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Review paper

Association between red meat consumption and cancer risk

Marija Boskovic[1], Milan Z. Baltic[1]

A b s t r a c t: Cancer is leading cause of mortality worldwide. It is assumed that cancer risk is mainly affected by environmental factors including diet habits. Among other food, it is considered that red meat consumption is linked to increased risk of cancer. Formation of mutagens, heterocyclic aromatic amines, and polycyclic aromatic hydrocarbons, mainly depend on cooking methods and is the highest in pan fried and barbecued meat. Fat, N-nitroso compounds and haeme iron which are also found in meat and meat products are considered to influence the occurrence of cancer. Colorectal cancer is the most often associated with red meat consumption, but studies show that meat eating may increase the risk for other types of cancer including breast cancer, gastric cancer, prostate cancer, pancreatic cancer, lung cancer, laryngeal cancer and bladder, kidney, and endometrial tumours.

Keywords: *red meat, PAH, HAA, colorectal cancer, diet and lifestyle habits.*

Introduction

Changes in dietary habits and lifestyle have resulted in civilisation diseases including coronary heart disease, obesity, hypertension, type 2 diabetes, cancers, autoimmune disease and osteoporosis, among which, cancer is leading cause of mortality worldwide (*Carrera-Bastos et al*., 2011; *Stewart and Wild*, 2014). As reported by a WHO (World Health Organization) update from 2014, approximately 14 million new cases of cancer and 8.2 million cancer-related deaths were reported in 2012 (*Stewart and Wild*, 2014). According to the same report, it is expected in the next two decades that cancer cases per year will rise from 14 million in 2012 to 22 million (*Stewart and Wild*, 2014). The most frequent types of cancer which cause mortality are lung (1.59 million deaths), liver (745 000 deaths) and stomach (723 000 deaths) cancer followed by colorectal (694 000 deaths) breast (521 000 deaths) and oesophageal cancer (400 000 deaths). Serbia is a developing country where stress, pollution, irregular diet and bad eating habits, lack of physical activity and insufficient sleep are part of everyday life. All of this has led to increasing cancer prevalence in Serbia. WHO data shows that cancer mortality rate in Serbia was 160.1 cases per 100,000 population in 2000, and 170.8 cases per 100,000 population in 2012 (*Stewart and Wild*, 2014). In 2012, only Zimbabwe, Hungary, Armenia and Mongolia had higher cancer mortality rates than Serbia. Cancer risk is influenced 5% by genetic factors, while the remaining impact is due to environmental factors, of which 30 to 35% represents diet (*Baena Ruiz and Salinas Hernández*, 2014). Meat, especially red meat, and meat products are the types of foods most often associated with some types of cancer, which highlights the need to discuss the role of meat in cancer aetiology. In October 2015, the Working Group from the International Agency for Research on Cancer (IARC) classified consumption of processed meat as "carcinogenic to humans" (Group 1) (*International Agency for Research on Cancer*, 2015). This classification was based on their being sufficient evidence to associate meat product (i.e. processed meat) consumption with colorectal cancer as well as with stomach cancer. Taking into account substantial epidemiological data showing a positive association between consumption of red meat and colorectal cancer, the IARC classified consumption of red meat as "probably carcinogenic to humans" (Group 2A).

Carcinogens from red meat

Fat, heterocyclic aromatic amines (HAAs), polycyclic aromatic hydrocarbons (PAHs), N-nitroso compounds (NOc) and haeme iron are substances

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isolated from unprocessed or processed red meat which are considered to influence the occurrence of cancer (*Higgs*, 2000; *Williamson et al.*, 2005; *Ferguson*, 2010; *Corpet*, 2011; *Baena Ruiz and Salinas Hernández*, 2014; *Boskovic et al.,* 2015). It is also suggested that intake of red meat, as a high energy food, could have an impact on obesity, which presents a major risk factor for cancer (*World Cancer Research Fund/American Institute for Cancer Research,* 2007; *Ferguson*, 2010). Although the exact mechanism by which meat influences the increased cancer risk is not completely known, there are a few proposed hypotheses. It is supposed that intake of fat, responsible for the high energy density of meat, has an important role in carcinogenesis because it increases insulin resistance, and impacts on higher production of secondary bile acids, which act as aggressive surfactants for the mucosa (*Ferguson*, 2010; *Corpet,* 2011; *Baena Ruiz and Salinas Hernández*, 2014).

NOc are produced by the reaction of nitrite and nitrogen oxides with secondary amines and N-alkylamides (*Lijinsky*, 1999; *Ferguson*, 2010). NOc can be formed endogenously after consumption of red and processed meat or could be found in some processed meats, although some authors suggested that N-nitrosamines produced from additives in processed meat have low carcinogenic potential because of small amounts in which they are found (*Higgs*, 2000; *Demeyer et al.*, 2008; *Santarelli et al.*, 2008; *Ferguson*, 2010). It was showed that haeme iron may catalyse the formation of NOc from natural precursors in the gastrointestinal tract (*Santarelli et al.*, 2008). Haeme iron may play a critical role in understanding links between red meat and particularly colorectal cancer risk, since the higher haeme iron content is one of the major differences between red meats and white meats including poultry and fish, consumption of which is not associated with increased risk of cancer (*Egeberg*, 2013). Iron increases cell proliferation in the mucosa, through lipoperoxidation and/or cytotoxicity of faecal water (*Sesink et al.*, 1999; *Ferguson,* 2010). Furthermore, haeme iron may act as a prooxidant, and induce lipid oxidation and also cause DNA damage (*Tappel*, 2007; *Egeberg*, 2013). While some studies found iron to be carcinogenic metal, other authors consider that it is more probable that iron has a co- carcinogenic effect (*Huang*, 2003; *Ferguson*, 2010).

The method of cooking red meat also influences the risk of cancer (*Berjia et al.*, 2014). Although meat cooking, especially at high temperatures, reduces the risk of foodborne diseases, this practice causes formation of chemical compounds including carcinogens and mutagens (*Navarro et al.*, 2004; *WHO/FAO*, 2004; *Jägerstad and Skog*, 2005; *Badry*, 2010; *Aaslyng et al.*, 2013). The most common are HAAs and PAHs (*Shin et al.*, 2007; *Kimura et al.*, 2007; *Hogg*, 2007; *Larsson et al.*, 2009; *Ferguson*, 2010; *Berjia et al*., 2014; *Boskovic et al.*, 2015). These genotoxic substances act directly on DNA, cause point mutations, deletions, insertions and initiate carcinogenesis process for a number of cancers (*Baena Ruiz and Salinas Hernández*, 2014; *Baltic and Boskovic*, 2015; *Boskovic et al.*, 2015). Their formation depends on meat type, temperature, and method of cooking (barbecuing/grilling, frying/broiling, roasting/baking) (*Jägerstad and Skog*, 2005; *Badry*, 2010; *Aaslyng et al.*, 2013; *Berjia et al.*, 2014; *Boskovic et al.*, 2015). High amounts of 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine are found in very well cooked chicken and bacon, while 2-amino-1,7-dimethylimidazo [4,5-g] quinoxaline was found in very well done pan-fried beef and steak, and in beef gravy (*Ferguson*, 2010). A recent study conducted by *Berjia et al.* (2014) showed that barbecued meat is associated with the formation of the highest concentrations of HAAs and PAHs, followed by fried meat and roasted red meat. Time of cooking has a great role in the HAAs production. Thus meat cooked for extended period mainly at higher temperatures contains a higher concentration of HAAs, which is why very well cooked meat is associated with cancer risk (*Knize et al.,* 1994; *Navarro et al.*, 2004; *Sugimura et al.*, 2004; *Felton and Knize*, 2006; *Ni et al.,* 2008; *Ferguson*, 2010).

The type of marinade also affects the formation of HAAs in meat (*Joshi et al.*, 2015). Results obtained from some studies showed that use of Asian-type marinades reduces HAA formation, while BBQ (barbecue) sauce marinade increased formation of these compounds (*Nerurkar et al.* 1999; *Viegas et al.*, 2012).

Red meat and colorectal cancer

Colorectal cancer is the third most common cancer in men and the second most common cancer in women, but it is also the cancer type most often associated with red meat consumption (*Ishibe et al.,* 2002; *Le Marchand et al.,* 2002; *Butler et al.*, 2003; *Gunter et al.,* 2005; *Wu et al.,* 2006; *Cross et al.,* 2007; *Wyness et al.,* 2011*; Kim et al.,* 2013; *Berjia et al.,* 2014).

In the meta-analysis conducted by *Norat et al.* (2002) based on case-control and cohort studies published between 1973 and 1999, it was concluded that high consumption of red and processed meat was associated with an increased risk of colorectal

cancer, while total meat intake, including poultry intake, was not associated with increased cancer risk. In a meta-analysis involving almost 8,000 cases from 19 prospective studies, *Larsson and Wolk* (2006) found consistent associations between high consumption of red and processed meat and an increased colorectal cancer risk. In this meta-analysis, they also found that high intake of red and processed meat is most associated with an increase of rectal cancer, followed by distal and proximal colon cancer, respectively. The relationship between red and processed meat consumption and increase the risk of colorectal cancer was also reported by *Norat et al.* (2005). *Chan et al.* (2011) reported that consumption of red and processed meats, assessed separately, were associated with increased risk of colorectal and colon cancers, but no statistically significant association with rectal cancer was found. This finding was different than those observed when red and processed meats were combined into a single food item.

A meta-analysis of 14 prospective studies where 725,258 subjects and 7,743 cases of colorectal cancer were evaluated, during 5 to 20 years, found no association between red meat and colorectal cancer (*Cho and Smith-Warner*, 2004; *McNeill and Van Elswyk*, 2012). Also, findings from a prospective study conducted by *Oba et al.* (2006) in Japan showed no association between red meat consumption and colon cancer risk, while a high intake of processed meat was associated with increased risk. These studies indicate that fresh meat *per se* is not carcinogenic and that, as well as other types of cancer, the risk of colorectal cancer is mainly associated with the method of cooking and carcinogens produced during meat processing and preparation. Higher risks were observed for darkly browned surfaces produced by barbecuing or iron pan cooking at temperatures above 150°C, and this risk was explained due to the formation of HAAs, while there was no association found with roasted and boiled meat (*Navarro et al.*, 2004). The incidence of meat consumption also has an impact on risk of colorectal cancer. *Norat et al.* (2002) found that consumption of 120 g per day of red meat (compared with no consumption) was associated with a 24% increase in risk, while consumption of 30 g per day of processed meat (compared with no consumption) increased the risk by 36% (*Williamson et al.,* 2005). Results obtained from thirteen studies by *Sandhu et al.* (2001) showed that a daily increase of 100 g in total or red meat intake was associated with a significant 12 to 17% increase in colorectal cancer risk (*Williamson et al.,* 2005). Results from meta-analysis conducted by *Smolińska and Paluszkiewicz* (2010) showed that the frequency of red meat consumption is a crucial risk factor for colon and rectum carcinogenesis, even more important than the amount of consumed meat. In the same study, it was found that risk of rectal and colon cancer increased with consumption of red meat more than once daily and that red meat intake in amounts higher than 50 g day^{-1} could lead to colon cancer, while the dependence between red meat consumption of over 50 g daily and rectal cancer was not conclusive (*Smolińska and Paluszkiewicz*, 2010). Moreover, some studies showed that exposure to meat carcinogens increase the risk of colorectal cancer only in genetically susceptible individuals (*Le Marchand et al.*, 2002).

Apart from haeme iron, PAHs and HAAs discussed above, some findings suggest that changes in gut microbiota also influenced colorectal cancer risk. Bacterial fermentation induced by large amounts of undigested proteins and compounds formed from amino acids during bacterial metabolism caused changes in colon epithelial homeostasis which may be predisposing factors for colorectal cancer (*Kim et al.,* 2013). Further, some studies showed that meat from different origins had differing impacts on colorectal cancer. *Egeberg et al.* (2013) did not find an association between all red meat consumption and risk of colon cancer, but they observed that intake of higher amounts of beef and lamb is related to elevated colorectal cancer risk. This could be explained due to the higher amount of haeme iron in lamb and beef compared to other types of meat.

Red meat and other types of cancer

Breast cancer (*Cho et al.,* 2006; *Steck et al.*, 2007; *Larsson et al.*, 2009; *Berjia et al.*, 2014), gastric cancer (*Zhu et al.*, 2013) prostate cancer (*Cross et al.*, 2005; *Koutros et al., 2008*; *John et al.*, 2011; *Berjia et al.*, 2014), pancreatic cancer (*Anderson et al.*, 2002; *Li et al.,* 2007; *Stolzenberg-Solomon et al.*, 2007; *Berjia et al.*, 2014), lung cancer (*Dosil-Diaz et al.,* 2007), laryngeal cancer (*Oreggia et al.*, 2001; *Bosetti et al.,* 2002) as well as bladder, kidney, and endometrial tumours are also associated with meat intake (*Ferguson*, 2010; *Béjar et al.*, 2012; *Baena Ruiz and Salinas Hernández*, 2014). Scientific data about the association between meat consumption and breast cancer are inconsistent. Breast cancer is associated with some meat components including meat-derived haeme iron, NOc, HAAs and PAHs (*Cho et al.*, 2006; *Kabat and Rohan*, 2007; *Lauber and Gooderham*, 2007; *Kallianpur et al.*, 2008; *Larsson et al.,* 2009). It is important to mention that oestrogen receptor-positive breast tumours are etiologically different from receptor-negative tumours.

