm I (autumn). The highest mortality rate (28.37%) and the least average live weight (1470g), daily gain (39g), daily feed intake (84.33g) as well as carcass (1223.3 ± 116.38g) and breast weight (427.70 ± 53.44g) were obtained at broilers from farm II (summer).

Cholesterol and total lipid content in broiler meat

Distribution of results for cholesterol and total lipids contents (% and mg/100g, respectively) in the raw and heat-prepared chicken meat (breast and drumstick), from both of the farms during periods of investigation was presented in Figures 1-4.

Total lipid content (%), (Figure 1; Table 3) was lower in samples of raw breast meat of broilers from farm II in summer (3.05 ± 1.11) and in autumn (2.61 ± 0.77) in comparison to those from Farm I (5.53 ± 0.61, in the summer and 4.2 ± 1.15, in the autumn).

The difference between total lipid in raw breast meat samples from the both farms in autumn was significant ($p < 0.001$), (Table 3). Obtained results regarding total lipid content in raw drumstick samples from Farm II were significantly lower ($p < 0.001$) only in summer (5.19 ± 0.45%) in comparison to Farm I (9.63 ± 0.64%). Also, there was statistically significant difference ($p < 0.001$) between total lipid contents in drumstick originated from Farm II, in the summer (5.19 ± 0.45%) and the autumn (8.16 ± 2.26%), (Table 3).

Heat-processed breast and drumstick (Figure 2) of broilers from Farm II contained lower total lipid content in the summer (3.57 ± 0.15% and 10.54 ± 0.22%, respectively) and in the autumn (3.72 ± 0.61% and 11.11 ± 2.04%, respectively) in comparison to meat samples of broilers from Farm I (in the summer: 6.08 ± 0.34% and 14.73 ± 0.55%, respectively); in the autumn: 6.25 ± 0.78% and 13.37 ± 0.95%-autumn, respectively). These differences were not statistically

Table 1. Composition (%) of feed mixtures for broiler nutrition
Tabela 1. Sastav smeša (%) za ishranu brojlera

Ingredient/Sastojak (%)	Starter (0-15 day)/ Starter (od 0-15 dana)				Grower (16-32 day)/ Grover (od 16-32 dana)				Finisher (33-38 day)/ Finišer (od 33-38 dana)			
	Farm/Farma											
	Ia	IIa	Ib	IIb	Ia	IIa	Ib	IIb	Ia	IIa	Ib	IIb
Corn/Kukuruz	55.2	55.12	50.06	49.45	56.03	56.03	39.08	49.6	61.1	55.8	55.8	52.75
Soybean meal (46% CP)/ Sojina sačma (46% SP)	34.8	34.8	18	/	30.9	30.9	/	/	25.4	29.8	/	/
Soybean meal (44% CP)/ Sojina sačma (44% SP)	/	/	15	33.60	/	/	31.60	29.4	/	/	23.5	24.5
Wheat grain/Pšenica	3.0	3.0	6.0	6.0	6.0	6.0	20.0	10.0	6.0	6.0	10	10
Sunflower meal (33% CP)/ Suncokretova sačma (33% SP)	/	/	/	/	/	/	/	/	/	/	/	3.0
Yeasts/Kvasac	/	/	1.5	/	/	/	/	/	/	/	/	/
Gluten/Gluten	/	/	/	2,0	/	/	/	2.0	/	/	2.0	/
Sunflower oil/ Suncokretovo ulje	1.65	1.65	2.5	2.62	2.4	2.4	4.42	3.45	3.05	3.45	3.45	4.45
Mono-calcium phosphate/ Monokalcijum fosfat	1.75	1.75	1.70	1.75	1.52	1.52	1.45	1.52	1.32	1.47	1.30	1.30
Limestone/Kreda	1.65	1.65	1.67	1.72	1.5	1.5	1.55	1.55	1.32	1.37	1.37	1.37
Salt/So	0.13	0.13	0.15	0.13	0.21	0.21	0.21	0.19	0.10	0.16	0.21	0.14
Sodium-bicarbonate/ Natrijum-bikarbonat	0.21	0.21	0,17	0.20	0.13	0.13	0.10	0.23	0.32	0.19	0.10	0.33
L – Lysine/Lizin	0.27	0.27	0.29	0.36	0,02	0.02	0,08	0.11	0.16	0.05	0.24	0.11
DL-Methionine/Metionin	0.28	0.28	0.30	0.28	0.15	0.15	0.17	0.15	0.15	0.11	0.14	0.,15
Treonine/Treonin	0.12	0.12	0.13	0.14	/	/	0.03	0.03	0.05	/	0.07	0.06
Choline 60%/Holin 60%	0.13	0.13	0.13	0.14	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin + mineral premix/ Vitaminsko-mineralni premiks 0,5%	0.60	0.60	0,60	0,60	0.60	0.60	0.50	0.50	0.50	0.50	0.50	0.50
Betafin/Betafin	0.06											
Cocci-diostat/ Kokcidiostatici	0.02		0.05		0.02		0.05		/			
Analyzed nutrient content (%)/Sadržaj hranljivih materija (%)												
Crude protein/Sirovi protein	22.32	22,82	22,46	22.25	19.24	20.39	20.1	20.62	18.71	18.83	19.12	19.82
Water/Voda	11.07	10.23	11.50	13.19	10,69	10.73	11.40	12.79	10.30	11.46	11.11	11.57
Crude fiber/Sirova vlakna	1.15	1.68	1.57	1.88	1.73	1.50	1.66	1.75	1.34	1.55	1.63	2.68
Fat/Mast	4.75	4.48	4.56	4.60	4.69	4.30	4.92	4.90	6,32	5,95	6.10	6.20
Ash/pepeo	5.48	5.80	5.63	5.61	5.32	6.04	5.52	5.21	4.62	4.88	4.98	5.44
Ca	0.82	0.95	0.86	0.88	0.74	1.03	0.86	0.81	0.81	0.74	0.78	0.81
Available P/Usvojivi P	0.71	0.70	0.68	0.65	1.73	0.69	1.05	0.73	0.58	0.70	0.64	0.63

Table 2. Growth performances and slaughter characteristics of broilers from two farms (I and II) during two periods of investigation, summer (a) and autumn (b), ($\overline{X}$ Sd)

Tabela 2. Proizvodni rezultati i klanične karakteristike brojlera sa dve farme (I i II) tokom dva perioda istraživanja, leto (a) i jesen (b), ($\overline{X}$ Sd)

Parameter (range)/ Parametar (opseg)	**Farm/Farma**			
	I^a (n=12)	**II^a (n=12)**	**I^b (n=12)**	**II^b (n=12)**
Average live weight (g)*/ Prosečna telesna masa (g)	2190	1470	2240	2220
Average daily gain (g)/ Prosečni dnevni prirast (g)	57	39	57	52
Average daily feed intake (g)/ Prosečni dnevni unos hrane (g)	103.9	84.33	100.51	106.1
Feed : gain (g:g)/ Konverzija hrane	1.82	2.16	1.76	2.04
Mortality rate (%)/ Mortalitet (%)	5.34	28.37	3.19	4.38
Age at slaughter (d)/ Uzrast na klanju (d)	38	38	39	38
Carcass weight (g)/ Masa trupa (g)	1279.45 ±174.02 (1021.7−1681.2)	1223.3±116.38 (1055−1486.4)	1764.87±227.83 (1498.8−2226.7)	1400.70±130.01 (1186−1712.7)
Breast weight (g)/ Masa grudi (g)	452.5±80.17 (339−628)	427.70±53.44 (337.1−547.2)	647.95±78.70 (510−775.1)	470.62±61.15 (393.4−614.1)
Drumstick weight (g)/ Masa bataka (g)	389.89±51.19 (316.8−494)	392.9±39,20 (354−472.7)	536.40±88.41 (443.2−703)	476.78±48.20 (398.6−572.4)

* – weight at farm level/ * – masa na nivou farme; n – number of samples/ n – broj uzoraka

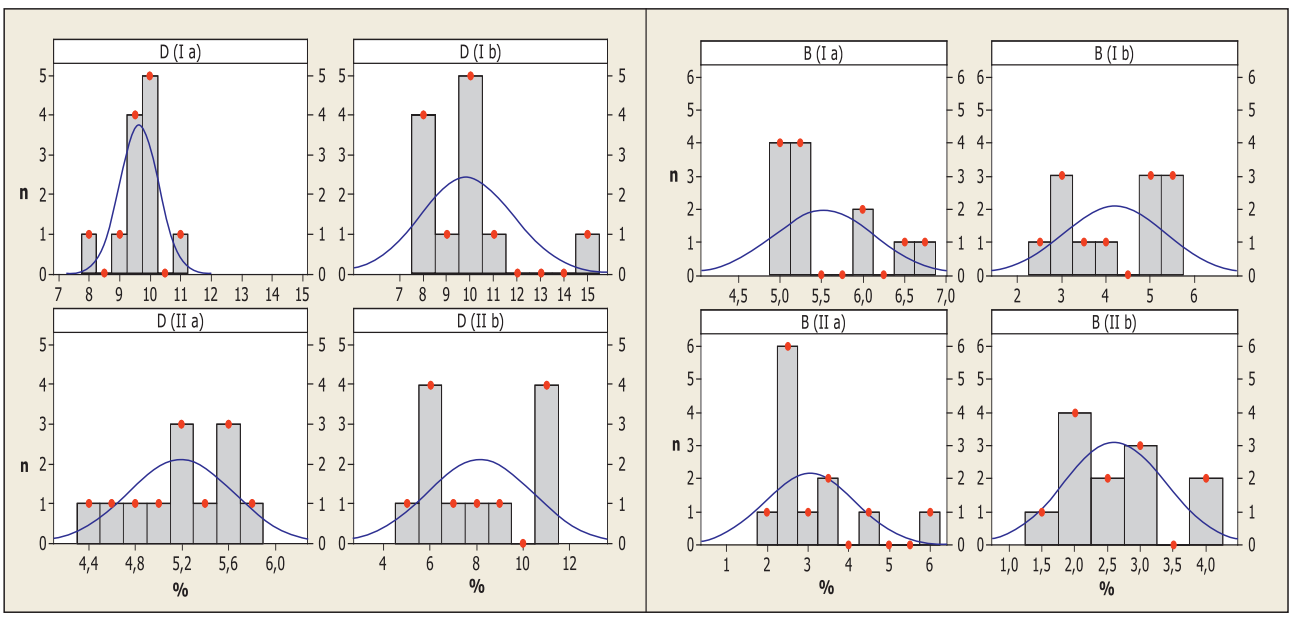

Figure 1. Distribution of total lipid content (%) in raw drumstick (D) and raw breast (B) meat with skin from farm I and II during summer (a) and autumn (b), n – number of samples

Slika 1. Distribucija sadržaja ukupne masti (%) u sirovom bataku (D) i sirovom belom mesu (B) sa kožom sa farme I i farme II tokom leta (a) i jeseni (b), n – broj uzoraka

significant ($p < 0.05$) within this raw meat samples, but they did differ significantly ($p < 0.01$) in the relation to roasted drumstick meat (collected in the autumn) of broilers from Farm I and Farm II in which were determined 13.37±0.95% and 11.10±2.04% of total lipid, respectively. Many authors have been determined the fat content either in meat with or without skin. *Holcman et al*. (2003) found the total lipids levels in breast muscle-plus-skin and leg muscle-plus skin as average 7.0% and 13.1%, respectively. *Qiao et al*. (2002) reported significantly lower total fat content in skinless broiler breast meat (1.21-1.25%). *Zlender and Gasperlin* (2005) found 6% and 0.9% of total lipids in breast meat with skin and without skin, respectively. In drumstick meat samples with skin and without skin, average values of total lipids were 12% and 0.9%, respectively. Furthermore, a number of researchers emphasized the effect of sex and its influence upon the protein and fat content in broiler meat. For example a higher fat content in female broilers was reported by *Sanz et al*. (1999) and *Haro* (2005).

Cholesterol content (mg/100g), (Figure 3) in the raw breast meat originated from Farm I and Farm II were: in the autumn (53.9±6.23; 46.97±9.05, respectively) and in the summer (62.1±12.57; 49.53±5.62, respectively). There was significant difference ($p < 0.001$) in cholesterol content in raw breast meat of broilers in the summer between two farms (Table 3). In raw drumstick meat, the average cholesterol content in the samples from Farm I and Farm II, were: 70.24±6.35; 65.05±8.76 in the autumn and 83.95±9.20; 60.92±8.68, in the summer, respectively.

These differences were significant in regard to cholesterol levels in drumstick samples collected from Farm I, in the summer to those collected from Farm II in the autumn and also between samples collected from both of the farms in the summer ($p < 0.001$), (Table 3). In roasted breast meat (Figure 4) originated from Farm I, cholesterol content was 70.32±7.53 mg/100g (the autumn) and 87.37±8.02 mg/100g (summer). The cholesterol levels measured in roasted drumsticks taken from the same farm were: 75.23±8.15 mg/100g (autumn) and 78.92±20.35 mg/100g (summer). On the other hand, roasted breast and drumstick meat taken from Farm II in regard to cholesterol content was 66.24±6.02 mg/100g and 91.6±7.25 mg/100g (autumn), respectively and 64.33±10.06 mg/100g and 81.31±10.19 mg/100g (summer), respectively.

The statistically significant differences ($p < 0.05$) were registered between the cholesterol content in the chicken raw breast meat accros the farms in the autumn and in the summer. Cholesterol content registered in chicken raw breast samples from Farm II were at lower level in comparison to Farm I. In the summer and in the autumn, measured levels of cholesterol in raw and heat processed samples of breast meat collected from Farm II were lower ($p < 0.01$). Concerning the

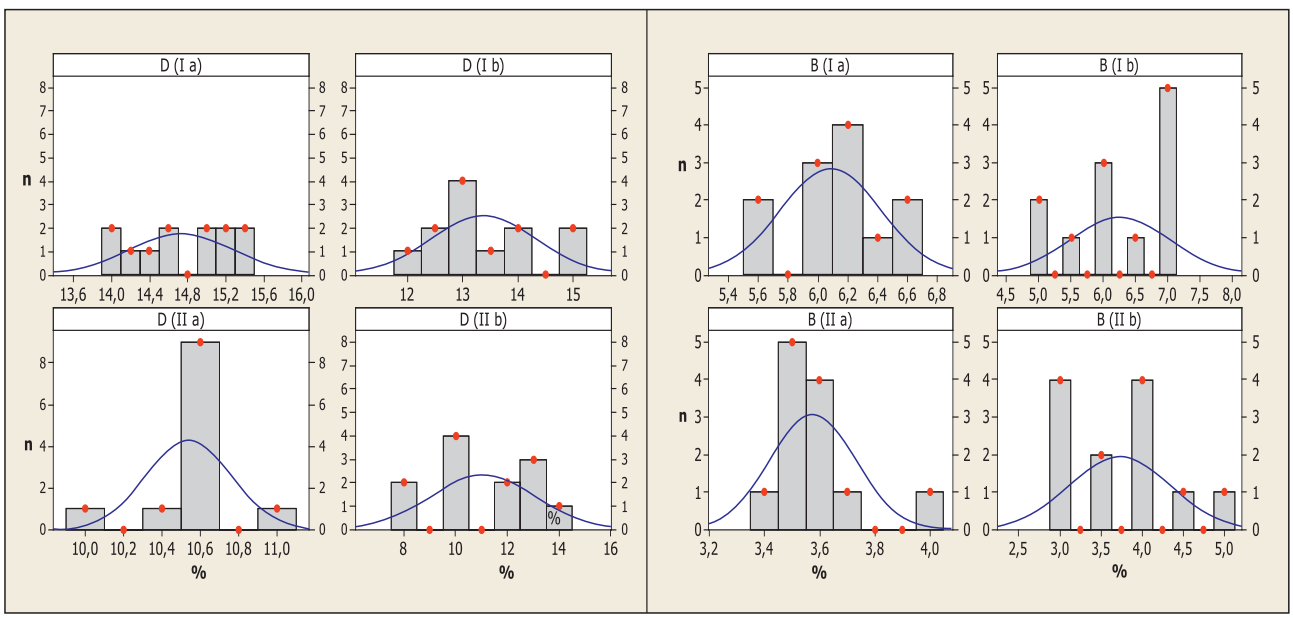

Figure 2. Distribution of total lipid content (%) in heat-processed drumstick (D) and breast (B) meat with skin from farm I and II during summer (a) and autumn (b), n– number of samples

Slika 2. Distribucija sadržaja ukupne masti (%) u termički tretiranom bataku (D) i belom mesu (B) sa kožom sa farme I i farme II tokom leta (a) i jeseni (b), n– broj uzoraka

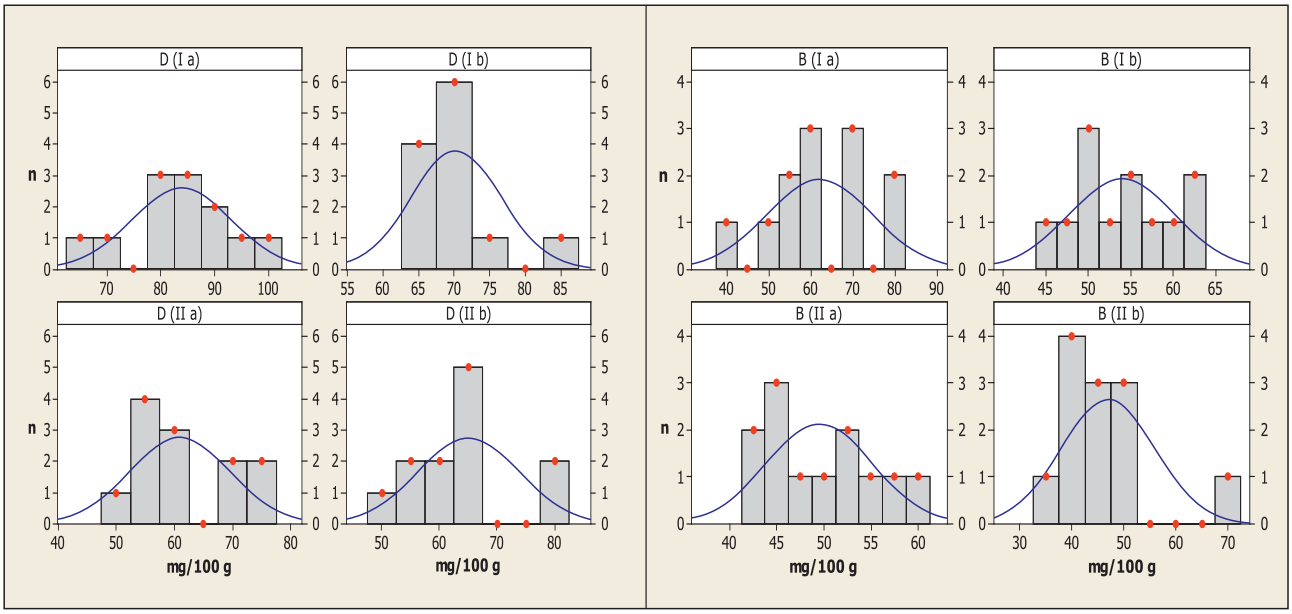

Figure 3. Distribution of cholesterol content (mg/100g) in raw drumstick (D) and raw breast (B) meat with skin from farm I and II during summer (a) and autumn (b), n – number of samples

Slika 3. Distribucija sadržaja holesterola (mg/100g) u sirovom bataku (D) i sirovom belom mesu (B) sa kožom sa farme I i farme II tokom leta (a) i jeseni (b), n – broj uzoraka

cholesterol content in raw and heat processed drumstick meat collected from both farms, siginifcant differences ($p<0.001$) were registered between the raw samples accros the farms in the autumn.

Obtained results are similar to data of cholesterol content (mg/100g) given in study of *Honikel* (1995), that found out 61.45 in raw white meat with skin and 84.65 in raw drumstick with skin. In the mentioned study, it is stated that the chicken meat with skin contains, besides higher cholesterol content also ten times higher fat content (breast meat without skin 0.70%, the breast with skin 6.20%;

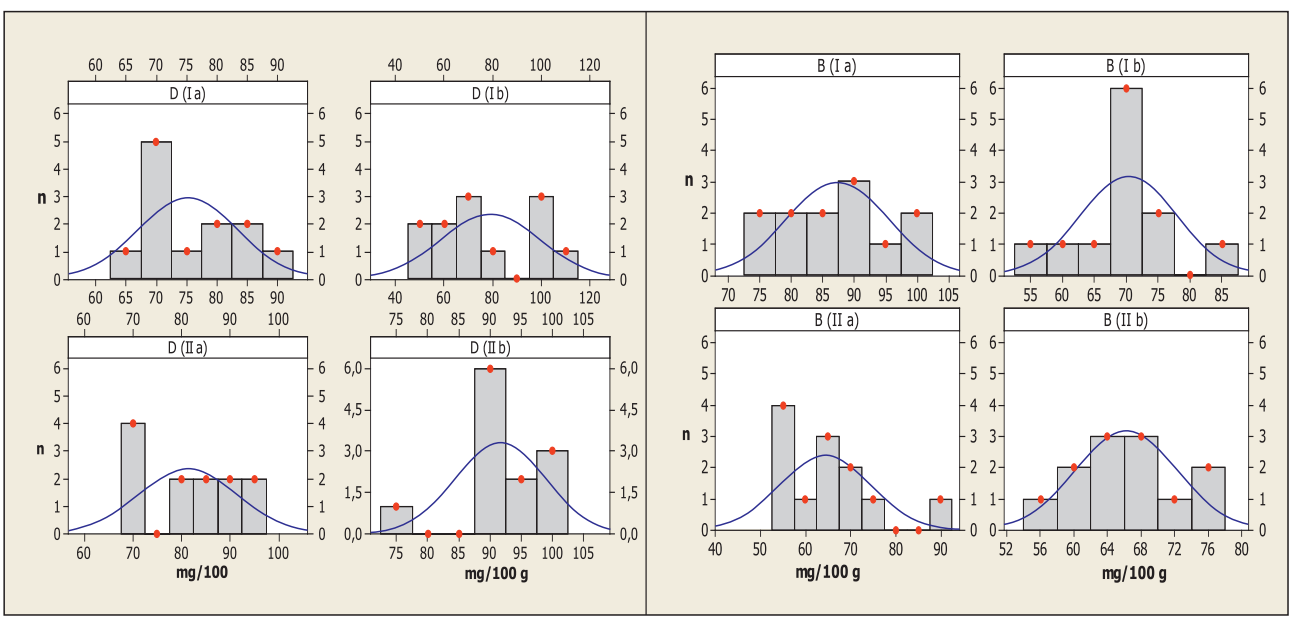

Figure 4. Distribution of cholesterol content (mg/100g) in heat-processed drumstick (D) and breast (B) meat with skin from farm I and II during summer (a) and autumn (b), n – number of samples

Slika 4. Distribucija sadržaja holesterola (mg/100g) u termički tretiranom bataku (D) i belom mesu (B) sa kožom tokom leta (a) i jeseni (b), n – broj uzoraka

drumstick meat with skin 15.10% and drumstick meat without skin 6.45% fat). Our results support the fact, generally adopted, that cooked and processed meat products usually have a greater cholesterol content than raw meat because of moisture loss wherein cholesterol being retained in the tissues (*Baggio and Bragagnolo*, 2006), despite the fact that some amount of cholesterol also being lost during the cooking. According to *Swize et al*. (1992), the migration of cholesterol from fat tissues to muscle tissues was used as an explanation for greater cholesterol content in cooked meat.

Our results are in accordance with results of *Piironen et al*. (2002), who reported 56.2 mg/100g cholesterol and 1.5% total lipid content in breast fillet and 84 mg/100g cholesterol and 11.2% total fat content in leg and thigh. In the mentioned study, it is stated that cholesterol content is not in correlation with fat content, and because of that (like in the case of the meat from livestock), consumption of chicken meat with reduced fat content does not imply that cholesterol intake also will be reduced. But, our results showed the total lipid content in drumstick meat that was higher than in breast meat, were accompanied with a higher cholesterol level.

However, it is more difficult to compare the cholesterol content of poultry meat with its content in beef and pork because of, when being analyzed chicken meat with skin, cholesterol content is always higher, approximately from 80 to more than 100 mg/100g (*Bragagnol*o, 2009).

It has been shown that total lipid content in raw skinless chicken breast (1.2%) was less than in raw chicken breast with skin (8.9%), (*Pereira and Vicente*, 2013; *Komprda et al.*, 2003). Additionally, significant differences in cholesterol and lipid content have reported between muscle types due to differences in their fiber types (*Bragagnolo*, 2009). According to literature data (*Chizzolini et al*., 1999; *Bragagnolo*, 2009), raw poultry meat has approximately 27 to 90 mg cholesterol/100g and cooked poultry meat contains around 59 to 154 mg/100g. According to *Archuleta* (2003), observing quantity of 3 ounces, total lipids in roasted skinless breast and thigh were 3g and 9g, respectively, while cholesterol content was 73 mg and 80 mg, respectively. A significant factor that influences the cholesterol content in the poultry meat is a type of retail cut, because of the difference between dark and white chicken meat and the presence of skin. Poultry skin has the greatest cholesterol content compared with poultry meat or poultry fat. Cholesterol content of visible fat and breast meat is similar to or lower than dark meat (*Komprda et al*., 2003). Moreover, the difference in cholesterol content between white and dark poultry meat is more pronounced than that between white muscles (predominantly glycolytic) and red muscles (predominantly oxidative) of beef and pork (*Browning et al*., 1990; *Sinclair et al*., 2010). *Van de Bovenkamp and Katan* (1981) also suggested that the high cholesterol content of chicken skin that had been reported up to that date was erroneous, and they reported an analytical value for chicken skin of 71 mg/100g of raw wet tissue, while *Dinh et al.* (2011) has been reported higher concentration of cholesterol in raw and cooked chicken skin (more than 100 mg/100g).

Cholesterol content of the animal tissues can be influenced by the composition of the feed mixtures such as the ratio of polyunsaturated fatty acids, especially alpha-linolenic acid ratio (*Komprda et al*., 2003). *Crespo and Esteve-Garcia* (2001) suggested that dietary fatty acid profile plays an important role in lipid deposition and metabolism and results of lower abdominal fat in broilers fed on PUFA. They also showed that this fatty acid (coming from altered lipid ingredient in feed mixture through sunflower, tallow, lard) could cause an inhibition of lipogenesis, redistribution of lipids in the body or higher energy expenditure despite their higher digestibility respect to SFA. According to *Wang et al*. (2006), cholesterol content in chicken meat can be altered by varying the composition of diet, age and gender. However, many variables, such as broiler provenience, age, sex, nutrition, rearing size, carcass dressing and type of meat, could affect the nutritional value of meat and also can induce small or large differences in the obtained results (*Bogosavljevic-Boskovic et al*., 2010). Our results support the generally accepted fact that at decreasing rate of moisture the total fat content increases (*Woolsey and Paul*, 1969). In fact, content of lipids and cholesterol were always higher in roasted meat than in raw ones, due to loss of water during the heat treatment. According to *Chizzolini et al*. (1999), calories and cholesterol per gram, therefore, normally increase on a wet tissue basis, but the picture is obviously different when it is expressed on the basis of a dry matter.

With respect to the nutritional aspects and obtained results for cholesterol content in examined meat samples (mean value of results for autumn and summer), a two hundred gram portion of roasted chicken drumstick meat with skin represents 51% (Farm I) and 58% (Farm II) of the upper limit of daily cholesterol intake (300 mg), (*American Heart Association*, 2004). On the other hand, it is 52% (Farm I) and 43% (Farm II) in the case of chicken roasted breast meat with skin.

Large differences in cholesterol and total lipid content can be explained by possible other factors. In fact, in the summer, it was noted that growth

performances for broilers from both of the farms were very different: average daily gain 57g (Farm I) and 39g (Farm II); average daily feed intake 103.9 g (Farm I) and 84.33 g (Farm II); feed: gain 1.82 g:g (Farm I) and 2.16 g:g (Farm II); mortality on the farm 5.34% (I) and 28.37% (II). In the autumn, the differences in these growth performances between farms were smaller, but did exist: average daily gain 57g (Farm I) and 52g (Farm II); average daily feed intake 100.5 g (Farm I) and 106.1 g (Farm II); feed: gain 1.76 g:g (Farm I) and 2.04 g:g (Farm II); mortality on the farm 3.19% (I) and 4.38% (II). These data show much worse growth performances for broilers on the Farm II comparing to Farm I, probably due to the problems in lipid metabolism, liver function and *de novo* synthesis of cholesterol, which might have influenced the levels of cholesterol and total lipid content in the meat of broilers from this farm (Figures I and II). Also, the observed differences could be associated with metabolic differences, higher competitiveness among males, different fat deposition, different nutritional requirements and higher hormonal effect in female broilers (*Tumova and Teimouri*, 2010).

Obtained differences in content of total lipid and cholesterol in the examined samples might be related to nutritional stress imposed by lower feed intake or some other reasons. *Komprda et al*. (2003) reported that total lipid content increased linearly and significantly in breast and thigh chicken tissues with the increasing live weight at the given age. Cholesterol content significant decreases in total muscle lipids while total lipid content in the muscle tissue increases, and in fact, there is decreasing trend in cholesterol content by increasing live weight.

Coefficients of correlation (r) in our trial were conducted in order to establish the relationship between total lipids and cholesterol content in breast and drumstick and carcass weight (Table 3). There were no– to moderate significant relationships between examined parameters.

For meat samples of the chickens from Farm I, in the summer, it was established a low significant positive correlation between cholesterol content in drumstick and carcass weight (0.41) and moderate positive correlation between cholesterol content in breast meat and carcass weight (0.51). There was a moderate significant relationship between total lipid content and carcass weight in samples of chicken breast from Farm I in the autumn (0.72). In the chicken meat samples from Farm II, there were registered moderate significant negative correlation between carcass weight and cholesterol content in drumstick and breast (-0.66 and -0.58, respectively), in the summer, while relationships

Table 3. Coefficient of correlation *(r)* between carcass weight (g), total lipid (%) and cholesterol (mg/100g) content

Tabela 3. Koeficijent korelacije *(r)* između mase trupa brojlera (g), sadržaja ukupne masti (%) i sadržaja holesterola (mg/100g)

Farm I, Summer	**Carcass weight** $\bar{X}$ 1279.5 g
	r
Total lipid – drumstick[d]	–0.001
Cholesterol – drumstick[a, e]	0.41
Total lipid – breast [b, f]	0.05
Cholesterol – breast [g]	0.51
Farm I, Autumn	**Carcass weight** $\bar{X}$ 1764.9 g
	r
Total lipid – drumstick	–0.49
Cholesterol – drumstick[a]	0.28
Total lipid – breast [b, h]	0.72
Cholesterol – breast	0.004
Farm II, Summer	**Carcass weight** $\bar{X}$ 1223.3 g
	r
Total lipid – drumstick[c, d]	0.02
Cholesterol – drumstick[e]	–0.66
Total lipid – breast [f]	0.17
Cholesterol – breast [g]	–0.58
Farm II, Autumn	**Carcass weight** $\bar{X}$ 1400.7 g
	r
Total lipid – drumstick[c]	–0.03
Cholesterol – drumstick	0.58
Total lipid – breast [h]	–0.51
Cholesterol – breast	–0.49

Legend/ Legenda: [a:a; b;b: c:c: d:d; e:e; f:f;; h:h] p0,001, [g:g] $p<0,001$
Chemical parametars in the same column followed by the same letters differ significantly/Hemijski parametri u istoj koloni sa istim slovnim oznakama se značajno razlikuju.

between cholesterol content in drumstick and carcass weight and total lipid content in breast meat and carcass weight were classified as significantly moderate positive and negative (0.58 and -0.51, respectively), in autumn.

Conclusion

The present experiment provides data of total fat and cholesterol content in fresh and roasted breast and drumstick chicken meat most commonly consumed in Serbia.

With the aim of getting more reliable results, content of total lipid and cholesterol have to be discussed in the light of other factors, such as genotype (breed), gender, feed composition, slaughtered age. Apart from these parameters, reduce in fat and cholesterol content in meat could be achieved with the use of various growth promoters, as well as with correct preparation of meat (trimming of fat, cooking methods, etc.).

Taken together the results presented here as well as high values for standard deviation, coefficient of variation and its wide range (big disparity for cholesterol contents, particularly) for examined samples of chicken meat shows that further investigations have to include greater number of samples of broilers meat from both of the farms.

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Sadržaj holesterola i ukupne masti u sirovom i termički tretiranom komercijalno proizvedenom pilećem mesu sa dve farme

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R e z i m e: Cilj ovih istraživanja je bio ispitivanje sadržaj holesterola i ukupne masti u sirovom i pečenom pilećem mesu brojlera poreklom sa dve komercijalne farme (Farma I i Farma II) u Vojvodini. Program ishrane brojlera i recepture za smeše za njihovu ishranu uradene su na bazi preporuka proizvodača hibrida ROSS, kako je predstavljeno u tabeli 1. Nakon tova od 39 dana, brojleri (po 12 jedinki sa svake farme), približno iste završne mase, zaklani su i obrađeni na liniji klanja, zatim ohlađeni, uobičajenim tehnološkim postupom. Od svake jedinke su uzeti uzorci belog mesa (Mm. pectoralis major et minor) i bataka (Mm. of regio tibio-femoralis) sa kožom, za određivanje sadržaja masti i holesterola u sirovom i pečenom mesu. Sadržaj ukupne masti je određen ekstrakcijom masti sa petroletrom nakon kisele hidrolize uzorka (SRPS ISO 1443/1992). Sadržaj holesterola je određen nakon direktne saponifikacije uzorka (bez prethodne ekstrakcije masti) tehnikom visokoefikasne tečne hromatografije (HPLC/PDA), prema metodi Maraschiello i dr. (1996).

Sadržaj ukupne masti je bio manji u uzorcima sirovog belog mesa pilića sa Farme II, u letnjem (3,05%) i jesenjem periodu (2,61%), u odnosu na sadržaj masti u uzocima sa Farme I (5,53%, leto i 4,20%, jesen). U uzorcima bataka sa Farme II sadržaj ukupne masti je bio statistički značajno manji ($p < 0,001$) samo u letnjem periodu (5,19%) u poređenju sa rezultatima sa Farme I (9,63%).

U uzorcima termički treiranog belog mesa i bataka koji su pripadali pilićima sa Farme II utvrđen je manji sadržaj ukupne masti u letnjem (3,57% i 10,54%, respektivno) i jesenjem periodu (3,72% i 11,11%, respektivno) u odnosu na uzorke sa Farme I (6,08% i 14,73%, respektivno, leto i 6,25% i 13,37%, respektivno, jesen). Razlike u sadržaju ukupne masti u uzorcima termički tretiranih bataka uzorkovanim u jesenjem periodu (13,37%, Farma I i 11.10%, Farma II) pokazale su se statistički značajne ($p < 0,01$). Sadržaj holesterola (mg/100g) u sirovom belom mesu je bio 53,90 (jesen) i 62,10 (leto), Farma I; 46,97 (jesen) i 49.53 (leto), Farma II. Utvrđena je statistički značajna razlika ($p < 0,001$) u sadržaju holesterola u uzorcima sirovog belog mesa pilića sa obe farme, u letnjem periodu. U sirovom bataku, prosečni sadržaj holesterola (mg/100g) iznosio je 70,24, u jesen i 83,95, u leto (Farma I) i 65,05, u jesen i 60,92, u leto, (Farma II). Dobijene razlike u sadržaju holesterola u uzorcima sirovih bataka su bile statistički značajne u uzorcima sa Farme I, u letnjem i jesenjem periodu i sa obe farme, u letnjem periodu ($p < 0,001$).

U pečenom mesu prosečan sadržaj holesterola (mg/100g) je bio između 70,32 (jesen) i 87,37 (leto), za belo meso i od 75,23 (jesen) do 78,92 (leto), za batak (Farma I) i između 64,33 (leto) i 66,24 (jesen), za belo meso i od 81,31(leto) do 91,6 (jesen), za batak (Farma II). Dobijeni rezultati ukazuje da je sadržaj ukupne masti i holesterola bio manji u sirovom i pečenom belom mesu sa kožom u odnosu na sirovo i pečeno crveno meso sa kožom. Dalja istraživanja je potrebno sprovesti na većem broju uzoraka mesa, a sadržaj ukupne masti i holesterola se mora sagledati uzimajući u obzir faktore kao što su rasa (heritabilnost), pol, starost, masa životinja za klanje i adekvatna ishrana. Pored toga, primena određenih dodataka u ishrani brojlera, način obrade i pripremanja mesa može značajno da smanji sadržaj masti i holesterola u mesu.

Ključne reči: *pileće meso, holesterol, ukupne masti.*

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Study on consumer evaluation of cooked sausages

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A b s t r a c t: The mapping method in the design of internal map is one of the novel and effective ways to evaluate consumer preferences. Internal map allows determining and visualizing differences in consumer preferences to foods that are being compared. It also helps to link together different information about the food item and consumer attitude to it.

The aim of this study is to investigate the possibilities of internal map's instruments to assess consumer attitude to cooked sausages. Five samples of cooked sausage "Doktorskaya", produced according to GOST R 521096-2011, which were purchased in department stores of Moscow and Moscow region (samples No 332 and No 326), the city of Kirov and Kirov region (samples No 347, No 312 and No 303), were chosen for research. Fifty one respondents took part in the consumer testing. All respondents were divided into groups: two groups according to gender (male, 19.6%; female, 80.3%), and two groups according to age (20 to 25 years, 76.5%; 26 to 45 years, 23.5%). All the description of sensory and other specific characteristics were discussed among the consumer groups previous to panel testing. Hedonic and additional information was collected with the aid of questionnaires. The results of consumer assessments were analysed together with additional attributes (demographic and other). The significance of the data obtained was checked with ANOVA. The data interpretation was carried out on two principal components, which explain over 70% of all data divergence.

In the first stage main sensory characteristics were analysed: appearance, colour, flavour, consistency. In the second stage some specific characteristics were analysed: "meaty" flavour, "smoky" aroma, aroma of spices, "spicy" taste and "saltiness". The obtained data demonstrated that samples No 332, No 326 and No. 347 were the leaders in terms of preference of their specific organoleptic characteristics.

It is evident that internal preference maps facilitate the analysis of the consumer panel testing results of both sensory and specific characteristics.

Summarizing the results of the study on the consumer evaluation of product quality, it can be concluded that the use of internal preference maps allows obtaining of the complex visualized information not only about overall preference of a product but also about preference of the individual organoleptic attributes and specific characteristics with regard to the market segments.

Statistical analysis of the results of consumer preferences by mapping method enables to: (1) study product classes, highlight a direction and strength of liking or disliking of a product by the specific groups of population, determine the best and similar products by organoleptic properties; (2) study consumer requirements; (3) determine a direction of product modifications to optimize its organoleptic characteristics and improve perception by consumers; – assess consumer reaction on newly developed products; (4) establish the link between the product organoleptic characteristics and consumer preferences.

The obtained information can be used by marketing services when developing a new product strategy and launching it to a market, studying a market structure, strengths and weaknesses of competitors, needs and motivation of buyers, the targeted work with a specific buyers' segment and so on.

***Key words**: consumer evaluation, cooked sausages, mapping method, internal preference maps, consumer preferences.*

Introduction

The most important target for a product manufacturer is the production of competitive products with high consumer acceptance. One of the ways to achieve the set goal is to implement modern methods of sensory analysis which, in addition to the product quality evaluation, can also be successfully used for correction of its organoleptic properties according to consumer preferences. Thus, sensory examinations are carried out using the methods of consumer evaluation based on the study of consumer impressions of organoleptic characteristics of a given product (*Meilgaard et al.*, 1999; *Šarčević et al,* 2011). Various methodologies of the approach to investigating consumer preferences and analysis of the obtained results help not only to understand the consumer attitudes toward a product, but also reveal the main purchase motivation. In the analysis of the obtained data, the multidimensional methods of statistical analysis are widely used; for example, the mapping method, which makes it possible to visualize the relationships of the sensory data array by two or three dimensional diagrams, so called the internal preference maps (*Resano et al.,* 2009; *Santa Cruz et al*., 2003).

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An internal preference map presents a model reflecting relationships of consumer liking and disliking, and a product being positioned, its organoleptic characteristics, packaging and so on. The revealed relationships are indicated by the corresponding arrows as a vector directed from the zero point toward the product samples, for which consumer preferences are investigated (*Meullenet et al*, 2007; *van Kleef*, 2006). So, the described method makes it possible to establish relationships of consumer liking of a product depending on the age, gender, purchase activity, and a respondent's income. Analysis of such data gives an opportunity to improve prediction and more precisely interpret the obtained results (*Guinard et al.,* 2001; *Šarčević et al,* 2013).

This study presents the detailed visualization of the relationships at the level of consumer liking – a single sensory property with several additional attributes.

The applied mapping method includes questionnaire, criteria for a product and respondent selection and method of creating a map based on the data obtained as a result of the survey. The aim of this study was to evaluate the possibilities of using consumer methods of competing product evaluation based on the analysis of the internal preference maps. As an example, the analysis of the internal preference maps revealing only a part of the methodology for consumer quality evaluation is presented.

Material and methods

Five samples of cooked sausage "Doktorskaya" produced according to GOST R 521096-2011 and sold in Moscow and Moscow region (samples No 332 and No 326), the city of Kirov and Kirov region (samples No 347, No 312 and No 303) were chosen for the present study. Fifty one respondents took part in the consumer testing. The respondents were divided into groups: two groups according to the gender (male, 19.6%; female, 80.3%), and two groups according to the age (20 to 25 years, 76.5%; 26 to 45 years, 23.5%). Before tasting, consumers were informed about the aim of the investigation. Organoleptic properties and specific characteristics (so called, descriptors) used for evaluation of cooked sausage quality were discussed with consumers (*Kuznetsova et al.*, 2014.). After tasting the products, the respondents filled in the questionnaires, which contained the general and specialized sections. In the general section, consumers were asked to express their preferences regarding the tested samples according to the main organoleptic attributes (appearance, taste, aroma, consistency and so on), and in the specialized section according to the specific characteristics (saltiness, taste of spices, aroma of smoking fume and so on). A graphic hedonic scale was used to assess the intensity of the organoleptic characteristics. The obtained data were analysed by multidimensional statistical methods.

Since the method of mapping is oriented to the imaging of relationships in a group of products, it is necessary to consider upon interpretation of the results that products with similar organoleptic properties are located on a map close to each other, and those that are different are at a significant distance. Vectors characterizing consumer reactions have different lengths, which reflect degrees of consumer liking.

Results and discussion

The results of consumer preference of the group of cooked sausages are presented in Fig. 1-3. The data characterizing preferences regarding the sausage appearance and colour (Fig 1a, 1 b) suggest the differentiated consumer perception of each sample (the samples are located far from each other). The figure shows that most of the vectors reflecting consumer liking of the product are directed toward the area located between samples No. 326 and No. 332. Analysis of the established relationships demonstrated that no sausage sample corresponded in full measure to the consumer liking and the most desirable for them was the combination of the appearance and colour characteristics typical of sausage samples No. 326 and No. 332. The obtained information can serve as a guide for a producer in optimization of appearance and colour of a product being manufactured.

The visualization of the preferences regarding attribute "taste of sausages" suggests that the preference spectrum for the majority of consumers is directed toward the area located between samples No. 332 and No. 347 (Fig. 1 c, d) and preference regarding the attributes "aroma" and "consistency" toward samples No. 332 and No. 326. Samples No. 303 and No. 312 were in the area of disliking for almost all organoleptic attributes.

The data presented in Fig. 1 a-e demonstrate no specific directions or clusters of preferences, which suggests the absence of a clear gradation dependent on age.

Appearance and colour of sample No. 326 were more attractive for the middle-aged people but they preferred sample No. 347 in terms of taste.

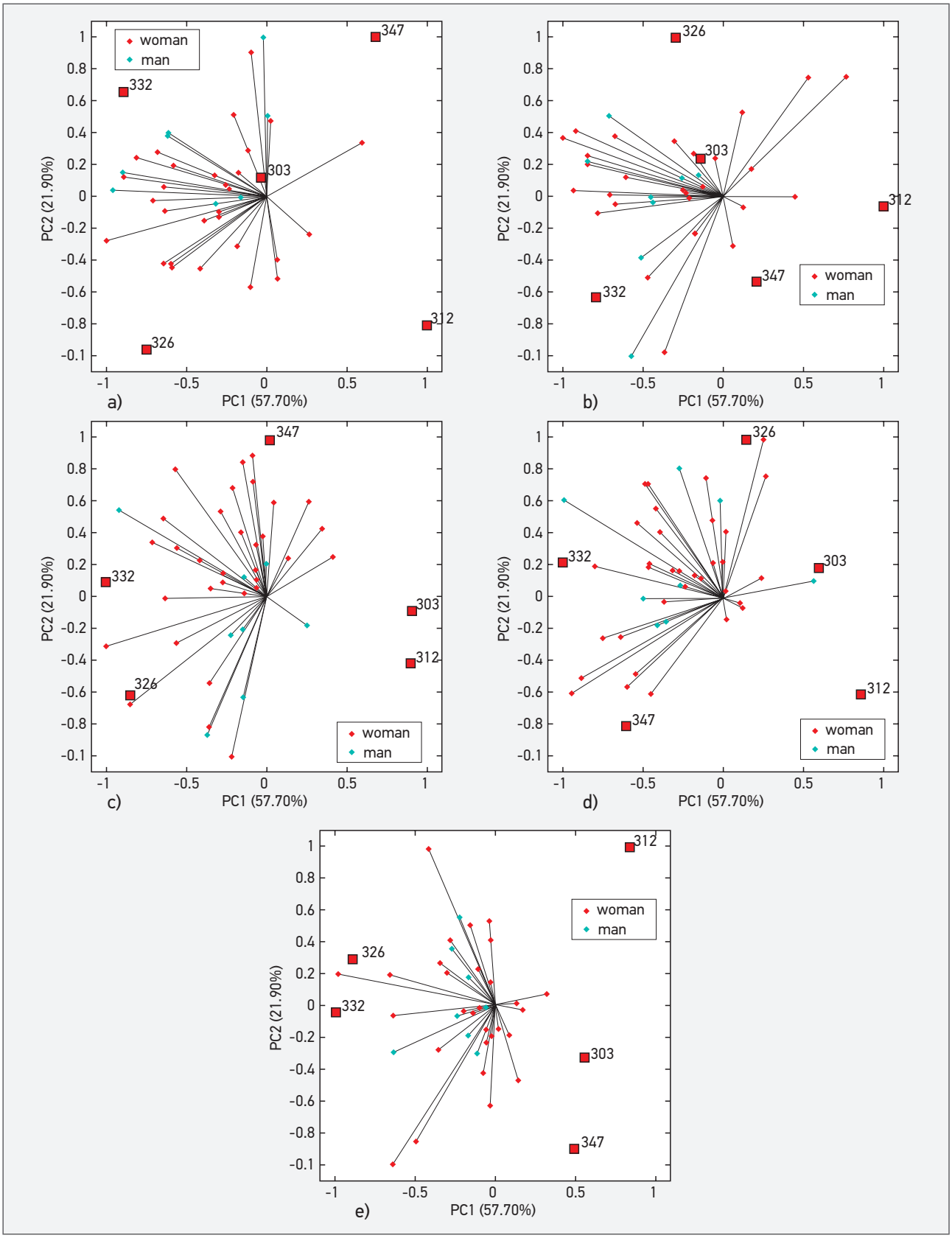

Figure 1. Internal preference maps by attributes: a) appearance; b) cut-surface colour c) taste; d) aroma; e) consistency.

Slika 1. Interne/unutrašnje mape prema osobinama: a) izgled; b) boja preseka; c) ukus; d) aroma; e) konzistencija

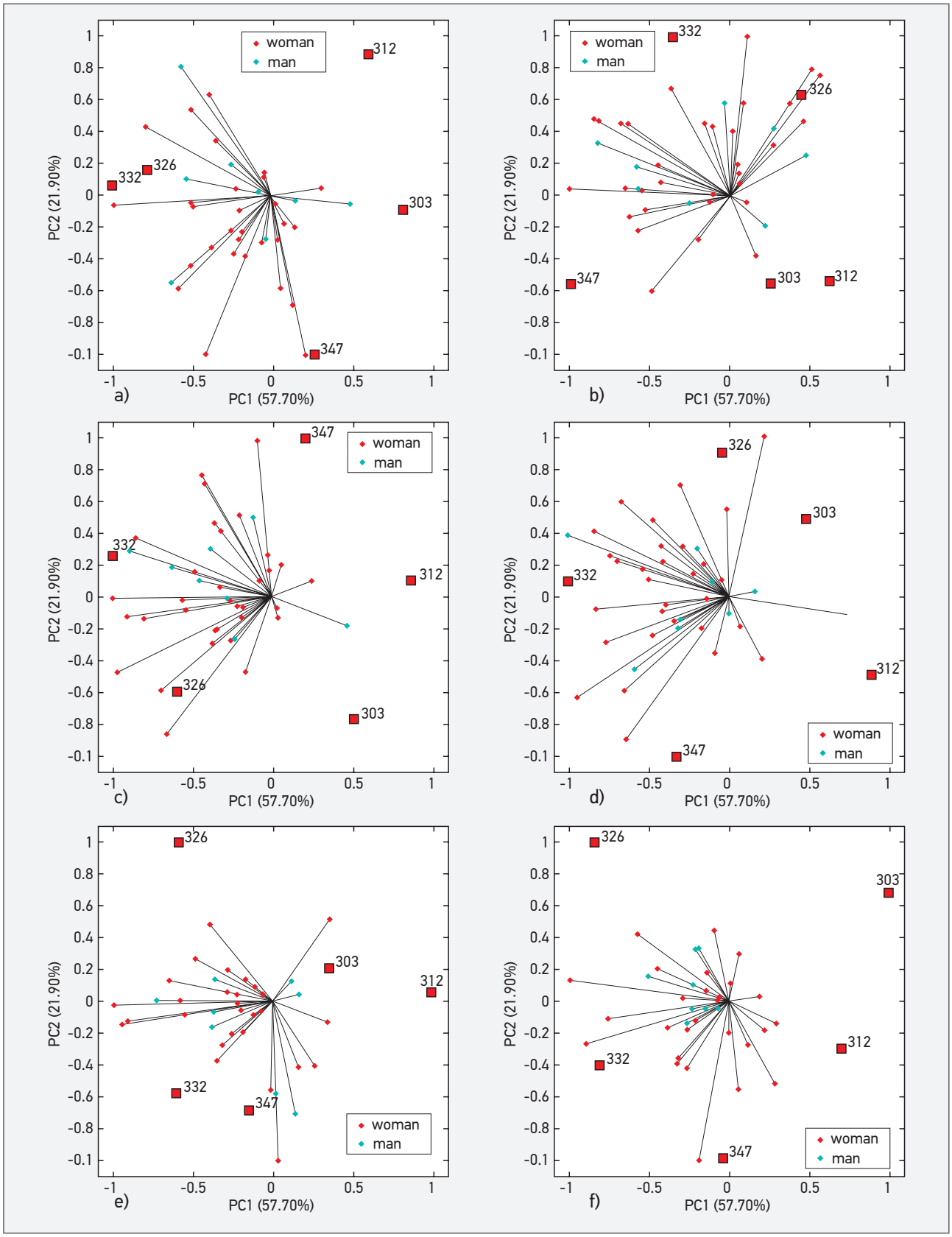

Figure 2. Internal preference maps by sensory attributes: a) "meaty taste", b) "meaty aroma", c) "aroma of smoking", d) "aroma of spices", e) "aste of spices", f) "saltiness" for cooked sausages Doktorskaya.

Slika 2. Interne/unutrašnje mape prema senzornim osobinama: a) „mesnati ukus", b) „mesnata aroma", c) „aroma dimljenog proizvoda", d) „aroma začina", e) „začinjenost", f) „slanoća" kuvanih kobasica Doktorskaya.

The differences in liking depending on the frequency of product consumption were established.

Thus, the absolute leaders in terms of consumer preference are samples No. 332 and No. 326. However, sample No. 347 cannot be assigned to the preference outsiders because the consumers of the older age group liked it mostly as they are used to that sausage. They have consumed it over a long period of time.

The second stage of the investigations consisted in the analysis of consumer preferences with regard to the specific organoleptic characteristics such as "meaty" taste and aroma, "aroma of smoking" and "aroma of spices", "taste of spices" and "saltiness", which are important attributes for assessment of its competitiveness.

Analysis of consumer preferences regarding "meaty" taste for cooked sausages showed that samples differed significantly from each other in their perception, with the exception of samples No. 332 and No. 326 (Fig. 2a). The proximity of the location of the latter two to each other suggests that consumers do not feel any significant differences between the samples in terms of this organoleptic characteristic and perceive them equally. Most of liking was directed towards samples No. 326, No. 332 and No. 347.

The aroma of smoking, its intensity and various hints can affect both positively and negatively the appeal of a product. Excessive intensity or unpleasant hint plays an important role in the competitiveness of a product.

The preference map shows that the consumers perceived products differently (Fig. 2c). The majority of the consumers liked the aroma of samples No. 326, No. 332 and No. 347, while samples No. 312 and No. 303 were in the disliking zone. The similar picture shows the perception for sausage "aroma of spices" (Fig. 2d).

Analysis of preferences according to attribute "saltiness" (Fig. 2e) shows that the spectrum of liking is distributed between samples No. 326, No. 332 and No. 347. If we consider liking of this characteristic with regard to the additional attributes (for example, gender), the group of male respondents was recognizably distinct with their preferences located in the direction toward samples No.332 and No. 326, which are saltier.

The obtained data demonstrated that samples No 332, No 326 and No. 347 were the leaders in terms of liking for the specific organoleptic characteristics.

Conclusion

Summarizing the results of the study on the consumer evaluation of product quality, it can be concluded that the use of internal preference maps allows obtaining of the complex visualized information not only about overall liking of a product but also about liking of the individual organoleptic attributes and specific characteristics with regard to the market segments.

Statistical analysis of the results of consumer preferences by mapping method enables to:

- study product classes, highlight a direction and strength of liking or disliking of a product by the specific groups of population, determine the best and similar products by organoleptic properties;
- tudy consumer needs;
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- assess consumer reaction on newly developed products;
- establish relationships of product organoleptic characteristics and consumer preferences.

The obtained information can be used by marketing services when developing of a new product strategy and launching it to a market, studying a market structure, strengths and weaknesses of competitors, needs and motivation of buyers, the targeted work with a specific buyers' segment and so on.

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Studija o proceni kvaliteta kuvanih kobasica od strane potrošača

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R e z i m e: Metod mapiranja u dizajnu internih mapa je jedan od novih i efikasnih načina za procenu stava potrošača prema proizvodima. Interna mapa omogućava da se utvrde i vizualizuju razlike u preferencama potrošača prema hrani koja se poredi. Mapa, takođe, pomaže da se povežu različite informacije o proizvodu i stavu potrošača prema njemu.

Cilj ove studije bio je da se istraže mogućnosti interne mape radi procene stava potrošača prema kuvanim kobasicama. Pet uzoraka kuvane kobasice „Doktorskaya" koja je proizvedena u skladu sa GOST R 521096-2011, a koji su kupljeni u prodavnicama u Moskvi i moskovskom regionu (uzorci br. 332 i br. 326), i u gradu Kirov i Kirovskom regionu (uzorci br. 347, br. 312 i br.303) su izabrani za istraživanje. Pedeset jedan ispitanik je učestvovao u testiranju. Ispitanici su bili podeljeni u grupe: dve grupe prema polu (muškarci, 19,6%; žene, 80,3%), i dve grupe prema uzrastu (20 do 25 godina, 76,5%, 26 do 45 godina, 23,5%). Opisi senzornih i drugih specifičnih karakteristika su razmatrani među potrošačima u grupama pre panel testiranja. Hedonističke i dodatne informacije su prikupljene uz pomoć upitnika. Rezultati ocene potrošača su analizirani, zajedno sa dodatnim atributima (demografskim i dr.). Značajnost dobijenih podataka je testirana upotrebom ANOVA. Podaci su tumačeni pomoću dve glavne komponente, koje opisuju više od 70% ukupne varijabilnosti podataka.

U prvoj fazi, analizirane su glavne senzorne osobine: izgled, boja, ukus i konzistentnost. U drugoj fazi, analizirane su neke specifične karakteristike: aroma mesa, aroma dima, aroma začina, „ljut" ukus i „slanost". Dobijeni podaci su pokazali da su uzorci br. 332, br. 326 i br. 347 imali vodeću poziciju u smislu sklonosti potrošača ka specifičnim organoleptičkim osobinama proizvoda.

Evidentno je da interna mapa preferenci omogućava analizu rezultata panel testiranja potrošača, kako senzornih, tako i specifičnih karakteristika.

Sumirajući rezultate studije o evaluaciji kvaliteta proizvoda od strane potrošača, može da se zaključi da upotreba interne mape preferenci omogućava dobijanje kompleksne vizuelne informacije, ne samo o ukupnom ukusu nekog proizvoda, već i o sklonosti potrošača ka pojedinačnim organoleptičkim osobinama i specifičnostima, a u vezi sa segmentima tržišta.

Statistička analiza rezultata preferenci potrošača metodom mapiranja omogućava: (1) ispitivanje kategorija proizvoda, označavanje pravaca i sklonosti ili odsustvo sklonosti ka nekom proizvodu, određivanje najboljih proizvoda i proizvoda sličnih organoleptičkih svojstava; (2) ispitivanje zahteva potrošača; (3) određivanje pravca modifikovanja proizvoda u cilju optimizovanja organoleptičkih osobina i poboljšavanja percepcije potrošača; procenu stava potrošača o novorazvijenim proizvodima; (4) uspostavljanje veze između organoleptičkih osobina proizvoda i preference potrošača.

Dobijene informacije mogu koristiti marketinškim službama prilikom izrade strategije novih proizvoda i njihovog plasmana na tržište, proučavajući tržišne strukture, prednosti i slabosti konkurenata, potreba i motivacija kupaca, ciljani rad sa segmentom specifičnih kupaca, i tako dalje.

Ključne reči: *procena potrošača, kuvane kobasice, metod mapiranja, interna mapa preferenci, preference potrošača.*

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Changes in the quality of goat meat in the production of smoked ham

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A b s t r a c t: The quality of fresh goat meat can be defined strictly in terms of physical and chemical properties, or in terms of consumer perception. In Serbia, there is not enough information about the quality of goat meat and goat meat products, such as smoked ham. The aim of this study was to determine differences in the basic chemical composition, colour, fatty acids composition, volatile compounds in fresh meat and smoked ham (musculus gluteus superficialis). The meat was obtained from the population of Serbian White goat, five or six years old. ISO methods were implemented in order to determine the quality of these parameters.

Statistically significant difference ($p < 0.05$) was determined between values of protein, fat, moisture, ash, pH value, fatty acids and volatile compounds determined in fresh meat and finished product (smoked ham). It is assumed that the complex chemical and biochemical processes occurring during production (growing, curing, smoking, drying) resulted in statistically significant differences between the quality parameters in fresh meat and smoked ham. There was a statistically significant difference ($p < 0.05$) between the values of capric acid, lauric acid, myristic acid, pentadecanoic acid, pentadecenoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and gadoleic acid identified in the thigh meat prepared for curing and smoking in compared to value of the fatty acids identified in the final product (smoked ham).

Key words: *goats, meat quality, maturation, curing, smoking.*

Introduction

The general definition of meat quality, including goat meat quality, is referring to meat safety (pathogenic microorganisms, toxins, heavy metals, pesticides and antibiotics residues, etc.), physical and chemical properties of meat and palatability (*Webb et al*., 2005; *Casey and Webb*, 2010). Parameters which define the quality of goat meat, as noted by *Webb et al.* (2005), are discovered and continually redefined. Goat meat quality depends on the biological factors including the age and sex of the animal, as well as other factors such as pre-slaughter stress , slaughter techniques and carcass cooling and freezing practices.

Physiological state of live animals and post-mortem biochemical changes in muscle, fat and fibrous tissue have a direct impact on the meat palatability. Animal feeding affects quality of the meat by muscle growth, muscle and fat ratio, fat accumulation and the fatty acid composition (*Casey and Webb,* 2010). Goat meat is an important source of proteins worldwide, especially in developing countries (*Biswas et al*., 2007). It has about the same nutritional value as sheep meat (contains more proteins and less fat compared to sheep meat). *Anaeto et al.* (2010) has considered that goat meat is easier to digest as a result of its molecular structure. Because goat meat contains low amount of saturated fatty acids and cholesterol, according *Anaeto et al*. (2010), it presents a healthier alternative compared to other types of red meat. According to the same author, polyunsaturated fatty acids are prevalent in goat meat and diet rich in unsaturated fatty acids is correlated with a reduced risk of stroke and coronary heart disease, which indicates important role of goat meat in human diet. Regardless of the nutritional value, goat meat is still less appreciated because of specific taste which is even more present in older animals (*Ivanović et al*., 2011).

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The fatty acids in the muscle tissue affect meat quality, including tenderness, color, stability of lipid and flavour (*Wood et al.,* 2004). In the transformation of fatty acids substances are formed which directly affect the smell and taste of goat meat. Slightly rancid odor is caused by hexanal which comes mainly from linoleic and arachidonic acid (*Martin et al.,* 2002). Other volatile aldehydes such as heptanal, octanal, nonanal and decanal derive mainly from oleic acid (*Machiels et al*., 2004). Fatty acids have been specifically implicated in sheep and goat flavors. 4-ethylocatanoic fatty acid is mainly responsible for strong smell of goat meat. This acid was detected in goat meat, lamb and mutton, as well as in cheeses made from milk from these species. In addition to fatty acids, taste and aroma are also affected by other compounds: hydrocarbons, aldehydes, ketones, alcohols, furans, thiophenes, pyrrols, pyrazines, oxazoles, thiazoles, and sulfurous compounds (*Todaro et al*., 2004). Goat meat has a significant role in human nutrition because it contains essential amino acids such as lysine, threonine and tryptophan. Goat breeding and goat meat consumption, despite mentioned qualitative composition, are determined by religion, tradition and customs as well as market and consumer habits (*Ivanović et al.,* 2009). In Serbia, there are not enough information about the quality of goat meat and goat meat products such as smoked ham. The aim of this study was to determine differences in the basic chemical composition, colour, fatty acids composition, volatile compounds of fresh meat and smoked ham (*m. gluteus superficialis*), which come from the Serbian population of white goat breed, aged five or six years.

Material and methods

Twenty culled Serbian white goats, 5−6 years old were used. All animals were selected from private farms in the rural area of Stara Planina Mountain. The goats were raised during the same period . Facilities for housing of goats were built of mixed solid materials and covered with ceramic tiles, with conditions that were satisfactory for goat breeding. The floor was stuffed soil and covered by thick layer of wheat straw. Watering was ad libitum.

The diet for goats during the winter consisted of hay which was collected from natural pastures (3.5 kg/day per animal) and concentrate (0.25 kg/day per animal). In the summer months, the goats were pastured and fed with concentrate in the amount of 0.25 kg/day. The concentrate was made of maize meal, wheat bran with added sodium chloride and premix.

The animals were slaughtered in the experimental slaughter house of the Institute for Animal Husbandry. The carcasses were processed in the way common for industrial production, and cooled at 4°C for 48 hours.

Processed goat hams with associated bones were dry salted using about 6% nitrite salt (99.5% sodium chloride and 0.5% sodium nitrite). Hams were kept in nitrite salt for 30 days at 5°C. During the salting period they were rotated every two days. Desalting was carried out in cold water for 24 h, the water was changed four times. Hams were cold-smoked for 45 days on moderate air circulation, humidity 70−78%. The smoke temperature did not exceed 20°C. During the first 10 days, the smoking was carried out every day for 2 hours, but between the 10th and 45th day it was done every two days for 2 hours. After the smoking period, hams were air dried (18−20°C) for another 45 days.

The material used for the determination of chemical composition, fatty acids and volatile compounds was *m. gluteus superficialis*. Moisture content was determined according to ISO 1442:1997, fat content according to ISO 1443:1973 and ash content according to ISO 936:1998. The protein content was calculated from nitrogen content multiplied with 6.25 using ISO 937:1978, sodium chloride content was determined according to ISO 1841-1:1996, pH value according to ISO 2917:1999 and nitrite content according to ISO 2918:1975.

AOAC method (1996, 2001) was applied for fat extraction from tissue, methylation with boron trifluoride reagent and GC determination. Analysis of FAMEs was performed by an internal standard method using a gas chromatograph (GC6890N, Agilent Tech., USA) with column DB−23 (60m × 0.25mm ID, 0.15 μm) and comparing with standard mix of FAMEs 37 (Supelco, USA).

Volatile compounds analysis was conducted by Likens-Nickerson extraction procedure (*Likens et al.,* 1964) and by gas chromatographic-mass spectral analysis using an GCMS-QP2010 Ultra (EIMS, electron energy = 70 eV, scan range = 30−350 amu, and scan rate = 3.99 scans/s) with SUPELCOWAX® 10 Capillary GC Column (30 m x 0.25 mm ID, particle size 0.25 μm). The carrier gas was helium with a flow rate of 1 mL/min, and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 min at 40°C, and subsequently programmed from 40°C to 120°C at a rate of 3°C/min and at a rate of 10°C/min from 120°C to 250°C where it was held for another 5 min. Identification of the peaks was based on comparison of their mass spectra with the spectra of the WILEY library and in

addition, in some cases, by comparison of their retention times with those of standard compounds.

The colour was measured on the fresh and smoked meat cuts (*musculus superficial gluteal*), from the right side of each carcass. CIE L*a*b* colour coordinates (CIE Colorimetry, 1986) were determined using Minolta Chromameter CR 400 (Minolta Co. Ltd., Osaka, Japan) in D-65 lighting, with standard angle of 2 degrees of shelter and 8 mm aperture of the measuring head. These results were expressed in CIE L*a*b* and were given as the mean values: L* (psychometer light), a* (psychometer tone) and b* (psychometer chroma).

Data obtained in this study were analysed by descriptive and analytical statistical parameters: mean value (M), standard deviation (SD) by using MS Excel 2003 and analysis of variance (ANOVA). The differences between the averages were compared by t-test at the level of significance of 95%.