Red meat consumption could increase the risk, especially of hormone receptor-positive breast cancers. This was confirmed in Nurses' Health Study II which showed that a high intake of red meat by premenopausal women was associated with a significantly increased risk of ER+/PR+ breast cancer but not ER–/PR– breast cancer (*Cho et al.*, 2006; *Larsson et al.*, 2009). *Larsson et al.* (2009) conducted an epidemiologic study on 61,433 Swedish women to try to determine if there was any association of meat intake with incidence of breast cancer as defined by oestrogen receptor and progesterone receptor status. During 17.4 years, 2,952 incident cases of invasive breast cancer were determined, but statistical analyses showed that consumption of neither fresh red meat nor processed red meat had any significant association with risk of breast cancer. *Missmer et al.* (2002) also found no significant association between total meat, red or white meat consumption (all considered separately) and breast cancer risk. These results are in accordance with those of the NIH-AARP Diet and Health Study as well as other studies (*Fraser*, 1989; *Ambrosone et al.*, 1998; *Gertig, et al.,* 1999; *Missmer et al.*, 2002; *Larsson et al.*, 2009).

Along with smoking and drinking alcohol, consumption of red or processed meat is consider to be a predisposing factor for gastric cancer (*González et al.*, 2013). High levels of salt are used in the production of some processed meats, and there are data that salt and salt-rich foods probably increase the risk of gastric cancer (*D'Elia et al*., 2012; *González et al.*, 2013) which can explain the link between meat products and gastric cancer. Meta-analysis based on epidemiological studies showed that consumption of red and/or processed meat increased gastric cancer risk (*Zhu et al.*, 2013). However, the same authors noted that further investigation should be conducted in order to confirm the association, especially for red meat. Other authors did not find associated between red and processed meat consumption with increased risk of gastric cancer, but they reported increased risk for oesophageal squamous cell carcinoma (*Keszei et al.*, 2012).

In a population-based case-control study *Anderson et al.* (2002) did not find a significant association between pancreas cancer and grilled/barbecued or fried red meat intake, but when fried meat was consumed, the risk of cancer was elevated. These results are consistent with those reported by *Bosetti et al.* (2013), while *Polesel et al.* (2010) found that eating red meat is associated with pancreas cancer.

In studies carried by *Brennan et al.* (2000) and *Alavanja et al.* (2001), it was found that meat intake influenced the incidence of lung cancer. Contrary to these results, *Norat et al.*, (2005) found that consumption of pork meat has a positive effect on lung cancer. This result was not confined only to meat *per se*, but to the fact that this meat is often eaten with green leafy vegetables, and often tends to be consumed with red wine, which contains resveratrol and tannins and has a protective effect.

An association between red meat and laryngeal cancer was reported by *Oreggia et al.* (2001). They reported that red meat and total meat intake were associated with a strong increase in laryngeal cancer risk, but this effect disappeared after adjusting for total fat intake. In the same study, HAAs had an impact on laryngeal cancer risk, but no association with salted meat was reported, although some authors did find that connection and attributed it to the presence of nitrosamines (*Oreggia et al.*, 2001). This association between red or processed meat and risk of laryngeal cancer was confirmed in the study conducted by *Bosetti et al*. (2002).

Moreover, a meta-analysis of 15 prospective studies found no positive association between consumption of red or processed meat and prostate cancer risk (*Alexander et al.*, 2010).

Discussion and recommendations

Although consumption of red meat could be associated with some types of cancer, the fact that meat contains anticancer components which are also essential in human nutrition, such as selenium, zinc, omega-3 fatty acids, vitamins B_6, B_{12}, D and folic acid, indicates that meat should not be eliminated from the human diet, but meat intake should be reduced to the recommended level of up to 500g per week (cooked weight) and little, if any, processed meat (***World Cancer Research Fund/ American Institute for Cancer Research*****,** 2007; *Chan et al.*, 2011; *Pérez-Cueto and Verbeke*, 2012; ***World Cancer Research Fund***, 2012; *Baena Ruiz and Salinas Hernández*, 2014; *Baltic and Boskovic*, 2015). Moreover, some peptides obtained from meat and fish possess anti-cancer properties, inhibit cell proliferation and have cytotoxic effects against tumour cells (*Shahidi and Zhong*, 2008; *Ryan et al.*, 2011; *Najafian and Babji*, 2012; *Udenigwe and Aluko*, 2012; *Baltic et al*., 2014). Red meat also contains other compounds with beneficial effects on human health, including carnitine, coenzyme Q10 and creatinine (*Schmid*, 2009). Moreover, red meat, especially beef, has a better ratio of n6:n3 fatty acids and more vitamins (A, B_6, and B_{12}) and mineral substances (Fe and Zn) than does white meat

(*Oostindjer et al.*, 2014). Many authors agree that red meat is not carcinogenic *per se* and that risk of cancer is influenced by cooking practices and dietary patterns in which meat is consumed (*Biesalski*, 2005; *Ferguson*, 2010). Cooking methods such as barbecuing and frying should be avoided, as well as excessive external brownness or charring of meat during preparation, while baking, boiling, and stewing do not produce high levels of carcinogens (HAAs and PAHs) (*Anderson et al.*, 2002). Also, it was established that not only nitrate but also other chemical additives used in meat products to extend shelf life have adverse effects on human health and there is a need for more natural additives, such as natural plant extracts. Plant extracts exhibit antimicrobial and antioxidative properties, have positive effects on meat safety and quality and also prolong shelf life (*Boskovic et al.*, 2013). Phytochemicals reduce the formation of nitrosamines in the human body when consumed simultaneously with meat products (*Chung et al.,* 2013; *Oostindjer et al.*, 2014). It is suggested that addition of garlic or curcumin have cancer protective effects (*Shu et al.*, 2010; *El-Bayoumy et al.*, 2011; *Oostindjer et al.*, 2014), which is why it could be beneficial if they can be added to a marinade or in meat dishes.

Since in general, civilisation diseases are influenced by different factors, eating and lifestyle habits should be considered as the main predisposing factors for various diseases, including cancer. People's habits and diets have changed during recent decades. It has been observed in various studies that individuals who consume high amounts of red or processed meat can also commonly consume more energy rich food products, including sugar-rich drinks and condiments, eat less vegetables, drink more alcohol, take less vitamins and are less physically active (*Alexander*, 2013; *Oostindjer et al*., 2014).

Conclusion

Although lately associated with number of diseases and conditions, because of the nutritional value of red meat, as well as the presence of minerals and vitamins, red meat still has a useful role in the human diet, and positively impacts on human health when it is consumed in moderate amounts after recommended cooking methods, and within the context of a well balanced diet.

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Effects of phytobiotics on Cobb broiler production results, meatiness and chemical composition

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A b s t r a c t: In order to achieve better results for fattening broilers, various additives can be added to feed, including phytobiotics (phytogenic additives). Phytobiotics protect young broilers' health based on the principles of competitive exclusion and improved usability of nutrients, growth and feed efficiency. Due to the importance and actuality of this topic, it is scientifically justified and interesting to examine the effects of using natural growth stimulators on the performance of intensively bred broilers, as well as the suitability of these compounds for nutritional, health and economic aspects of broiler production.

In this study, 120 Cobb 500 broilers were divided into two groups, one control without and one experimental with a mixture of phytobiotics in their feed. At the beginning of the study, all broilers were of equal body mass. In some phases of fattening and at the end (after 42 days), body mass and total gain of the broilers receiving phytobiotics were significantly higher in than control broilers ($p<0.05$ and $p<0.01$, respectively). Total consumption of feed for the whole fattening period was higher in the control than in the phytobiotic-recieving broilers. A better feed conversion rate was determined in the broilers receiving phytobiotics than in the control broilers ($P<0.05$). The carcass meatiness was also improved in the broilers receiving phytobiotics ($p<0.01$). There was no significant difference between control and phytobiotic-receiving broilers with regard to meat chemical composition.

Keywords: *phytobiotics, production performance, feed conversion, meatiness, meat chemical composition.*

Introduction

The use of biologically active compounds as replacements for antibiotics has now been a current topic for some time. Instead of antibiotics and other drugs, contemporary animal feed production strives to use bioactive ingredients to maintain health and welfare and reduce stress effects from the environment on the immune systems and production results of farm animals in intensive production. Eubiosis in the digestive tract of animals is one of the most important factors for maintaining the health of animals and, therefore, production of high quality and safe animal-origin food. Phytobiotics (phytogenic additives) are secondary metabolites of plants with proven antimicrobial effects (*Lawrence and Reynolds,* 1984; *Windisch et al.,* 2008.). Phytobiotics' positive effects are based on maintaining and preserving eubiosis between microorganisms in the digestive tract (*Bakkali et al.,* 2008; *Windisch et al.,* 2008; *Stojkovic et al.,* 2013). Proper nutrition and hygienic conditions are very important for eubiosis maintenance. Phytobiotics, when added to poultry feed, reduce poultry immune stress (anti-inflammatory and antioxidant activity, antimicrobial, antiviral and anticoccidial effects) and increase intestinal availability for absorption of essential nutrients (enhancing flavour and food intake, stimulating secretion of digestive enzymes, increasing motility of stomach and intestine) (*Burt,* 2004; *Platel and Srinivasan,* 2004; *Giannenas et al.,* 2005; *Aksit et al.,* 2006; *Kirkpinar et al,* 2010; *Gregacevic et al.,* 2014). Phytobiotics may have potential in promoting production performance and impact on productivity and, thus, could be considered as natural growth stimulators (*Hashemi and Davoodi,* 2010). Although phytobiotics are a relatively new group of food additives, they are attracting a lot of attention in the animal feed production industry. Compared with synthetically derived antibiotics and inorganic chemical substances, phytobiotics are natural, proven less toxic and do not produce residues. Therefore, they could become ideal feed additives and successfully replace antibiotics as growth promoters in food

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(*Hashemi et al.,* 2008; *Gregacevic et al.,* 2014). The objective of this study was to determine the effect of phytogenic additives in broiler feed on the production performance of broilers, carcass meatiness and chemical composition, as well as the suitability of these compounds for use in intensive breeding of commercial broilers from nutritional, health and economic aspects.

Materials and Methods

One day old market broilers (n=120), of Cobb 500 provenance, were housed on a registered farm. Chickens were of both sexes, with average weight 46.59±3.48 g. Broilers were divided into two groups of 60 broilers each. Control group C received feed without added phytobiotics, while experimental group P had phytobiotics containing cumin, mint, clove and anise, at 150 g t^{-1}, in their feed. Broilers were fattened for 42 days, divided into three phases, during each of which they received appropriate feed for that phase. The first phase was 0−10 days, the second was 11−20 days and the third was 21−42 days.

Housing, keeping and feeding of broilers

Broilers were housed in a commercial broiler house on the floor. Before broilers were introduced, the floor was mechanically cleaned and sanitary washed and disinfection of equipment and floor was conducted with a biodegradable, broad spectrum agent. The pad was strewn with sawdust of 12 cm thickness. The broiler house was heated appropriately and all hygienic and microbial conditions were according to the breeder's guidelines. Broilers were introduced and fed with mixtures recommended for Cobb provenance broilers. Three mixtures were used that completely met the needs of broilers at the three phases of fattening (Table 1) (*NRC,* 1994). Feed and water were supplied *ad libitum*.

Production results

Broilers were weighed on electronic scales (accuracy 1g) at the start and after each phase (on days 1, 10, 11, 20, 21 and 42) of fattening. Average body mass was calculated at the end of each fattening phase and at the beginning and the end of the study.

Table 1. Ingredients and chemical composition of broiler diets (%)

Ingredients or chemical composition	Feed mixture for phase I	Feed mixture for phase II	Feed mixture for phase III
Ingredients			
Corn	50.85	44.15	44.95
Wheat	–	10.00	15.00
Soybean grits	15.00	17.00	20.00
Soybean meal	12.40	1.00	1.00
Soybean cake	17.00	23.30	14.70
Monocalcium phosphate	1.20	1.00	0.90
Cattle chalk	1.60	1.60	1.60
Cattle salt	0.35	0.35	0.35
Premix[a]	1.00	1.00	1.00
Lysine	0.20	0.20	0.10
Methionine	0.20	0.20	0.20
Adsorbent	0.20	0.20	0.20
Chemical composition			
Moisture	8.04	9.38	9.98
Proteins	24.98	22.17	20.91
Fat	6.09	7.03	5.44
Cellulose	2.04	2.16	2.38
Ash	5.45	4.88	4.76

Legend:[a]; Premix composition per kg: Vit. A 1,300,000 IJ, Vit. D3 250,000 IJ, Vit. E 3,000 mg, Vit. K3 300 mg, Vit. B1 250 mg, Vit. B2 800 mg, Vit. B6 350 mg, Vit. B12 2 mg, Biotin 10 mg, Ca-pantothenate 1,500 mg, Niacin 3,000 mg, Choline chloride 13,750 mg, Betaine 10,000 mg, Folic acid 100 mg, Vit. C 2,000 mg, Fe 4,000 mg, Cu 800 mg, Mn 8,000 mg, Zn 5,000 mg, I 75 mg, Se 15 mg, Co 25 mg, Helmox (antioxidant) 10,000 mg, Clinacox 0.2% 20,000mg.