Results and discussion

The results of chemical composition and pH value of fresh goat meat and ham are shown in Table 1.

Results presented in Table 1 showed that there was a statistically significant difference ($p < 0.05$) between the values of protein, fat, moisture, ash and pH value in fresh goat meat and the value of protein, fat, moisture, ash and pH value determined in the finished product (smoked ham). Sodium chloride and nitrites were determined only in smoked ham.

The results of the fatty acid composition in *m. gluteus superficialis* of goat meat and smoked ham from these goats are presented in Table 2.

There were statistically significant differences ($p < 0.05$) between the values of capric acid, lauric acid, myristic acid, pentadecanoic acid, pentadecenoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and gadoleic acid identified in the thigh meat prepared for curing and smoking compared to value of the fatty acids identified in the final product (smoked ham). The ratio of unsaturated/saturated fatty acids was 0.83 in fresh meat and 0.55 in smoked ham.

Table 3 shows the results obtained by analysing the presence of specific volatile substances in fresh meat and smoked ham.

Some volatile compounds, such as, benzene, ethylbenzene, phenol, 2-methyl-phenol and 2-methoxy-phenol were not detected in fresh goat meat while 2-pentanol and 1-octen-3-ol were not detected in smoked ham.

In this study no statistically significant differences ($p > 0.05$) were found between butanoic acid and octane. There were statistically significant differences ($p < 0.05$) between other volatile compounds determined in fresh meat and smoked ham (table 3).

Colour parameters (L* a* b*) of fresh meat samples taken from a goat leg and samples of

Table 1. The basic chemical composition and pH value of fresh goat meat and smoked ham
Tabela 1. Osnovni hemijski sastav i pH vrednost svežeg kozijeg mesa i dimljene šunke

Fresh meat/Sveže meso			Smoked ham/Dimljena šunka		
Parameter/ Parametar	n	M ± SD	Parameter/ Parametar	n	M ± SD
Protein/Protein, %	20	20.1 ± 0.8^a	Protein/Protein, %	20	37.9 ± 0.9^b
Fat/Mast, %	20	3.5 ± 0.7^a	Fat/Mast, %	20	16.1 ± 1.0^b
Moisture/Vlaga, %	20	74.9 ± 0.8^b	Moisture/Vlaga, %	20	39.1 ± 0.7^a
Ash/Pepeo, %	20	1.04 ± 0.04^a	Ash/Pepeo, %	20	5.6 ± 0.2^b
Sodium chloride/ Natrijum-hlorid, %	20	nd	Sodium chloride/ Natrijumhlorid, %	20	4.7 ± 0.1
Nitrites/Nitriti, mg/kg	20	nd	Nitrites/Nitriti, mg/kg	20	27.0 ± 1.0
pH value/ pH vrednost	20	5.71 ± 0.06^a	pH value/ pH vrednost	20	5.52 ± 0.04^b

Legend/Legenda: [a, b] Means within the same row with different superscripts differ significantly ($p < 0.05$), nd – not determined/[a,b] srednje vrednosti u istom redu sa različitim znakom se značajno razlikuju ($p < 0,05$), nd – nije određeno

Table 2. The fatty acid composition (% of total fatty acids) of fresh goat meat and smoked ham
Tabela 2. Sastav masnih kiselina (% od ukupnih masnih kiselina) svežeg kozijeg mesa i dimljene šunke

Fatty acids/Masne kiseline, n = 20	M ± SD	
	Fresh meat/Sveže meso n = 20	Smoked ham/Dimljena šunka n = 20
Capric acid/Kaprinska kiselina (C10:0)	0.30 ± 0.05[a]	0.46 ± 0.08[b]
Lauric acid/Laurinska kiselina (C12:0)	1.15 ± 0.12[a]	1.54 ± 0.15[b]
Myristic acid/Miristinska kiselina (C14:0)	9.30 ± 1.15[a]	11.03 ± 1.22[b]
Pentadecanoic acid/Pentadekanska kiselina (C15:0)	2.46 ± 0.30[a]	3.31 ± 0.45[b]
Pentadecenoic acid/ Pentadekenska kiselina (C15:1)	0.20 ± 0.05[b]	0.12 ± 0.02[a]
Palmitic acid/Palmitinska kiselina (C16:0)	25.95 ± 2.13[a]	29.28 ± 2.50[b]
Palmitoleic acid/Palmitoleinska kiselina (C16:1)	4.24 ± 0.40[b]	2.38 ± 0.45[a]
Heptadecanoic acid/Heptadekanska kiselina (C17:0)	1.35 ± 0.15[a]	2.05 ± 0.22[b]
Stearic acid/Stearinska kiselina (C18:0)	13.70 ± 1.32[a]	16.35 ± 1.65[b]
Oleic acid/Oleinska kiselina (C18:1)	36.20 ± 2.80[b]	31.15 ± 2.55[a]
Linoleic acid/Linolna kiselina (C18:2)	3.40 ± 0.30[b]	1.45 ± 0.14[a]
Linolenic acid/Linolenska kiselina (C18:3)	1.21 ± 0.15[b]	0.30 ± 0.10[a]
Arachidic acid/Arahidonska kiselina (C20:0)	0.32 ± 0.05[a]	0.45 ± 0.10[b]
Gadoleic acid/Gadoleinska kiselina (C20:1)	0.21 ± 0.05[b]	0.10 ± 0.05[a]
Σ SFA (Saturated Fatty Acid/Zasićene masne kiseline)	54.53±5.27[a]	64.47±6.37[b]
Σ MUFA(Monounsaturated fatty acids/Mononezasićene masne kiseline)	40.85 ± 3.30[b]	33.75 ± 3.07[a]
Σ PUFA (Polyunsaturated fatty acid/Polinezasićene masne kiseline)	4.61 ± 0.45[b]	1.75 ± 0.24[a]
USFA/SFA (Saturated fat/Zasićena mast)	0.83	0.55

Legend/Legenda: [a, b] Means within the same row with different superscripts differ significantly ($p < 0.05$)/[a,b] srednje vrednosti u istom redu sa različitim znakom se značajno razlikuju ($p < 0,05$)

smoked ham originated from the same leg are presented in Table 4.

In this study, statistically significant differences ($p < 0.05$) were found for lightness (L*) as well as for redness (a*) and yellowness (b*).

Meat has heterogeneous composition, which is specific for each type, and varies depending on many factors, therefore it is difficult to define the quality of the meat. Meat quality is affected by breed, gender, productivity and adaptation to stress, environment, management, nutrition, body weight and health condition at the time of slaughter, slaughter methods and post-slaughter carcass practices. In addition, meat products, in this case smoked ham, are manufactured in different ways and therefore it is difficult to compare the results represented by different authors. Previously, we examined chemical and sensory characteristics of meat from Bunte Deutsche Edelziege and Balkan goat breed (*Ivanović et al.,* 2011) and meat quality of Serbian White goat and Balkan goat (*Ivanović et al.,* 2014). The results of chemical composition (total protein, fat, water, ash) and pH value of fresh meat presented in Table 1 are consistent with the results we obtained in the previous study, which related to the population of Serbian White goat (*Ivanović et al*., 2014). Our findings related to the fresh meat (Table 1) are also consistent with the results obtained by *Paleari et al.* (2008). These authors investigated the composition of meat from goat crosses (Frisa × Frontalasca) aged 2-3 years. Goats were reared in similar conditions as goats in our experiment (during summer season they were on pasture and during the winter kept inside facilities). *Ding et al.* (2010) investigated the quality of the meat from Guanzhong Dairy breed and three genotypes thereof. Our results relating to fresh meat, water, protein and ash are in agreement with the results of *Ding et al.* (2010) for the breed Guanzhong Dairy, however not in accordance regarding the fat.

Table 3. Volatile compounds of fresh goat meat and smoked ham quantified by GC/MS (μg/kg)
Tabela 3. Isparljiva jedinjenja u svežem kozijem mesu i u dimljenoj šunci kvantifikovana GC/MS (μg/kg)

Volatile compounds/Volatilna jedinjenja n=20	M ± SD	
	Fresh meat n = 20	Smoked ham n = 20
Aldehydes/aldehidi		
3-methylbutanal/3-metilbutanal	1.20 ± 0.21[a]	3.11 ± 0.40[b]
Pentanal/pentanal	3.08 ± 0.58[b]	1.48 ± 0.30[a]
Hexanal/heksanal	16.07 ± 1.14[b]	5.96 ± 1.05[a]
Heptanal/heptanal	2.31 ± 0.28[b]	1.06 ± 0.18[a]
Benzaldehyde/benzaldehid	0.24 ± 0.05[a]	0.61 ± 0.09[b]
Octanal/oktanal	1.77 ± 0.24[b]	0.37 ± 0.07[a]
Nonanal/nonanal	2.98 ± 0.35[b]	0.52 ± 0.12[a]
Ketones/Ketoni		
2,3-butanedione/2,3-butanedion	0.30 ± 0.08[a]	9.53 ± 0.11[b]
2-butanone/2-butanon	3.65 ± 0.33	n.d.
2-pentanone/2-pentanon	0.14 ± 0.03[a]	0.72 ± 0.35[b]
3-hydroxy-2-butanone/3-hidroksi-2-butanon	0.17 ± 0.04[a]	22.25 ± 1.75[b]
2-heptanone/2-heptanon	0.30 ± 0.05[b]	0.24 ± 0.05[a]
2,3-octanedione/2,3-oktanedion	0.23 ± 0.05[a]	0.39 ± 0.08[b]
Heterocyclic compounds/heterociklična jedinjenja		
2,6-dimethylpyrazine/2,6-dimetilpirazin	0.11 ± 0.03[a]	0.25 ± 0.05[b]
Aromatic hydrocarbons/Aromatični vodougljenici		
Benzene/benzen	n.d.	0.46 ± 0.10
Methylbenzene/metilbenzen	0.13 ± 0.03[a]	8.98 ± 1.23[b]
Ethylbenzene/etilbenzen	n.d.	0.43 ± 0.09
Phenols/Fenoli		
Phenol/fenol	n.d.	1.22 ± 0.25
2-methyl– phenol/2-metil-fenol	n.d.	0.40 ± 0.08
2– methoxy-phenol/2-metoksi-fenol	n.d.	1.28 ± 0.25
Alcohols/Alkoholi		
1-penten-3-ol	0.22 ± 0.04[a]	1.64 ± 0.31[b]
2-pentanol	0.17 ± 0.03	n.d.
3-methyl-1-butanol	0.15 ± 0.03[a]	1.49 ± 0.30[b]
1-pentanol	1.16 ± 0.20[b]	0.61 ± 0.12[a]
Furfurol	0.16 ± 0.04[a]	1.15 ± 0.22[b]
1-octen-3-ol	1.07 ± 0.14	n.d.
Organic acids/Organske kiseline		
Acetic acid/Sirćetna kiselina	0.29 ± 0.05[a]	3.63 ± 0.55[b]
Butanoic acid/Butanoinska kiselina	0.65 ± 0.10[NS]	0.72 ± 0.12[NS]
3-methyl-butanoic acid/3-metil-butanoinska kiselina	0.10 ± 0.03[a]	1.66 ± 0.27[b]
Alkanes/Alkani		
Hexane/heksan	0.27 ± 0.05[a]	5.87 ± 0.95[b]
Heptane/heptan	0.15 ± 0.03[a]	0.82 ± 0.38[b]
Octane/oktan	0.81 ± 0.15[NS]	0.84 ± 0.15[NS]
Nonane/nonan	0.15 ± 0.03[a]	0.43 ± 0.08[b]
Alkenes/Alkeni		
1-octene	0.19 ± 0.04[a]	0.56 ± 0.10[b]

Legend/Legend[a, b] Means within the same row with different superscripts differ significantly ($p < 0.05$), NS – not statistically significant difference, n.d-Not determined/[a,b] srednje vrednosti u istom redu sa različitim znakom se značajno razlikuju ($p < 0,05$), NS – nije statistički značajno; nd – nije određeno

Table 4. Colour of fresh goat meat and smoked ham expressed in CIE L*a*b* system
Tabela 4. Boja svežeg kozijeg mesa i dimljene šunke izražena u CIE L*a*b* sistemu

Parameter/Parametar, n = 20	M ± Sd	
	Fresh meat/Sveže meso	Smoked ham/Dimljena šunka
Lightness/Svetla boja – L*	34.1 ± 2.2[b]	30.1 ± 2.0[a]
Redness/Crvena boja – *a**	20.9 ± 1.8[b]	17.1 ± 1.5[a]
Yellowness/Žuta boja – *b**	5.2 ± 1.1[b]	3.3 ± 0.9[a]

Legend/Legenda:[a, b] Means within the same row with different superscripts differ significantly ($p < 0.05$)/[a,b] srednje vrednosti u istom redu sa različitim znakom se značajno razlikuju ($p < 0,05$)

In our studies, the most represented fatty acids in fresh meat were, in the following order, oleic acid, palmitic acid, stearic acid, myristic acid and palmitoleic acid. The percentages of these fatty acids in smoked ham are little different (Table 2). Statistically significant differences in regard tio the fatty acid composition in fresh meat and smoked ham are the result of manufacturing process (maturation, curing, smoking, drying). Fatty acid composition in meat and milk of ruminants depends on breed and feeding (*Grubić et al*., 2005; *Ivanović et al.,* 2012). Lipids from the diet are hydrolyzed in the rumen of ruminants. Unsaturated fatty acids from food are biohydrogenated by microorganisms from rumen. As a result, ruminants absorb predominantly saturated fatty acids, which is why the food that originates from ruminants contains mainly saturated fatty acids. Our results showed that the total saturated fatty acids participate with 54.53% ± 5.27 in fresh meat and 64.47 ± 6.37 in smoked ham. The USFA/SFA ratio in fresh meat was 0.83 and in smoked ham 0,55. The results obtained in the present study for oleic acid, palmitic acid and stearic acid in fresh meat are in accordance with the results obtained by *Paleari et al.* (2008), while for the smoked ham are consistent only for oleic acid, which is understandable, because the production process is not the same. Our results regarding the total SFA, MUFA and PUFA contents are also consistent with the results of previously mentioned authors . The results obtained for fresh meat, that are related to percentage of oleic acid, palmitic acid, stearic acid and myristic acid are in agreement with the results obtained by *Mushi et al*. (2008), but not in agreement with the results from same study relating to the total SFA, MUFA and PUFA content.

it The presence of volatile compounds was determined in the analysed samples within the following groups: aldehydes, ketones, heterocyclic compounds, aromatic hydrocarbons, phenols, alcohols, organic acids, alkanes (Table 3). By analysing the samples of fresh meat, two compounds from the group of aromatic hydrocarbons (benzene and ethylbenzene) and compounds from the group of phenols (phenol, 2-methyl– phenol and 2– methoxyphenol), which were identified in smoked ham, were not determined. By analysing the samples of smoked ham, the presence of mentioned compounds was determined, however, in the group of ketones, 2-butanone was not determined, and in the group of alcohols, 2-pentanol and 1-octen-3-ol compounds were not identified. The compounds identified in smoked ham probably were formed as a result of smoking.

Aldehydes were the most common groups of compounds identified in the analysed samples. Hexanal, 16.07 ± 1.14 μg/kg in fresh meat and 5.96 ± 1.05 μg/kg in smoked ham, was the most common type of aldehyde. Hexanal mainly comes from linoleic and arachidonic acid (*Martin et al*., 2002). Our results regarding the aldehyde in smoked ham are in agreement with results from study conducted by *Paleari et al.* (2008). Values of aldehyde in the fresh meat do not agree with results obtained by *Kang et al.* (2013), but are in agreement with ones obtained by *Villalobos-Delgado et al.* (2014). These authors have examined the fresh sheep meat during production process. Aldehydes in general are major sources of volatile fractions obtained from ruminant meat (*Vasta and Priolo*, 2006). According to *Mottram* (1998), aldehydes are compounds which are formed as a result of lipids oxidation. They may significantly contribute to the overall taste of the product because of their low levels of olfactory perception.

The second most present group of compounds are ketones. 2-butanone is mainly determined in fresh meat, while its presence was not determined in the smoked ham. Most common ketones found in smoked ham were 3-hydroxy-2-butanone and 2,3-butanedione. Type and amounts of ketones, as well as aldehydes, in smoked ham in our study are in agreement with the resultes obtained by *Paleari*

et al. (2008), while the ones found in fresh meat are contrary to the results obtained by *Kang et al.* (2013), but agree with results from study conducted by *Villalobos-Delgado et al.* (2014). Detection of the ketones in the meat is generally correlated with type of diet. It has been found that 2,3 – octanedione is present in a higher amount in meat from the animals fed with grass (*Vasta and Priolo,* 2006).

The results in our study referring to aromatic hydrocarbons obtained for smoked ham are in agreement with the results from study conducted by *Paleari et al*. (2008). In fresh meat two of three compounds were not detected (Table 3).

Phenols were not detected in fresh meat while they were present in small amounts in smoked ham. Also organic acids were present in a small percentage in fresh meat (Table 3), although they are responsible for the distinct taste of goat meat. Their level in final product was slightly higher, especially amount of acetic acid. Our results for acetic acid in smoked ham are in agreement with the results from study conducted by *Paleari et al.* (2008). Other compounds such as alcohols, alkanes and alkenes were detected in very low concentrations, but they probably have synergistic effects with other compounds and can affect the smell and the taste of goat meat and meat products.

Statistically significant differences between the parameters which determine the colour of fresh meat and smoked ham were expected because the processes during the production of smoked ham (maturation, curing and smoking) leads to discoloration. The results obtained in this study (Table 4) relating to fresh meat are in agreement with our previous research (*Ivanović et al.,* 2014) but not with the results of *Madruga et al.* (2008). The results of measurements of colour in fresh meat obtained in the study conducted by *Teixeira et al.* (2011), referring to the parameter L* are in agreement with our results, while the parameters a* and b* are not compatible.

Conclusion

The statistical difference between individual fatty acides in fresh and smoked meat lead to complex chemical and biochemical processes during technological production (maturation, brine, smoking, drying). As result of these processes, some volatile compounds, which were present in fresh meat, were probably synthesized in the whole group of other compounds that are present only in smoked meat. All the changes that have occurred, have led to significant differences ($p < 0.05$) in colour between samples of fresh and smoked meat.

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Promene kvaliteta mesa koza u procesu dobijanja dimljene šunke

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R e z i m e: Kvalitet svežeg mesa koza može se definisati strogo u smislu fizičkih i hemijskih osobina, ili u smislu percepcije potrošača. U Srbiji se malo zna o kvalitetu kozjeg mesa i proizvoda od kozjeg mesa, kao što je dimljena šunka. Cilj ovog istraživanja bio je da se utvrde razlike u osnovnom hemijskom sastavu, boji, sastavu masnih kiselina, volatilnih materija u svežem mesu i dimljenoj šunki (m. superficial gluteal). Meso je dobijeno klanjem koza iz populacije Srpske bele koze, starih pet-šest godina. Za određivanje navedenih parametara kvaliteta korišćene su ISO metode.

Između utvrđenih vrednosti proteina, masti, vode, pepela, pH vrednosti, masnih kiselina i isparljivih materija utvrđenih u svežem mesu i gotovom proizvodu (dimljena šunka) postojala je statistički značajna razlika ($p < 0,05$). U svežem mesa nisu utvrđena dva jedinjenja iz grupe aromatičnih ugljovodonika i jedinjenja iz grupe fenola. Pretpostavlja se da su složeni hemijski i biohemijski procesi tokom proizvodnje (zrenje, salamurenje, dimljenje, sušenje) doveli do statistički značajne razlike između ispitivanih parametara kvaliteta u svežem i dimljenom mesu. Utvrđena je statistički značajna razlika ($p < 0,05$) između vrednosti kaprinske kiseline, laurinske kiseline, miristinske kiseline, pentadekanske kiseline, palmitinske kiseline, palmitoleinske kiseline, heptadekanske kiseline, stearinske kiseline, oleinske kiseline, linolne kiseline, linolenske kiseline, arahidonske kiseline i gadoleinske kiseline u svežem mesu pripremljenom za sečenje i dimljenje u odnosu na vrednosti ovih masnih kiselina identifikovanih u gotovom proizvodu (šunka).

***Ključne reči**: koze, meso, kvalitet, zrenje, salamurenje, dimljenje.*

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Primena sledljivosti u proizvodnji *zlatarskog sira*

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S a d r ž a j: U ovom radu su dati elementi sledljivosti koji moraju biti primenjeni tokom tradicionalnog procesa proizvodnje i distribucije zlatarskog sira. Aktivnosti koje se sprovode radi dobijanja oznake geografskog porekla proizvoda moraju obavezno da sadrže i ovaj mehanizam upravljanja rizikom u sistemu bezbednosti hrane. Stoga, imperativ u osnovnim načelima proizvodnje zlatarskog sira podrazumeva obezbeđivanje pouzdanog sistema koji omogućuje praćenje celokupnog toka proizvodnje u cilju identifikovanja i otklanjanja potencijalnog rizika po zdravlje potrošača, a time, posredno, i zaštite njihovog zdravlja. Prikazani mehanizmi su u funkciji potvrde geografskog porekla, te podizanja nivoa kvaliteta i bezbednosti ovoga tradicionalnog proizvoda, odnosno smanjenja zdravstvenog rizika kod potrošača, što na direktan način doprinosi njegovoj boljoj konkurentnosti i otvaranju puteva na domaćem i tržištima u okruženju.

Ključne reči: *sledljivost, zlatarski sir, geografsko poreklo, bezbednost.*

Uvod

„Od polja do trpeze“ u Italiji, „od farme do viljuške“ u Engleskoj ili „od proizvođača do potrošača“ u Nemačkoj, sinonimi su opšteg načela na kome se temelji novi integralni sistem bezbednosti hrane u Evropskoj uniji. Načelo upućuje na to da sigurnost hrane započinje, najpre, na nivou primarne proizvodnje, počev od proizvodnje na poljoprivrednom zemljištu i u staji, a završava se konzumiranjem hrane od strane krajnjeg potrošača. Ovim sistemom određena je odgovornost svih učesnika u čitavom integrisanom lancu proizvodnje, prerade i distribucije hrane. Istovremeno, ovim sistemom se uvažava i podstiče proizvodnja tradicionalne hrane i hrane sa geografskim poreklom.

U poslednjih nekoliko godina, sistem sledljivosti prehrambenih proizvoda je privukao pažnju mnogih istraživača, iz nekoliko razloga (*Jeansen-Vullers i dr.,* 2003): prvo, primena sledljivosti, prema Uredbi Evropske unije (Regulation EC, No 178/2002), je postala zakonska obaveza; drugo, industrija hrane nastoji da obezbedi elemente sledljivosti kao strateški alat za povećanje poverenja potrošača, uz istovremeno, poboljšanje poslovnog imidža kompanija i samog prehrambenog proizvoda.

Princip sledljivosti, kao integralni deo sistema bezbednosti hrane, ukazuje na zahtev da svi subjekti u poslovanju sa hranom, bez obzira jesu li proizvođači, prerađivači ili uvoznici, osiguraju da se sva hrana, ali i životinje koje se uzgajaju za proizvodnju hrane, kao i hrana za životinje i sastojci hrane za životinje, može pratiti kroz celi prehrambeni lanac, od polja do trpeze. Svaki subjekat u poslovanju s hranom mora biti u mogućnosti da identifikuje svoga dobavljača, kao i subjekta kojeg je on sam snabdeo, zbog čega je pristup poznat pod nazivom „korak nazad – korak napred“.

Sledljivost (eng. *traceability*) predstavlja sposobnost pronalaženja i praćenja prehrambenih proizvoda, hrane za životinje, životinja za proizvodnju hrane, kao i supstanci koje su namenjene ili se očekuje da budu unete u prehrambene proizvode ili stočnu hranu, tokom svih faza proizvodnje, obrade i snabdevanja (Regulation EC, No 178/2002). Drugim rečima, to je dokumentovani sistem aktivnosti kojim se reguliše i kontroliše kvalitet proizvoda, kao i njihove karakteristike, čime se obezbeđuje sigurnost prilikom njihovog konzumiranja, ali sprečava i

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moguća prevara potrošača u prehrambenim lancima (*Furness i Osman*, 2003; *Coff i dr.,* 2007).

Evropska regulativa (EC) 178/2002, kojom se definišu opšta pravila uspostavljanja i funkcionisanja sistema sledljivosti hrane u Evropskoj uniji, stupila je na snagu 1. januara 2005. godine, kao deo Opšteg zakona o hrani u Evropskoj uniji (General Food Law). Podsticaji za uvođenje strogih pravila sledljivosti u lancu hrane imali su za osnovu izraženu zabrinutost potrošača u pogledu njene bezbednosti (*Van Rijswijk i Frewer*, 2008), nivo higijene tokom postupaka izrade prehrambenih proizvoda (*Furness i Osman*, 2003), doslednost u poštovanju etičkih normi tokom procesa proizvodnje (*Beekman*, 2007; *Van Rijswijk i Frewer,* 2008), zastupljenost elemenata njene autentičnosti (*Furness i Osman*, 2003), kao i aspekti održivosti ekološke proizvodnje (*Van Rijswijk i Frewer*, 2008).

Obezbeđivanje adekvatnog sistema sledljivosti, koji omogućuje praćenje toka proizvodnje *zlatarskog sira* (počev od muznih krava od čijeg se mleka proizvodi, preko hrane koja služi za njihovu ishranu, pa do same kontrole mleka i konkretnog postupka izrade sira), predstavlja nužni alat u procesu upravljanja rizikom od nastanka potencijalne opasnosti po zdravlje potrošača (*Moe,* 1998; *Guidelines for Food Traceability*, 2008). Dosadašnja iskustva u našoj zemlji su pokazala da u ovoj oblasti nema adekvatno razrađenih mehanizama na osnovu kojih bi potrošači imali dovoljno poverenja u kvalitet i bezbednost hrane koju konzumiraju. Ukoliko bi se razvio adekvatan vid sledljivosti hrane, kroz sve faze njene proizvodnje, prerade i prometa, pa do trenutka njene upotrebe, kupcima (direktnim korisnicima) bi u značajnoj meri bio podignut nivo poverenja prema takvim proizvodima, a samim tim i spokojstvo tokom konzumiranja (*Opara,* 2003; *Turubatović i dr.,* 2011; *Zakon,* 2009).

Trenutno, na tržištu Republike Srbije ne postoji način da se utvrdi poreklo poljoprivrednih proizvoda koji se mogu naći na pijacama i u marketima širom zemlje. Našem potrošaču su potpuno nepoznati domaći proizvođači hrane, kao i njeno geografsko poreklo, i što je najvažnije – način na koji je ta hrana tretirana (*Guidelines for Food Traceability*, 2008). Omogućiti da informacije o sledljivosti hrane budu dostupne kroz sve faze njene proizvodnje, prerade i distribucije, stvara uslove za njeno stabilno pozicioniranje na domaćem i međunarodnom tržištu.

Zlatarski sir je jedan od najznačajnijih predstavnika domaćih autohtonih belih sireva u salamuri, koji se proizvodi na području Zlatara po tradicionalnoj tehnologiji izrade, koja se prenosi sa generacije na generaciju. Dobijanjem oznake geografskog porekla stiče uslove da bude prepoznatljiv kao zaštićeni proizvod na tržištu (izdvaja se i razlikuje od drugih, njemu sličnih, sireva), dobija dodatnu vrednost, povećava se nivo njegove konkurentnosti, postaje zaštićen od kopiranja i zloupotreba, cena mu postaje viša i stabilna uz istovremeno povećanje mogućnosti bolje prodaje i ulaska u velike trgovinske lance, itd. Međutim, postupak zaštite je neprihvatljiv bez adekvatno primenjenog i prilagođenog sistema sledljivosti u lancu njegove proizvodnje.

Cilj rada je bio da se prikaže predlog koncepta sledljivosti u proizvodnji z*latarskog sira*, koji bi za rezultat imao dokazivanje porekla, te podizanje nivoa njegovog kvaliteta i bezbednosti, a samim tim i jačanje poverenja potrošača uz smanjenje rizika od neželjenih akcidentnih situacija usled njegovog konzumiranja.

I – *Zlatarski sir* kao proizvod sa registrovanom oznakom geografskog porekla

Autohtoni sirevi su proizvodi od mleka nastali u određenom geografskom podneblju kao rezultat dugogodišnjeg razvoja tradicionalne proizvodnje. Svest o značaju ovakve proizvodnje potpomognuta je rastućom potražnjom organske i visokokvalitetne hrane sa oznakama geografskog porekla, čija se konkurentnost i cena, u poređenju sa konvencionalnim proizvodima, značajno, iz dana u dan, povećava. Danas, autohtoni sirevi, predstavljaju obeležje jednog naroda, država i regija tj. bogatstvo i deo materijalne baštine svake zemlje (*Ostojić i Topisirović,* 2008; *Vesković-Moračanin i dr.,* 2012d).

Zlatarski sir je jedan od najznačajnijih predstavnika domaćih autohtonih belih sireva u salamuri. Proizvodi se od nekuvanog punomasnog kravljeg mleka (shema 1) na teritoriji opštine Nova Varoš i manjim delom teritorije opština Prijepolje i Sjenica (*Maćej i dr.,* 2006; *Vesković-Moračanin i dr.,* 2012a; *Vesković-Moračanin i dr.,* 2012b; *Vesković-Moračanin i dr.,* 2012c). Autentičnost sireva zlatarskog podneblja, u odnosu na ostale sireve istog tipa, ali drugih regija, bazirana je na osobenostima i raznolikosti prisutnih autohtonih mikroorganizama, prvenstveno bakterija mlečne kiseline (BMK), koje su nosioci mlečne fermentacije i procesa zrenja sireva (*Vesković-Moračanin i dr.,* 2013a; *Vesković–Moračanin i dr.,* 2013b). Na specifičnost ove vrste sira najviše utiče klima, geografski položaj, zemljište, voda, botanički sastav prirodnih livada i pašnjaka, rasa i način uzgoja mlečne stoke, kao i tradicionalne navike i običaji lokalnog stanovništva *(Vesković-Moračanin i dr.,* 2012a; *Vučić i dr.,* 2008; *Ostojić i Topisirović,* 2006).

Sirovo punomasno kravlje mleko/
Raw whole cow's milk

Blago zagrevanje (oko 35^{0}C)/
Slight warming (about 35°C)

Podsiravanje mleka sirilom („majom")/
Coagulation milk by rennet

Obrada gruša – rezanje/
Cutting the coagululum

Odlivanje surutke/
Drainage of whey

Samopresovanje / Presovanje grude/
Autopressing / Pressing

Sečenje grude u kriške/
Cutting curd in chease slices

Slaganje kriški u drvene kačice/
Putting chease slices in wooden kits package

Soljenje kriški kuhinjskom solju/
Salting slices with dry common salt

Zrenje sira/
Ripening cheese

Shema 1. Autohtona tehnologija proizvodnje zlatarskog sira

Diagram 1. Indigenous production technology of Zlatar cheese

Zlatarski sir pripada grupi „sireva u salamuri", prema sadržaju mlečne masti grupi „punomasnih" sireva, a prema sadržaju vode u bezmasnoj materiji grupi „mekih" sireva (*Ostojić i Topisirović,* 2006; *Pravilnik,* 2010). U pogledu senzorskih svojstava *zlatarski sir* je standardizovanog kvaliteta, sa jasno definisanim karakteristikama (*Mijačević i Bulajić,* 2007; *Elaborat,* 2013): bele do beložute boje, blagog mlečno-kiselog mirisa, zbijene strukture preseka sa prisutnim malim šupljinama (ili bez šupljina), izraženo prijatnog ukusa fermentisanog sira, ne previše slan i kiseo, bez prisustva gorčine. Pri žvakanju sira oseća se prijatna aroma koja se dugo zadržava i kremasta struktura koja se topi. Kriške sira su četvrtastog oblika (10–12 ×10–12 cm) ili kružnog isečka sličnih dimenzija, dok je debljina kriške od 1–1,5 cm.

II – Sledljivost u proizvodnji *zlatarskog sira*

Primena postupka sledljivosti tokom proizvodnje hrane bazirana je, kako na zakonskoj regulativi iz oblasti bezbednosti, tako i nametnutom potrebom u poslovanju i u komunikaciji sa trgovinskim lancima snabdevanja, konkurentskom prednošću, smanjenim troškovima potencijalnog opoziva proizvoda, religijskim pitanjima i sl. (*Lazarević i dr.,* 2012). Stoga primenu postupka sledljivosti u proizvodnji i prometu *zlatarskog sira* na nacionalnom nivou, treba posmatrati i kao sticanje ulaznice za plasman na svetsko tržište hrane.

Zlatarski sir, kao proizvod sa registrovanom oznakom geografskog porekla, po pravilu, ima veću vrednost za potencijalnog potrošača nego drugi, njemu slični, sirevi. Ta dodatna vrednost i prepoznata atraktivnost proizlazi iz vrednosti njegovih sastojaka, načina proizvodnje i vrednosti koju on prenosi. Međutim, oznaka geografskog porekla *zlatarskog sira* ne znači samo zaštitu njegovog porekla, već i obavezu da ovaj sir mora imati i određena, tačno definisana svojstva, koja će proizvod uvek povezivati sa visokim parametrima kvaliteta.