Total gain per phase was calculated from the difference of body mass at the beginning and end of each phase. Total and daily gain was calculated based on the duration of each phase and the study. Quantity of feed eaten and wastage were measured at the end of each phase. Feed conversion rates were calculated from consumption and gain data for each phase, as well as for the whole study.

Carcass meatiness

Each individual broiler was measured before and after slaughter and chilling. Carcass yield was calculated from the live weight before slaughter and chilled carcass weight. Breasts and drumsticks with thighs were measured on electronic scales (±0.05 g) and their participation in the total carcass weight was calculated.

Chemical composition

The chemical composition (water content, protein, fat and minerals) of meat from breasts and thighs with drumsticks from both groups was determined 48 h after slaughter. Values were measured using standard reference methods (*SRPS*, 1992a; *SRPS,* 1992b; *SRPS,* 1998; *SRPS,* 1999).

Statistical analysis

Statistical analysis of the results was conducted using the software GraphPad Prism Version 5.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Mean values were calculated and the groups were compared with one-way ANOVA with Tukey's multiple comparison statistical test. Values of $p<0.05$ and $p<0.01$ were considered significant. The results are expressed as mean ± SD.

Results and Discussion

The average body mass of the Cobb broilers per day and total gain per phase by group are shown in Table 2.

The positive impact of the phytobiotics on broiler body mass can be seen in Table 2. Broilers from the control group (without phytobiotics) had significantly lower body mass ($p<0.05$ and $p<0.01$) than experimental group on days 20 and 42. Those results were in agreement with the results of other authors. According to *Soltan et al.* (2008), anise seed addition (0.5−0.75 g kg^{-1}) in feed for the broilers over 42 days resulted in higher body weight and gain. *El Tazi et al.* (2014) added different concentrations (0, 0.5, 0.75 and 1.0 %) of black pepper (*Piper nigrum* L.) to broiler feed. At the end of that study, the broilers with 1% black pepper in their feed had the highest body mass. The lowest body mass was measured in broilers without phytobiotics. *Qamar et al.* (2015) added different phytobiotics to broilers' drinking water and found significantly ($p<0.05$) the highest body mass in those broilers compared to others which did not intake phytobiotics.

Body mass of broilers is a good indicator of nutritive value and hygienic quality of feed, but total gain is better. Total gain usually is shown by phase of fattening or by whole fattening period. Our results (Table 2) showed that broilers receiving phytobiotics had significantly better total gain ($p<0.01$) than control broilers at the end of the fattening period. This is in agreement with the results of many authors, such as *Ghasemi et al.* (2014), *El Tazi et al.* (2014) and *Murugesan et al.* (2015). They all found that phytobiotics in broiler feed had positive impacts on broilers' total gain. *Cross et al.* (2007) found that adding thyme essential oils (1 g kg^{-1}) to broiler feed resulted in significantly higher total gain in those broilers.

Table 2. Average body mass (g) of Cobb broilers per day and total gain (g) per phase by group

Average body mass (g)			Total gain (g)		
Day	C	P	Phase (days)	C	P
1	46.80±3.27	46.33±3.57	1–10	207.83±16.26	211.03±23.90
10	254.63±16.78	257.37±23.97	11–20	489.43±80.59	520.77±51.56
20	744.07±81.95^{a}	778.37±44.24^{a}	21–42	1590.23±173.84	1682.60±189.60
42	2334.30±147.78^{A}	2460.97±190.89^{A}	1–42	2287.50±147.61^{A}	2414.63±190.12^{A}

Legend: Means within a row with a common superscript letter differ significantly: $^{a}p<0.05$; $^{A}(p<0.01)$

Table 3. Average daily feed consumption (g), total feed consumption (kg) and feed conversion (kg) during the fattening of broilers

	Daily feed consumption (g)		Total feed consumption (kg)		Total feed consumption (kg)	
Phase (days)	C	P	C	P	C	P
1–10	44.82	42.00	0.448	0.420	0.448	0.420
11–20	95.58	95.67	0.956	0.957	0.956	0.957
21–42	159.44	152.22	3.348	3.197	3.348	3.197
1–42	113.15	108.89	4.752	4.573	4.752	4.573

Results of average daily and total feed consumption and feed conversion are shown in Table 3.

Summing up the results (Table 3), phytobiotics in feed reduced the overall consumption of feed in relation to feed without phytobiotics. The same effect was seen by *Hashemi et al.* (2014) and *El Tazi et al.* (2014). Some authors (*Ocak et al.,* 2008; *Amad et al.,* 2011; *Banjo,* 2012; *Gadzirayi et al.,* 2012; *Qamer et al.,* 2015) showed that addition of phytobiotics to broiler feed or water had no impact on feed consumption. It should be noted that in similar studies, differences in phytobiotic use (composition and quantity) occur, producing differing results and effects on appetite and feed consumption.

Feed conversion, as well as the interaction of feed consumption and gain, is one of the best indicators of feed quality and cost of production. Our phytobiotic-receiving broilers had better feed conversion in all phases of fattening, than control broilers (without phytobiotics). *El Tazi et al.* (2014) also found similar results in broilers receiving different concentrations of black pepper in their feed. Adding different phytobiotics, such as garlic (*Allium sativum*), thyme (*Thymus vulgarus*), or echinacea (*Echinacea purpurea*) to broiler feed results in better feed conversion (*Aji et al.,* 2011; *Rahimi et al.,* 2011 and *Khan et al.,* 2012)

Average meatiness of carcasses resulting from the broilers is shown in Table 4.

The broilers with phytobiotics in feed produced more meaty carcasses than control broilers without phytobiotics (Table 4). Carcass yield was better in the phytobiotic broilers than in the control broilers. The same results were obtained by *Oleforuh-Okoleh et al.* (2014) – better carcass yield occurred in broilers which received phytobiotics in feed. *Zhang et al.* (2005) and *Abou-Elkhair et al.* (2014) also produced better carcass yields in broilers with phytobiotics

Table 4. Carcass yield, cold carcass weight, weight of breasts and thigh with drumsticks and their participation in carcass on in carcass of the broilers

Group	Carcass yield (%)	Cold carcass weight (g)	Breast		Thigh with drumstick	
			(g)	(%)	(g)	(%)
C	78.26	1826.82±200.60^{a}	618.61±86.41^{A}	34.93	491.42±43.61^{A}	27.85
P	79.18	1948.66±161.99^{a}	698.57±50.84^{A}	36.43	544.36±30.49^{A}	28.38

Legend: Means within a column with a common superscript letter differ significantly: ^{a} $p<0.05$; ^{A} $p<0.01$

Table 5. Chemical composition of broiler breast meat and thigh with drumstick meat (%)

Parameters	Breast meat		Thigh with drumstick meat	
	C	P	C	P
Protein	22.10±3.04	21.86±0.75	18.85±0.92	18.73±1.08
Water	73.42±2.50	73.85±1.31	72.07±1.81	73.35±3.20
Fat	3.49±1.08	3.25±0.92	8.10±1.70	6.90±2.11
Ash	0.99±0.03	1.04±0.02	0.98±0.05	1.02±0.03

added to feed. In our study, broilers receiving phytobiotics had better cold carcass weight than control broilers ($p<0.05$). The same effect was reported by *Oleforuh-Okoleh et al.* (2014) and *Erener et al.* (2011). Breast and thigh with drumstick weights and their participation in the carcass depends on many factors such as genetic, feed, fattening period, sex and age (*Bogosavljevic-Boskovic et al.,* 2011). We found a positive impact of the phytobiotics in feed on breast and thigh with drumstick weights ($p<0.01$), and on their participation in carcasses. *Khattak et al.* (2014) found similar effects in Ross 308 broilers.

The chemical composition of broiler breast meat and thigh with drumstick meat is shown in Table 5.

Chemical composition of meat depends on different factors such as provenance of broilers, sex, age, nutritional status and part of the carcass (*Strakova et al.,* 2002; *Suchy et al.,* 2002; *Araujo et al.,* 2004). There was no significant difference in chemical composition of the breast or thigh with drumstick meat between our broiler groups (Table 5). Results from some authors (*Al-Beitawi and El-Ghousein,* 2008; *Sarker et al.,* 2010) showed that phytobiotics in feed had an impact on chemical composition of the resultant meat.

Conclusion

Phytobiotics added to broiler feed had a positive impact on all production results and meatiness in the broilers. Broilers which received phytobiotics had higher body mass and total gain, lower feed consumption, better feed conversion and produced more meaty carcasses than broilers which did not consume phytobiotics.

Using phytobiotics in feed for poultry achieves similar effects as does the use of antibiotics, but phytobiotics do not result in tissue residues and they do not have a withdrawal period. Therefore, phytobiotics could become ideal feed additives and successfully replace antibiotics as growth promoters in broiler feed.

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Carcass performance of Simmental and Holstein Friesian beef cattle in Serbia

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A b s t r a c t: The aim of this study was to determine the slaughter weight, hot carcass weight, cold carcass weight, dressing percentage, chilling loss, forequarter weight, hindquarter weight and participation of forequarters and hindquarters of the beef cattle breeds Simmental (males, females and cattle from repurchase) and Holstein Friesian. A total of 100 animals were studied. After slaughter, carcass parameters were measured individually. The results show that the Holstein Friesian cattle had a significantly lower ($p < 0.01$) average slaughter weight, hot carcass weight, cold carcass weight and dressing percentage than Simmental beef cattle. Also, females had a significantly lower average cold carcass weight than males ($p < 0.05$). Male Simmental beef cattle had significantly higher ($p<0.01$) average forequarter and hindquarter weights than female Simmental beef cattle and Holstein Friesian cattle.

Keywords: *Carcass performance, cattle, Simmental beef, Holstein Friesian beef.*

Introduction

Improving carcass performance and meat quality traits are the main objectives of most research carried out in the beef production area. Meat quality is an important criterion that influences consumers' decisions to purchase beef (*Baltic and Boskovic*, 2015; *Djordjevic*, 2016). Beef meat contains about 23% protein, 2.8% fat, 73% water and 1.2% mineral matter. The energy value of beef meat is 494 KJ (116 kcal) per 100 g (*Williams*, 2007). Numerous factors, such as race, gender, age, diet and mode of production affect variations in the chemical composition of beef.

Meat production in Serbia is largely based on Simmental beef cattle, a dual purpose worldwide breed common in central Europe, slaughtered between 16–18 months and 600–700 kg live weight (*Sami et al.*, 2004; *Dokmanovic at al.*, 2014). Beef production in Serbia has decreased as result of permanent reductions in cattle numbers in the past twenty-five years (*Dokmanovic at al.*, 2014). In the European Union, a deficit of beef will amount to 600,000 tons (*Petricevic et al.*, 2015). According to data from the FAO (Food and Agriculture Organization of the United Nations), the average annual world beef consumption for 2010 was 9.4 kilograms per capita. The highest beef consumption per capita for 2010 was in Argentina (55.7 kg) followed by Brazil with 39.8 kg and the US, with 38.2 kg. In EU countries, annual consumption of beef meat for the year 2010 was 16.4 kg per capita. (*Anon.,* 2012).

Many studies (*Mandell, et al.*, 1998; *May et al.*, 1992; *Sinclair et al.*, 1998; *Maltin et al.*, 2001; *Moloney et al.*, 2001) have shown the relationships among different production factors (age, breed, gender, feeding plan, etc.) and carcass characteristics of beef cattle. The breed and feeding plan is considered as one of the most important factors that affects meat production.

The objective of this study was to evaluate the carcass performance of Simmental and Holstein Friesian beef cattle in Serbia.

Materials and Methods

Experimental grouping of cattle

Simmental beef cattle (n=90) and Holstein Friesian beef cattle (n=10), all approximately 16 months old, were classified by gender and breed into four groups (Table 1). Group I (males), group II (females) and group III (repurchased from different

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Table 1. Experimental grouping of studied cattle

Simmental cattle			Holstein Friesian cattle
Group I	**Group II**	**Group III**	**Group IV**
Males	Females	Repurchase	Males
n=50	n=30	n=10	n=10

origins) were Simmental beef cattle, while group IV comprised Holstein Friesian beef cattle.

Carcass analysis

Male cattle were fasted 18 h before slaughter. Final live weights were recorded. Cattle were slaughtered at one of four commercial facilities. After slaughter, the hot and cold weights of the carcasses were measured. Carcasses were cooled for 24 h at 4°C. Dressing proportions were calculated as the ratio of cold carcass weight to final live weight. Chilling loss was calculated as the ratio of hot carcass weight to cold carcass weight. The carcasses were divided between the 12th and 13th rib interface into forequarters and hindquarters (*Baltic*, 1994).

Statistical analysis

Statistical analysis of the results was conducted using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Each parameter was described by mean and standard deviation (SD). One-way ANOVA with Tukey's test was performed to assess the significance of differences among various groups. Values of $p<0.05$ and $p<0.01$ were considered significant.

Results and Discussion

Table 2 shows the carcass performance of the four cattle groups. Average group slaughter weight was between 461.3±48.68 kg (group IV) and 586.9±75.40 kg (group I). The breed ($p<0.01$) and gender ($p<0.05$) significantly affected the slaughter weight, as higher weights were recorded for group I (male Simmental beef cattle) than group II (female Simmental beef cattle) and group IV (Holstein Friesian beef cattle). The effect of breed was more pronounced than the effect of gender. Group IV cattle, the Holstein Friesians, were significantly lighter at slaughter ($p<0.01$) than the other three groups, but differences between the Simmental cattle in groups I, II and III were less significant. The average hot carcass weight was between 250.7±28.80 kg (group IV) and 333.2±53.44 kg (group I). Group IV, the Holstein Friesians, had significantly lower hot carcass weights than Simmentals (groups I, II and III; $p<0.01$). The hot carcass weight of Simmental male cattle was significantly lower than the hot carcass weight of Simmental female cattle ($p<0.05$). Similar results were seen with cold carcass weight (Table 2).