Za proizvođače *zlatarskog sira* sistem sledljivosti podrazumeva potvrdu njegove autentičnosti kroz dokumentovani sistem praćenja i dokaza porekla, počev od primarne proizvodnje, ulaznih sirovina, pa do gotovog proizvoda. Adekvatno uspostavljenim sistemom označavanja obezbeđuje se uspostavljanje veze između konkretno proizvedenog proizvoda i njegovog porekla. Drugim rečima, sistem sledljivosti mora da funkcioniše na način da svakoga trenutka da odgovor na sledeća pitanja: koja su muzna grla u laktaciji dala neophodnu sirovinu (mleko) za proizvodnju *zlatarskog sira*; koja i kog porekla je hrana upotrebljena za ishranu muznih krava; kao i koliko je mleka, najpre, proizvedeno a potom prerađeno u *zlatarski sir*.

Označavanje muznih grla i hrane koja se koristi u njihovoj ishrani

Muzna grla koja se koriste za dobijanje mleka namenjenog za proizvodnju *zlatarskog sira* moraju da potiču sa definisanog geografskog područja. Kretanja i migracije ovih životinja, iz različitih regija, moraju biti organičena i svedena na najmanju meru. Takođe, mlečna grla moraju da budu zdrava i obeležena u skladu sa važećim nacionalnim propisima.

Evidencija grla koja daju mleko za proizvodnju zlatarskog sira/ *Records on animals that produce milk for Zlatar cheese production*					
Poljoprivredno gazdinstvo/*Agricultural farm*: **Ime i prezime**/*Name and surname*:					
Mesec proizvodnje (sedmica)/*Month of production (week)*					
Identifikacioni broj grla/ *Identification number of animals*		**Zdravstveni status**/ *Health status*	**Laktacija**/ *Lactation*		**Potpis**/ *Signature*
			Da/Yes	Ne/No	
1.					
2.					

Obrazac 1. Evidencija grla koja daju mleko za proizvodnju zlatarskog sira (Elaborat, 2013)
Template 1. Records on animals that produce milk for production Zlatar cheese (Study, 2013)

Identifikacioni broj grla, koji se nalazi na ušnim markicama, predstavlja polaznu identifikaciju krava čije se mleko koristi u proizvodnji *zlatarskog sira*. Paralelno sa ovom informacijom vodi se evidencija i o zdravstvenom statusu životinja, kao i o količini pomuzenog mleka. Primer evidencije mlečnih grla u registrovanim domaćinstvima, koja se bave tradicionalnom proizvodnjom ovoga sira, dat je u naročito dizajniranom formularu/obrascu, koji je deo Elaborata o zaštitu oznake geografskog porekla ovog proizvoda (*Elaborat,* 2013) (Obrazac 1 – Evidencija grla koja daju mleko za proizvodnju *zlatarskog sira*).

Sistem sledljivosti u proizvodnji *zlatarskog sira* zasnovan je na poznavanju i dokumentovanju porekla i vrste hrane koja se koristi za ishranu muznih krava koje daju mleko namenjeno proizvodnji *zlatarskog sira*. Proizvođači sira treba u svojoj dokumentaciji da, kao obavezni element sistema sledljivosti, vode evidenciju o vrsti, poreklu i količini upotrebljene hrane. Ovaj vid zapisa je nužan s obzirom da se 5% koncentrovane hrane i žitarica nabavlja iz drugih područja, najčešće iz Vojvodine.

Evidencija proizvodnje mleka i zlatarskog sira

Primena sledljivosti u proizvodnji *zlatarskog sira*, u registrovanim domaćinstvima, podrazumeva i praćenje proizvodnje tj. dnevne muže mleka. Ukupnu količinu pomuzenog mleka, tokom jedne muže, neophodno je evidentirati u zapisu/obrascu, koji je tako dizajniran da se u njemu može prikazati, kako dnevna količina pomuzenog mleka, tako i količina mleka pomuzena u različitim periodima dana.

Isti zahtev se postavlja pred proizvođača i kada je u pitanju sam postupak izrade *zlatarskog sira*. Forma i priroda zapisa nije propisana, ali treba da je tako dizajnirana da se podaci mogu lako unositi, da su neophodne informacije pregledne i jasne, kao i da se u svakom trenutku može dobiti odgovor koliko je i od kojeg mleka proizvedena određena dnevna partija sira. S obzirom da je tehnologija izrade, odnosno, skladištenja *zlatarskog sira,* takva da se sir proizveden od jedne proizvodne partije (muže) mleka slaže u odgovarajuće kantice ili drvene kačice, princip sledljivosti u ovoj fazi proizvodnje nalaže da se ovakve ambalažne jedinice obeležavaju identifikacionim brojem na načina da direktno ukazuju na mleko od koje je proizvodne šarže pripremljen

Nadalje, jedna od osobenosti u tehnologiji izrade *zlatarskog sira* je i činjenica da nakon nekoliko dana (dva dana, najčešće) dolazi do njegovog „sleganja“, tako da je uobičajeno da se kantica dopunjava novom količinom sira. Ovaj korak u proizvodnji sira mora se, takođe, evidentirati u dokumentaciji ali, tako da se, svakog trenutka, može utvrditi u kojoj se kantici nalazi sir proizveden određenog datuma, kao i kog je datuma ta kantica bila dopunjena. Takođe, u praksi se često koristi ambalaža različite zapremine pri čemu dolazi do pojave da se sir proizveden različitih datuma slaže u istu ambalažnu jedinicu. Adekvatnim sistemom obeležavanja i dokumentovanja ne postoji opasnost u gubitku sledljivosti.

Deklaracija *zlatarskog sira*, kao lična karta proizvoda, sadrži informaciju koja ovaj sir odvaja od drugih sličnih, a to je da sir potiče sa definisanog

područja Zlatara, pružajući poruku potrošaču o njegovoj autentičnosti i autohtonosti. Istovremeno na deklaraciji proizvoda nalazi se identifikacioni broj sira koji ga prati do trenutka potrošnje, dajući mogućnost, u zavisnosti od potrebe, da se ustanovi dan, poreklo, kao i proizvedena i prodata količina sira.

Navedeni primeri sledljivosti više su usmereni na aspekte dokaza geografske pripadnosti *zlatarskog sira,* pri čemu prikazana dokumentacija ne daje odgovore na pitanja o elementima samog tehnološkog postupka izrade. Stoga, sledeća faza unapređenja principa sledljivosti u proizvodnji *zlatarskog sira* morala bi da uključi vođenje iscrpne evidencije, koja bi obezbeđivala, pored kontrole i evidencije porekla i količine sirovine, i mogućnost praćenja svih faza u proizvodnji sira, počev od postupka podsiravanja (vrsta i količina upotrebljene maje, temperature sirovog mleka i sl.) pa do samog procesa zrenja i skladištenja sira (temperatura, vlažnost vazduha, manipulacija sa grušom i sl.).

Zaključak

U postupku zaštite geografskog porekla *zlatarskog sira*, pored autentičnosti i osobenosti ovoga proizvoda, primena koncepta sledljivosti je imperativ u njegoj implementaciji. Preporučeni mehanizmi u načinu obezbeđivanja sistema sledljivosti, kao obavezni i nezaobilazni koraci u kontroli bezbednosti ovoga proizvoda, imaju za cilj, istovremeno, pružanje potpune informacije o njemu uz obaveznu garanciju kvaliteta i autentičnosti, kao i podizanje stepena poverenja kod potrošača. U suštini, primena sistema sledljivosti u proizvodnji i distribuciji *zlatarskog sira* ima za cilj da umanji proizvodnju i distribuciju nebezbednih ili proizvoda lošeg kvaliteta, što zauzvrat smanjuje mogućnost lošeg publiciteta, lične odgovornosti i dodatnih troškova povlačenja proizvoda iz prometa. Istovremeno, to je sistem sigurnosti koji obezbeđuje podršku u konkurentnosti i boljem pozicioniranju ovoga tradicionalnog proizvoda na domaćem i međunarodnom tržištu hrane.

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Implementation of traceability principle in the production of *Zlatar cheese*

Vesković-Moračanin Slavica, Stefanović Srđan, Šaponjić Milinko, Đukić Dragutin

S u m m a r y: The traceability elements that must be applied during the traditional process of production and distribution of Zlatar cheese are presented in this paper. The activities that are implemented in order to obtain label of a product with geographical origin necessarily include the mechanism of risk management in the food safety system. Therefore, it is imperative that the basic principles of production of Zlatar cheese involve the provision of a reliable system that allows tracing of the entire production flow in order to identify and eliminate the potential risks to consumer health, and thus, indirectly, to protect it. The mechanism shown are in function of the improvement of quality and safety of this traditional product, reduction of the health risk to consumers, which in a direct way contributes to its better competitiveness and opening roads in the local and surrounding markets.

Key words: *traceability, Zlatar cheese, geographic origin, safety.*

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Redukcija soli u ishrani ljudi – globalna strategija u 21. veku

Šarčević Danijela,[1] Lilić Slobodan[1], Vranić Danijela[1]

S a d r ž a j: Povećana upotreba prerađene hrane, rapidna urbanizacija i promene stila života promenili su navike u ishrani ljudi. U svakodnevnom životu postaje dominantna upotreba visoko energetske hrane, bogate zasićenim mastima, trans mastima, šećerom i natrijum-hloridom (so). Prekomeran unos soli u ishrani ljudi ima za posledicu drastičan porast kardiovaskularnih oboljenja, kao što su hipertenzija, srčani udar i dr.

Prema podacima Svetske zdravstvene organizacije (WHO), vodeći uzroci smrti u 21. veku su neinfektivna oboljenja, u koje spadaju i kardiovaskularna oboljenja. Kako je prekomeran unos kuhinjske soli, odnosno natrijuma, jedan od rizika za pojavu esencijalne hipertenzije i kardiovaskularnih oboljenja, WHO je donela preporuku da se globalni unos soli redukuje za 30% do 2025. godine.

Na osnovu analize različitih studija, u radu su izneti podaci koji pokazuju da je u 20. veku, kod populacije koja je unosila manje od 3 g soli dnevno, zabeleženo odsustvo hipertenzije. Danas prema podacima iz literature potrebe odraslog čoveka u soli, u cilju održavanja metaboličkih procesa, iznose 1,5 g, a prema podacima Američke asocijacije za srce zabeleženi dnevni unos je 8−15 g.

Cilj rada je bio analiza značaja redukcije soli u ishrani ljudi, kako bi se prevenirale bolesti srca i očuvalo javno zdravlje populacije. Kroz jedan širi teorijski okvir iznete su smernice i preporuke Svetske zdravstvene organizacije i sličnih međunarodnih kompetentnih institucija.

U radu su izneti podaci koji ukazuju na to da je moguće redukovati so u proizvodima od mesa, a da se pri tom ne utiče na kvalitet i održivost proizvoda.

Ključne reči: *so, kardiovaskularna oboljenja, očuvanje javnog zdravlja.*

Uvod

Povećana upotreba prerađene hrane, rapidna urbanizacija i promena stila života, promenili su navike u ishrani ljudi. Zbog toga je sve više u porastu konzumacija brze hrane, koja je lako dostupna i sve manje skupa. Međutim, ona je bogata zasićenim mastima, trans mastima, šećerom i natrijum-hloridom, odnosno kuhinjskom solju (u daljem tekstu so). Istovremeno, sa promenama navika u ishrani, ljudi sve manje konzumiraju voće i hranu bogatu vlaknima, koji predstavljaju važnu komponentu zdrave ishrane. Nasuprot natrijumu koji izaziva povećanje krvnog pritiska, voće i povrće bogati su kalijumom koji doprinosi snižavanju krvnog pritiska.

Prema podacima Svetske zdravstvene organizacije (WHO) vodeći uzroci prerane smrti u 21. veku su neinfektivne bolesti, uključujući bolesti srca i srčani udar. Na Svetskom danu srca, održanom u Ženevi 25. septembra 2014. godine, saopštena je podrška WHO vladama različitih zemalja da implementiraju „Globalni akcioni plan za smanjivanje neinfektivnih oboljenja“ koji sadrži devet globalnih ciljeva, uključujući i globalno smanjenje unosa soli za oko 30% do 2025. godine. WHO podržava vlade različitih zemalja da implementiraju „Globalni akcioni plan za smanjivanje neinfektivnih oboljenja“ koji sadrži devet globalnih ciljeva, uključujući i globalno smanjenje unosa soli za oko 30% do 2025. godine. Tom prilikom zemlje učesnice su pozvane da preduzmu sve aktivnosti, kako bi se implementirale preporuke o redukciji natrijuma u cilju smanjenja broja ljudi obolelih od kardiovaskularnih oboljenja. „Ukoliko se cilj smanjivanja unosa soli za 30% do 2025. godine postigne, mogli bi biti spaseni milioni života, čiji bi prekid uslovilo, bilo neko oboljenje srca, srčani ili moždani udar i ostala slična stanja“, izjavio je dr Oleg Chestnov, pomoćnik generalnog direktora za neinfektivna oboljenja i mentalno zdravlje WHO (*WHO's Guidelines Review Committee*, 2012).

Napomena: Prezentovani rezultati proistekli su iz rada na realizaciji projekta TR 31083 i III 46009 koje finansira Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

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Značaj redukcije soli u ishrani ljudi, kao i posledice koje prekomeran unos natrijuma može da ima po zdravlje čoveka, je u radu analiziran kroz jedan širi teorijski okvir koji uključuje smernice i preporuke Svetske zdravstvene organizacije i sličnih kompetentnih međunarodnih institucija.

Značaj soli tokom razvoja čovečanstva

Istorija korišćenja soli stara je koliko i istorija ljudskog roda. So je omogućavala kolonijalnu moć, izazivala ratove i bila jedna od prvih kategorija trgovačke razmene. Otkriće soli omogućilo je da hrana bude dostupna nezavisno od godišnjeg doba i kao i njen transport na veće udaljenosti. Najstariji rudnici na svetu nalazili su se u brdima u kojima je iskopavana, pakovana u kožne vreće koje su se tovarile na životinje, a razmenjivala se za ćilibar, zlato i bakar, ili su se njome isplaćivale plate i porezi.

Rimska imperija kontrolisala je cenu soli i korigovala je, od najveće, kada su zaradu koristili za vođenje ratova, do najniže, kada je siromašnom stanovništvu data mogućnost da je kupuje. Deo plate rimskih vojnika je, prema uobičajenom verovanju, bila so, a otuda i naziv plate u nekim jezicima, npr. u engleskom „salary“ što odgovara latinskoj reči „salarium“. Takođe, nekada su vojnici u Američkom građanskom ratu bili plaćani solju. Bila je jednako važna za Jevreje, Grke, Kineze i ostale narode antičkog doba. Sa razvojem Rima, gradili su se putevi koji su omogućavali lakši transport soli od Jadranskog mora, koje je bilo poznato po svom visokom salinitetu.

U to doba nastajali su poznati putevi soli kao što su „Via salaria“ u Italiji, „Salzstraβe“ od Lüneburga do Lübecka i „zlatna staza“ od Passaua do Böhmena.

So je transportovana i daleko do germanskih plemena, a u Africi 40 hiljada kamila je na putu dugom 400 milja prenosilo so koja je imala toliku vrednost da se nekada trampila i za robove.

Najstariji podaci o primeni soli u medicini datiraju 3000 godina pre nove ere i potiču od egipatskog graditelja i lekara Imothepa, koji navodi da so suši inficiranu ranu i da može sprečiti upalni proces, a primenu u medicini nastavlja i Hipokrat u staroj Grčkoj. Paracelzus uvodi so kao treći element pored sumpora i žive i prekida dualističku koncepciju alhemije i smatra da se samo posoljena hrana može dobro probaviti. On je jedan od prvih koji koristi slane kupke u lečenju kožnih oboljenja.

Potrebe čoveka u soli

Nekoliko miliona godina, preci ljudi konzumirali su manje od 1 g soli dnevno (*Blackburn i Prineas*, 1983; *Eaton i Konner,* 1985). To znači da su današnji ljudi programirani na pomenuti unos soli. Namensko dodavanje soli hrani počelo je pre oko 5000−10000 godina u početku razvoja poljoprivrede i dostiglo je prosečnih 10 g dnevno, a u evolutivnom smislu to je relativno skoro.

Oko 40 nekultivisanih plemena, koja žive u Južnoj Americi, Africi, Pacifiku i Arktiku, koristi manje od 3 g soli dnevno (*Denton,* 1982) i njihov pritisak se ne povećava sa starošću. Poznato je južnoameričko indijansko pleme Yanomamo na granici Venecuele i Brazila, koji imaju dnevni unos soli manji od 0,5 g i prosečan krvni pritisak kod muškaraca iznosi 105/70 mmHg i 95/60 mmHg kod žena.

Natrijum se u organizmu nalazi uglavnom u ekstracelularnoj tečnosti i utiče na održavanje balansa vode, funkciju nerava, kiselo-baznu ravnotežu i kontrakcije mišića. Iako je skoro neočekivano, smanjeni unos natrijuma može da dovede do grčenja mišića, mučnine, povraćanja, anorekcije i kome.

Unos soli uslovljen je, ne samo fiziološkim potrebama (sportisti), nego i navikama, koje se stiču još u ranom detinjstvu, kao i tradicijom u ishrani (podneblje, odnosno klimatski uslovi, priprema hrane, resursi stoke i sl.). Od ukupne dnevne količine kuhinjske soli, koja se u organizam unese putem uobičajenih količina hrane (jela pripremljena u domaćinstvu, hleb, pekarski proizvodi, sir), oko 20% potiče iz proizvoda od mesa (*Wirth*, 1991).

Prema nekim podacima, dnevna potreba u natrijumu odraslih osoba, za održavanje metaboličkih potreba, manja je od 1,5 g. Kod sportista su potrebe veće, pa čak prevazilaze 10 g dnevno, ukoliko se znojem gube velike količine natrijuma. Međutim, dnevni unos natrijuma često je veći od 5 g (www.healthline.com/hlbook/nut-sodium), odnosno 8−15 g natrijum hlorida (*Žarinov i Veselova*, 2003). Američka asocijacija za srce (American Heart Association) predlaže da hipertenzivne osobe ne bi trebalo da unose više od 1,5 g, a osobe sa kongestivnim srčanim tegobama ne više od 1 g natrijuma dnevno (www.intelihealth.com).

Osim pojave hipertenzije, prekomeran unos soli može da dovede do:

- direktnog rizika od srčanog udara (*Perry i Beevers*, 1992);
- hipertrofije leve komore (*Schmieder i Messerli*, 2000);

- retencije natrijuma u ekstracelularnoj tečnosti, odnosno do retencije vode i kliničkih i idiopatskih edema, naročito kod žena (*MacGregor i de Wardener*, 1997);
- smanjenja elastičnosti zida krvnih sudova, naročito arterija, nezavisno od krvnog pritiska (*Avolio i dr*., 1986);
- proteinurije, u prvom redu albumina, a time i do povećanog rizika za oboljenja srca i bubrega (*du Cailar i dr*., 2002);
- veće mogućnosti infekcije sa *Helicobacter pylori* i rizika od nastanka raka želuca (*Tsugane i dr*., 2004);
- povećanja urinarne ekskrecije kalcijuma i rizika od stvaranja kamena u bubregu (*Capuccio i dr.*, 2000), zatim rizika od smanjenja gustine kostiju, a shodno tome i od osteoporoze i kompresivnih fraktura, naročito kod žena u menopauzi (*Devine i dr*., 1995);
- eksacerbacije (pojačanje, produženje) astmatičnih napada (*Mickleborough i dr*., 2005);i
- povećanja HOMA (homeostasis model assessment) insulinske rezistencije kod pacijenata sa esencijalnom hipertenzijom, od kojih je većina sa umanjenom tolerancijom na glukozu (*Kuroda i dr*., 1999).

Ključne činjenice o unosu natrijuma hranom (*Salt reduction, Fact sheet N°393*):

- visok dnevni unos natrijuma (više od 2 g dnevno – ekvivalent 5 g soli) i nedovoljan unos kalijuma (manji od 3,5 g dnevno) doprinose pojavi povećanog krvnog pritiska i porastu rizika od koronarnih oboljenja i srčanog i moždanog udara;
- osnovni izvor natrijuma je so iz hrane, mada on može poticati i iz mononatrijum glutaminata, koji se koristi u mnogim delovima sveta kao dodatak;
- mnogi ljudi konzumiraju previše soli, obično 9−12 g dnevno, što je dvostruko više od maksimalno preporučenog unosa;
- unos soli manji od 5 g dnevno kod odraslih pomaže da se reguliše povećani krvi pritisak i smanjuje rizik od kardiovaskularnih oboljenja.

Redukcijom soli može da se unapredi zdravlje cele populacije. Procenjuje se da, ako se globalni unos soli smanji na preporučeni nivo, može biti spaseno oko 2,5 miliona života, a troškovi lečenja pali bi ispod prosečnog godišnjeg prihoda, odnosno bruto domaćeg proizvoda.

Smernice WHO (*WHO Guideline: Sodium intake for adults and children*, 2014)

WHO je predstavila, koristeći dokaze i informacije, određene smernice koje se odnose na natrijum i kalijum, a koristeći procedure navedene u Priručniku WHO za razvoj smernica (*WHO's Guidelines Review Committee*, 2012). Koraci u ovom procesu uključuju:

- identifikaciju prioritetnih pitanja i ishoda,
- nalaženje dokaza,
- procenu i sintezu dokaza,
- formulisanje preporuka,
- identifikaciju istraživačkih nedostataka, i
- planiranje diseminacije, implementaciju, evaluaciju uticaja i ažuriranje smernica.

U saopštenju za javnost od 31. januara 2013. godine (Ženeva), izneto je da je WHO usvojila nove smernice za unos natrijuma i kalijuma hranom, gde se navodi da bi odrasli trebalo da konzumiraju manje od 2 g natrijuma ili 5 g soli, i najmanje 3,5 g kalijuma dnevno. Osoba sa povećanim unosom natrijuma i smanjenim unosom kalijuma, mogla bi biti u riziku od porasta krvnog pritiska, sa povećanjem rizika od bolesti srca i srčanog i moždanog udara. „Povišeni krvni pritisak je veliki rizik za bolesti srca i srčani udar – brojni su slučajevi smrti i invalidnosti globalno“, rekao je dr Francesko Branca, direktor Svetske zdravstvene organizacije Departmana ishrane za zdravlje i razvoj. Dalje je izjavio, da ove smernice, takođe čine i preporuku za decu preko 2 godine, zato što deca sa povišenim krvnim pritiskom često postaju odrasli sa povišenim krvnim pritiskom. Smernice su značajan alat za eksperte koji se bave javnim zdravljem i one koji kreiraju politiku svake zemlje kada su u pitanju neinfektivna oboljenja. Mere javnog zdravlja za redukciju unosa natrijuma i povećanje unosa kalijuma, trebalo bi da uključe deklarisanje hrane, edukaciju potrošača, donošenje nacionalnih smernica u ishrani i pregovaranje sa proizvođačima hrane da, u svojim proizvodima, smanje sadržaj natrijuma.

Preporuke Svetske zdravstvene organizacije

Svetska zdravstvena organizacija preporučuje sledeće:

- redukciju unosa natrijuma u cilju snižavanja krvnog pritiska i smanjivanja rizika od kardiovaskularnih oboljenja, srčanog i moždanog udara i koronarnih bolesti srca kod odraslih (jaka preporuka);

- redukciju dnevnog unosa natrijuma na manje od 2 g što odgovara 5 g soli, kod odraslih (jaka preporuka);
- redukciju unosa natrijuma sa ciljem kontrole krvnog pritiska kod dece (jaka preporuka);
- preporučeni maksimalan dnevni unos natrijuma od 2 g kod odraslih, trebalo bi da bude kod dece podešen na osnovu nivoa energetskih potreba, a u odnosu na odrasle.

Preporuke se primenuju na sve individue, sa ili bez hipertenzije (uključujući trudnice i žene koje doje), osim u slučaju ljudi koji boluju od neke bolesti ili su na terapiji lekovima koji dovode do gubitka natrijuma i hiponatrijemije ili problema sa gubitkom vode u telu ili potreba kod ishrane sa nadzorom (npr. kod pacijenata sa srčanom manom ili onih sa dijabetesom tip I). U ovoj subpopulaciji, može postojati naročiti odnos između unosa natrijuma i zdravstvenog ishoda (*Paterna i dr.,* 2008; *Thomas i dr.,* 2011). Zbog toga, ovakvi slučajevi nisu bili razmatrani u pregledu dokaza i stvaranja smernica.

Ove preporuke komplementarne su sa smernicama WHO koje se tiču unosa kalijuma i trebalo bi da budu korišćenje u saglasnosti sa drugim nutritivnim smernicama i preporukama u cilju razvoja javnog zdravlja, nutritivnih programa i politike. Optimalni odnos natrijuma i kalijuma u ishrani je izvan obima ove smernice, ali ipak se preporučuje da ovaj odnos bude jedan prema jedan, u skladu sa zdravstvenim benefitima (WHO. *Diet, nutrition and the prevention of chronic disease. Report of a Joint WHO/ FAO Expert Consultation,* 2003). Sistematsko praćenje unosa soli je neophodno i zbog toga, što se redukcijom unosa soli smanjuje i unos joda, tako da mora da se osigura da individualni unos preporučenih količina natrijuma bude praćen dovoljnim unosom joda u organizam.

Uspešna implementacija ovih preporuka imala bi značajan uticaj na javno zdravlje smanjujući morbiditet i mortalitet, unapređenje kvaliteta života miliona ljudi i znatno smanjenje troškova lečenja i preventivnih mera (*WHO-Global Health risks: Mortality and burden of disease attributable to selected major risks*, 2009; *Murray i dr.,* 2003*; Mackay i Mensah*, 2004).

Globalna strategija ishrane, fizičke aktivnosti i zdravlja

Svetska zdravstvena skupština (World Health Assembly, WHA), usvojila je 2004. godine dokument pod nazivom „Globalna strategija ishrane, fizičke akitvnosti i zdravlja“. Strategija poziva vlade, Svetsku zdravstvenu organizaciju, međunarodne partnere, privatni sektor i društvo da preduzmu akcije na globalnom, regionalnom i lokalnom nivou i da njima podrže zdravu ishranu i fizičku aktivnost.

U 2010. godini, WHA odobrila je set preporuka za marketing hrane i bezalkoholnih pića za decu. Zemlje koriste ove preporuke u jačanju postojeće i dizajniranju nove politike u cilju smanjivanja uticaja marketinga „nezdrave“ hrane na decu.

U 2011. godini, svetski lideri posvetili su se smanjenju izloženosti ljudi nepravilnoj ishrani. Odluka je doneta političkom deklaracijom sa sastanka na visokom nivou Generalne skupštine Ujedinjenih nacija koja se odnosi na prevenciju i kontrolu neinfektivnih oboljenja.

U 2012. godini, WHA usvojila je šest globalnih nutritivnih ciljeva, uključujući smanjenje zaostatka u rastu, kaheksije i gojaznosti kod dece, podsticanje dojenja, odnosno ishrane majčinim mlekom, i redukcije anemije i nedovoljne telesne mase prilikom rođenja.

U 2013. godini, WHA je dogovorila devet globalnih dobrovoljnih ciljeva za prevenciju i kontrolu neinfektivnih oboljenja, koji uključuju zaustavljanje porasta dijabetesa i gojaznosti i 30% relativne redukcije soli do 2025. godine. Da bi se ovi ciljevi ostvarili date su smernice za države članice, WHO i agencije Ujedinjenih Nacija sadržane u dokumentu „Globalni akcioni plan za prevenciju i kontrolu neinfektivnih oboljenja 2013−2020“.

Mnoge zemlje beleže rapidan rast gojaznosti dece, tako da je WHO u maju 2014. godine osnovala komisiju za gojaznost dece. Komisija će sastaviti izveštaj za 2015. godinu, specificirajući koji pristupi i akcije će biti efektivnije u različitim kontekstima širom sveta.

Najnovije strategije Svetske zdravstvene organizacije za redukciju unosa soli

(*WHO−Fact sheed N°393, Salt reduction*, 2014)

Globalne strategije Svetske zdravstvene organizacije bazirane na činjenicama redukcije unosa soli uključuju:

- regulaciju i politiku obezbeđivanja toga da će proizvođači hrane i svi subjekti u poslovanju hranom koji je prodaju, smanjiti nivo soli u hrani i napicima,
- ugovori sa industrijom hrane koji će osigurati da proizvođači i prodavci hrane proizvode „zdraviju“ hranu (sa manje soli);
- negovanje okoline koja promoviše zdravu ishranu (smanjivanje unosa soli) na jav-

nim mestima, kao što su škole, bolnice, radna mesta i javne institucije;
- obezbeđivanje jasno deklarisanje hrane, kako bi svaki potrošač mogao potpuno da razume koji je sadržaj soli u proizvodu;
- implementacija preporuka Svetske zdravstvene organizacije u ishrani dece, u marketingu hrane i bezalkoholnih napitaka.
- Strategije za potrošače radi smanjenja unosa soli hranom uključuju:
- čitanje deklaracije proizvoda prilikom kupovine hrane da bi se proverio sadržaj soli u proizvodu;
- zahtevanje proizvoda sa manjim sadržajem soli prilikom kupovine pripremljene hrane;
- uklanjanje slanika i flaširanih sosova sa stola na kom se služi hrana;
- ograničavanje upotrebe soli koja se dodaje prilikom kuvanja na ukupnu maksimalnu količinu od pet kafenih kašičica soli tokom celog dana;
- limitiranje frekventnog unosa proizvoda sa velikim sadržajem soli;
- formiranje kod dece receptora za ukus ishranom sa većinom neprerađene hrane bez dodavanja soli;
- u zemljama sa deficijencijom joda potrebno je da sva so koja se koristi bude jodirana. Čak i konzumiranje malih količina jodirane soli i dalje će obezbediti dodatnu korist za zdravlje, osiguravajući pravilan kognitivni razvoj kod dece.

Unos natrijuma preko proizvoda od mesa

Sadržaj soli u proizvodima od mesa zavisi, u prvom redu, od tehnoloških opravdanih količina i, naravno, od uticaja na ukus slanosti. Postoje mnoge studije o sadržaju soli u različitim proizvodima od mesa (*Vranic i dr*., 2009). Najmanji sadržaj soli imaju barene kobasice i konzerve sa mesom u komadima. U barenim kobasica, sadržaj soli se nalazi u opsegu od 1,28−2,03 g/100 g, prosečno 1,66 g/100 g, dok se u konzervama nalazi u opsegu od 1,35−1,84 g/100 g, prosečno 1,67 g/100 g. U dimljenim proizvodima od mesa, sadržaj soli nešto je veći i iznosi od 1,66−3,11 g/100 g, odnosno prosečno 2,19 g/100 g. U suvim fermentisanim kobasicama, tehnološki opravdana količina soli je znatno veća i dodaje se 2,5−3,0%, jer se ovi proizvodi ne podvrgavaju toplotnoj obradi, pa so služi za održavanje mikrobiološke stabilnosti proizvoda. Sadržaj soli u ovoj vrsti kobasica iznosi 2,08−3,98 g/100 g, odnosno prosečno 2,61 g/100 g. Najveći sadržaj soli imaju suvomesnati proizvodi. Usled dugotrajnog procesa proizvodnje, odnosno salamurenja, ovi proizvodi se usoljavaju ili salamure sa 5−10% soli ili soli za salamurenje, što ima za posledicu smanjenje aktivnosti vode i sprečavanje razmnožavanja nepoželjnih mikroorganizama. Sadržaj soli u suvom mesu nalazi se u opsegu od 3,78−7,35 g/100 g, prosečno 5,09 g/100 g.

Sadržaj soli u proizvodima od mesa može da se smanji na više načina: Smanjivanjem dodatog natrijum-hlorida, supstitucijom dela NaCl drugim solima, upotrebom pojačivača ukusa i maskirajućih agenasa, dodavanjem začinskog bilja i ekstrakata začina, optimizacijom fizičke forme soli i alternativnim procesnim tehnikama, o čemu govore mnogi istraživači (*Ruusunen i Puolanne,* 2005; *Desmond,* 2006; *Sofos*, 1983; *Lilić*, 2000; *Terell*, 1983; *Guàrdia i dr.,* 2006; *Lilić i dr.*, 2008; *Lilić i dr*., 2014; *Lilić i dr*., 2014a; *Lilić i Matekalo-Sverak*, 2007; *Angus i dr*., 2005; *Claus i Sørheim*, 2006).

Na osnovu navedenog jasno je da postoje višestruke mogućnosti redukcije soli u proizvodima od mesa, a pošto so više ne predstavlja osnovni činilac održivosti proizvoda od mesa, smatra se da industrija mesa mora da počne sa sopstvenim programima redukcije soli i svojim društveno odgovornim ponašanjem doprinese javnom zdravlju (*Šarčević i dr.,* 2009). Istraživanja koja su na tom polju rađena u Republici Srbiji su oskudna, ali ono što je prisutno ukazuje na to da su navike u konzumiranju mesa i proizvoda od mesa kod školske dece uzrasta 7−18 godina, bazirane na brzoj hrani, sa visoko energetskim sadržajem masti i soli (*Šarčević i dr.,* 2013).