The present study was conducted to evaluate the carcass performance in Simmental and Holstein Friesian beef cattle in Serbia. Our results (slaughter weight, hot carcass weight and cold carcass weight) were in accordance with those reported by other authors for Simmental beef cattle (*Piasentier et al.,* 2009; *Zapletal et al.,* 2009).

According to statistical data in Serbia, the average weight of adult animals before slaughter during 1995 to 2000 was 478 kg and from 2006 to 2011 was 504 kg. Lower masses compared to our results could be due to the fact that in those data, cattle were not separated by age and sex (*Dokmanovic et*

Table 2. Beef carcass performances (X±SD) of beef cattle groups

Weight (kg)	Group			
	I (n=50)	**II (n=30)**	**III (n=10)**	**IV (n=10)**
Slaughter	$586.9^{A,a}$±75.40	$541.5^{B,a}$±55.76	568.6^{C}±41.67	$461.3^{A,B,C}$±48.68
Hot carcass	$333.2^{A,a}$±53.44	305.6^{Ba}±31.82	313.4^{C}±25.74	$250.7^{A,B,C}$±28.80
Cold carcass	$325.6^{A,a}$±53.17	$296.5^{B,a}$±31.28	305.0^{C}±25.25	$245.3^{A,B,C}$±28.35

Legend: Within a row and each parameter, values were compared; Means with a common superscript letter differ: A, B, C; $p<0.01$; a, b; $p<0.05$.

al., 2014). The weights of male yearlings (Domestic Simmental), slaughtered in a Cajetina slaughterhouse ranged from 499 kg to 604 kg, while females of the same breed ranged from 430 kg to 481 kg (*Drca*, 2009). *Janjic* (2004) found that the average weight of yearlings slaughtered in Vracevsnica ranged between 543 kg for male and 509 kg for female animals. In a Toplica slaughterhouse, the average weight of yearlings was 533 kg for male and 421 kg for female animals (*Dokmanovic et al.*, 2014). According to *Drca* (2009) the average carcass weight for male beef cattle ranged from 286 kg to 327 kg and for female from 230 kg to 266 kg. Male Simmental cattle (Germany) fed with a concentrate mixture had an average weight of 623 kg before slaughter, while those fed predominantly with with roughage weighed around 620 kg (*Nuernberg et al*., 2005). Holsteins fed with a concentrate mixture had an average weight of 619 kg before slaughter and those animals fed predominantly roughage weighed around 624 kg. Simmental animals were 495 days old and Holsteins were 394 days old (*Nuernberg et al*., 2005). Before slaughter, Belgian Blue cattle weighed 755 kg (males) and 740 kg (females), while the cooled carcass weights were 501 kg and 470 kg, respectively (*Fiems et al.,* 2003).

According to *Kamienieckog et al.* (2009), Charolais bulls, Charolais x Hereford crossbreeds, and Charolais x Simmental crossbreeds had before slaughter weights of 554 kg, 560 kg and 592 kg, respectively. Animals were 518, 547 and 518 days old, respectively.

Crossbreeding is widely used in the beef industry to increase a production. Many studies reported that carcass weight was higher in crossbreds compared to purebred beef cattle (*Kamieniecki el al.,* 2009). Positive heterosis for carcass weight has been found (*Neumann*, 2002; *Kamieniecki el al*., 2009).

Figure 1 shows the average dressing percentage of the four cattle groups studied. The dressing percentage was between 54.22% (group IV) and 56.56 % (group I). Dressing percentage was affected by cattle breed. Significant differences were found between groups IV (Holstein Friesian) and I (Simmental males) ($p<0.01$), and between groups IV (Holstein Friesian) and II (Simmental females) ($p<0.05$).

The average dressing percentage of Holstein Friesian cattle was significantly lower than Simmental cattle in the current study. Our dressing percentages accorded with those of *Waritthitham et al.* (2010) and *Sanudoa et al.* (2004). In research by *Chambaz et al.* (2003), Simmental beef cattle carcasses presented worse carcass conformation than Charolais and Limousin cattle, which were significantly heavier. *Fiems et al.* (2003) measured different dressing percentages in males and females.

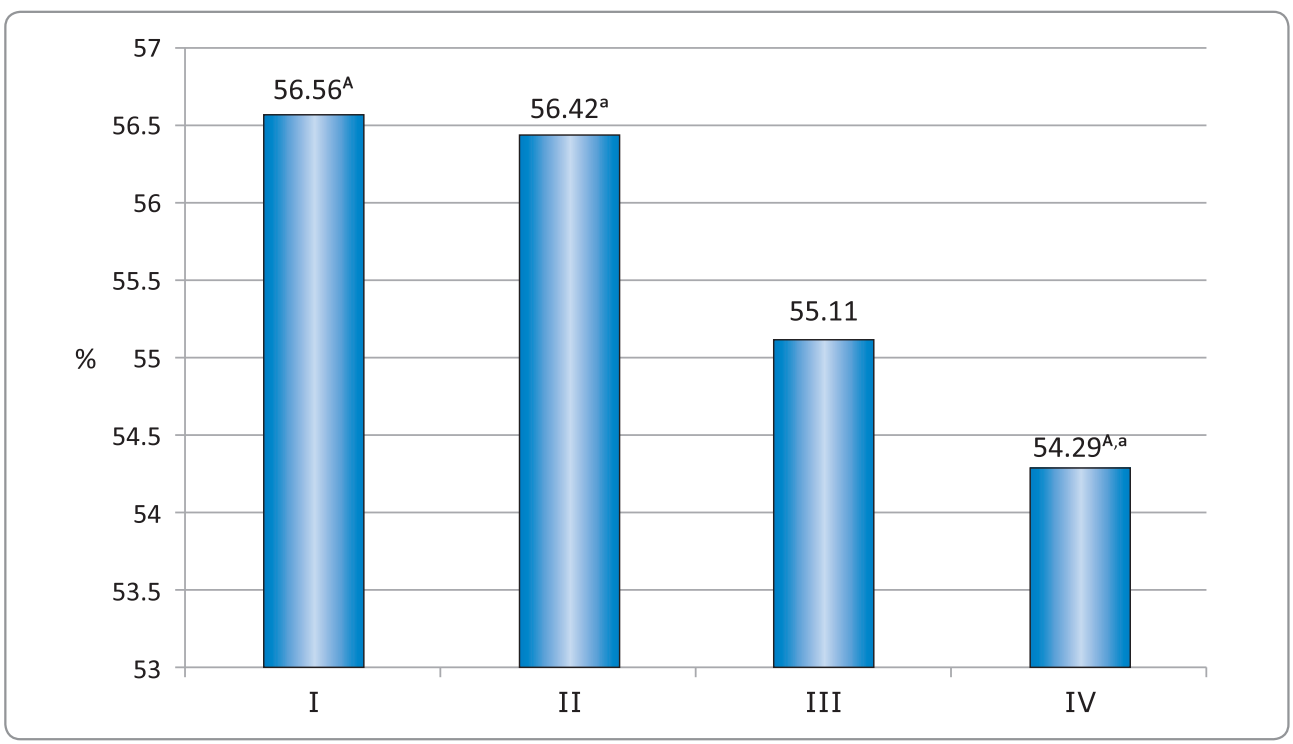

Figure 1. Dressing percentage calculated for the four cattle groups studied. Between groups, (^{A}; $p<0.01$, ^{a}; $p<0.05$)

They explained that age was probably of less importance, but the fact that the females had been gravid explained their lower dressing percentage. In present study, male and female Simmental beef cattle produced similar average dressing percentages (Figure 1).

Dressing percentages in well-fattened Shorthorn animals ranged from 65% to 68%, Herefords achieved 65%, Sedans ranged from 65% to 70%, Charolais produced over 60% and Aberdeen Angus reached 65% (fattened bulls 75%) (*Savic et al.,* 2007). Standard JUS EC1.022 is a local criterion for grading beef cattle, and includes dressing percentage (*Anon*., 1974). In young cattle aged 12 to 18 months, carcass yields (in this study termed dressing percentages) of both male and female cattle should be at least 56% for Class I, 54% for Class II and 50% for Class III cattle. Simmental beef cattle are Class III animals. Beef cattle older than 18 months (with a maximum of four permanent incisors with the exception of Class IA with two permanent incisors) should be classified as Class IA, so dressing percentage must be at least 58% (male and female). In our study, male Domestic Simmental cattle from our groups I and III would be classified as class IA if they had not more than two incisors. Groups V and VI beef cattle (female) would be classified as Class I animals, while animals from other groups of cattle would be classified as Class II (at least 54% yield) (*Anon*., 1974).

Drca (2009) reported that male Domestic Simmental type cattle from three different manufacturers in Serbia had dressing percentages between 54.20% and 55.40%, while females achieved between 53% and 55.40%. According to *Petricevic et al*. (2011), Domestic Simmental type bullocks of average weight of 500 kg had an average dressing percentage of 55.31% and beef cattle with an average weight of 600 kg had a dressing percentage of 56.30%. *Kamiemiecki et al.* (2009) found that Simmental x Charolais crossbreeds produced a dressing percentage of 58.5%.

Kamiemiecki et al. (2009) reported that the dressing percentage of male Charolais yearlings was 57.2% (average live weight of 523 kg at the age of 517 days), while male Charolais x Hereford crossbreeds had a dressing percentage of 56.9% (average live weight of 532 kg at age 547 days). *Fiems et al.* (2003) investigated conformation and dressing percentage (carcass yield, calculated on the weight of cold carcass) of double-muscled Belgian blue cattle. The average carcass yield for males was 66.6% (prior to slaughter weight 755 kg, the average age of 648 days), and for females was 63.8% (prior to slaughter weight 740 kg, the average age of the 1,822 days). According to *Stokovic et al.* (2013), male Simmentals had carcass yields of 59.92% (prior to slaughter, weight was 555 kg at an average age of 420 days). *Aleksic et al.* (2002) showed that male Domestic Simmental x Limousine

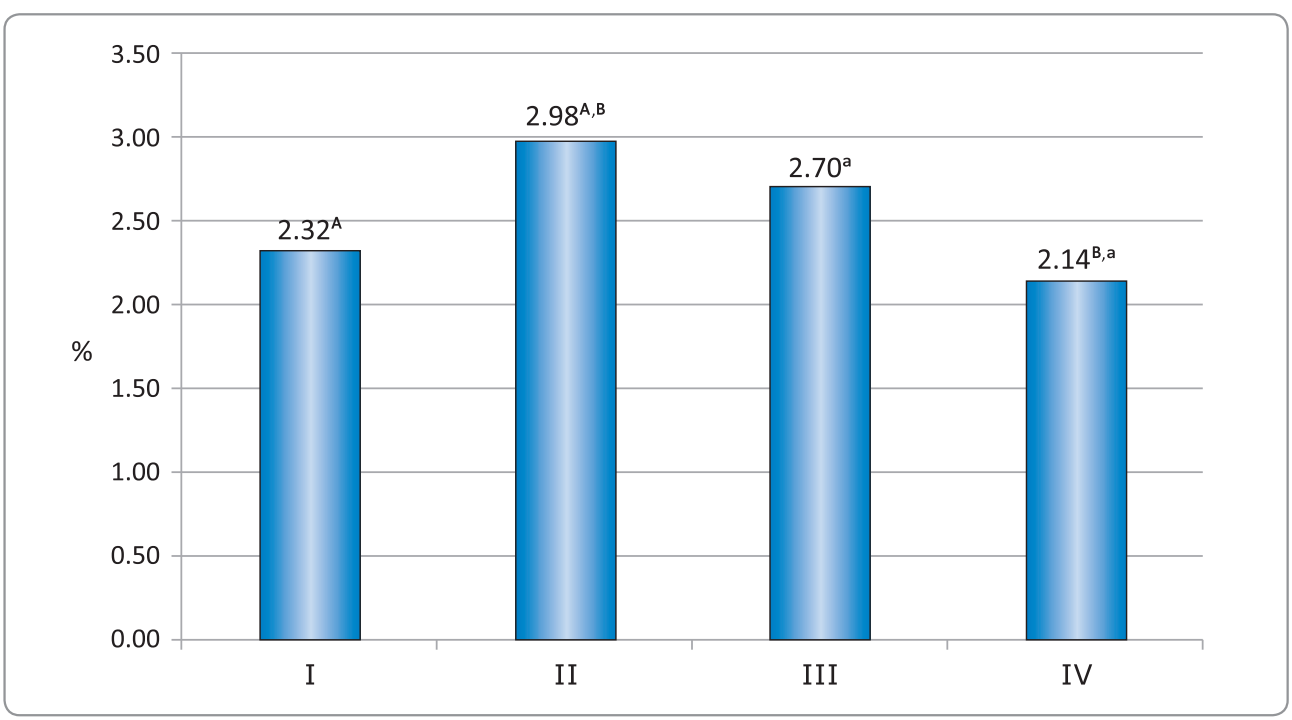

Figure 2. Chilling loss (%) ($^{A, B}$; $p<0.01$; ^{a} ; $p<0.05$)

Table 3. Weight of forequarters and hindquarters (X±SD) of beef cattle groups

Weight (kg)	Group			
	I (n=50)	II (n=30)	III (n=10)	IV (n=10)
Forequarter	$177.9^{A,B}$±30.39	$161.5^{A,C}$±16.08	167.3^{D}±12.49	$133.0^{B,C,D}$±13.84
Hindquarter	$147.8^{A,B}$±23.22	$135.0^{A,C}$±15.53	137.7^{D}±13.46	$112.3^{B,C,D}$±14.78

Within a row and each parameter values were compared; Means with a common superscript letter differ: A, B, C, D; $p<0.01$.

crossbreeds achieved a higher yield of 45.5% than male Domestic Simmentals. *Simoes et al.* (2005) showed that the yield was about 5% higher in animals weighing 650 kg (heavy breed) and 550 kg (lighter breed) prior to slaughter compared to animals with lower body mass (400 kg for the heavy breed and 300 kg for the lighter breed).

The average chilling loss of carcasses from the four groups of cattle is presented in Figure 2. Chilling loss was significantly higher in group II than in groups I and IV cattle ($p<0.01$). Group III cattle had significantly higher chilling loss than group IV cattle. However, carcass cooling conditions were not the same in the four slaughterhouses. In spite of that, our results were similar to those obtained previously (*Petricevic et al.,* 2011).

According to *Petricevic et al*. (2011), the chilling loss of heifer carcasses was 2.52% when cattle weighed 500 kg before slaughter and the warm carcass mass was 277 kg, and 1.72% when cattle weighed 600 kg before slaughter and the warm carcass mass was 334 kg. The chilling loss of male yearling carcasses, according to *Drca* (2009), was 2.33% to 2.94%, and for females, was between 3.01% and 3.36%. The author attributed this to males weighing more than females, as well as having better torso coverage with body fat. Chilling loss has great economic importance when the slaughter of a large number of animals is taken into consideration.

Average weights of forequarters and hindquarters of all cattle groups are shown in Table 3. Group I cattle had significantly higher forequarter and hindquarter weights than group II and IV cattle. Also, Simmental males (group I) had significantly higher forequarter and hindquarter weights than Simmental females (group II) ($p<0.01$). The Holstein Friesian cattle had significantly lower forequarter and hindquarter weights than Simmental beef cattle ($p<0.01$).

Figure 3 shows the participation of forequarters and hindquarters in the groups of Simmental

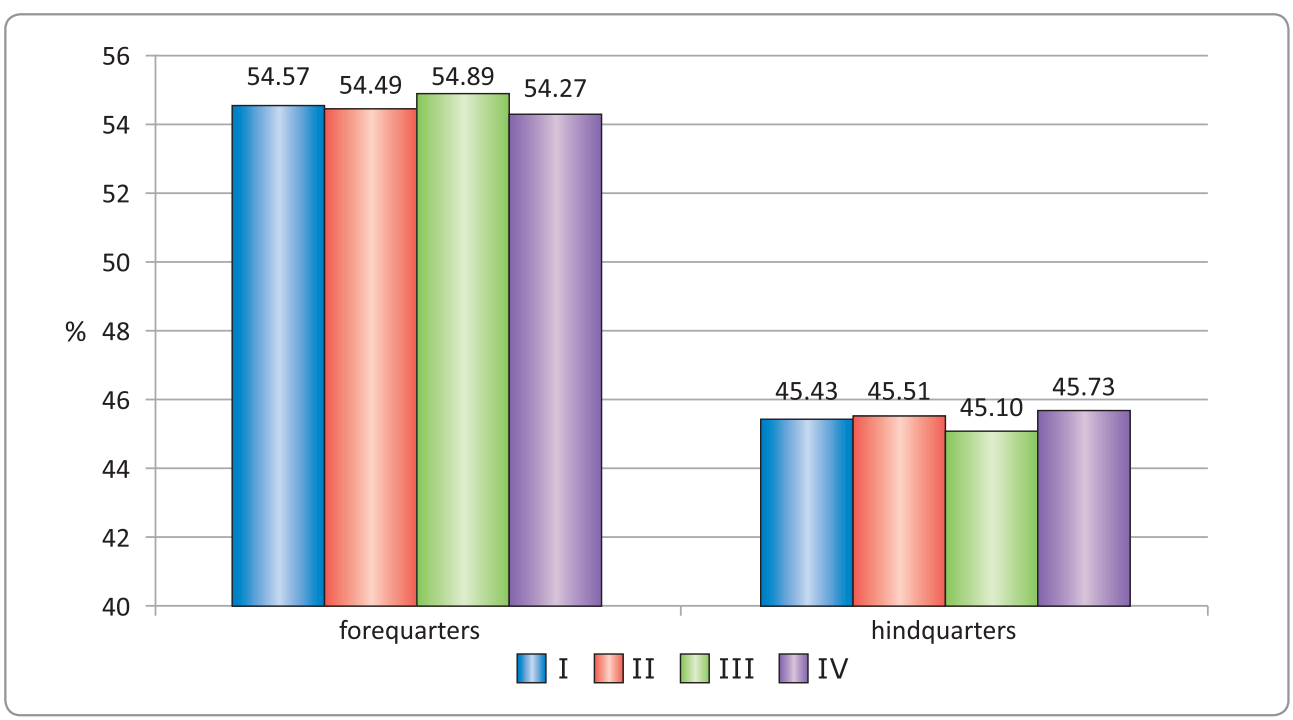

Figure 3. Participation of forequarters and hindquarters (X±SD) of cattle groups

and Holstein Friesian beef cattle. No significant differences were detected among the four groups, but participation of forequarters was higher in group III than in groups I, II and IV. The Holstein Friesian cattle had a higher participation of hindquarters than Simmental cattle.

In *Drca* (2009), participation of forequarters from male beef cattle was 47.48% and participation of hindquarters was 52.54%. Female beef cattle had a forequarter participation of 50.34% and hindquarter participation of 49.66%. There is generally a lack of data about mass and participation of forequarters and hindquarters. This may be due to the different methods of processing carcasses after cooling. The carcass cutting method is determined by the purpose of the quarters (distribution) and practices in different countries (*Baltic*, 1994).

Conclusion

The highest average weight before slaughter, and mass of hot and cold carcasses was measured in male Simmental beef cattle, followed by female Simmentals, then young male bulls of the same breed. The lowest average pre-slaughter weight was seen in Holstein Friesian cattle. The dressing percentage (carcass yield) was higher in beef cattle with higher weights before slaughter. Chilling loss of the studied carcasses was variable.

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Effect of dietary conjugated linoleic acid on chemical and fatty acid composition of pig skeletal muscle and subcutaneous adipose tissue

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A b s t r a c t: Relationships among conjugated linoleic acid (CLA) in pig nutrition, chemical composition of meat and fatty acids composition of meat and adipose tissue were determined in pigs (crossbreed Yorkshire x Landrace) (n=60) A commercial CLA preparation containing 60% of CLA isomers was added to the pig diet. SFA and MUFA in the control pig diet (with no added CLA) were significantly higher (p<0.01) than SFA and MUFA in the experimental pig diet. CLA supplementation in the pig diet significantly increased SFA and PUFA and decreased MUFA in both meat and adipose tissue. The same content of c9t11CLA and t10c12CLA was found in the supplemented pig diet. Both c9t11CLA and t10c12CLA were found in meat and adipose tissue of pigs consuming the experimental diet, but not in meat and adipose tissue of pigs consuming the control diet.

Keywords: *pig nutrition, chemical composition, fatty acids, meat, CLA.*

Introduction

The production of high quality pork has been a constant objective of the pig industry for many decades. The main goal is to obtain pigs with high lean percentage and good meat quality traits at the same time (*Baltic et al.*, 2011; *Dokmanovic et al.*, 2015; *Dokmanovic et al.*, 2016). Fat and fatty acids (FAs), whether in adipose tissue or muscle, importantly contribute to the various aspects of meat quality. Several nutritional attempts to modify fat and FAs in pigs have been attempted recently; one of them is the addition of conjugated linoleic acid (CLA) in the diet for growing/finishing pigs to increase CLA in muscle and adipose tissue (*Ivanovic et al.*, 2015; *Markovic et al.*, 2015). CLA is a group of positional and geometric isomers of linoleic acid (C18:2), which were first identified in rumen fluid as an intermediate of the biohydrogenation process. In synthetic CLA preparations, the c9,t11 and t10,c12 isomers are predominant (often in a 1:1 ratio). It appears that the c9t11 isomer has positive effects on some types of cancer by inhibiting tumourogenesis, while t10c12 isomer could be responsible for changes in fat deposition (*Pariza et al.*, 2000). In addition, dietary CLA seems to be highly deposited in body tissues of monogastric animals and as a result, in pork and meat products (*Bee*, 2001; *Corino et al.*, 2005).

Most reports in which the effect of CLA on FAs composition was evaluated were performed in muscle. Therefore, the aim of this study was to evaluate effect of CLA on chemical composition of meat and FA composition of meat and adipose tissue.

Materials and Methods

Animals and diets

Crossbreed Yorkshire x Landrace pigs, with initial body weight of 60 kg were divided into two groups of 30 pigs each and fed a standard mixture (*National Research Council*, 1998), formulated to meet maintenance and growth requirements of animals during their growth from 60 to 110 kg (fattening period of 60 days). Commercially prepared CLA (60%) (Lutalin™, BASF, Germany), was added to the feed of the experimental pigs at the recommended rate of 2% in the feed mixture. The nutrient composition of the diets is shown in Table 1.

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Table 1. Ingredients and chemical composition of the pig diets (%)

Ingredients	Control diet	Experimental diet
Corn	48	46
Barley	28	28
Soybean meal	16	16
Wheat bran	5	5
Lutalin	–	2
Di-Ca-P	0.60	0.60
Cattle chalk	1.20	1.20
Cattle salt	0.40	0.40
Premix	1.16	1.16
Chemical composition		
Moisture	12.28	12.22
Proteins	15.24	15.39
Fat	2.968	2.984
Crude fibre	4.088	4.206
Crude ash	2.64	2.78
Ca	0.77	0.77
P	0.52	0.52
ME-s	12.66	12.66
Lys	0.89	0.89
Met+Cyst	0.54	0.54

Legend: Control diet without addition of CLA); Experimental diet with addition of CLA); Premix composition (per kg): Vit. A 700000IJ; Vit. D3 100000IJ; Vit. E 1200 mg; Vit. K3 100 mg; B1 200mg; B2 250 mg; B6 150mg; B12 1.5 mg; Biotin 5 mg; Ca-pantothenate 1200 mg; Niacine 1500 mg; Choline chloride 50000 mg; Fe 10000mg; Cu 2000 mg; Mn 2500 mg; Zn 10000mg; J 90 mg; Se 10 mg; Co50 mg; Helmox (antioxidant) 10000 mg

Chemical methods

Complete mixtures of the two pig diets were sampled to determine the FA composition at the beginning of the study. Meat (*Longissimus thoracis et lumborum*) and fat samples were taken from ten pigs in experimental and control groups, after slaughtering, processing and chilling of carcasses, for chemical analysis and analysis of FA composition. Chemical analyses to determine protein, water, fat and mineral content were conducted according to AOAC methods (*AOAC*, 1990). Lipids from subcutaneous back fat were extracted by the procedure proposed by Bligh and Dyer (1959). Total lipids for FA determination were extracted from pig muscle tissue with a hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Germany). After evaporation of solvent until dryness under the stream of nitrogen, total lipids were converted to FA methyl esters (FAMEs) by trimethylsulphonium hydroxide. FAMEs were determined by using Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cyanopropyl HP-88 capillary column (100m x 0.25 mm x 0.20μm) (*Trbovic et al.*, 2011). Temperature of the injector and detector were 250°C and 280°C, respectively. The carrier gas was nitrogen at a flow rate of 1.33 mL min^{-1} and split ratio of 1:50. Injection volume was 1 μL. Column oven temperature was programmed in the range from 125°C to 230°C. The total run time was 50.5 min. FAMEs were identified on the basis of the relative retention time compared with the relative retention times of the individual compounds in the standard FAME mixture Supelco Component 37 FAME mix (Supelco, Bellefonte, USA). Quantification of FAs was determined relative to an internal standard, heneicosanoic acid, C21:0. The content of FAs is expressed as a percentage (%) of the total identified FAs.

Statistical Analysis

Statistical analysis of the results was conducted using software GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego CA, USA, www.graphpad.com. All parameters were described by descriptive statistics (mean±standard deviation). Student's t-test was used to determine the significance of differences between the control and experimental group. Values of $p<0.01$ and $p<0.05$ were considered significant.

Results and Discussion

Fatty acid composition of the animal diets

Significant differences between the FA composition of the diets for control and experimental pigs were found. SFA and MUFA were significantly higher ($p<0.01$) in the control pig diet compared to the experimental diet. Polyunsaturated FAs (PUFA), n-3 and n-6 were significantly higher ($p<0.01$) in the experimental diet. A more beneficial n-6/n-3 ratio was seen in the control pig diet ($p<0.05$) (Table 2).