Zaključak

Na osnovu podataka iznetih u radu, može da se zaključi, da je prekomerno unošenje soli, veoma opasno po zdravlje ljudi. U tom smislu, ohrabrujuća je inicijativa Svetske zdravstvene organizacije i sličnih međunarodnih institucija, koje su na osnovu analiza ponašanja potrošača i populacije raznih uzrasta, objavili sugestije i preporuke za redukciju unosa soli putem ishrane. Radi zaštite javnog zdravlja, države pokazuju interes za primenom ovih preporuka i strategija, ali je neophodno da se u ovaj proces uključe i proizvođači iz industrije hrane. Sinergetskim društveno odgovornim ponašanjem svih subjekata u društvu, od porodice, preko države, proizvođača hrane, do potrošača i medija, moguće je smanjiti rizik od pojave kardiovaskularnih oboljenja, kako kod odraslih, tako i kod dece. Podaci iz literature, koji se odnose na oblast industrije mesa,

ukazuju na to, da je moguće redukovati so u proizvodima od mesa i na taj način doprineti zdravlju populacije, a pri tom ne smanjiti kvalitet i održivost proizvoda. Očuvanje javnog zdravlja koje država i subjekti u poslovanju hranom svojim društveno odgovornim ponašanjem mogu da ostvare, je važan zadatak u prevenciji kardiovaskularnih oboljenja u Republici Srbiji, posebno ako se ima u vidu da je kultura ishrane u našoj zemlji bazirana na jakoj i začinjenoj hrani.

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Salt reduction in human diet – a global strategy for 21st century

Šarčević Danijela, Lilić Slobodan, Vranić Danijela

S u m m a r y: Increased use of processed foods, rapid urbanization and lifestyle changes have changed the eating habits of people. In everyday life, the use of high-energy foods rich in saturated fat, trans fat, sugar and sodium chloride (salt) becomes dominant. Excessive salt intake in the human diet has resulted in a drastic increase in cardiovascular diseases such as hypertension, heart attack and others.

According to the World Health Organization (WHO), the leading causes of death in the 21st century are non-infectious diseases, which include cardiovascular diseases. As the excessive intake of salt or sodium pose a risk for essential hypertension and cardiovascular diseases, WHO has adopted a recommendation that the global salt intake is reduced by 30% before 2025.

Based on the analysis of various studies, the authors presented data showing the absence of hypertension in the 20th century, in the population which consumed less than 3 g of salt per day. Today, according to the literature data, the salt requirement of an adult man, for the purpose of maintaining of metabolic processes, is 1.5 g, and according to the American Heart Association reported daily intake is 8-15 g.

The aim of the study was to analyze the importance of salt reduction in food consumption in order to prevent heart diseases and preserve the public health of the population. Through a broader theoretical framework the guidelines and recommendations are outlined of the World Health Organization and other international competent institutions.

The paper presents data that suggest that it is possible to reduce salt in meat products, without affecting the quality and sustainability of the product.

Key words: *salt, cardiovascular diseases, preservation of public health.*

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Uticaj soljenja i mariniranja na mikrobiološki status i hemijski sastav skuše upakovane u modifikovanu atmosferu

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S a d r ž a j: Cilj našeg rada je bio uticaj soljenja i mariniranja na mikrobiološki status i hemijski sastav skuše upakovane u modifikovanu atmosferu. Za potrebe ispitivanja skuša je podeljena u dve grupe, gde je prva grupa bila soljena skuša pakovana u MAP, a druga grupa je bila marinirana skuša pakovana u MAP. Marinade i so imaju pre svega uticaj na mikrobiološki status mesa pa je i cilj ovog istraživanja bio utvrđivanje pojedinih grupa bakterija (ukupan broj bakterija, bakterije mlečne kiseline i broj enterobakterija) tokom pedeset dana skladištenja. Soljenje, a posebno mariniranje, značajno utiče na promenu hemijskog sastava mesa ribe, budući da dolazi do značajnog smanjenja sadržaja vode, povećanja sadržaja masti i povećanja sadržaja pepela. Ovi postupci utiču i na smanjenja a_w vrednosti mesa ribe. U uzorcima marinirane ribe, utvrđen je značajno manji broj svih ispitivanih grupa bakterija, u odnosu na uzorke koji su bili tretirani slanim rastvorom.

Ključne reči: *ukupan broj bakterija, MAP, enterobakterije, bakterije mlečne kiseline, skuša, marinada.*

Uvod

Riba je, s obzirom na njenu hranljivu vrednost, oduvek predstavljala značajan deo ishrane ljudi u svetu. Iako, danas, znatan deo ove hrane potiče iz akvakulture u toplovodnim, nizijskim kao i u hladnovodnim ribnjacima, izlov ribe iz prirodnih staništa u Srbiji i dalje predstavlja značajan izvor ribe namenjen tržištu (*Simonović*, 2001). Sveža riba je namirnica koju karakteriše kratka održivost (pH< 5,2; a_w <0,95) i, zbog toga, mora da bude skladištena pri niskim temperaturama hlađenja (–1 do +3°C) (*Milijašević i dr.*, 2010). Riba koja se koristi za ishranu ljudi, pre svega, mora da bude odgovarajućeg kvaliteta i bezbedna za potrošača. Kontaminacija mesa ribe bakterijama može da bude direktna, kada mikroorganizmi potiču iz zagađene sredine, ili indirektna, kada je prisustvo bakterija u mesu ribe posledica kontaminacije ribe u toku manipulacije ribom, pa sve do postupaka u domaćinstvu (*Kilibarda i dr*., 2008). Neadekvatan izbor sirovine, nepažljiva manipulacija sirovinom u toku primarne obrade i nehigijenska proizvodnja mogu usloviti, sa jedne strane, kontaminaciju sirovine nepatogenim mikroorganizmima koji smanjuju kvalitet gotovog proizvoda, ali sa druge strane, što je značajnije sa aspekta zdravlja potrošača, pojavu patogenih mikroorganizama u gotovom proizvodu, što je, ujedno, i najznačajniji aspekt bezbednosti hrane kada su u pitanju proizvodi od ribe.

Soljenje, a posebno mariniranje, značajno mogu da utiču na promenu hemijskog sastava mesa ribe, budući da dolazi do značajnog smanjenja sadržaja vode, povećanja sadržaja masti i povećanja sadržaja pepela. Ovi postupci utiču i na smanjenje a_w vrednosti mesa ribe, a time i na smanjenje ukupnog broja bakterija. Efekat soli na održivost mesa ribe poznat je još iz davnih vremena kao i njegov uticaj na senzorne osobine gotovog proizvoda koji nije zanemarljiv. Soljenje ima konzervišući efekat, s obzirom da se u mesu ribe povećava procenat soli u vodenoj fazi, a smanjuje aktivnost vode, tj. količina

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vode dostupna mikroorganizmima (*Jittinandana i dr*., 2002; *Leroi i dr.*, 2000). Pored soljenja, kao postupak konzerviranja, danas se koristi i mariniranje. Osnovni efekat mariniranja zasniva se na delovanju kiselina i drugih sastojaka marinade na bakterije i enzime, a ovaj postupak utiče na mekoću mesa, promenu ukusa, teksturu proizvoda (promena strukture), što sve doprinosi specifičnom mirisu i ukusu. Soljenje i mariniranje značajno utiču na bakteriološki status ribe. Pored konzervišućeg efekta, na kvalitet i bezbednost ribe utiče i način pakovanja sirovine. Pakovanje hrane, ima za cilj da potrošačima pruži i osnovne informacije o ribi, a naročito o uslovima čuvanja i roku trajanja. Tehnologija pakovanja u modifikovnoj atmosferi sastoji se u primeni gasova u cilju održanja kvaliteta od proizvođača do potrošača, odnosno održavanja originalnih svojstava proizvoda (*Cutter,* 2002). Konzervišuće delovanje gasova primenjenih u pakovanju namirnica zasniva se na njihovoj sposobnosti da onemogućavanjem, ili usporavanjem razmnožavanja mikroorganizama, utiču na zaustavljanje, odnosno usporavanje procesa razlaganja koje prouzrokuju mikroorganizmi, ili fizičkohemijski agensi koji dubinski menjaju proizvod čineći ga neupotrebljivim za konzumiranje. Da bi se gasovi ispravno upotrebili moraju se dobro poznavati svojstva i uloge zaštitnih gasova, ali i karakteristike proizvoda koji se pakuje, kao na primer procenat sadržaja vlage, količine lipida, boja, pH vrednost, itd. Pakovanje u modifikovanoj atmosferi, uglavnom, zahteva primenu smeše najmanje dva gasa, a njihovi optimalni odnosi variraju u zavisnosti od vrste hrane. Najčešća kombinacija gasova koja se primenjuje kod pakovanja mesa su ugljen-dioksid i azot, pri čemu CO_2 utiče na bakteriološki status i kvalitet pakovane ribe, dok azot utiče samo na pakovanje (manji efekat skupljanja prevlake za pakovanje), bez efekata na mikroorganizme. Kiseonik se, takođe, može koristiti u smeši gasova s obzirom na činjenicu da njegovo prisustvo utiče na očuvanje prirodne boje mesa (*Sivertsvik i dr.,* 2002). Iako su i drugi gasovi, kao što su azot-oksid, sulfat-dioksid, etilen, hlor, ozon i propilen-oksid eksperimentalno korišćeni, oni se ne primenjuju u MAP tehnologiji, zbog bezbednosti, propisa i cene pakovanja *(Babić i dr.,* 2009).

Cilj našeg ispitivanja je bio da se ispita uticaj soljenja i mariniranja na mikrobiološki status i hemijski sastav skuše upakovane u modifikovanu atmosferu tokom 50 dana skladištenja pri 4 ± 1°C.

Materijal i matode rada

U eksperimentu je korišćena skuša konzumne veličine, mase od 350−400 grama, koja je obrađena na način uobičajen za industrijski objekat koji se bavi obradom ribe. Riba je podeljena u dve grupe, sa po 12 uzoraka u svakoj grupi. Za soljenje, mariniranje, odnosno, pakovanje korišćen je primarno obrađen trup ribe. Prva grupa je tretirana samo u slanom rastvoru (10% soli), a druga grupa je marinirana u marinadi koja se sastojala od 10% soli i 0,5% sirćetne kiseline. Tretiranje ribe trajalo je dvadeset četiri sata. Nakon toga, soljena, odnosno marinirana riba je pakovana u modifikovanu atmosferu gde je odnos gasova bio 40% CO_2 + 60% N_2. Za pakovanje uzoraka upotrebljena je mašina za pakovanje „Variovac“ (Variovac Primus, Zarrentin, Nemačka). Kao materijal za pakovanje korišćena je folija OPA/EVOH/PE (orijentisani poliamid/etilen vinil alkohol/polietilen, Dynopack, Polimoon, Kristiansand, Norveška) sa niskom propustljivošću za gasove (stepen propustljivosti za O_2 – 3,2 cm^3/m^2/dan pri 23°C; za N_2 – 1 cm^3/m^2/dan pri 23°C; za CO_2 – 14 cm^3/m^2/dan pri 23°C i za vodenu paru 15 g/m^2/dan pri 38°C). Svi uzorci su skladišteni pri istim, kontrolisanim, uslovima, na temperatura 4 ± 1°C. Na početku eksperimenta utvrđen je hemijski sastav a na svakih deset dana u toku pedeset dana, vršene su mikrobiološke analize pakovane skuše. Mikrobiološke metode su podrazumevale ispitivanje: ukupnog broja aerobnih mezofilnih bakterija prema standardu SRPS EN ISO 4833: 2008; mikrobiologije hrane i hrane za životinje – Horizontalna metoda za određivanje broja mikroorganizama – Tehnika brojanja kolonija na 30° C; ukupnog broj bakterija iz familije *Enterobacteriaceae* prema standardu SRPS ISO 21528-2:2009; mikrobiologije hrane i hrane za životinje − Horizontalna metoda za otkrivanje i određivanje broja *Enterobacteriaceae* − Deo 2: Metoda brojanja kolonija; bakterija mlečne kiseline prema standardnoj metodi ISO 15214:1998 (MRS, Merck). Hemijske analize su podrazumevale ispitivanje sadržaja: Vode − određivanjem gubitka mase pri sušenju homogenizovanog uzorka pri 105 ± 1°C do konstantne mase (JUS ISO 1442); Masti − metodom po Soxhletu, ekstrakcijom masti iz osušenog uzorka petrol etrom, destilacijom i sušenjem pri 105 ± 1°C do konstantne mase (JUS ISO 1443); Proteina − metodom po Kjeldalhu primenom uređaja firme „Tecator“ (JUS ISO 937); Pepela − sagorevanjem uzorka pri 550°C do konstantne mase (JUS ISO 936); Natrijum-hlorid − metodom po Volhardu (JUS ISO 1841-1).

Kao osnovne statističke metode korišćeni su deskriptivni statistički parametri (aritmetička

Tabela 1. Hemijski sastav uzoraka skuše
Table 1. The chemical composition of mackerel samples

Uzorak/ Sample	Sastojci/Ingredients (%)			
	Voda/Water	Mast/Fat	Proteini/Proteins	Pepeo/Ash
Sirova skuša/ Raw mackerel	$69,17^{aA} \pm 1,25$	$9,56^{AB} \pm 0,41$	$20,05 \pm 0,94$	$1,12^{AB} \pm 0,05$
Soljena skuša/ Salted mackerel	$66,24^{a} \pm 2,18$	$10,40^{AC} \pm 0,38$	$19,94 \pm 0,81$	$2,92^{AC} \pm 0,09$
Marinirana sluša/ Marinated mackerel	$65,13^{A} \pm 1,84$	$11,40^{BC} \pm 0,56$	$19,95 \pm 0,90$	$3,46^{BC} \pm 0,08$

Legenda/Legend: Ista slova A, B, C $p<0,01$; a $p<0,05$/the same letters A, B, C – $p<0,01$; a $p<0,05$

sredina, standardna devijacija, standardna greška, minimalna, maksimalna vrednost i koeficijent varijacije). Za ispitivanje signifikantnih razlika između tri i više posmatranih tretmana korišćen je grupni test, ANOVA, a zatim pojedinačni Tukey test. Statistička analiza dobijenih rezultata je urađena u statističkom paketu PrismaPad 5.00.

Rezultati i diskusija

Hemijski sastav uzoraka sirove, soljene i marinirane skuše prikazan je u tabeli 1. Sadržaj vode u mesu ribe bio je od 65,15 ± 1,84% (marinirana riba) do 69,17 ± 1,25% (sirova riba). Utvrđeno je da je sadržaj vode u mesu soljene ribe (66,24 ± 2,18%) bio statistički značajno manji ($p<0,05$) od sadržaja vode u mesu sirove ribe, kao i da je sadržaj vode u mesu marinirane ribe bio statistički značajno manji ($p<0,01$) od sadržaja vode u mesu sirove ribe. Sadržaj masti u mesu ispitivanih uzoraka sirove ribe bio je 9,56 ± 0,41%, u uzorcima soljene ribe 10,40 ± 0,38% i u uzorcima marinirane ribe 11,40 ± 0,56%. U svim slučajevima poređenja razlika između prosečnih vrednosti sadržaja masti bila je statistički značajna ($p<0,01$). Nisu utvrđene statistički značajne razlike između prosečnih sadržaja proteina ispitivanih uzoraka ribe (sirova, soljena, marinirana). Prosečan sadržaj pepela u ispitivanim uzorcima sirove ribe bio je 1,12 ± 0,05%, u uzorcima soljene ribe 2,92 ± 0,09% i u uzorcima marinirane ribe 3,46 ± 0,08% i u svim slučajevima poređena utvrđena je statistički značajna razlika ($p<0,01$).

Ukupan broj bakterija, tokom pedeset dana ispitivanja, bio je statistički značajno veći ($p<0,01$) u uzorcima koji su soljeni (tabela 2).

Rezultati ispitivanja ukupnog broja enterobakterija tokom pedeset dana skladištenja prikazani su u tabeli 3. Poređenjem rezultata utvrđeno je da je u uzorcima prve grupe (soljena skuša pakovana u MAP) (0,62 ± 0,07 log CFU/g) prosečan broj enterobakterija bio statistički značajno veći ($p<0,05$) u odnosu na broj enterobakterija u uzorcima druge grupe (marinirana skuša pakovana u MAP) (0,48 ± 0,07 log CFU/g). Nakon desetog dana ispitivanja, statistički značajna razlika ($p<0,05$) uočena je između ukupnog broja enterobakterija poređenjem uzoraka prve i druge grupe. Prosečan broj enterobakterija 20, 30, 40, i 50 dana ispitivanja bio je statistički značajno veći ($p<0,01$) u uzorcima koji su soljeni, a zatim pakovani u modifikovanu atmosferu.

Tabela 2. Ukupan broj bakterija u uzorcima skuše tokom skladištenja (log CFU/g)
Table 2. The total number of bacteria in the mackerel samples during storage (log CFU/g)

Grupa/ Group	Dani ispitivanja/Test days ($\overline{X} \pm SD$)					
	0.	10.	20.	30.	40.	50.
I	$2,33 \pm 0,20^{A}$	$2,65 \pm 0,10^{A}$	$3,27 \pm 0,08^{A}$	$3,87 \pm 0,12^{A}$	$4,78 \pm 0,15^{A}$	$5,00 \pm 0,14^{A}$
II	$1,97 \pm 0,14^{A}$	$2,10 \pm 0,09^{A}$	$2,38 \pm 0,08^{A}$	$2,73 \pm 0,08^{A}$	$3,42 \pm 0,15^{A}$	$3,52 \pm 0,08^{A}$

Legenda/Legend: Isto slovo A– $p<0,01$/the same letter A– $p<0,01$
I grupa: soljena skuša pakovana u MAP/Group I: salted mackerel packed in MAP
II grupa: marinirana skuša pakovana u MAP/Group II: marinated mackerel packed in MAP

Tabela 3. Ukupan broj enterobakterija u uzorcima skuše tokom skladištenja (log CFU/g)
Table 3. The total number of enterobacteria in the mackerel samples during storage (log CFU / g)

Grupa/ Group	Dani ispitivanja/Test days ($\overline{X}$ ± SD)					
	0.	10.	20.	30.	40.	50.
I	0,62 ± 0,07^{a}	1,05 ± 0,05^{a}	2,01 ± 0,11^{A}	2,86 ± 0,08^{A}	3,41 ± 0,14^{A}	3,38 ± 0,07^{A}
II	0,48 ± 0,07^{a}	0,87 ± 0,08^{a}	1,10 ± 0,09^{A}	1,35 ± 0,10^{A}	1,80 ± 0,14^{A}	1,71 ± 0,07^{A}

Legenda/Legend: Isto slovo A– $p<0,01$; a– $p<0,05$/the same letter A– $p<0,01$; a– $p<0,05$
I grupa: soljena skuša pakovana u MAP/Group I: salted mackerel packed in MAP
II grupa: marinirana skuša pakovana u MAP/Group II: marinated mackerel packed in MAP

Prosečan broj bakterija mlečne kiseline nultog dana ispitivanja bio je statisički značajno veći u uzorcima prve grupe (1,91 ± 0,14 log CFU/g) u odnosu na prosečan broj bakterija mlečne kiseline u uzorcima druge grupe (1,33 ± 0,10 log CFU/g) (tabela 4). Poređenjem dobijenih rezultata tokom svih pedeset dana ispitivanja, može da se konstatuje da je prosečan broj bakterija mlečne kiseline bio statistički značajno veći ($p<0,01$) u uzorcima soljene skuše u odnosu na marinirane uzorke skuše.

Interes potrošača za svežom, ohlađenom, polugotovom hranom sa produženom održivošću, usmerilo je brojna istraživanja na postupke konzerviranja kojima se može kontrolisati rast bakterija, da bi proizvod bio bezbedan i da bi bio održiv duže vreme (*Sallam,* 2007). Kvarenje ribe može da se spreči različitim postupcima konzervisanja (*Sivertsvik i dr.,* 2007). Hemijski sastav mesa ribe zavisi, pre svega, od vrste ribe ali ima varijacija i unutar same vrste. Te varijacije zavise od starosti, veličine, pola, sezone izlova (*Silva i Scamol,* 2000). U stvari, varijacije u hemijskom sastavu ribe zavise od ishrane, migracije i polnog ciklusa. Proizvođači imaju direktan interes za hemijski sastav ribe, kao polazni materijal za preradu (*Duyar i Eke*, 2009). Mariniranje utiče na promene hemijskog sastava mesa ribe. Sadržaj vode u soljenoj i mariniranoj ribi je prema našim rezultatima znatno manji od sadržaja vode u sirovoj ribi (tabela 1). Promena sadržaja vode uticala je na promene odnosa ostalih sastojaka mesa ribe, pa je tako zabeleženo u soljenoj i mariniranoj ribi povećanje sadržaja masti (10,40% soljena riba, 11,40% marinirana riba i 9,56% sirova riba). Soljenjem, odnosno, mariniranjem povećava se sadržaj pepela u mesu (soljena riba 2,92%, marinirana ribe 3,46% i sirova riba 1,12%) pa razume se i sadržaj soli.

Mariniranje kao postupak konzerviranja je zasnovan tretman mešanja sa rastvorom koji sadrži so, začine, kiseline, limunov sok i doprinosi dobroj prihvatljiosti za različite proizvode od mesa (*Duyar i Eke*, 2009). Poznato je da se mariniranjem menja sposobnost vezivanja vode, smanjuje kalo toplotne obrade i poboljšava tekstrura. Rast bakterija kod mariniranih proizvoda posledica je pada pH vrednosti a ponekad i prisustva antibakterijskih supstanci (*Poligne i Collignan,* 2000). Održivost ribe i proizvoda od ribe u MAP-u (*Modified atmosphere packaging*) može da se produži pri čemu održivost zavisi od izvora (stanja) sirovine, temperature, odnosa gasova i materijala za pakovanje. U literaturi postoje brojni podaci o uticaju MAP-a na ribu i proizvode od ribe (*Goulas i Kontamines,* 2005; *Lopez-Caballero i dr.,*

Tabela 4. Ukupan broj bakterija mlečne kiseline u uzorcima skuše tokom skladištenja (log CFU/g)
Table 4. The total number of lactic acid bacteria in the mackerel samples during storage (log CFU/g)

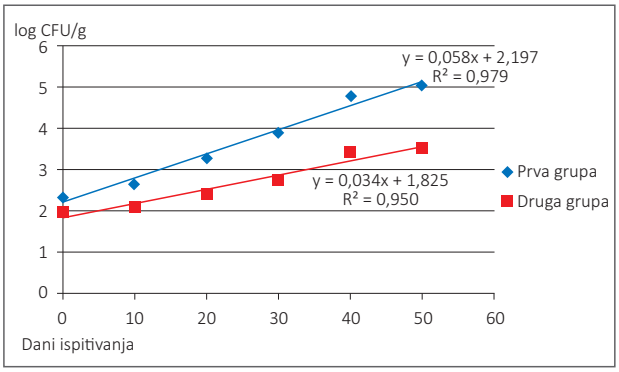
Grupa/ Group	Dani ispitivanja/Test days ($\overline{X}$ ± SD)					
	0.	10.	20.	30.	40.	50.
I	1,91 ± 0,14^{A}	2,01 ± 0,15^{A}	2,66 ± 0,13^{A}	3,46 ± 0,15^{A}	5,67 ± 0,10^{A}	5,73 ± 0,12^{A}
II	1,33 ± 0,10^{A}	1,60 ± 0,09^{A}	1,95 ± 0,10^{A}	2,41 ± 0,11^{A}	3,56 ± 0,15^{A}	3,61 ± 0,35^{A}

Legenda/Legend: Isto slovo A− $p<0,01$/the same letter A− $p<0,01$
I grupa: soljena skuša pakovana u MAP/ Group I: salted mackerel packed in MAP
II grupa: marinirana skuša pakovana u MAP/Group II: marinated mackerel packed in MAP

2002; *Özogul i dr.,* 2004). Održivost skuše na ledu je oko sedam dana i zato se traže mogućnosti produženja održivosti (*Stamatis i Arkoudelos*, 2007). U MAP-u se održivost skuše može produžiti i do 12 dana.

Tehnološki aspekti pakovanja u MAP se dugo izučavaju (*Günsen i dr.,* 2011). U pakovanjima sa CO_2 rast *Shewanella Putrefaciens* je veoma inhibiran. Nasuprot tome, gram-negativan organizam *Photobacterium Phosphoreum* je identifikovan kao mikroorganizam odgovoran za kvar (*Günsen i dr.,* 2011). MAP u kombinaciji sa hlađenjem može da bude vrlo efektivan metod konzervisanja koji omogućava produženje održivosti i očuvanje kvaliteta svežih, hlađenih namirnica kao što je crveno meso, meso živine, voće, povrće, pasta, riba (*Günsen i dr.,* 2011). Plodovi voda se razlikuju od ostalih vrsta mesa, pa i po tome što su podložni kako mikrobiološkom tako i hemijskom kvaru (*Pastoriza,* 1996). Kvar ribe počinje odmah nakon smrti, odnosno izlova. To je rezultat brojnih promena koje su uzrokovane aktivnošću bakterija i enzima. Ukupan broj bakterija može da bude pokazatelj kvara. Smatra se da ukupan broj bakterija od 10^8 CFU/ml i ukupan broj sulfitoredukujućih bakterija od 10^6 CFU/ml još uvek nije znak kvara ribe (*Stamatis i Arkoudelos,* 2007). Porast broja enterobakterija je zabeležen u obe grupe ispitivanih uzoraka ali je porast bio znazno veći u uzorcima koji su bili soljeni (grafikon 2). Takođe, poređenjem bakterija mlečne kiseline i ukupnog broja bakterija, može se uočiti da je porast ovih bakterija bio znatno veći u soljenim uzorcima, a zatim pakovani u MAP (grafikon 1 i grafikon 3).

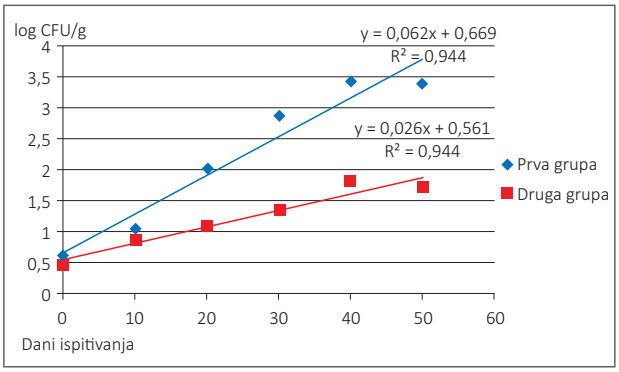
Hlađenje je najvažnija strategija koja usporava rast bakterija i produživost ribe. MAP ili vakuum mogu dodatno da produže održivost (*Günsen i dr.,* 2011). Pozitivan efekat CO_2 na održivost ribe je dobro dokumentovan (*Hansen,* 2008; *Mendes i Goncalves*, 2008) i u MAP-u CO_2 redukuje rast nekih bakterija (*Pseudomonas* i *Shewanella*) a neke bakterije su tolerantne (*Photobacterium Phosphoreum)*. Kombinacija organskih kiselina i pakovanja u atmosferi sa CO_2 u potpunosti inhibira rast bakterija ako je njihov broj 10^3 CFU/g kod lososa za četrnaest dana. Do sličnih rezultata došli su i drugi autori (*Hansen*, 2008; *Mendes i Goncalves*, 2008; *Manju i dr.*, 2007; *Sallam,* 2007; *Sallam*, 2008). Upotreba istovremeno MAP-a i organskih kiselina sa CO_2 ima bolji efekat nego upotreba samo organskih kiselina ili samo pakovanje u MAP-u sa CO_2. Ovo je potvrđeno i našim ispitivanjima. Sirćetna kiselina ima bolji antibakterijski efekat od limunske kiseline a kombinacija ove dve kiseline ima bolji efekat od pojedinačnog delovanja jedne od ovih kiselina. Nije međutim zapažen značajno bolji efekat ako se kombiniju ove dve kiseline i pakovanje sa CO_2.



Legenda/Legend: Dani ispitivanja/test days;
Prva grupa/Group I; Druga grupa/Group II

Grafikon 1. Prosečan ukupan broj bakterija u uzorcima skuše tokom skladištenja (log CFU/g)

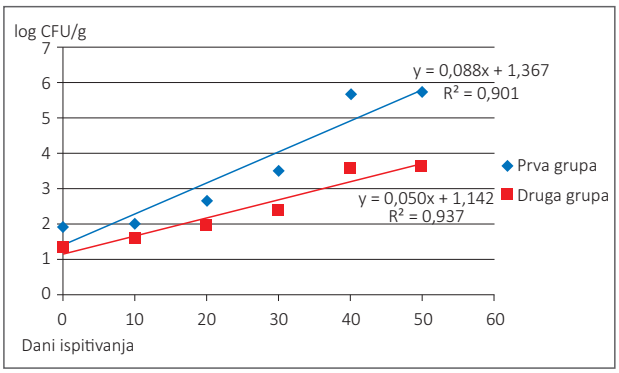
Graph 1. The average total number of bacteria in the mackerel samples during storage (log CFU/g)



Legenda/Legend: Dani ispitivanja/test days;
Prva grupa/Group I; Druga grupa/Group II

Grafikon 2. Prosečan broj enterobakterija u uzorcima skuše tokom skladištenja (log CFU/g)

Graph 2. The average number of enterobacteria the mackerel samples during storage (log CFU/g)



Legenda/Legend: Dani ispitivanja/test days;
Prva grupa/Group I; Druga grupa/Group II

Grafikon 3. Prosečan broj bakterija mlečne kiseline u uzorcima skuše tokom skladištenja (log CFU/g)

Graph 3. The average number of lactic acid bacteria in the mackerel samples during storage (log CFU/g)

Zaključak

Mariniranje, kao jedan od načina konzerviranja ribe, ima poseban uticaj na činioce od značaja za bezbednost i kvalitet skuše. Rezultati ispitivanja pokazuju da mariniranje ribe, u našem slučaju, skuše, a zatim pakovanje u modifikovanu atmosferu imaju najpovoljniji efekat na parametre kvaliteta ribe. U uzorcima marinirane ribe, a posebno u uzorcima ribe pakovane u modifikovanu atmosferu, utvrđen je značajno manji broj svih ispitivanih grupa bakterija u odnosu na uzorke koji su bili tretirani slanim rastvorom.

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The effect of salting and marinating on microbiological status and chemical composition of mackerel packed in the modified atmosphere

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S u m m a r y: The aim of our study was the effect of salting and marinating on microbiological status and chemical composition of mackerel packed in the modified atmosphere. For the purposes of the testing, mackerel were divided into two groups where the first group consisted of salted mackerel packed in MAP and the other group was marinated mackerel packed in the same way. Marinades and salt primarily have impact on microbiological status of meat and the aim of this study was to identify certain groups of bacteria (total bacteria, lactic acid bacteria and the number of Enterobacteriaceae) during the fifty days of storage. Salting and especially marinating, significantly affect the change in the chemical composition of fish meat, since it leads to a significant reduction in water content, an increase in fat content and increased ash content. These processes affect the reduction of aw value of fish meat. In the samples of marinated fish, especially fish samples packaged in the modified atmosphere, significantly lower bacteria count was determined in the tested group relative to the samples which were treated with brine, and were packed under modified atmosphere.

Key words: *total number of bacteria, MAP, enterobacteriaceae, lactic acid bacteria, mackerel, marinade.*

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Mogućnosti supstitucije natrijum-hlorida nekim hloridnim solima u procesu proizvodnje suvog svinjskog mesa

Lilić Slobodan[1], Borović Branka[1], Vranić Danijela[1]

S a d r ž a j: Unos natrijuma umnogome prevazilazi nutritivne preporuke, naročito u modernim i industrijski razvijenim zemljama. Od ukupne dnevne količine kuhinjske soli, koja se u organizam unese putem uobičajenih količina hrane (jela pripremljena u domaćinstvu, hleb, pekarski proizvodi, sir), oko 20% potiče iz proizvoda od mesa. Istraživanja u studiji o sadržaju soli u proizvodima od mesa na tržištu Srbije, pokazuju da je najveći prosečan sadržaj natrijum-hlorida utvrđen u suvomesnatim proizvodima (5,09%). Ovi rezultati su očekivani s obzirom da se oni ne obrađuju toplotom, a da je, uz ostale činioce, ova količina soli potrebna da bi se ovakav proizvod učinio mikrobiološki stabilnim. Najčešći način za redukciju natrijuma u proizvodima od mesa predstavlja parcijalna supstitucija natrijum- hlorida drugim hloridnim solima, uglavnom kalijum-hloridom. U radu su prikazane mogućnosti supstitucije natrijum hlorida nekim hloridnim solima u procesima salamurenja, sušenja i zrenja mesa. Posebna pažnja posvećena je mikrobiološkim promenama tokom ovih procesa, kao i nekim fizičko-hemijskim i enzimskim promenama.