The CLA isomers c9t11CLA and t10c12CLA were detected in the diet with CLA (the experimental diet). The content of c9t11CLA was 2.57±0.02%, while that of t10c12CLA was 2.55±0.01 (Table 3). CLA, in nature, originates mainly from bacterial isomerisation or/and biohydrogenation of PUFA in the rumen and from desaturation of trans FAs in the adipose tissue and mammary gland. In the rumen, the bacterial microbiota produces the enzymes linoleate isomerase and CLA-reductase which convert unsaturated FAs in fat metabolism to CLA or important intermediate CLA precursors, enabling the end product, stearic acid. The endogenous synthesis from trans-vaccenic acid was also

Table 2. Fatty acid composition in feed (% of total fatty acids)

Fatty acid, %; ratio	Control diet ($\overline{X}$±SD)	Experimental diet ($\overline{X}$±SD)
SFA	22.39^{A}±0.40	17.93^{A}±0.27
MUFA	38.31^{A}±0.20	25.71^{A}±0.24
PUFA	39.00^{A}±0.60	56.36^{A}±0.32
n-3	2.37^{A}±0.07	3.37^{A}±0.22
n-6	36.87^{A}±0.55	47.84^{A}±0.18
n-6/n-3	15.54^{a}±0.31	20.00^{a}±0.21
C14:0	0.13^{A}±0,01	0.09^{A}±0.004
C15:0	0.04±0,004	0.04±0.008
C16:0	16.44^{A}±0,27	14.57^{A}±0.23
C17:0	–	0.22±0.03
C18:0	4.48^{A}±0.08	2.21^{A}±0.03
C20:0	0.46±0.04	0.45±0.04
C22:0	0.58^{A}±0.007	0.21^{A}±0.009
C24:0	0.25^{A}±0.008	0.21^{A}±0.005
C16:1	0.09^{A}±0.009	0.11^{A}±0.005
C18:1	38.22^{A}±0.21	25.43^{A}±0.24
C18:2 n-6	36.60^{A}±0.16	47.27^{A}±0.58
C20:2 n-6	0.02±0.002	0.02±0.003
C20:3 n-3	1.47^{A}±0.22	2.37^{A}±0.07
C18:3 n-6	0.21^{A}±0.009	0.58^{A}±0.007
c9t11CLA	**ND**	**2.57±0.02**
t10c12CLA	**ND**	**2.55±0.01**
c9t11CLA+ t10c12CLA	**ND**	**5.12±0.03**

Legend: $^{A, B, C}$Same letters indicate significant difference of $p<0.01$; ^{a}Same letters indicate significant difference of $p<0.05$; ND not detected

Table 3. Chemical composition of meat from pigs fed different diets (mean±standard deviation)

Parameters (%)	Pigs fed control diet[A]	Pigs fed experimental diet[B]
Moisture	69.00±0.14	69.49±0.01
Proteins	21.74±0.03	21.66±0.09
Fat	8.32[a]±0.08	7.90[a]±0.05
Ash	0.94±0.01	0.95±0.01

Legend: [A]Control diet without addition of CLA; [B] Experimental diet with added CLA; [a] same letters indicate significant difference of $p<0.05$

documented in humans but the predominant source of CLA seems to be the dietary CLA intake via meat and meat products as well as milk and dietary products. The commercial CLA preparation used in the current study contains equal amounts of c9t11CLA and t10c12CLA isomers (*Eggert et al.*, 2001). The efficiency of CLA enrichment on products of animal origin (meat, milk, eggs) varies primarily depending on the species and concentration of CLA in diet (*Wiegand et al*., 2001; *Joo et al.*, 2002; *Wachira et al.*, 2002; *Kott et al.*, 2003; *Lauridsen et al.*, 2005; *Bee et al.*, 2008; *Cordero et al.*, 2010; *Markovic et al.,* 2013; *Tous et al.*, 2013).

Chemical composition of meat

The chemical composition of the meat (protein, fat, water and ash) is shown in Table 2. The moisture content in meat from control animals (consuming the control diet) was 69.00±0.14% and in meat from experimental pigs (consuming the experimental diet) was 69.49±0.01%. Meat from control animals contained 21.74±0.03% protein, while meat from experimental animals contained 21.66±0.09% protein. The fat content in meat from control and experimental pigs was 8.32±0.08% and 7.90±0.05%, respectively, with a significant difference between groups ($p<0.05$) (Table 3). The ash content in meat from the control and experimental groups was approximately the same, being 0.94±0.01% and 0.95±0.01%, respectively. These results were similar to those of other studies (*Lawrie,* 1991; *Dokmanovic et al.,* 2015; *Djordjevic et al.,* 2016).

Fatty acid composition of meat and adipose tissue

The content of SFA and PUFA in the meat and adipose tissue of control pigs (those consuming a control diet) was significantly higher ($p<0.01$) than in experimental pigs. The MUFA content was significantly lower ($p>0.05$) in the meat and adipose tissue of control pigs. There were no significant differences in the contents of n-3 and n-6 FAs between the meat of control and experimental pigs. The significant differences between SFA, MUFA and PUFA are shown in Tables 4 and 5.

The meat of pigs fed the experimental diet contained 2.37±0.01% of c9t11CLA and 1.19±0.01% of t10c12CLA (Table 4), while adipose tissue contained 2.86±0.07% of c9t11CLA and 83±0.01% of t10c12CLA1 (Table 5). CLA was not detected in the meat or adipose tissue of pigs consuming control pigs.

Previous studies have observed an increase of SFA and a reduction of MUFA in subcutaneous backfat due to dietary CLA (*Bee*, 2001; *Demaree et al*. 2002; *Smith et al*., 2002). In the first studies with pigs, dietary CLA increased lean tissue deposition and decreased fat deposition (*Ostrowska et al.*, 1999). Comprehensive reviews on the effects of CLA on growth performance and carcass fat deposition in pigs have been published by *Corino et al.* (2005) and *Bee et al.* (2008). In general, the effects of adding CLA to pig diets were inconclusive. Inconsistency could be attributed to the pig breeds used in different studies or sources of CLA, the dietary fat content or feeding duration (*Tous et al*., 2013).

A higher amount of CLA was used in order to increase the level of CLA in meat. *Bee* (2001) reported that CLA-enriched oil (2%) supplemented in the diet of Swiss Large White pigs weighting from 70 to 105 kg resulted in a measurable CLA content (14.9 mg g^{-1} of FAs) in the adipose tissue, compared with non-detectable CLA levels in the pigs with linoleic acid-enriched oil or lard supplements.

Several reports indicate that CLA supplementation increases the amount of saturated FAs (C14:0, C16:0, and C18:0) and decreases the MUFA fraction (mainly C18:1) in pig tissues by down-regulating the D9-desaturase activity (*Bee*, 2001; *Eggert et al*., 2001; *Thiel-Cooper et al.,* 2001; *Smith et al*., 2002; *Lauridsen et al*., 2005).

It is known that the CLA can contain at least 28 different isomers, but only the two major isomers

(9c,11t CLA and 10t,12c CLA) have been evaluated in most studies performed in pigs, although the rest could also have some important roles in metabolism (*Tous et al.*, 2013). The CLA supplements used in the studies below generally consisted of a mixture of a limited number of CLA isomers (c9t11CLA and t10c12CLA). Glaser *et al*. (2002) analysed muscle tissue (*m. Longissimus dorsi*) of Large White pigs fed a barley–wheat–soybean meal-based diet with 6% of high-oleic sunflower oil or different amounts (1.85%, 3.70%, 5.55%) of partially hydrogenated rapeseed oil (high in trans FAs), from 30 to 103 kg. They reported increased amounts of CLA in muscle tissue of pigs fed the diet with added partially hydrogenated rapeseed oil (3.8, 6.4, and 8.5 mg CLA g^{-1} of FAME) and 0.9 mg g^{-1} of FAME in the sun-flower oil control pigs.

Cordero *et al.* (2010) found increased SFA (mainly C16:0) in pig meat after ingestion of dietary CLA. This shift towards greater saturation in all tissues and a decrease in MUFA in muscle could lead to reduced lipid oxidation of the adipose tissue and improve the meat technological properties, but could have a hypercholesterolaemic effect for the consumer. These changes in saturation reflect a reduction of Δ-9 desaturase activity by CLA (*Smith et al*., 2002). However, oleic acid was more reduced by CLA than palmitoleic acid, indicating that the inhibition of Δ-9 desaturase may be less pronounced for palmitic acid (*Smith et al*., 2002).

Table 4. Fatty acid composition in muscle derived from pigs consuming a control or experimental diet (% of total fatty acids)

Fatty acid, %; ratio	**Control diet** ($\overline{X}$±SD)	**Experimental diet** ($\overline{X}$±SD)
SFA	43.08^{A}±1.38	49.51^{A}±1.07
MUFA	46.57^{A}±1.88	37.35^{A}±0.28
PUFA	9.95^{a}±0.60	12.79^{a}±0.92
n-3	0.47±0.04	0.48±0.03
n-6	9.36±0.57	8.39±0.90
n-6/n-3	19.91^{a}±2.60	17.48^{a}±1.97
C14:0	1.08^{A}±0.01	2.01^{A}±0.07
C15:0	0.04±0.008	0.05±0.01
C16:0	27.22^{A}±0.24	29.98^{A}±1.16
C17:0	0.33±0.05	0.32±0.03
C18:0	14.72^{A}±1.08	17.45^{A}±0.16
C20:0	0.24^{a}±0.03	0.20^{a}±0.01
C16:1	2.38^{A}±0.24	3.40^{A}±0.36
C18:1	43.26^{A}±1.70	33.95^{A}±0.59
C20:1	0.93±0.08	ND
C22:1+C20:4	0.33±0.04	0.36±0.01
C18:2 n-6	8.83±0.54	7.91±0.85
C18:3 n-3	0.35^{A}±0.02	0.30^{A}±0.02
C20:2 n-3	0.42±0.06	0.39±0.04
C20:3 n-6	0.11^{A}±0.01	$0,09^{A}$±0.01
C20:3 n-6	0.12^{A}±0.01	$0,18^{A}$±0.01
c9t11CLA	**ND**	**2.37±0.01**
t10c12CLA	**ND**	**1.19±0.01**
c9t11CLA+ t10c12CLA	**ND**	**3.56±0.71**

Legend: ^{A}Same letters indicate significant difference of $p < 0.01$; ^{a}Same letters indicate significant difference of $p < 0.05$; ND not detected

Table 5. Fatty acid composition in adipose tissue derived from pigs consuming a control or experimental diet (% of total fatty acids)

Fatty acid, %; ratio	Control diet ($\overline{X}$±SD)	Experimental diet ($\overline{X}$±SD)
SFA	41.68^{A}±0.38	50.54^{A}±0.96
MUFA	44.62^{A}±0.99	31.28^{A}±0.35
PUFA	13.70^{A}±0.78	18.18^{A}±0.71
n-3	0.50±0.06	0.54±0.03
n-6	13.11±0.71	12.71±0.68
n-6/n-3	18.69^{a}±1.55	17.37^{a}±0.54
C14:0	1.03^{A}±0.04	2.24^{A}±0.16
C15:0	0.06^{A}±0.01	0.09^{A}±0.01
C16:0	25.63^{A}±0.48	29.64^{A}±0.98
C17:0	0.43^{a}±0.07	0.54^{a}±0.08
C18:0	14.26^{A}±0.40	17.82^{A}±0.25
C20:0	0.28±0.02	0.27±0.02
C16:1	2.02^{A}±0.09	1.60^{A}±0.10
C18:1	41.42^{A}±0.93	29.53^{A}±0.36
C20:1	0.99±0.04	ND
C22:1+C20:4	0.29^{A}±0.02	0.16^{A}±0.04
C18:2 n-6	12.15±0,71	11.79±0.64
C18:3 n-3	0.53^{A}±0,03	0.58^{A}±0.02
C20:2 n-6	0.59±0,01	0.61±0.03
C20:3 n-6	0.16^{A}±0,01	0.11^{A}±0.01
C20:3 n-3	0.16^{A}±0,001	0.14^{A}±0.001
c9t11CLA	**ND**	**2.86±0.07**
t10c12CLA	**ND**	**1.83±0.01**
c9t11CLA+ t10c12CLA	**ND**	**4.69±0.65**

Legend: ^{A}Same letters indicate significant difference of $p<0.01$; ^{a}Same letters indicate significant difference of $p<0.05$; ND not detected

Feeding gilts a conventional corn–soybean meal diet supplemented with either 1% CLA oil or 1% sunflower oil from 75 to 120 kg live weight resulted in a higher CLA concentration in the *m. Longissimus dorsi* of gilts fed a diet with added CLA oil (5.5 vs. 0.9 mg g^{-1} of FAs) (*Eggert et al.,* 2001). Similar results (4.4 vs. 0.8 mg g^{-1} of FAME in *m. Longissimus dorsi*) were recently published by *Lauridsen et al*. (2005) who supplemented the diet of 100 Danish barrows either with 0.5% CLA or 0.5% sunflower oil from 40 to 100–130 kg live weight.

Demaree et al. (2002) fed pigs from the 17th day of age with corn-soybean meal diets supplemented with either tallow or corn oil with or without 3% CLA for 35 days and found CLA content of 27.3 and 23.0 mg g^{-1} of FAs in *m. Longissimus dorsi* (corn oil with CLA and tallow with CLA, respectively). The CLA content in meat could be further enhanced if CLA supplementation is combined with additional dietary fat (*Markovic et al.*, 2015).

Conclusion

Addition of the recommended amount of dietary CLA for conventional genotype pigs (Yorkshire x Landrace) increased the CLA in meat and adipose tissue. Also, the addition of CLA to the pig diet increased the SFA and PUFA contents and reduced the MUFA content in both meat and adipose tissue. The results from this study showed that pig products can be enriched with CLA to provide a significant increase in the level of functional lipids, which could have positive influences on human health.

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Proximate composition, water activity and sodium and potassium content in dry fermented sausages with reduced salt content

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A b s t r a c t: The aim of this study was to examine the influence of partially reducing sodium chloride in dry fermented sausages by adding potassium chloride and ammonium chloride in different amounts, on the proximate composition, water activity and sodium and potassium content in the final products. Control sausages were produced with 3% sodium chloride. In group 1 and 2 sausages, sodium chloride was partially replaced by potassium chloride, and in group 3 and 4 sausages, sodium chloride was partially replaced by ammonium chloride. The lowest moisture content and the highest fat content were determined in the sausages in which sodium chloride was partially substituted with 30% potassium chloride. Due to this, the water activity in these sausages was the lowest. The highest ash content was determined in the sausages in which sodium chloride was partially substituted with potassium chloride, in amounts of one third and one half, and in which the moisture content was the lowest as well. It could not be determined whether the protein and collagen contents were influenced by the partial replacement of sodium chloride with potassium chloride or ammonium chloride or whether the determined differences were the result of the raw material used. The highest potassium content and the lowest sodium content were determined in the sausages where sodium chloride was partially replaced with one half of potassium chloride. According to the added amount of potassium chloride, the sodium/potassium ratio in these sausages was favourable.

Keywords: *sodium chloride reduction, dry fermented sausages, proximate composition, water activity, collagen content, sodium, potassium.*

Introduction

From a health point of view, an excessive intake of meat products, because of their significant sodium and fat content, cannot be recommended for some population groups. Most of these products are manufactured with sodium chloride and are important sources of sodium in the diet (*Muguerza et al*., 2004). According to these authors, major difficulties occur when developing low-salt and low-fat dry fermented sausages, because salt and fat have important functions in the quality of these products. Sodium chloride has an important influence on the final taste of dry fermented sausages and also plays an important role in microbial stability. Fat is necessary for the development of sensory properties such as texture, juiciness and flavour.

Potassium chloride is the one of the main sodium chloride replacers in the production of dry fermented sausages. However, such replacement is limited because of the negative effects of potassium chloride on the sensory characteristics of dry fermented sausages. *Gou et al.* (1996) used potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and found important flavour defects occurred when substitution with these salts was larger than 40% of the original salt weight. *Guàrdia et al.* (2006) found the overall acceptability of products decreased when potassium lactate, glycine and potassium chloride were used at levels higher than 30%, 20% and 40%, respectively. In contrast, *Askar et al.* (1993) concluded there was no difference in the overall acceptability of dry fermented sausages in which sodium chloride was replaced with 50% of potassium chloride or potassium lactate.

Ibañez et al. (1997) did not find statistical differences between sausages produced with 3% sodium chloride and sausages produced with 1.5% sodium chloride and 1% potassium chloride. *Gimeno et al.* (1998) used a mixture of 1% sodium chloride, 0.55% potassium chloride, 0.23%

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magnesium chloride and 0.46% calcium chloride to replace the traditional amount of 2.6% sodium chloride in chorizo, and found out that sensorial acceptability was decreased mainly due to the lower salty taste.

In all of the investigations cited above, the authors did not establish any safety problems related to the growth of undesirable microorganisms, but rather, the taste of these dry fermented sausages was described as less salty and more bitter. According to *Ruusunen and Puolanne* (2005), the lowest sodium chloride content in dry fermented sausages is 2.5%, particularly in salamis. Sausages with less salt content are not firm enough and cannot be easily sliced.

The aim of this study was to examine the influence of partially substituting sodium chloride with potassium chloride and ammonium chloride in different amounts in dry fermented sausages on the proximate composition, water activity and sodium and potassium content of the final product.

Materials and Methods

Sausage production

Five groups of sausages were produced. Pork for production of control group sausages was cured only with nitrite curing salt, while sausages from groups 1 to 4 were cured with various salt mixtures, according to Table 1. Meat and fat were minced to a granulation of 6 mm, mixed with salt and salt mixtures and then filled into pig small intestine, 22–24 mm in diameter. Smoking, drying and fermentation lasted for 21 days in a smoking house.

Determination of proximate composition, collagen content and water activity

The proximate composition was carried out by determining the moisture (*SRPS ISO*, 1998a), total protein (*SRPS ISO*, 1992), total fat (*SRPS ISO*, 1998b) and total ash contents (*SRPS ISO*, 1999). Collagen[2] was determined by multiplying the hydroxyproline content by eight (*SRPS ISO*, 2002). Water activity (a_w) was measured according to ISO (2004).

Determination of sodium and potassium content

Sausage (approximately 0.3 g) was transferred into Teflon vessels and 5ml nitric acid (p.a. Sigma) and 1.5ml hydrogen peroxide (30%, p.a., Merck) were added. Digestion was conducted using a microwave program with three steps as follows: 5 min from room temperature to 180°C, 10 min hold 180°C, 20 min vent. After cooling at room temperature, the digested sausage samples were quantitatively transferred into disposable flasks and diluted to 100ml with deionised water (Elga).

Analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS). Measurements were performed using the instrument iCap Q (Thermo Scientific, Bremen, Germany), equipped with collision cell and operating in kinetic energy discrimination (KED) mode. The isotope detected was ^{23}Na.

Torch position, ion optics and detector settings were adjusted daily using tuning solution (Thermo Scientific Tune B), in order to optimise measurements and minimise possible interferences. For

Table 1. Composition of sausages, g

Group	Raw material	Sodium chloride	Potassium chloride	Ammonium chloride	Sodium nitrite
C	Pork shoulder, 2400 Fat, 600	90.00	–	–	0.4500
1	Pork shoulder, 2400 Fat, 600	60.00	30.00	–	0.4500
2	Pork shoulder, 2400 Fat, 600	45.00	45.00	–	0.4500
3	Pork shoulder, 2400 Fat, 600	45.00	–	30.00	0.3750
4	Pork shoulder, 2400 Fat, 600	60.00	–	7.50	0.3375

qualitative analysis of the samples, a five-point calibration curve (including zero) was constructed for each isotope in the concentration range of 0.1 to 2.0 mg L^{-1}. An additional line of the peristaltic pump was used for on-line introduction of multi-element internal standard (^{6}Li, ^{45}Sc – 10 ng mL^{-1}; ^{71}Ga, ^{89}Y, ^{209}Bi – 2 ng mL^{-1}), covering a wide mass range. Concentrations of each measured isotope were corrected for response factors of both higher and lower mass internal standards using the interpolation method.

The quality of the analytical process was controlled by analysis of a standard reference material (SRM 1577c, National Institute of Standards and Technology, Gaithersburg, MD, USA). Measured concentrations were within the range of the certified values for all isotopes.

Statistical evaluation

The results were statistically evaluated using Microsoft Excel 2010 and presented as mean ± SD. Differences between averages of examined parameters were determined at the level of 0.05 and 0.01 by Student's t-test.

Results and Discussion

Sausages from all groups were sensorially acceptable, and their overall acceptability was evaluated favourably despite statistical differences from the overall acceptability of control sausages (*Lilic et al*., 2016).

Results of proximate composition, a_w and collagen content are shown in Table 2.

There was no difference in moisture content of control sausages and group 4 sausages (26.43±0.29 and 26.39±0.49%, respectively), but control sausages contained more moisture than group 1, 2 and 3 sausages ($p\leq0.01$; moisture content of 20.49±0.48, 22.95±0.21 and 24.59±0.23%, respectively). The moisture content of group 1 sausages was significantly lower than group 2 and 3 sausages ($p\leq0.05$). Consequently to the lowest moisture content, the a_w in group 1 sausages was the lowest (0.711±0.012), and was significantly lower ($p\leq0.01$) than the a_w determined in control and group 4 sausages (0.788±0.005 and 0.782±0.011, respectively). The a_w of group 1 sausages was significantly less than the a_w determined in group 2 and 3 sausages ($p\leq0.05$). According to these results, it is clear that adding potassium chloride as partial replacer of sodium chloride in amounts of one third and one half of the total amount of added salt significantly decreased a_w in dry sausages ($p\leq0.01$).

The fat content in control, group 3 and group 4 sausages (39.47±2.21%, 37.71±1.77% and 37.40±0.37%, respectively) was not significantly different, while it was higher ($p\leq0.05$) in group 1 and 2 sausages in comparison with sausages from other groups (42.43±0.39% and 41.92±0.35%, respectively). The highest fat content, determined in group 1 sausages, corresponded to the lowest moisture content in this group of sausages, which is expected because the content of these two components vary depending on each other in meat products (*Hui*, 2012). There was no statistical difference between protein content in control, group 1 and group 2 sausages (28.30±1.54, 29.93±0.38 and 28.75±0.84%, respectively), while group 3 and 4 sausages (33.58±2.03%

Table 2. Water activity, proximate composition and collagen content of sausages, mean±SD, n=6

Group	Water activity	Moisture, %	Fat, %	Protein, %	Ash, %	Collagen,%
C	$0.788\pm0.005^{b,x}$	26.43 ± 0.29^{x}	39.47 ± 2.21^{a}	28.30 ± 1.54^{a}	$5.48\pm0.52^{a,x}$	2.48 ± 0.40^{x}
1	$0.711\pm0.012^{a,y}$	$20.49\pm0.48^{a,y}$	42.43 ± 0.39^{b}	29.93 ± 0.38^{a}	6.71 ± 0.18^{y}	3.08 ± 0.07^{y}
2	$0.744\pm0.006^{b,y}$	$22.95\pm0.21^{b,y}$	41.92 ± 0.35^{b}	28.75 ± 0.84^{a}	6.39 ± 0.24^{y}	2.43 ± 0.22^{x}
3	$0.756\pm0.018^{b,y}$	$24.59\pm0.23^{b,y}$	37.71 ± 1.77^{a}	33.58 ± 2.03^{b}	$4.35\pm0.08^{b,x}$	2.71 ± 0.24^{x}
4	$0.782\pm0.011^{b,x}$	26.39 ± 0.48^{x}	37.40 ± 0.37^{a}	31.63 ± 1.05^{b}	$4.78\pm0.11^{b,x}$	2.46 ± 0.24^{x}

Legend: ^{a,b} Numbers with different superscript letters are significantly different ($p\leq0.05$); ^{x,y} Numbers with different superscript letters are significantly different ($p\leq0.01$)

Table 3. Sodium and potassium content and sodium/potassium ratio in sausages, mean±SD, n=6

Group	Sodium, mg/kg	Potassium, mg/kg	Sodium/Potassium ratio
C	16084.15±1156.50^{x}	4947.17±312.86^{x}	3.25
1	14620.78±475.22^{y}	13945.21±331.33^{y}	1.05
2	9847.71±847.30^{z}	16587.90±955.03^{z}	0.59
3	10706.42±459.37^{z}	5191.01±179.57^{x}	2.06
4	14197.06±11.73^{y}	5066.77±161.09^{x}	2.80

Legend: ^{x,y,z} Numbers with different superscript letters are significantly different ($p \leq 0.01$)

and 31.63±1.05%, respectively) contained more protein ($p \leq 0.05$) than sausages from other groups. The collagen content of group 1 sausages was significantly higher ($p \leq 0.01$) than that of sausages from other groups, probably due to differences in the raw meat used.

The sodium and potassium content and sodium/potassium ratio of the sausages are presented in Table 3. Sodium content was highest in control sausages as a consequence of the largest amount of added sodium chloride. Potassium content was highest in group 1 and 2 sausages, which corresponded to the added amount of potassium chloride. Excessive sodium intake in the human diet is one of the main causes of essential hypertension, and so it is very important to know the sodium/potassium ratio in foods (*Perez and Chang*, 2016). A diet high in sodium and low in potassium produces a biological interaction in the kidneys, resulting in excessive sodium and insufficient potassium concentrations in the human body. These biologic changes result in vascular smooth muscle cell contraction, followed by an increase in peripheral vascular resistance and higher blood pressure and finally hypertension (*Adrogué and Madias*, 2007). Some authors (*Drewnowski et al*., 2015) investigated the dietary intake of sodium and potassium as well as the sodium/potassium ratio and concluded that the main problem is not an insufficient intake of potassium, but rather, a poor sodium/potassium dietary ratio. According to new guidelines by WHO (2013), adults should consume less than 2000 mg of sodium and at least 3510 mg of potassium every day.

Conclusion

The lowest moisture content and the highest fat content were determined in the sausages in which sodium chloride was partially substituted with 30% potassium chloride. Due to this, the a_w in these sausages was the lowest.

The highest ash content was determined in the sausages where sodium chloride was partially substituted with potassium chloride in amounts of one third and one half, and in which the moisture content was the lowest as well.

The highest potassium content and the lowest sodium content were determined in the sausages where sodium chloride was partially replaced with one half of potassium chloride. According to the amount of potassium chloride added, the sodium/potassium ratio in these sausages was favourable.