Ključne reči: *suvo svinjsko meso, natrijum-hlorid, kalijum-hlorid.*

Uvod

U današnje vreme postoje mnogi značajni dokazi koji ukazuju da je konzumiranje hrane u uskoj povezanosti sa zdravljem i svi trendovi nutricionizma vode ka tome da se u hrani smanji sadržaj masti, šećera i soli. Povećan unos natrijuma može biti krucijalan za razvoj hipertenzije, i takva pojava se i zapaža u modernim društvima, naročito kod starijih osoba (*McCarty,* 2004). Unos natrijuma umnogome prevazilazi nutritivne preporuke, naročito u modernim i industrijski razvijenim zemljama. Osnovni izvor natrijuma u prehrambenim proizvodima je poreklom iz natrijum-hlorida, odnosno iz kuhinjske soli. Unos kuhinjske soli uslovljen je, ne samo fiziološkim potrebama (sportisti), nego i navikama, koje se stiču još u ranom detinjstvu, kao i tradicijom u ishrani (podneblje, odnosno klimatski uslovi, priprema hrane, resursi stoke i sl.). Od ukupne dnevne količine kuhinjske soli, koja se u organizam unese putem uobičajenih količina hrane (jela pripremljena u domaćinstvu, hleb, pekarski proizvodi, sir), oko 20% potiče iz proizvoda od mesa (*Wirth*, 1991).

So u proizvodima od mesa izaziva slanost (*Ruusunen i Puolanne*, 2005) i zajedno sa mastima doprinosi još nekim senzorskim karakteristikama. Povećanje slanosti je izraženije u proizvodima sa više masti, a u proizvodima sa većim sadržajem proteina osećaj slanosti je manji. Jedna od najvažnijih funkcija soli u proizvodima od mesa je solubilizacija funkcionalnih miofibrilarnih proteina, što aktivira proteine da povećaju hidraciju i sposobnost vezivanja vode (Water holding capacity − WHC) i, shodno tome, poboljšanje teksture proizvoda. Povećanje WHC u mesu smanjuje gubitak mase tokom kuvanja i doprinosi većoj sočnosti i mekoći proizvoda od mesa. Postoje dve hipoteze o ulozi soli u WHC mesa (*Ruusunen i Puolanne*, 2005). Prema *Hammu* (1986) joni hlora imaju tendenciju da penetriraju u miofilamente uzrokujući njihovo rastvaranje, dok *Offer i Trinick* (1983) tvrde da natrijumovi joni formiraju jonski „oblak“ oko filamenata. Oni baziraju svoju hipotezu na selektivnom vezivanju jona hlora za miofibrilarne proteine. Rastvoreni miofibrilarni proteini formiraju lepljivi eksudat na površini komadića mesa, koji se na taj način vezuju tokom

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toplotne obrade proizvoda. Matriks proteina koagulisanih toplotom vezuju u „klopku“ slobodnu vodu. Tako, na primer, u emulgovanim proizvodima od mesa kao što su barene kobasice, rastvoreni proteini, u formi kontinuirane faze, predstavljaju film oko kapljica masti i vode.

Sadržaj soli u barenim kobasicama, nalazi se u opsegu od 1,6−2,4%, u suvim fermentisanim kobasicama 3,5−5,0%, u dimljenim proizvodima 3−4%, dok je u suvomesnatim proizvodima nešto veći i iznosi 4−7%, nekada i više (*Čavoški i dr.,* 1990). U studiji *Vranić i dr*. (2009), od analiziranih uzoraka proizvoda od mesa, najveći prosečan sadržaj natrijum-hlorida utvrđen je u suvomesnatim proizvodima (5,09%), što je i očekivano s obzirom da se oni ne obrađuju toplotom, a da je, uz ostale činioce, ova količina soli potrebna da bi se ovakav proizvod učinio mikrobiološki stabilnim.

Mogućnosti smanjenja soli u proizvodima od mesa

Ruusunen i Puolanne (2005) i *Desmond* (2006), govore o mogućnostima smanjenja sadržaja soli u proizvodima od mesa, što se može postići na više načina: (1) smanjivanjem dodatog natrijum hlorida (*Sofos*, 1983; *Lilić*, 2000); (2) zamenom dela NaCl drugim solima (*Sofos*, 1983; *Terell*, 1983; *Guàrdia i dr*.., 2006; *Lilić i dr.*, 2008; *Lilić i dr*., 2014; *Lilić i dr*., 2014a).; (3) upotrebom pojačivača ukusa i maskirajućih agenasa (*Desmond*, 2006); (4) kombinacijom navedenih postupaka (*Sofos*, 1983; *Terell*, 1983); (5) dodavanjem začinskog bilja i ekstrakata začina u proizvode od mesa (*Lilić i Matekalo-Sverak*, 2007); (6) optimizacijom fizičke forme soli (*Angus i dr.*, 2005); i (7) alternativnim procesnim tehnikama (*Claus i Sørheim*, 2006).

Kalijum-hlorid je načešće korišćen supstituent soli, međutim potpuna zamena nije moguća, jer već kod 50% supstitucije dolazi do pojačavanja gorkog ukusa i smanjivanja slanosti. Upotreba kalijumovih soli je često osporavana zbog moguće osetljivosti izvesnog dela populacije, kao što su ljudi oboleli od dijabetesa tip I, hronične renalne insuficijencije, od poslednjeg stadijuma bubrežnih oboljenja, ljudi sa srčanom i nadbubrežnom insuficijencijom (*FSAI,* 2005). Dietary Guidelines for Americans (2005) navode da ishrana bogata kalijumom slabi efekte soli na krvni pritisak i preporučuju dnevni unos kalijuma od 4,7 g.

Na tržištu se već nalaze dijetalne soli koje su mešavina natrijum-hlorida i kalijum- hlorida, obično uz dodatak L-lizin hidrohlorida, koji maskira gorak ukus soli i pospešuje izlučivanje natrijuma iz organizma (*Ruusunen i dr*., 2002).

U kuvanim šunkama natrijum hlorid se može supstituisati kalijum hloridom i 50% bez uticaja na senzorske karakteristike (*Frye i dr*., 1986). U šunkama, korišćenje smeše 70% NaCl i 30% KCl, odnosno smeše 70% NaCl i 30% $MgCl_2$, nemaju uticaj na ukus, miris, mekoću i opštu prihvaljivost u poređenju sa šunkama proizvedenim samo sa NaCl (*Collins*, 1997). *Gou i dr*. (1996) utvrdili su da ne postoje razlike u teksturi suvih fermentisanih kobasica, prilikom parcijalne supstitucije natrijum-hlorida, ali da se gorak ukus oseti već kod 30% dodatog KCl. Oni takođe navode da supstitucija od 40% sa KCl i kalijum laktatom u sušenom mesu ne dovodi do nepoželjnih karakteristika u ukusu.

Prema navodima *Ruusunen i Puolanne* (2005) redukcija soli u fermentisanim kobasicama nije moguća ispod 2% zbog nemogućnosti postizanja dovoljno niske aktivnosti vode koja obezbeđuje mikrobiološku stabilnost ovih proizvoda.

Neki proizvodni procesi razvijeni su tako da se salamura ubrizgava u meso, koja sadrži KCl u kombinaciji sa kalcijum-citratom, kalcijum-laktatom, laktozom, dekstrozom, kalijum fosfatom, askorbinskom kiselinom i natrijum nitritom (*Riera i dr*., 1996).

Fosfati su takođe uspešni u redukciji soli u proizvodima, mada deluju sinergistički sa natrijum-hloridom. Oni povećavaju WHC povećavanjem jonske jačine, kada slobodne grupe sa negativnim nabojem omogućavaju da proteini vezuju više vode (*Trout i Schmidt*, 1984). Međutim i fosfati su nosioci natrijuma. Tako natrijum polifosfat sadrži 31,24% natrijuma u poređenju sa 39,34% koliko ga ima u natrijum hloridu, međutim njegova upotreba je ograničena na oko 0,5% u proizvodu.

Ruusunen i dr. (2002) utvrdili su da je moguća proizvodnja bolonja kobasice i kuvane šunke sa manje soli (1,0−1,4%) i da je moguće smanjenje sadržaja natrijuma korišćenjem kalijumovih soli. Postoji još jedna mogućnost da se nadomeste tehnološki poželjne osobine NaCl, a to je upotreba ingredijenata kao što su vlakna, hidrokoloidi i skrobovi, koji omogućavaju formiranje gela i proteinskih koagulata (*Collins*, 1997). Jedna od mogućnosti smanjenja soli u proizvodima od mesa je i upotreba smeša pojačivača ukusa i maskirajućih agenasa. Postoji mnogo ovakvih komercijalnih smeša koje obično sadrže ekstrakt kvasca, laktate, mononatrijum-glutamat i nukleotide. Pojačivači ukusa deluju aktivirajući receptore u usnoj šupljini koji pomažu da se kompenzuje redukcija soli u proizvodu (*Brandsma*, 2006).

Pojedini autori (*Pasin i dr*., 1989) utvrdili su da je moguće smanjiti NaCl do 75% u kuvanim

kobasicama, kombinujući KCl sa komercijalnom mešavinom 5'-ribonukleotida inozin monofosfat i guanozin monofosfat. Bilo kakvo dodavanje mononatrijum-glutaminata dovodi do opadanja prihvatljivosti ukusa proizvoda i za 50% usled pojačavanja gorkog ukusa poreklom od soli kalijuma. Linguagen, USA kompanija, patentirala je bloker gorkog ukusa, adenozin 5'-monofosfat, koji deluje tako što blokira aktivaciju gustducina u ćelijama receptora za ukus i, shodno tome, prevenira stimulaciju nerva koji inerviše receptore ukusa (*McGregor*, 2004). Ovaj bloker može se koristiti za unapređenje ukusa prilikom korišćenja kombinacija KCl i NaCl.

Na tržištu se nalazi još preparata kao što su „NeutralFresh" koji uklanja metalni, gorak ukus KCl i daje ukus kao natrijumova so, zatim i „Magifique Salt-Away" i „Mimic", koji maskiraju gorki i metalni karakter KCl kao i „SaltTrim".

Druge kombinacije kao što su lizini i ćilibarna kiselina koriste se kao supstituenti (*Turk*, 1993). Ove supstance imaju slan ukus i neke antimikrobne i antioksidativne karakteristike i mogu se koristiti da zamene do 75% soli. Za tehnološke karakteristike, odnosno vezivanje vode mogu da se koriste i fosfati, skrobovi i gume.

Gou i dr. (1996) proučavali su efekat glicina i kalijum-laktata kao supstituenta soli i utvrdili da je moguća supstitucija 40% NaCl nekim od ovih jedinjenja, koja u većoj količini daju neprihvatljiv sladak ukus. U sušenom mesu moguća je supstitucija do 40% kalijum- hloridom i kalijum-laktatom bez značajne razlike u ukusu, dok je 30% maksimalna količina za glicin.

Postoje i derivati mikoproteina (Mycoscent) pomoću kojih se može smanjii sadržaj natrijum-hlorida za 50% u biskvitima i snack proizvodima i za 25% smanjiti sadržaj natrijuma u pikantnim jelima. Mycoscent 400 je prirodan izvor ribonukleotida i glutaminske kiseline, a ukusa je na bujon i može biti korišćen za ukus kuvanog mesa (*Mycoscent*, 2005). Poznati su i autolizati kvasca koji naročito suzbijaju gorak ukus KCl, kao što su „Provesta" preparati, „Aromild" i „Maxaromeselect". Problemi sa autolizatima su ti što imaju izražen ukus na bujon, što nije poželjno u nekim proizvodima, a neki od njih imaju tipičan originalan umami ukus. Pojedinim tehnološkim postupcima teži se da se ovi preparati optimizuju za proizvode od mesa sa neutralnim ukusom i optimalnim umami efektom.

Stepen slanosti zavisi i od fizičke forme soli. So u ljuspicama se pokazala funkcionalnijom u pogledu vezivanja vode, povećavanja pH vrednosti, povećanja rastvorljivosti proteina u model sistemima emulzija (*Campbell*, 1979). So u ljuspicama je bolje i brže rastvorljiva nego so u granulama i to može biti problem kada se u formulama ne koristi voda, tako da so u ljuspicama može biti dobra za proizvode u koje se ne dodaje voda, kao što je suvo meso. Leatherhead Food International je istraživala optimizaciju fizičke forme soli i pratila promene fizičke forme soli koja postaje raspoloživija i samim tim može biti korišćena u manjoj količini. Ovo uključuje povećanje efikasnosti soli, menjanje strukture i modifikovanje percepcije soli (*Angus i dr*., 2005).

Promene mikrobioloških parametara

So nema direktan antimikrobni efekat. Inhibitorni efekat soli zasniva se na snižavanju aktivnosti vode u mesu. U izvesnim koncentracijama soli, osmozom se bakterijskih ćelija gubi voda, što usporava ili potpuno zaustavlja njihov rast. Relativno visoke koncentracije soli su potrebne za inhibiciju rasta mikroorganizama. Granične koncentracije soli iznose 5% za *Clostridium botulinum* tip E i *Pseudomonas fluorescens*, 6% za *Shigela* i *Klebsiella* vrste, 8% za *E. coli*, *Salmonella* spp., *Bacillus cereus*, *Clostridium botulinum* tip A i *Clostridium perfringens*, 10% za *Clostridium botulinum* tip B i *Vibrio parahaemolyticus*, 15% za *Bacillus subtilis* i bakterije familije *Streptococcaceae*, 18% za *Staphylococcus aureus*, 25% za *Penicillium* i *Aspergillus* plesni i 26% za *Halobacterim halobium*, *Bacterium prodigiosum* i *Spirillium* vrste (*Prändl*, 1988).

Alińo i dr. (2009), ispitivali su efekat parcijalne supstitucije natrijum-hlorida kalijum- hloridom u količini do 70% na fizičko-hemijske i mikrobiološke parametre suvog svinjskog mesa posle salamurenja i sušenja. Mikrobiološkim analizama aerobnih mezofilnih bakterija, halotolerantnih bakterija i mlečnokiselinskih bakterija, nije utvrđena zavisnost ispitivanih mikrobioloških parametara od primenjene formulacije soli, pri čemu su predominantnu mikrofloru činile halotolerantne i mlečnokiselinske bakterije. Prema istraživanjima *Yamanaka i dr.* (2005), natrijum-hlorid indukuje selektivno razmnožavanje halotolerantnih i mlečnokiselinskih bakterija i suzbija rast i razmnožavanje koliformnih bakterija. Nalaz laktoza pozitivnih bakterija familije *Enterobacteriaceae* i fekalnih koliformnih bakterija u nivou manjem od 3 cfu/g, u svim ispitivanim eksperimentalnim grupama, govori o tome da nije bilo značajnih razlika u smislu primenjenih tretmana sa smešama različitih udela natrijum-hlorida i kalijum-hlorida. *Listeria* spp. bile su utvrđene u 17%, računajući na sve uzorke suvog mesa, nezavisno od tretmana, a najveća količina je bila 1460 cfu/g, što prevazilazi njihov poželjan broj do 100 cfu/g (*Risk*

Assessment Drafting Group, 2004). Prevalenca koagulaza pozitivnih stafilokoka i *B. cereus* bila je 33%, i to 2230 cfu/g i 1660 cfu/g. *Yamanaka i dr.* (2005), utvrdili su da su u mesu pre salamurenja bile prisutne samo Gram negativne bakterije (*Vibrio*, *Acinetobacter*, *Pseudomonas* i bakterije familije *Enterobacteriaceae*), ali tokom salamurenja, broj Gram pozitivnih bakterija (*Micrococcus*, *Staphylococcus* i *Pediococcus*) raste, dok broj Gram negativnih bakterija postepeno opada. Bakterije roda *Staphylococcus* imaju snažnu tolerancu na visoke koncentracije natrijum- hlorida (više od 10%) u poređenju sa bakterijama roda *Micrococcus*, što objašnjava činjenicu prisustva stafilokoka na kraju procesa salamurenja (*Molina i dr*., 1989; *Silla i dr*., 1989; *Yamanaka i dr*., 2005). *Clostridium perfringens*, sulfitoredukujuće klostridije, *Salmonella* spp. i *Shigella* spp. nisu bile detektovane u analiziranim uzorcima, bez obzira na korišćenu smešu soli. Čak *Strong i dr*., još 1970. godine izveštavaju da kalijum-hlorid inhibira rast *Clostridium perfringens* više nego natrijum-hlorid. Dobijeni rezultati *Yamanaka i dr*. (2005) ukazuju da kalijum-hlorid može da bude iskorišćen kao supstituent natrijum--hlorida u proizvodnji suvog svinjskog mesa i da to ne predstavlja rizik sa mikrobiološke tačke gledišta, što su potvrdili i *Boziaris i dr*. (2007) u in vitro uslovima. Međutim, različite studije pokazuju da je teško uraditi bilo kakvu predikciju u antimikrobnom delovanju soli i mešavina soli, zbog toga što to zavisi od mnogih faktora, kao što su temperatura, pH vrednost, inicijalna kontaminacija sirovine i vrste mikroorganizama prisutnih u mesu (*Gimeno i dr*., 1999, 2001; *Ibáñez i dr.,* 1995, 1996).

Lorenzo i dr. (2015), utvrdili su da se ukupan broj bakterija značajno menja ($p < 0,001$) tokom četiri različita perioda soljenja. Veći ukupan broj bakterija (6,16 log cfu/g) utvrđen u svežem mesu mogao bi biti u vezi sa većom kontaminacijom komada mesa i značajnijim razmnožavanjem tokom zrenja suvog mesa u komorama. Na kraju procesa proizvodnje, značajne razlike ($p < 0,05$) utvrđene su između suvog mesa različitih grupa, pri čemu je najveći broj bakterija utvrđen u suvom mesu koje je soljeno mešavinom natrijum hlorida i kalijum-hlorida (50:50). Ovi podaci su u saglasnosti sa rezultatima *Raccacha i Henninena* (1997) koji su utvrdili da korišćenje kalcijum-hlorida ima veći uticaj na inhibiciju rasta aerobnih mezofilnih bakterija, u odnosu na korišćenje natrijum-hlorida i kalijum-hlorida u istim količinama. Međutim, neki autori (*Aliño i dr*., 2010; *Blesa i dr*., 2008) nisu našli značajne razlike u ukupnom broju aerobnih mezofilnih bakterija prilikom korišćenja različitih vrsta hloridnih soli.

Supstitucija natrijum-hlorida drugim solima ima uticaj na povećanje broja halotolerantnih bakterija (*Lorenzo i dr*., 2015). Suvo meso proizvedeno sa mešavinom natrijum-hlorida i kalijum-hlorida u podjednakim količinama i suvo meso u kome je natrijum- hlorid supstituisan sa 25% kalijum-hlorida, 20% kalcijum-hlorida i 10% magnezijum-hlorida, sadržalo je značajno veći ($p < 0,05$) broj halotolerantnih bakterija u odnosu na ostale grupe. Dobijeni rezultati nisu u saglasnosti sa podacima *Aliño i dr*. (2010) koji su utvrdili manji broj halotolerantnih bakterija kada je sadržaj natrijum-hlorida iznosio manje od 50%. Nasuprot njima, *Yamanaka i dr*. (2005) ukazuju da natrijum-hlorid indukuje selektivno razmnožavanje halotolerantnih i mlečnokiselinskih bakterija, a suzbijaju rast koliformnih bakterija.

U pogledu broja kvasaca, primećuje se statistički značajna razlika ($p < 0,01$) između tretmana (*Lorenzo i dr*., 2015), tako da je najmanji broj kvasaca sadržalo suvo meso na kraju proizvodnje, izrađeno samo uz dodatak natrijum-hlorida, što ukazuje da su kvasci blago osetljivi na povećan sadržaj natrijum-hlorida. Ipak, u poslednjim stadijumima soljenja, kvasci se intenzivno razmnožavaju, što doprinosi stvaranju poželjnih senzorskih karakteristika suvog mesa, usled njihove proteolitičke i lipolitičke aktivnosti (*Purriños i dr*., 2013) i njihove uloge u stvaranju isparljivih jedinjenja (*Purriños i dr*., 2012).

Fizičko-hemijske promene

U pogledu sadržaja vlage, u suvom mesu, *Lorenzo i dr*. (2015) utvrdili su značajne razlike ($p < 0,05$) između različitih tretmana, naročito kod onih gde je, kao supstituent, korišćen kalijum-hlorid, što se može pripisati njegovom ometajućem delovanju na odavanje vode iz mesa, tokom sušenja i zrenja, što potvrđuju *Aliño i dr*. (2009a). *Armenteros i dr*. (2012), nasuprot prethodno navedenom, nisu utvrdili značajne razlike ($p > 0,05$) u sadržaju vlage u suvom mesu soljenom različitim solima u odnosu na tradicionalno soljeno meso. Međutim *Wu i dr*. (2014) ukazali su da je sadržaj vlage u bekonu gde je kao supstituent korišćen kalijum-hlorid u količini od 70%, značajno veći ($p < 0,05$) od onog u bekonu soljenom samo natrijum-hloridom.

Aktivnost vode opada tokom celog procesa sušenja i zrenja mesa (*Lorenzo i dr*., 2015), i na kraju proizvodnje, najveći pad aktivnosti vode ($p < 0,001$) zabeležen je u suvom mesu u kome je natrijum-hlorid bio supstituisan kalijum-hloridom u količini od 50%, ali je, takođe, utvrđeno da je to posledica nešto većeg gubitka vode tokom sušenja, što je pokazala i pozitivna korelacija aktivnosti vode i smanjenja sadržaja vlage.

Prisustvo kalijum-hlorida u mesu ubrzava period soljenja, potreban da se postigne ciljani sadržaj hlorida u dubljim slojevima mesa (*Aliño i dr*., 2010), pri čemu se vreme skraćuje sa smanjenim sadržajem kalijuma. Ovo potvrđuju i rezultati prethodnih istraživanja (*Aliño i dr*., 2009, 2009a; *Blesa i dr*., 2008) kod kojih je utvrđeno da kalijumovi joni penetriraju lakše u mišićno tkivo u odnosu na druge katjone. Potrebno je još mnogo istraživanja da bi se objasnila ponašanje kalijumovih jona u različitim proporcijama, naročito pri mešavini od 75% natrijum hlorida i 25% kalijum hlorida, čime je potrebo iznenađujuće kratko vreme da se postigne ciljna koncentracija hlorida u mesu. Dodavanje kalcijum hlorida i magnezijum hlorida pri istom sadržaju kalijuma produžava period soljenja. Katjoni kalcijuma i magnezijuma koji su elektronegativniji u poređenju sa katjonima kalijuma i natrijuma, čvrsto se vezuju za polarne grupe proteina i jačaju proteinske interakcije (*Xiong i Brekke*, 1991), što usporava proces penetracije soli u meso.

Aliño i dr. (2010a) utvrdili su da nema značajnih razlika u masi između šunki soljenih samo natrijum-hloridom i onoj proizvedenoj sa mešavinama soli. Tokom soljenja, primećuje se viši stepen penetracije kalijuma u odnosu na kalcijum i magnezijum koji teže prodiru u meso. Prisustvo kalijum--hlorida odlaže pad aktivnosti vode, a u prisustvu kalcijum-hlorida i magnezijum-hlorida usporava se penetracija soli i, shodno tome, snižavanje aktivnosti vode. Ovo ukazuje da je potrebno produžiti period soljenja do postizanja određene aktivnosti vode, koja je slična aktivnosti vode tokom tradicionalnog soljenja. To se dobro može videti iz podatka da je „post-salting period“ pri korišćenju kombinacije natrijum-hlorida i kalijum- hlorida trajao 76 dana, a pri dodavanju kalcijum-hlorida i magnezijum-hlorida 86 dana. Supstitucija natrijum-hlorida sa 50% kalijum-hlorida nema značajne efekte na kinetiku soli u odnosu na formulacije sa 100% natrijum-hlorida.

Korak koji prati proces soljenja u proizvodnji španske šunke pod nazivom „post-salting period“ odvija se pri temperaturi od oko 3°C sa ciljem sprečavanja rasta mikroorganizama, posebno Clostridium botulinum (*Ventanas i Cava*, 2001). Najrizičniji deo šunke koji može podleći mikrobiološkom kvaru su dublje partije mesa u okolini femoralne arterije zbog najniže koncentracije soli, najvećeg sadržaja vlage i visoke aktivnosti vode (*León-Crespo i dr*., 1997; *Barat i dr*., 2005).

Ipak, i pri supstituciji natrijum-hlorida drugim hloridnim solima, *Blesa i dr*. (2008) nisu utvrdili značajne promene broja mikroorganizama pri različitim formulacijama ovih soli.

Enzimske promene

Studije nekih istraživača (*Rico i dr*., 1991; *Toldrá i dr*., 1993) ukazuju da natrijum- hlorid indukuje inhibitorni efekat na aktivnost katepsina B i katepsina B+L. Količina od 50−70% kalijum-hlorida u mešavinama soli za salamurenje značajno favorizuje aktivnost katepsina B i katepsina B+L ($p < 0,05$) u poređenu sa mesom tretiranim samo natrijum- hloridom. Aktivnost katepsina H ne menja se značajno tokom soljenja mesa različitim vrstama hloridnih soli. Rezultati ovih istraživanja ukazuju da parcijalna supstitucija natrijum- hlorida kalijum hloridom od 50% dovodi do povećane aktivnosti katepsina B i katepsina B+L, što rezultuje produženjem procesa proteolize. Aktivnost dipeptidil peptidaze je različita pri korišćenju različitih smeša za soljenje mesa, osim aktivnosti dipeptidil peptidaze II koja nije pod uticajem dodavanja različitih hloridnih soli. Aktivnost dipeptidil peptidaze I značajno je veća ($p < 0,05$) u mesu soljenom samo natrijum-hloridom u poređenju sa mesom koje je soljeno i drugim vrstama soli. Aktivnost dipeptidil peptidaze III opada značajno ($p < 0,05$) sa porastom udela kalijum-hlorida u smeši, dok se aktivnost dipeptidil peptidaze IV ne nalazi pod uticajem dodavanja različitih vrsta hloridnih soli.

Aktivnost aminopeptidaza, takođe se nalazi pod uticajem tretmana mesa različitim vrstama hloridnih soli (*Armenteros i dr*., 2009). Arginil aminopeptidaza pokazuje povećanu aktivnost u suvom mesu gde je natrijum hlorid supstituisan sa 50%, odnosno 70% kalijum- hlorida, a leucin aminopeptidaza u mesu gde je natrijum-hlorid supstitusan sa 70% kalijum hlorida. Suprotno njima, metionil aminopeptidaza više je inhibirana pri povećanju sadržaja kalijum-hlorida u smeši za soljenje mesa. Efekti supstitucije natrijum-hlorida kalijumomhloridom ne pokazuje pravilnosti i jasan odnos između tretmana različitim hloridnim solima u slučaju aktivnosti alanil aminopeptidaze, u poređenju sa mesom tretiranim samo natrijum- hloridom. Autori su, takođe, utvrdili da se najznačajnije promene dešavaju na sarkomplazminim proteinima prilikom tretmana mesa osim natrijum-hlorida i drugim hloridnim solima, dok se razlike u miofibrilarnim proteinima ne zapažaju. Elektroforeogrami miofibrilarnih proteina pokazuju intenzivnu degradaciju veza između miozina i aktina tokom perioda zrenja suvog mesa (*Toldrá et al*., 1993). Nasuprot tome, elektroforetski profili sarkoplazminih proteina pokazuju veću gustinu veza u suvom mesu soljenom sa parcijalnom supstitucijom natrijum-hlorida drugim hloridnim solima, u odnosu na meso soljeno samo natrijum hloridom.

Primena različitih hloridnih soli u smešama za soljenje mesa ne utiče značajno na lipolitičke procese u suvom mesu, tako da je ukupan sadržaja zasićenih, mononezasićenih i polinezasićenih masnih kiselina uglavnom sličan (*Armenteros i dr*., 2009), što je u saglasnosti i sa nalazima drugih autora (*Countron-Gambotti i Gandemer*, 1999), koji su u svojim eksperimentima izvršili parcijalnu supstituciju natrijum hlorida kalijum hloridom u količini od 50%.

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Possibilities for substitution of sodium chloride with some salts in the production of dried pork

Lilić Slobodan, Borović Branka, Vranić Danijela

S u m m a r y: Sodium intake greatly exceeds nutritional recommendations, particularly in the modern and industrialized countries. From total daily amount of table salt that is consumed through common amounts of food (prepared meals, bread, bakery products, cheese), around 20% originates from meat products. In the study of the salt content in meat products from Serbian markets, the highest sodium chloride content is determined in dry meat products (5.09%) which is expected, because these types of products are not heat treated and because of that salt is necessary for microbiological stability. The common way to reduce the sodium content in meat products is partial substitution of sodium chloride with other chloride salts, mainly with potassium chloride. In this paper are presented the possibilities of substitution of sodium chloride with some chloride salts in the curing proces and during drying and fermentation of dried meat. The particular attentnion is dedicated to microbiological changes during these processes as well as some physico-chemical parameters and enzymatic changes.

***Key words**: dried pork, sodium chloride, potassium chloride.*

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Određivanje PAH4 jedinjenja u dimljenom mesu i dimljenim proizvodima od mesa – razrada metode

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S a d r ž a j: Dimljeno meso i proizvodi od mesa zauzimaju važno mesto u ishrani stanovništva u Srbiji. S obzirom, da tokom dimljenja tj. procesom sagorevanja drveta nastaju policiklični aromatični ugljovodonici (Polycyclic Aromatic Hydrocarbons, PAH), koji su klasifikovani kao karcinogena i mutagena jedinjenja, proučavanje ovih jedinjenja uvek zaokuplja pažnju javnosti. Na osnovu podataka, koji su rezultat novih bioloških i toksikoloških naučnih istraživanja, evropska naučna komisija o zagađivačima u lancu ishrane je u junu 2008. godine predložila da se suma sadržaja benzo[a]pirena, benzo[a]antracena, benzo[b]fluorantena i hrizena, tj. PAH4 jedinjenja koristi kao marker prisustva drugih PAH jedinjenja u različitoj hrani. Ovi predlozi postali su deo zakonske regulative, kako Evropske unije, tako i Srbije. Zakonska regulativa Srbije, koja je u saglasnosti sa propisima EU, od 1. septembra 2014. godine definiše maksimalno dozvoljenu količinu (MDK) za sumu sadržaja PAH4 jedinjenja (12 µg/kg), kao i za sadržaj benzo[a]pirena (2 µg/kg) u dimljenom mesu i proizvodima od mesa. U ovom radu razvijena je metoda za određivanje benzo[a]pirena, benzo[a]antracena, benzo[b]fluorantena i hrizena tj. PAH4 jedinjenja u dimljenom mesu i proizvodima od mesa. Za ekstrakciju lipida i lipofilnih jedinjenja iz uzoraka korišćena je ubrzana ekstrakcija pomoću rastvarača. Ekstrakcija na čvrstoj fazi je korišćena kao postupak za uklanjanje molekula lipida iz ispitanih uzoraka. Identifikacija i kvantifikacija benzo[a]pirena, benzo[a]antracena, benzo[b]fluorantena i hrizena rađena je korišćenjem visokoefikasne tečne hromatografije sa fluorescentnim detektorom (HPLC-FL). Primenjeni su različiti uslovi HPLC analize (mobilna faza, HPLC kolona, temperatura peći, protok mobilne faze) u cilju postizanja optimalnih uslova za kvalitativnu i kvantitativnu analizu PAH4 jedinjenja.

Ključne reči: *PAH4 jedinjenja, benzo[a]antracen, hrizen, benzo[b]fluoranten, benzo[a]piren.*

Uvod

Policiklični aromatični ugljovodonici, tj. PAH jedinjenja, (Polycyclic Aromatic Hydrocarbons, PAH) pripadaju grupi perzistentnih organskih zagađivača (Persistent Organic Pollutants, POP), tj klasi organskih jedinjenja koja su, u različitoj meri, otporna na fotolitičku, biološku i hemijsku degradaciju.

Najznačajniji prirodni izvori nastanka ovih jedinjenja su šumski požari i vulkanske erupcije (*Nikolaou i dr.,* 1984). Najvećim delom PAH jedinjenja dospevaju u životnu sredinu od antropogenih izvora zagađenja, kao što je automobilski saobraćaj (*Lim i dr.,* 2007), sagorevanje organskog materijala na poljoprivrednim zemljištima (*Conde i dr.,* 2005), sagorevanje različite vrste drveta (*Djinovic-Stojanovic i dr.,* 2013), industrija nafte (*Rao i dr.,* 2008), itd. Sagorevanje drveta tokom procesa dimljenja mesa je najznačajniji izvor kontaminacije ove hrane PAH jedinjenjima. Sadržaj PAH jedinjenja u dimljenim proizvodima od mesa, zavisi od više faktora, kao što su vrsta drveta, dostupnost kiseonika tokom procesa dimljenja, temperatura na kojoj se generiše dim i vreme dimljenja (*SCF,* 2002). Takođe, utvrđeno je, da je u različitim vrstama mesa dimljenim pod istim uslovima sadržaj PAH jedinjenja različit (*Djinovic i dr.,* 2008).

Proučavanje policikličnih aromatičnih jedinjenja u dimljenom mesu i proizvodima od mesa zaokuplja posebnu pažnju naučne javnosti, s obzirom na činjenicu da su neka PAH jedinjenja klasifikovana kao karcinogena i mutagena (*IARC,* 2010). Svetska zdravstvena organizacija, u okviru Internacionalne agencije za istraživanje raka (International Agency for Research on Cancer, IARC), proučava biološku aktivnost različitih jedinjenja. Na osnovu dobijenih rezultata, izdaju se monografije (*IARC,* 1987, 1989, 1996, 2010) u kojima se proučavana

Napomena: Rezultati su proistekli iz rada na realizaciji projekata ev. br. III 46009, koje finansira Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

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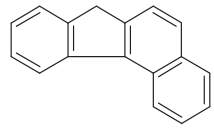
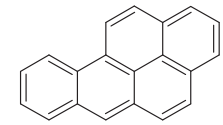
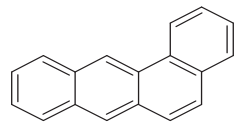
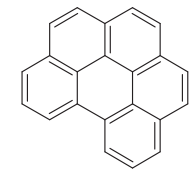
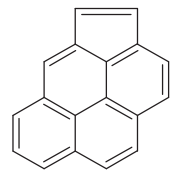
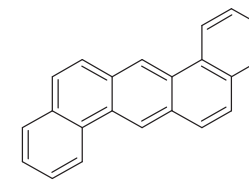
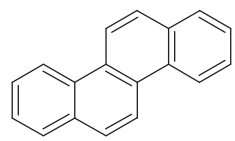
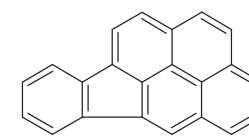
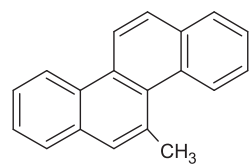
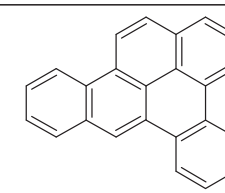
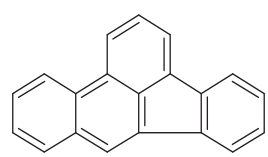
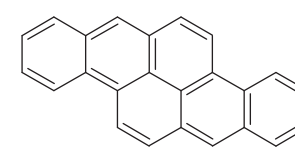
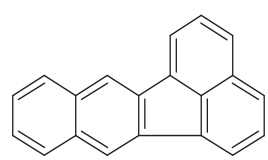
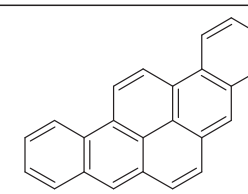
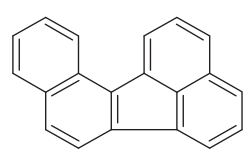
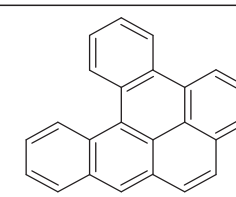
Benzo[c]fluoren/ benzo [c]fluorene, **BcL,** -		Benzo[a]piren/ benzo[a]pyrene, **BaP** Grupa/Group 1	
Benzo[a]antracen/ benz[a]anthracene, **BaA** Grupa/Group 2B		Benzo[g,h,i]perilen/ Benzo[g,h,i]perylene, **BgP** -	
Ciklopenta[c,d]piren/ Cyclopenta[c,d]pyren, **CPP** Grupa/Group 2A		Dibenzo[a,h]antracen/ Dibenz[a, h]anthracene, **DhA** Grupa/Group 2A	
Hrizen/Chrysene, **CHR** Grupa/Group 2B		Indeno[1,2,3-cd]piren/ Indeno[1,2,3-cd]pyrene, **IcP** Grupa/Group 2B	
5-metilhrizen/ 5-methyl- chrysene, **5MC** Grupa/Group 2B		Dibenzo[a,e]piren/ Dibenzo[a,e]pyrene, **DeP** -	
Benzo[b]fluoranten/ Benzo [b]fluoranthene, **BbF** Grupa/Group 2B		Dibenzo[a,h]piren/ Dibenzo[a,h]pyrene, **DhP** Grupa/Group 2B	
Benzo[j]fluoranten/ Benzo [j]fluoranthene, **BjF** Grupa/Group 2B		Dibenzo[a,i]piren/ Dibenzo[a,i]pyrene, **DiP** Grupa/Group 2B	
Benzo[k]fluoranten/ Benzo [k]fluoranthene, **BkF** Grupa/Group 2B		Dibenzo[a,l]piren/ Dibenzo[a,l]pyrene, **DlP** Grupa/Group 2A	

Slika 1. Nazivi, strukturne formule i skraćenice 16 EU prioritetnih PAH jedinjenja.
Figure 1. The names, structural formulas and abbreviations of 16 EU priority PAHs

jedinjenja klasifikuju u četiri grupe karcinogenosti po čoveka (Grupa 1− Karcinogena jedinjenja; Grupa 2A− Verovatno karcinogena jedinjenja; Grupa 2B− Moguće karcinogena jedinjenja; Grupa 3− Jedinjenja koja nisu klasifikovana kao karcinogena i Grupa 4− Verovatno nekarcinogena jedinjenja). Na slici 1 dati su nazivi, strukturne formule, skraćenice i relativne molekulske mase (Mr) 16 PAH jedinjenja koje je Komisija Evropske unije označila kao prioritetna (16 EU prioritetna PAH jedinjenja) (*European*

Tabela 1. MDK za PAH jedinjenja
Table 1. MRLs for PAH compounds

Tačka/ ***Item***	**Proizvod/** ***Product***	**Benzo[a]piren MDK, [µg/kg]/** ***Benzo[a]pyrene, MRL, [µg/kg]***	**Suma benzo[a]pirena, benzo[a]antracena, benzo[b] fluorantena i hrizena, MDK, [µg/kg]/** ***Sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b] fluoranthene and chrysene, MRL, [µg/kg]***
6.1.1.	Ulja i masti (isključujući kakao puter i kokosovo ulje) namenjena za neposrednu ljudsku potrošnju ili kao sastojak u hrani/ *Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or as an ingredient in food*	2,0	10,0
6.1.2.	Kakao u zrnu ili proizvodi od kakao zrna/ *Cocoa beans or cocoa beans products*	5,0	35,0 do 31.3.2015. godine/*35.0 before 31st March 2015*; 30,0 od 1.4.2015. godine/*30.0 from 1st April 2015*.
6.1.3.	Kokosovo ulje namenjeno za neposrednu ishranu ljudi ili kao sastojak u hrani/ *Coconut oil intended for direct human consumption or as an ingredient in food*	2,0	20,0
6.1.4.	Dimljeno meso i dimljeni proizvodi od mesa/ *Smoked meat and smoked meat products*	5,0 do 31.8.2014. godine/*5.0 before 31st August 2014*; 2,0 od 1.9.2014. godine/*2.0 from 1st September 2014*	30,0 do 31.8.2014. godine/*30.0 before 31st August 2014*; 12,0 od 1.9.2014. godine/*12.0 from 1st September 2014*
6.1.5.	Meso dimljene ribe i dimljeni proizvodi ribarstva, osim proizvoda iz tačke 6.1.6. i 6.1.7. Maksimalne količine za dimljene rakove važe za mišićno meso sa dodacima i grudi , a u slučaju dimljenih kraba i rakova sličnim krabama (*Brachyura* i *Anomura*) se odnosi na mišićno meso iz dodataka/ *The meat of smoked fish and smoked fishery products, other than products referred to in point 6.1.6. and 6.1.7. Maximum amounts of smoked crabs apply for muscle meat with additives and breast, and in the case of smoked crab and crayfish similar to crabs (Brachyura and Anomura) refers to muscle meat from supplements*	5,0 do 31.8.2014. godine/*5.0 before 31st August 2014*; 2,0 od 1.9.2014. godine/*2.0 from 1st September 2014*	30,0 do 31.8.2014. godine/*30.0 before 31st August 2014*; 12,0 od 1.9.2014. godine/*2.0 from 1st September 2014*
6.1.6.	Dimljene papaline i konzervirane dimljene papaline (*Sprattus sprattus*); školjke (sveže, ohlađene ili zamrznute); termički obrađeno meso i termički obrađeni proizvodi od mesa namenjeni za neposrednu ishranu ljudi/ *Smoked sprats and canned smoked sprats (Sprattus sprattus); shellfish (fresh, chilled or frozen); heat-treated meat and heat treated meat products intended for direct human consumption*	5,0	30,0
6.1.7.	Školjke (dimljene)/ *Shells (smoked)*	6,0	35,0

Commission, 2005, 2006). Takođe, kod svakog PAH jedinjenja na slici 1 navedeni su podaci o trenutnoj IARC klasifikaciji karcinogenosti (*IARC,* 2010).

Evropska naučna komisija o zagađivačima u lancu ishrane je 9. juna 2008. godine od radnog tela Evropske unije o bezbednosti hrane (EFSA, European Food Safety Authority) usvojila novo mišljenje o policikličnim aromatičnim ugljovodonicima u hrani, gde je zaključeno da se benzo[a]piren više ne može smatrati pogodnim markerom za proveru prisustva PAH jedinjenja u hrani (*EFSA,* 2008). Predloženo je da suma sadržaja PAH4 ili PAH8 jedinjenja bude pokazatelj prisustva PAH jedinjenja u hrani. Grupa PAH4 obuhvata: BaP, BaA, BbF i CHR, dok grupa PAH8 obuhvata: BaP, BaA, BbF, CHR, BkF, BgP, DhA i IcP. EFSA je na osnovu rezultata naučnih ispitivanja u vezi sa kontaminacijom hrane PAH jedinjenjima zaključila da suma PAH8 jedinjenja ne bi obezbedila mnogo više informacija u poređenju sa sumom PAH4 jedinjenja (*EFSA,* 2008) i predložila da se suma sadržaja benzo[a]pirena, benzo[a]antracena, benzo[b]fluorantena i hrizena, tj. PAH4 jedinjenja koristi kao marker prisustva drugih PAH jedinjenja.

Regulativa Komisije Evropske unije broj 835/2011 (*European Commission,* 2011) od 19. avgusta 2011. godine propisala je maksimalno dozvoljene količine (MDK) za benzo[a]piren, kao i za sumu benzo[a]pirena, benzo[a]antracena, benzo[b]fluorantena i hrizena u različitim vrstama hrane. Pravilnik (*Sl. glasnik RS,* br. 25/2010, 28/2011 i 20/2013) koji je bio na snazi do 12. marta 2014. godine nije definisao MDK vrednost za sumu PAH4 jedinjenja u dimljenom mesu i dimljenim proizvodima od mesa, dok je MDK vrednost za benzo[a]piren bila 5 µg/kg. Aktuelni Pravilnik (*Službeni glasnik RS, br.* 29/14), koji je u potpunosti u saglasnosti sa Regulativom Komisije Evropske Unije broj 835/2011 (*European Commission,* 2011) u pogledu MDK vrednosti za PAH jedinjenja, definišu se MDK vrednosti za sumu PAH4 jedinjenja, kao i za BaP u dimljenom mesu i dimljenim proizvodima od mesa (tabela 1). Prema ovom Pravilniku (*Službeni glasnik RS,* br. 29/14), koji je stupio na snagu 13. marta 2014, vrednost MDK za sumu PAH4 jedinjenja do 31. avgusta 2014. godine iznosila je 30 µg/kg, dok je vrednost MDK za BaP iznosila 5 µg/kg. Novi, rigorozniji zahtevi važe od 1. septembra 2014. godine, gde MDK vrednost za sumu PAH4 jedinjenja iznose 12 µg/kg, a MDK vrednost za BaP iznosi 2 µg/kg.

Dimljeni proizvodi od mesa zauzimaju važno mesto, kako u proizvodnji, tako i u ishrani stanovništva u Srbiji. Cilj ovog rada bio je razvijanje metode za istovremenu analizu (identifikaciju i kvantifikaciju) benzo[a]antracena, hrizena, benzo[b]fluorantena i benzo[a]pirena u dimljenom mesu i dimljenim proizvodima od mesa u skladu sa zahtevima Pravilnika (*Službeni glasnik RS,* br. 29/14) koji je stupio na snagu 13. marta 2014.

Materijal i metode

Reagensi i ostali materijali

Svi korišćeni rastvarači bili su HPLC čistoće. U toku eksperimentalnog rada korišćeni su: acetonitril (Sigma Aldrich, Germany), metilen-hlorid (J.T. Baker, USA), n-heksan (Sigma Aldrich, Germany), voda HPLC čistoće (Sigma Aldrich, Germany), smeša za sušenje (poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide), cross-linked) (Sigma Aldrich, Germany), blanko mast (Oma's SCHMALZ, Schachinger, Germany), glass Fiber Filter _ Cellulose (Dionex, 19.8 mm, 100 PCS), mega SPE kolonice (punjene sa 5 g silika faze, 20 mL) (Phenomenex, USA), PTFE filter (Whatman) veličine pora 1 µm.

Standardi benzo[a]antracena (BaA), hrizena (CHR), benzo[b]fluorantena (BbF) i benzo[a]

Tabela 2. Sadržaji pojedinačnih PAH4 jedinjenja (µg/kg) na različitim MDK nivoima obogaćenosti blanko uzoraka masti.

Table 2. The contents of individual PAH4 compounds (µg/kg) at different MRL levels of enriched blank samples of fat.

MDK/MRL	Sadržaj (µg/kg)/Content (µg/kg)				
	BaA	CHR	BbF	BaP	suma PAH4/ *PAH4 sum*
0,25	0,75	0,75	1	0,5	3
0,5	1,5	1,5	2	1	6
1	3	3	4	2	12
1,5	4,5	4,5	6	3	18

pirena (BaP) bili su analitičke čistoće proizvođača Dr. Ehrenstorfer, Germany, kupljeni u čvrstom stanju. Standardni rastvori pravljeni su rastvaranjem čvrstih supstanci u acetonitrilu.

Priprema uzoraka za HPLC analizu i HPLC analiza

Ubrzana ekstrakcija pomoću rastvarača (Accelerated solvent extraction, ASE)

S obzirom na činjenicu da ne postoje uzorci dimljenih proizvoda od mesa koji ne sadrže PAH jedinjenja, blanko uzorci masti su korišćeni za obogaćivanje PAH jedinjenjima. Obogaćeni blanko uzorci masti ubrzano su ekstrahovani n-heksanom na aparatu ASE 200 Dionex (Sunnyvale, USA), na temperaturi od 100 °C i pritisku od 10 MPa, tokom dva sukcesivna ciklusa (statično vreme – 10 min, vreme čišćenja – 120 s). Uzorci masti su obogaćivani na različitim MDK nivoima (0,5 MDK, 1 MDK i 1,5 MDK). MDK za sumu PAH4 jedinjenja od 1. septembra 2014. godine iznosi 12 µg/kg, a za benzo[a]piren 2 µg/kg (tabela 1). U tabeli 2 dati su sadržaji pojedinačnih PAH jedinjenja, na različitim MDK vrednostima, na kojima su obogaćivani blanko uzorci masti.

Ekstrakcija na čvrstoj fazi (Solid phase extraction, SPE)

Ekstrakcija na čvrstoj fazi je korišćena kao postupak za uklanjanje molekula lipida iz ispitanih uzoraka. Prečišćavanje je urađeno pomoću mega SPE kolonica, zapremine 20 mL, koje sadrže 5 g silika faze. Od pripremljenog uzorka nakon ASE ekstarkcije uzeto je 1 ml rastvora i naneto na kolonu, nakon ispiranja i kondicioniranja mega SPE kolonice (*Moret & Conte*, 2002). PAH4 smeša se eluira sa smešom n-heksan / dihlormetan 70:30 (v/v). Rastvarač iz eluata je uparen u struji azota na 40 °C, a suvi ostatak je rekonstituisan u 500 µl acetonitrila.

HPLC analiza

Pripremljen rastvor PAH4 jedinjenja nakon ekstrakcije na čvrstoj fazi, analiziran je visokoefikasnom tečnom hromatografijom koristeći fluorescentni detektor (High-Performance Liquid Chromatographic, Fluorescence detector, HPLC/FL). HPLC uređaj (Shimadzu, Japan) se sastoji od pumpe (Solvent Delivery Module LC-20AB), autosamplera (SIL-20A/20AC), peći (Column Oven CTO-20°/20AC) i fluorescentnog detektora (Spectrofluorometric detector RF-10Ax). U toku rada korišćene su različite kolone i biće specificirane u delu rezultati i diskusija.

Rezultati i diskusija

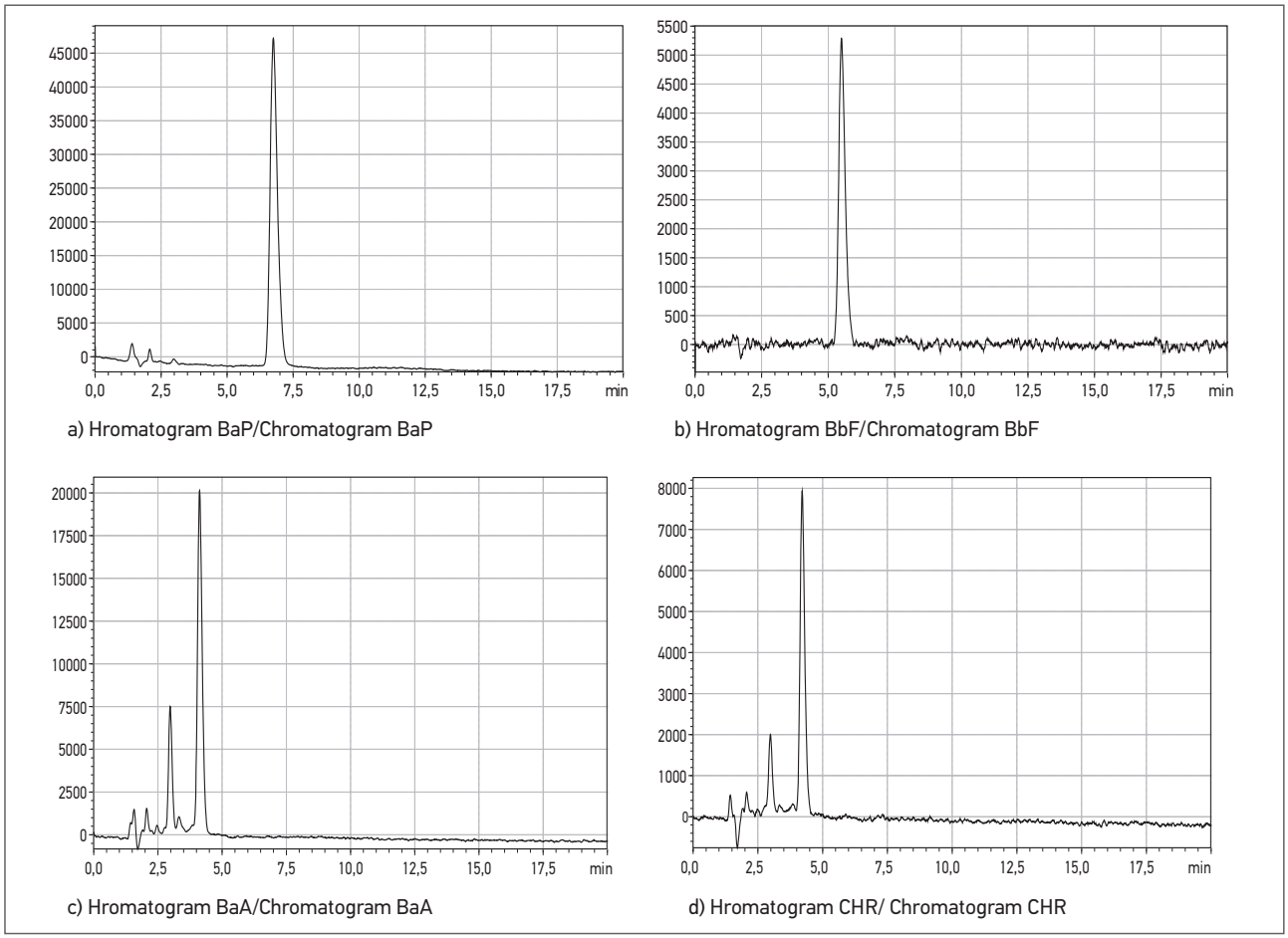
Rezultati razvijanja metode za određivanje PAH4 jedinjenja u dimljenom mesu i dimljenim proizvodima od mesa prikazani su u vidu hromatograma uz odgovarajuću diskusiju. Pored hromatograma dati su uslovi HPLC analize pod kojima su dobijeni dati hromatogrami.

HPLC analiza-1

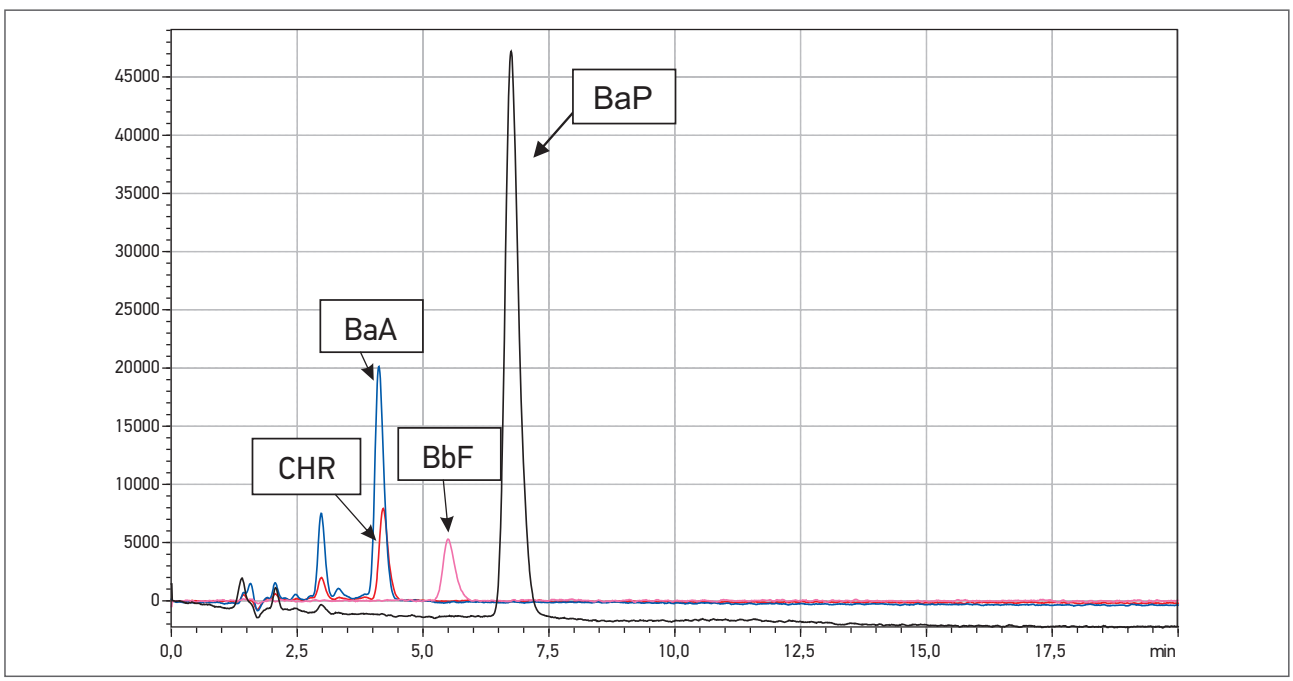
HPLC analiza-1 izvođena je pri uslovima koji su prikazani u tabeli 3.

Tabela 3. Uslovi pri kojima je izvođena HPLC analiza-1
Table 3. Conditions in which the HPLCanalysis-1 was performed

***Kolona/*Column**	**Pathfinder AS, Silica 100, 5.0 µm RP, 150 x 4,6 mm**
Vreme trajanja analize/ Duration of the analysis (min)	10
Injektovana zapremina/ Injected volume (µl)	50
Protok mobilne faze/ Flow of mobile phase (ml/min)	1,2
Pritisak/pressure (MPa)	0,0–35,0
*Talasne dužine (*nm*) (ekscitacija/emisija)*/ Wavelengths (nm) (excitation /emission)	BaA i CHR – 275/385, BaP – 260/410, BbF – 256/446
*Temperatura peći/*Oven temperature (ºC)	25–85
*Mobilna faza/*Mobile phase	ACN



Slika 2. Hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-1
Figure 2. The chromatograms of individual PAH4 compounds at the conditions of HPLC analysis-1



Slika 3. Poređenje hromatograma BaP, BbF, BaA i CHR pri uslovima HPLC analize-1
Figure 3. Comparison of chromatograms BaP, BbF, BbA and CHR in conditions of HPLC analysis-1

Na slici 2 prikazani su hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-1, dok je na slici 3 prikazano poređenje hromatograma BaP, BaF, BaA i CHR.

Na osnovu dobijenih hromatograma pojedinačnih jedinjenja (slika 2), vidi se da su pod primenjenim uslovima HPLC analize-1, pikovi zadovoljavajućeg intenziteta, kao i da su retenciona vremena svih jedinjenja manja od 10 minuta. Međutim, poređenjem hromatograma ovih jedinjenja (slika 3) uočava se da se pikovi BaA i CHR preklapaju, što ukazuje na to da se ova dva jedinjenja u smeši pod primenjenim uslovima HPLC analize ne mogu razdvojiti i kvantifikovati. Iz tih razloga neophodno je bilo promeniti uslove HPLC analize u cilju pojedinačne identifikacije i kvantifikacije PAH4 jedinjenja.

HPLC analiza-2

HPLC analiza-2 izvođena je pri uslovima koji su prikazani u tabeli 4.

Na slici 4. prikazani su hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-2, dok je na slici 5. prikazano poređenje hromatograma BaP, BbF, BaA i CHR.

Promenom mobilne faze, tj. uvođenjem polarnije mobilne faze (ACN/H_2O, 70/30, v/v) u odnosu na HPLC analizu-1, dobijaju se znatno lošiji rezultati, koji se ogledaju u nemogućnosti indentifikacije BaA i BbF (slike 4a, 4b). Takođe, pik BaP je niskog inteziteta (slika 4c) i preklapa se sa pikom CHR (slika 5). Neustaljenost bazne linije je, takođe, nedostatak koji se javlja primenom gore navedenih uslova. Na osnovu dobijenih rezultata može se zaključiti da smeša acetonitrila i vode nije dobro odabrana mobilna faza, pri datim HPLC uslovima analize. Iz tih razloga acetonitril je ponovo korišćen kao mobilana faza, ali je promenjen protok i temperatura peći kao što je prikazano u uslovima HPLC analize-3.

HPLC analiza-3

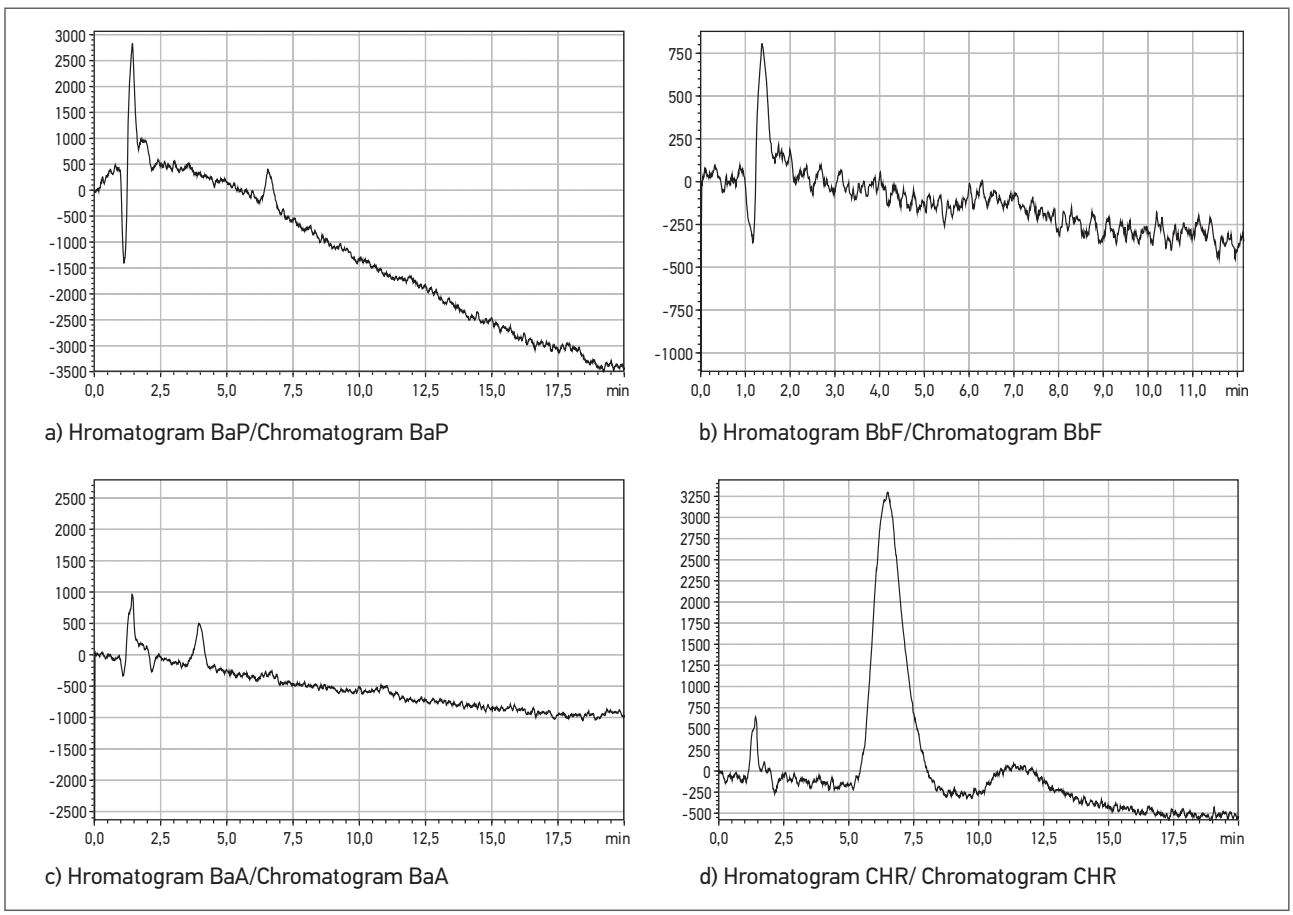
HPLC analiza-3 izvođena je pri uslovima koji su prikazani u tabeli 5.

Na slici 6 prikazani su hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-3, dok je na slici 7 prikazano poređenje pojedinačnih hromatograma BaP, BbF, BaA i CHR.

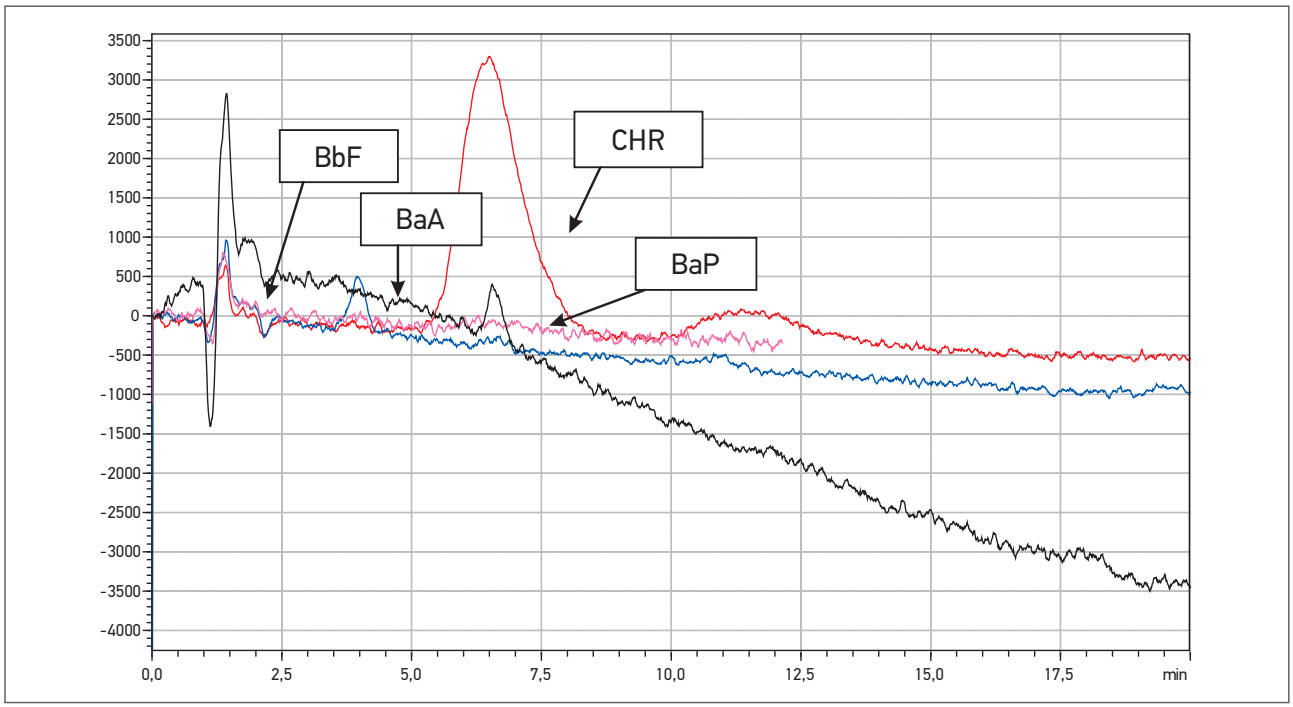
Analizom dobijenih hromatograma pojedinačnih PAH4 jedinjenja može se zaključiti da su intenziteti pikova na zadovoljavajućem nivou. U poređenju sa hromatogramima dobijenim pod uslovima HPLC analize-1 u kojoj je korišćena ista mobilna faza, primećuje se sa su retenciona vremena pomerena ka višim vrednostima (slika 6), što je posledica smanjenog protoka mobilne faze. Upoređivanjem hromatograma sva četiri PAH jedinjenja, primećuje se da se pikovi koji potiču od BaA i CHR preklapaju, što onemogućava njihovu identifikaciju i kvantifikaciju (slika 7). Iz svega navedenog može se zaključiti da uslovi HPLC analize-3 nisu odgovarajući za uspešnu analizu PAH4 jedinjenja, pa je u skladu sa tim bilo neophodno dalje menjati uslove analize.

Tabela 4. Uslovi pri kojima je izvođena HPLC analiza-2
Table 4. Conditions in which the HPLC analysis-2 was performed

***Kolona/*Column**	**Pathfinder AS, Silica 100, 5.0 µm RP, 150 x 4,6 mm**
Vreme trajanja analize/ Duration of the analysis (min)	10
Injektovana zapremina/ Injected volume (µl)	50
Protok mobilne faze/ Flow of mobile phase (ml/min)	1,2
Pritisak/pressure (MPa)	0,0–35,0
*Talasne dužine (*nm*)* (ekscitacija/emisija)/ Wavelengths (nm) (excitation / emission)	BaA i CHR – 275/385, BaP – 260/410, BbF – 256/446
*Temperatura peći/*Oven temperature *(*ºC)	25–85
*Mobilna faza/*Mobile phase	ACN/H_2O (70/30, v/v)



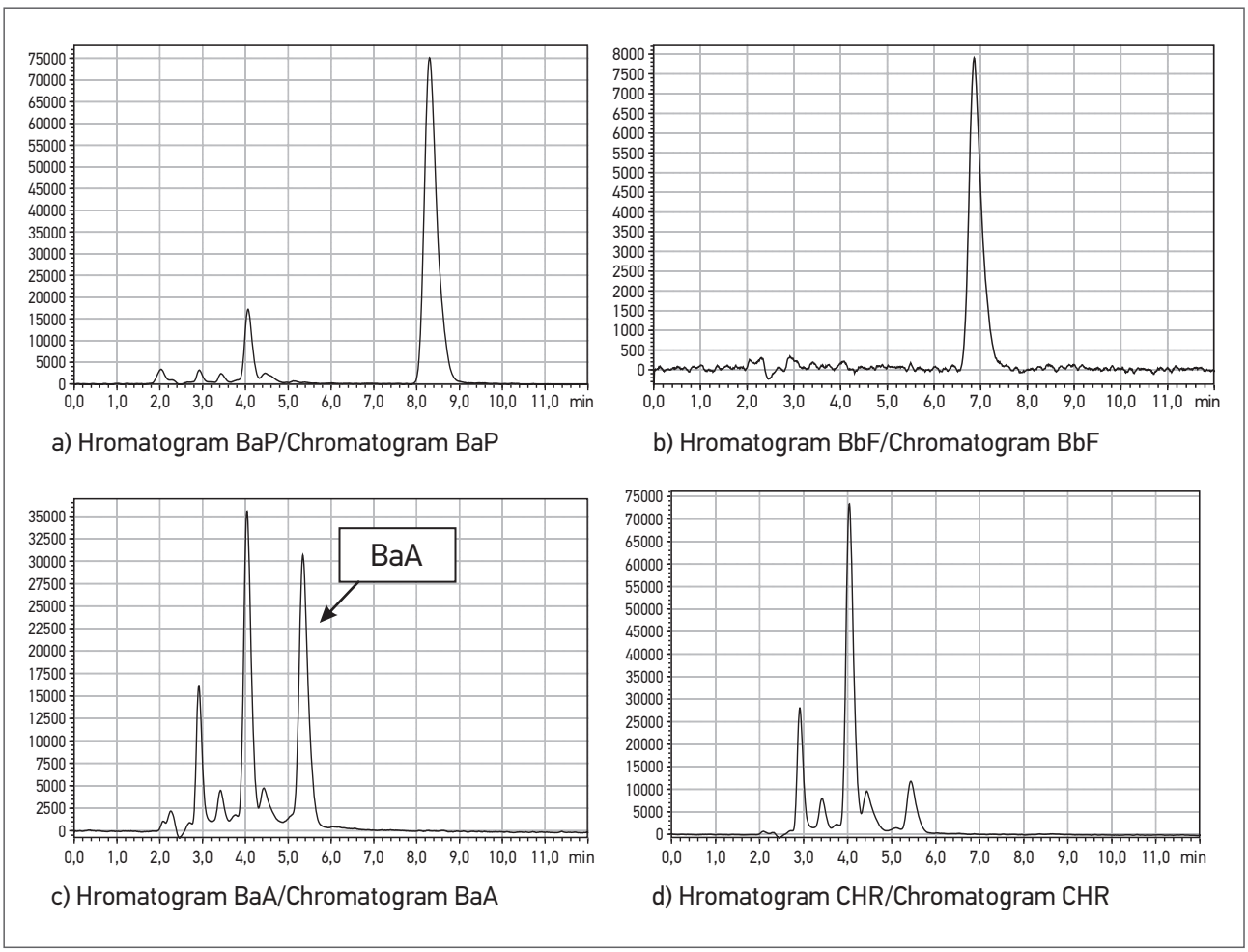
Slika 4. Hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-2
Figure 4. The chromatograms of individual PAH4 compounds at the conditions of HPLC analysis-2



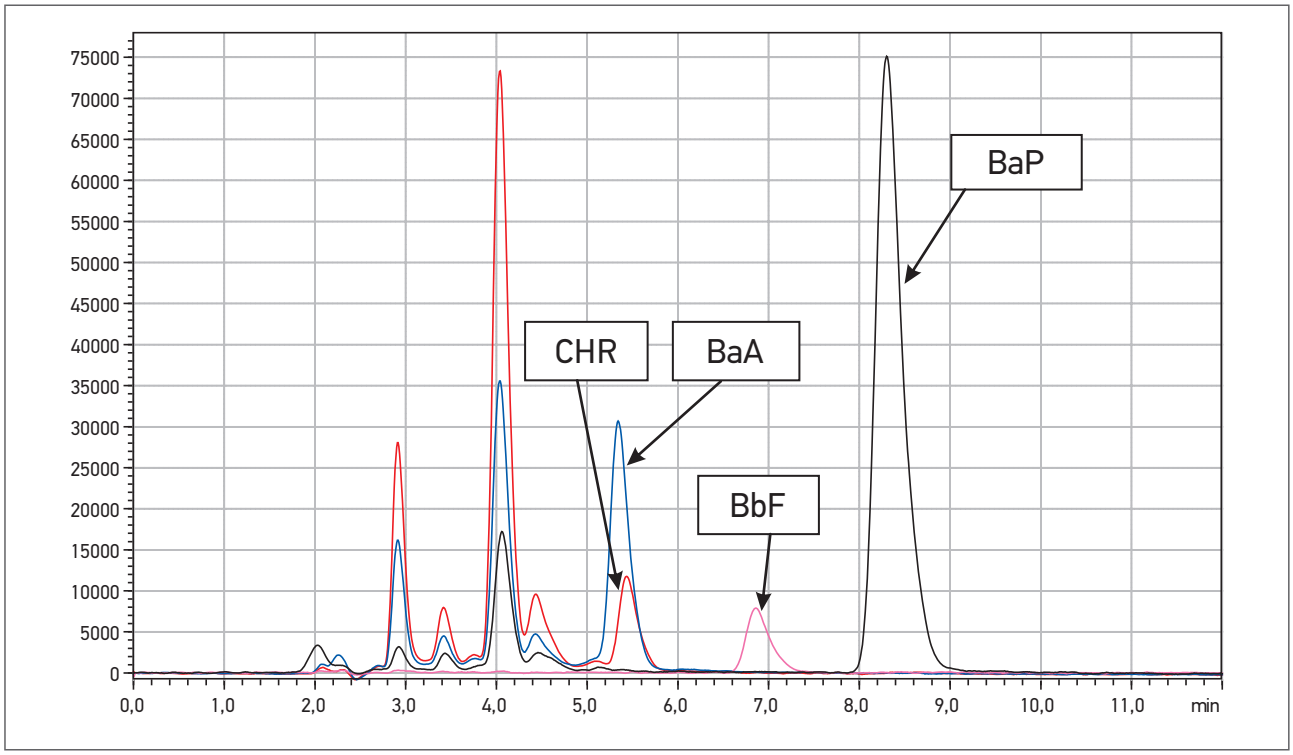
Slika 5. Poređenje hromatograma BaP, BbF, BaA i CHR pri uslovima HPLC analize-2
Figure 5. Comparison of chromatograms BaP, BbF, BbA and CHR in conditions of HPLC analysis-2

Tabela 5. Uslovi pri kojima je izvođena HPLC analiza-3
Table 5. Conditions in which the HPLC analysis-3 was performed

***Kolona/*Column**	**Pathfinder AS, Silica 100, 5.0 µm RP, 150 x 4,6 mm**
Vreme trajanja analize/ Duration of the analysis (min)	10
Injektovana zapremina/ Injected volume (µl)	50
Protok mobilne faze/ Flow of mobile phase (ml/min)	0,8
Pritisak/pressure (MPa)	0,0–35,0
*Talasne dužine (*nm*) (ekscitacija/emisija)/* Wavelengths (nm) (excitation / emission)	BaA i CHR – 275/385, BaP – 260/410, BbF – 256/446
*Temperatura peći/*Oven temperature *(*ºC)	35–85
*Mobilna faza/*Mobile phase	ACN



Slika 6. Hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-3
Figure 6. The chromatograms of individual PAH4 compounds at the conditions of HPLC analysis-3



Slika 7. Poređenje hromatograma BaP, BbF, BaA i CHR pri uslovima HPLC analize-3.
Figure 7. Comparison of chromatograms BaP, BbF, BbA and CHR in conditions of HPLC analysis-3

HPLC analiza-4

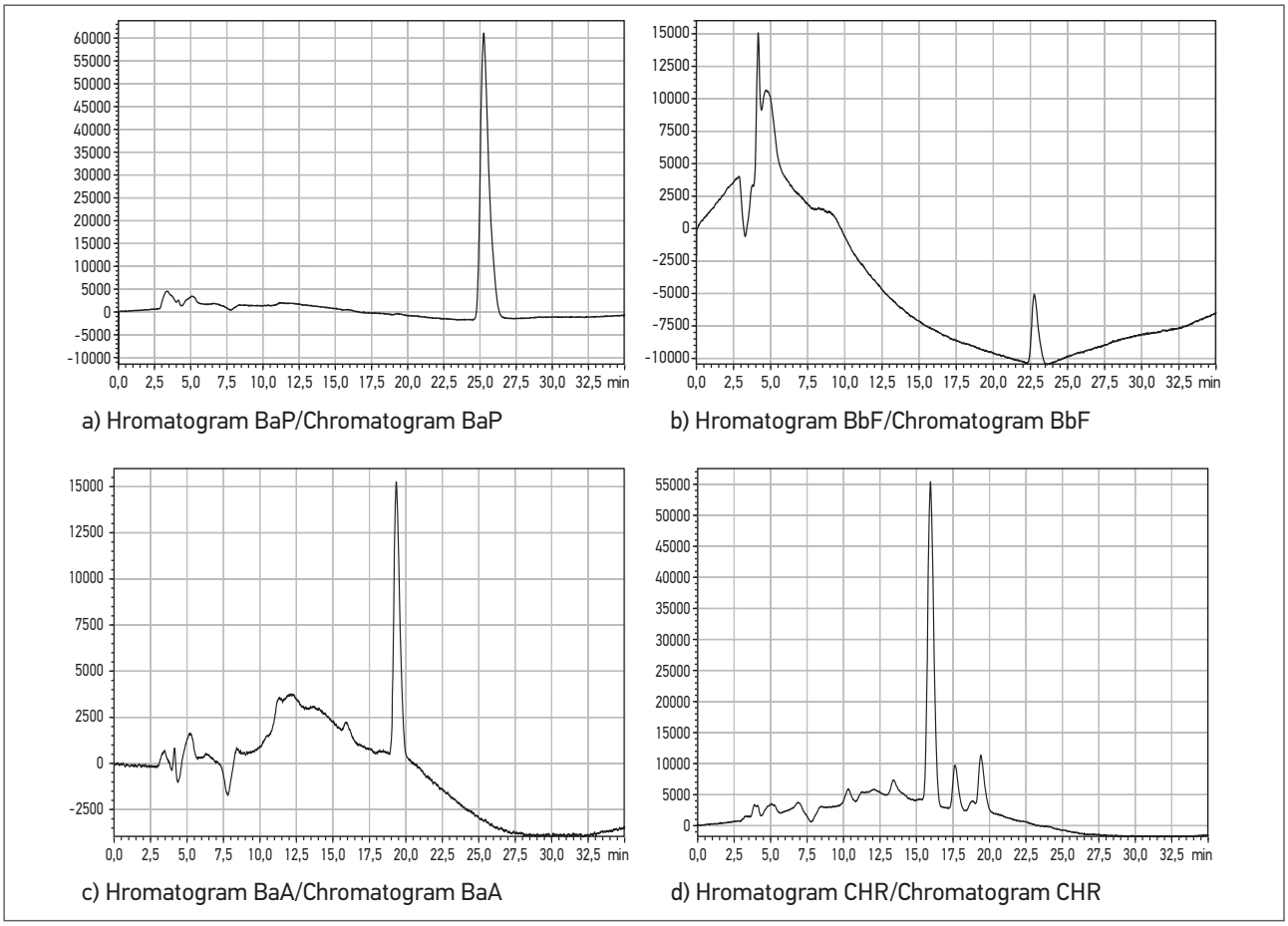
HPLC analiza-4 izvođena je pri uslovima koji su prikazani u tabeli 6.

Na slici 8 prikazani su hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-4, dok je na slici 9 prikazano poređenje hromatograma BaP, BbF, BaA i CHR.

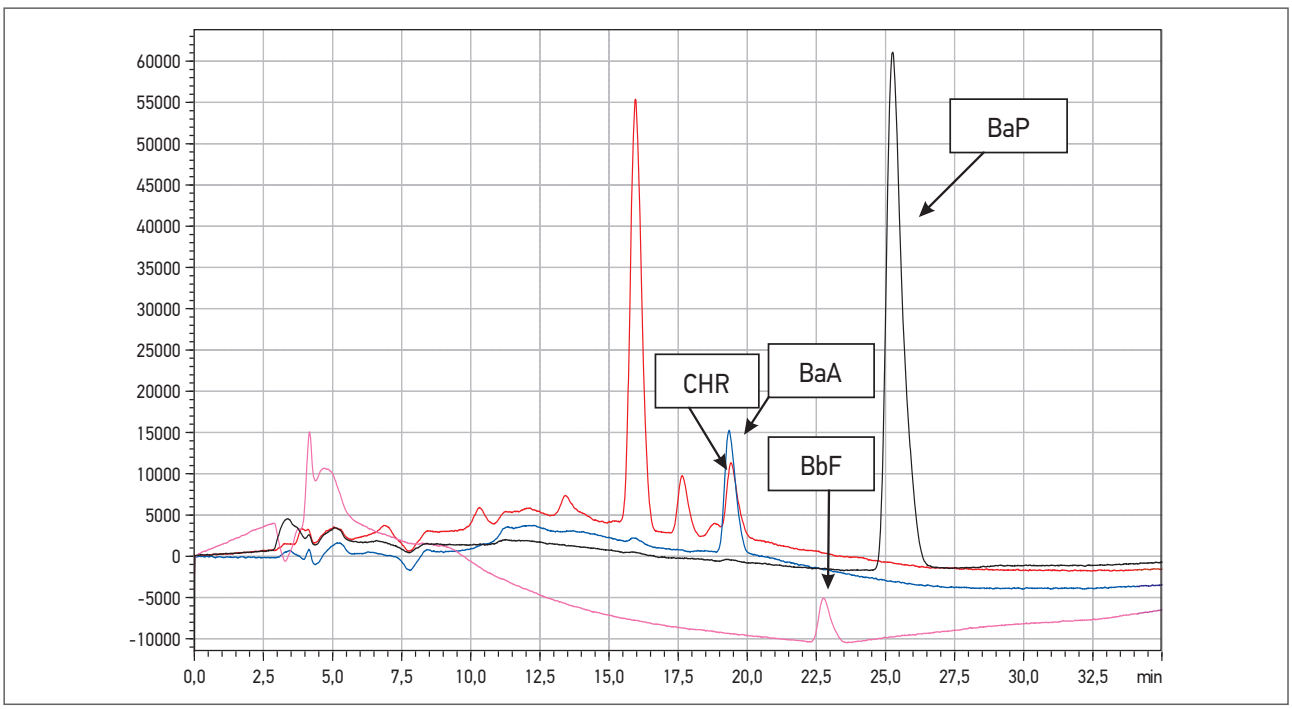
Povećanje polarnosti mobilne faze (A (30%) − H_2O/MeOH, 20/80, v/v; B (70%) − ACN) i smanjenje protoka (0,65 ml/min) uz korišćenje iste kolone nije rezultiralo do razdvajanja željenih jedinjenja (slika 9). Zato je bilo potrebno promeniti kolonu i izabrati takve uslove koje će omogućiti uspesno razdvajanje BaA, BbF, CRH i BaP.

Tabela 6. Uslovi pri kojima je izvođena HPLC analiza-4
Table 6. Conditions in which the HPLC analysis-4 was performed

***Kolona*/Column**	**Pathfinder AS, Silica 100, 5.0 µm RP, 150 x 4,6 mm**
Vreme trajanja analize/ Duration of the analysis (min)	30
Injektovana zapremina/ Injected volume (µl)	50
Protok mobilne faze/ Flow of mobile phase (ml/min)	0,65
Pritisak/pressure (MPa)	0,0–35,0
*Talasne dužine (*nm*) (ekscitacija/emisija)*/ Wavelengths (nm) (excitation / emission)	BaA i CHR – 275/385, BaP – 260/410, BbF – 256/446
Temperatura peći/Oven temperature (ºC)	35–85
Mobilna faza/Mobile phase	A (30%) – H_2O/MeOH (20/80, v/v), B (70%) – ACN



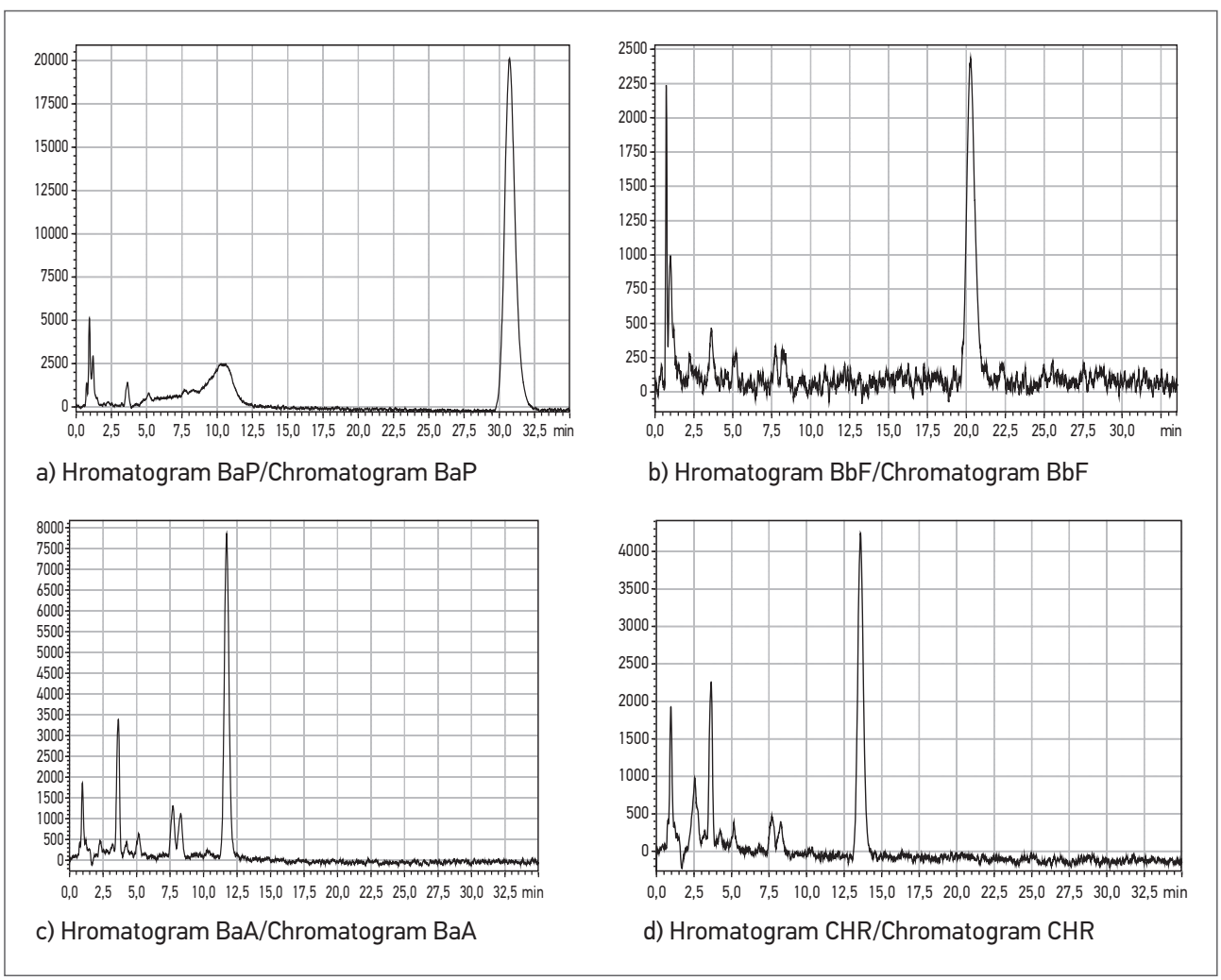
Slika 8. Hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-4
Figure 8. The chromatograms of individual PAH4 compounds at the conditions of HPLC analysis-4



Slika 9. Poređenje hromatograma BaP, BbF, BaA i CHR pri uslovima HPLC analize-4
Figure 9. Comparison of chromatograms BaP, BbF, BbA and CHR in conditions of HPLC analysis-4

Tabela 7. Uslovi pri kojima je izvođena HPLC analiza-5
Table 7. Conditions in which the HPLC analysis-5 was performed

***Kolona/*Column**	**Envirosep PP 5 µm PP, LC column 125 × 4.6 mm**
Vreme trajanja analize/ Duration of the analysis (min)	35
Injektovana zapremina/ Injected volume (µl)	50
Protok mobilne faze/ Flow of mobile phase (ml/min)	1,2
Pritisak/pressure (MPa)	0,0–35,0
*Talasne dužine (*nm*) (ekscitacija/emisija)*/ Wavelengths (nm) (excitation / emission)	BaA i CHR – 275/385, BaP – 260/410, BbF – 256/446
*Temperatura peći/*Oven temperature (ºC)	25–85
*Mobilna faza/*Mobile phase	ACN/H_2O (70/30, v/v)



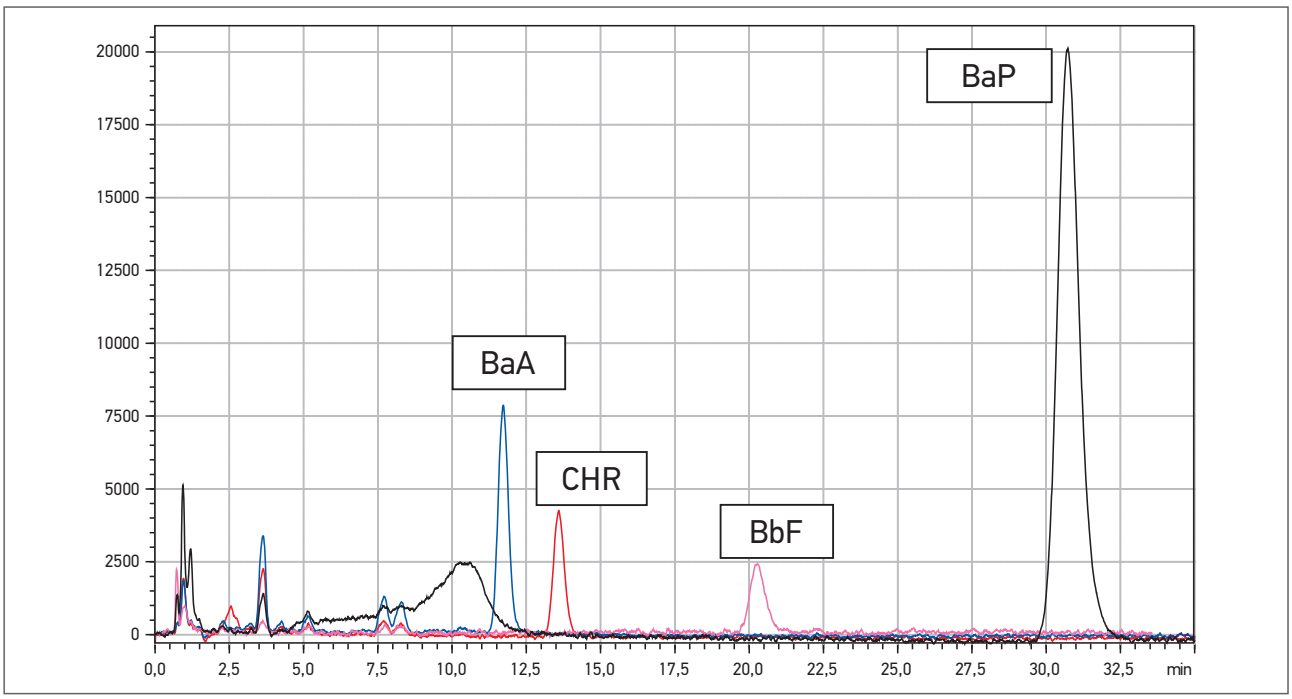
Slika 10. Hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-5
Figure 10. The chromatograms of individual PAH4 compounds at the conditions of HPLC analysis-5

HPLC analiza-5

HPLC analiza-5 izvođena je pri uslovima koji su prikazani u tabeli 7.

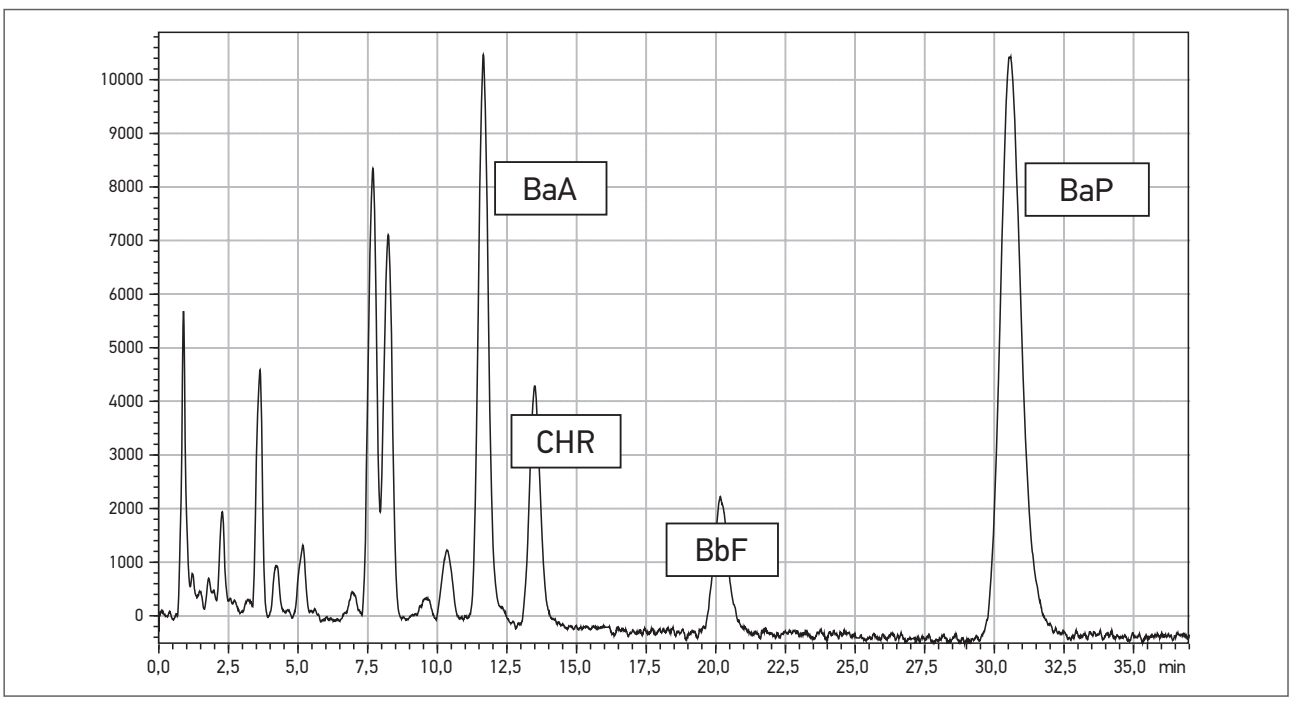
Na slici 10 prikazani su hromatogrami pojedinačnih PAH4 jedinjenja pri uslovima HPLC analize-5, dok je na slici 11 prikazano poređenje hromatograma BaP, BbF, BaA i CHR.

Poređenjem hromatograma pojedinačnih standarda BaA, CHR, BbF i BaP (Slika 11), koji su dobijeni pri uslovima HPLC analize-5, uočava se da se



Slika 11. Poređenje hromatograma BaP, BbF, BaA i CHR pri uslovima HPLC analize-5

Figure 11. Comparison of chromatograms BaP, BbF, BbA and CHR in conditions of HPLC analysis-5



Slika 12. Hromatogram smeše PAH4 jedinjenja pri uslovima HPLC analize-5

Figure 12. The chromatogram of the mixture of PAH4 compounds in conditions of HPLC analysis-5

pikovi ovih jedinjenja ne preklapaju. Analizom smeše PAH4 jedinjenja pri istim uslovima analize (slika 12), analizirana jedinjenja su uspešno razdvojena i mogu se kvantifikovati. Uslovi HPLC analize-5 se razlikuju od ostalih, pre svega, po koloni (Envirosep PP). Kolona Envirosep PP uspešno razdvaja BaA i CHR (slika 12), što se u prethodnim uslovima HPLC analize nije moglo postići.

Na osnovu dobijenih rezultata može se zaključiti da se BaA, CHR, BbF i BaP, pri uslovima HPLC analize-5, uspešno mogu identifikovati i kvantifikovati (Slika 12.) i da se ovi uslovi HPLC analize mogu primeniti u procesu validacije metode za određivanje PAH4 jedinjenja u dimljenim proizvodima od mesa.

Zaključak

U cilju određivanja PAH4 jedinjenja u dimljenom mesu i dimljenim proizvodima od mesa, tokom procesa razrade metode zaključeno je da se benzo[a]antracena, hrizena, benzo[b]fluorantena i benzo[a]pirena (PAH4 jedinjenja) mogu uspešno izolovati iz uzoraka, a zatim identifikovati i kvantifikovati. Razrada ove metode je neophodan korak u procesu validacije metode.

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Determination of PAH4 compounds in smoked meat and meat products – elaboration of the method

Đinović-Stojanović Jasna, Stišović Jelena, Popović Aleksandar, Nikolić Dragica, Janković Saša

S u m m a r y: Smoked meat and smoked meat products represent a significant part of the human diet in Serbia. The process of smoking, i.e. wood combustion, is one of the most important sources of polycyclic aromatic hydrocarcarbons (PAHs). Considering the carcinogenic and mutagenic properties of some PAHs, these compounds have been in the spotlight of scientific interest. The EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM Panel) reviewed the available data on occurrence and toxicity of PAHs in food. In 2008, the CONTAM Panel concluded that benzo[a]pyrene is not a suitable indicator for the occurrence of PAHs in food. Based on the currently available data relating to occurrence and toxicity, the CONTAM Panel concluded that the sum of benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b]fluoranthene (PAH4) are the most suitable indicators of PAHs in food.

These proposals have become part of the legislation both of EU and Serbia. From 1st September 2014, maximum residue limits (MRL) both for benzo[a]pyrene (2 µg/kg) and sum of PAH4 compounds (12 µg/kg), in smoked meat and meat products, were defined by the legislation of Serbia, which is in accordance with EU regulation.

In this paper, the method has been developed for the determination of benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b] fluoranthene (PAH4 compounds) in smoked meat and smoked meat products. Accelerated solvent extraction (ASE) was used for extraction of lipids and lipophilic compounds. Solid Phase Extraction (SPE) was used in order to remove lipids from analysed samples. High-performance liquid chromatographic with fluorescence detection (HPLC-FL) was applied for identification and quantification of benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b]fluoranthene. Different conditions of HPLC-FL analysed were applied (mobile phase, HPLC column, oven temperature, flow) in order to achieve optimal conditions for qualitative and quantitative analysis of PAH4 compounds.

Key words: *PAH4 compounds, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene.*

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Rad prihvaćen: 25.11.2014.

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