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Original scientific paper

Cadmium in pheasant tissues as a bioindicator of environmental pollution in 23 Serbian districts

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A b s t r a c t: Concentrations of cadmium (Cd) were measured in leg muscle and liver of pheasants (n=316) from 23 different Serbian regions under the Serbian National Residue Monitoring Program during 2013-2015. Levels of Cd in pheasant tissues were determined by inductively-coupled plasma mass spectrometry (ICP-MS). Most of the pheasants examined had Cd in their tissues. In the third year of the residue monitoring program, the Cd concentrations detected in leg muscles were nearly double the levels detected in the first year. The highest Cd levels in muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts. All leg muscle samples had Cd concentrations below permitted maximum residue levels (MRL=0.05 m kg^{-1}). However, fifteen liver samples had Cd levels that exceeded the permitted MRL (0.50 mg kg^{-1}). The number of non-compliant pheasant liver samples increased over the years. The lowest mean Cd level in pheasant livers was measured in birds from Morava district (0.035 mg kg^{-1}), while the highest was in birds from Branicevo district (0.574 mg kg^{-1}). This monitoring program shows that the Cd levels measured in pheasants, which are suitable bioindicators of environmental pollution, indicate that environmental pollution with Cd is increasing.

Keywords: *pheasants, cadmium, leg muscle, liver, Serbian districts.*

Introduction

Industrial development has played an important role in environmental pollution, including in heavy metal toxicity in the biosphere, leading to severe environmental and health hazards. Different heavy metals are found in the environment as natural components. However, heavy metals primarily get into foods of animal origin and the bodies of human consumers due to anthropogenic (industrial, agricultural, traffic) activities (*Tchounwou et al*., 2012). Heavy metals are not biodegradable, but are accumulated in living organisms and metabolised mostly to more toxic, rarely to less toxic derivatives by biochemical processes (*Lehel et al.*, 2016). Cadmium (Cd) is one of the most toxic heavy metals and poses a significant health risk to humans (*Järup et al.*, 1998). Pollution of the environment and contamination of animals with Cd is a problem in most countries (*Hecht et al.*, 1984; *Stawarz et al.*, 2003). When released into the atmosphere by smelting, mining or some other processes, Cd compounds can be associated with respirable-sized airborne particles and can be carried long distances. It moves easily through soil layers and enters the food chain via plant uptake (*Alexander et al.,* 2009**)**. Cd is also found in meat, especially offal such as liver and kidney (*MacLachlan et al.*, 2016; *Ertl et al.*, 2016; *Wua et al.*, 2016). According to the US poison and disease registry (*Priority List of Hazardous Substances*, 2015), Cd ranks seventh among toxic substances for human health hazard. The International Agency for Research on Cancer (*IARC,* 2012) declared Cd and Cd compounds carcinogenic to humans.

Game, including game birds, as a representative of wildlife, is considered as a suitable bioindicator of potential environmental pollution with Cd (*Mochizuki et al.,* 2002). Levels of Cd in domestic animals and game are generally very different, because game species have the freedom to choose their food. Their diet depends on the seasonal availability of certain types of food and they feed over a wide area and mainly live longer than domestic animals, whose nutrition is uniform and controlled, and thus, livestock have lower levels of Cd (*Kramárová et al.,* 2005; *Toman et al.,* 2005). The accumulation of toxic substances in the tissues of game birds is studied almost world-wide (*Kramárová et al*., 2005; *Toman et al.,* 2005; *Mochizuki et al.,* 2002; *Petrovic and Jankovic*, 2008). *Lazarus et al.* (2014) reported a

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high percentage of free-living game liver and kidney samples exceeding the Croatian national legislative limits for Cd and lead, and recommended that people should limit consumption of offal from certain game species. Also, children, pregnant and lactating women should avoid eating game offal altogether.

Serbia has a long hunting tradition with a large number of well-kept hunting grounds, where pheasants are among the most popular species hunted. According to the *Statistical Yearbook of the Republic of Serbia* (2015), 146,000 pheasants were shot during 2013. The aim of this study was to establish the distribution of Cd in pheasant tissues, as a suitable bioindicator of environmental pollution, from Serbian districts.

Materials and Methods

Concentrations of Cd were measured in leg muscle and liver of pheasants (n=316) from different Serbian regions within the Serbian National Residue Monitoring Program in 2013 (n=134), 2014 (n=98) and 2015 (n=84). The pheasants were acquired from 23 of the 29 districts in Serbia during regular hunting seasons (Figure 1). Pheasants were chosen for this biological monitoring campaign based on the specificity of the districts and their availability in every specific location.

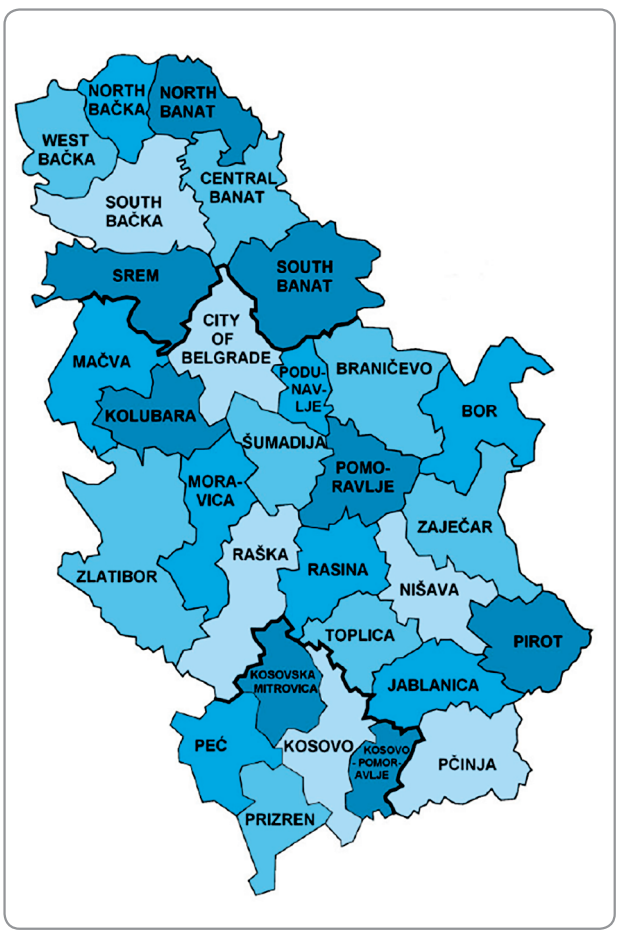

Figure 1. Map of 29 Serbian districts

Frozen samples of pheasant leg muscle and liver were thawed at 4ºC for a day before analysis, then homogenized. Approximately 0.5 g of homogenized tissue was transferred into a teflon vessel with 5 mL nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MA, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from room temperature to 180°C, 10 min hold at 180°C, 20 min ventilation. After cooling, the digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 mL with deionized purified water (Purelab DV35, ELGA, Buckinghamshire, UK).

Analysis of the ^{111}Cd isotope was performed by inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). For quantitative analysis of the samples, a five-point calibration curve (including zero) was constructed for Cd isotope. Multielement internal standard (^{6}Li, ^{45}Sc at 10 ng mL^{-1}; ^{71}Ga, ^{89}Y, ^{209}Bi at 2 ng mL^{-1}) was introduced online by an additional line through the peristaltic pump.

The quality of the analytical process was verified by analysis of the certified reference material NIST 1577c (Gaithersburg, MD, USA). Reference material was prepared in the same manner as the samples, using microwave digestion. Replicate analyses were in the range of certified values.

Statistical analysis of data was performed using software Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). One way analysis of variance – ANOVA – and Tukey's HSD test were used to compare Cd concentrations between leg muscles as well as between livers from the different Serbian districts. Differences were considered significant if $p<0.05$.

Results and Discussion

The Cd concentrations measured in leg muscle and liver of the examined pheasants are summarised in Tables 1 and 2, respectively. For calculation, when the concentration of Cd was below the limit of detection (LOD, LOD=0.001 mg kg^{-1}), that value was assumed to be equal to one half of the LOD (1/2 LOD).

Table 1. Concentration (mg kg^{-1}) of cadmium in pheasant leg muscle by district

District	n[a]	Min-max	(Mean±SD)[b]
South Backa	4	< LOD-0.007	0.004±0.002
Belgrade	14	0.009-0.018	0.008±0.006
South Banat	5	< LOD-0.018	0.007±0.006
Rasina	7	< LOD-0.008	0.003±0.002
Sumadija	9	0.002-0.010	0.005±0.003
Morava	6	< LOD-0.004	0.002±0.001
Kolubara	9	< LOD-0.017	0.008±0.005
Nis	9	< LOD-0.017	0.005±0.005
North Banat	9	< LOD-0.025	0.007±0.008
Branicevo	3	< LOD-0.012	0.007±0.005
Jablanica	6	< LOD-0.006	0.003±0.002
Bor	6	< LOD-0.013	0.005±0.005
Raska	9	< LOD-0.012	0.005±0.004
Pomoravlje	6	< LOD-0.011	0.003±0.004
Zlatibor	12	0.002-0.037	0.007±0.010
Macva	6	< LOD-0.039	0.008±0.015
Podunavlje	9	< LOD-0.049	0.010±0.016
Zajecar	5	0.002-0.012	0.005±0.004
North Backa	4	< LOD-0.008	0.003±0.003
South Banat	3	0.005-0.009	0.007±0.002
Srem	9	< LOD-0.005	0.002±0.002
West Backa	10	< LOD-0.004	0.001±0.001
West Banat	1	< LOD	< LOD

Legend: [a]n – number of samples; [b](Mean±SD) – mean value ± standard deviation

Table 2. Concentration (mg kg^{-1}) of cadmium in pheasant livers by district

District	n[a]	Min-max	(Mean±SD)[b]	n1[c] (year)
South Backa	4	0.245-0.421	0.374±0.102	
Belgrade	14	0.046-0.839	0.353±0.242	3 (2015)
South Banat	5	0.094-0.370	0.212±0.115	
Rasina	7	0.140-0.329	0.216±0.065	
Sumadija	9	0.076-0.348	0.196±0.102	
Morava	6	0.014-0.082	0.035±0.025	
Kolubara	9	0.092-1.054	0.408±0.278	1 (2014)
Nis	9	0.093-0.246	0.130±0.048	
North Banat	9	0.028-0.335	0.144±0.099	
Branicevo	3	0.106-1.162	0.574±0.538	1 (2015)
Jablanica	6	0.068-0.811	0.287±0.267	1 (2014)
Bor	6	0.113-0.916	0.363± 0.325	2 (2014)
Raska	9	0.042-1.104	0.353±0.373	1 (2013), 1 (2015)
Pomoravlje	6	0.046-0.143	0.101±0.036	
Zlatibor	12	0.060-0.998	0.291±0.307	1 (2013), 1 (2015)
Macva	6	0.087-0.391	0.217±0.110	
Podunavlje	9	0.204-0.865	0.404±0.236	1 (2014), 2 (2015)
Zajecar	5	0.022-0.170	0.105±0.061	
North Backa	4	0.078-0.248	0.127±0.082	
South Banat	3	0.209-0.462	0.366±0.137	
Srem	9	0.053-0.373	0.168±0.096	
West Backa	10	0.036-0.187	0.091±0.043	
West Banat	1		0.059	

Legend: [a]n – number of samples; [b](Mean±SD) – mean value ± standard deviation; [c]n1– number of non-compliant samples

The concentration of Cd in leg muscles was within the range <0.00–0.049 mg kg^{-1}. During our monitoring, the number of muscle samples in which Cd was detected increased. In 2013, 2014 and 2015, Cd was detected in 43.28%, 65.31% and 83.33% of all pheasant leg muscle samples, respectively. The highest Cd levels in muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts (Table 1). However, there were no statistically significant differences in Cd concentrations in pheasant leg muscles between different districts. All leg muscles from pheasants sampled in this study had Cd concentrations below maximum residue levels (MRL; 0.050 mg kg^{-1}) for Cd in game muscle according to national legislation (*Serbia*, 2014). Mean Cd concentrations in pheasant leg muscle from all districts (0.005 mg kg^{-1}) determined in this study were lower than those in shot pheasants (0.019 mg kg^{-1}) reported by *Koréneková et al.* (2008).

While Cd concentrations in muscles are generally low, liver and kidney accumulate higher concentrations (*Massányi et al.,* 1995; *Massányi and Uhrín*, 1996; *Toman et al.,* 2005). In the current study, the mean concentration of Cd in pheasant leg muscle (0.005 mg kg^{-1}) was significantly lower than in liver (0.254 mg kg^{-1}). The lowest mean Cd concentration in pheasant liver was measured in birds from the Morava district (0.035 mg kg^{-1}) while the highest concentrations were detected in Branicevo district birds (0.574 mg kg^{-1}). Nevertheless, there were no significant differences in Cd concentrations in pheasant livers from the different districts. According to national legislation (*Serbia*, 2014), the MRL for Cd in game liver is 0.500 mg kg^{-1}. Fifteen pheasant liver samples analysed during three years (2013–2015) exceeded this level, and so were non-compliant (Table 2). The highest Cd levels detected were 1.162, 1.054 and 1.104 mg kg^{-1} in pheasant livers from Branicevo, Kolubara and Raska districts, respectively. The number of non-compliant pheasant liver samples increased over the years. In 2013, only two non-compliant livers were detected, from Raska and Zlatibor districts. The next year, we found five non-compliant pheasant livers from four different districts (two from Bor, and one each from Kolubara, Branicevo and Jablanica), which was 10.2% of the non-compliant samples of all game in 2014. In 2015, 19.05% of the pheasant livers examined were non-compliant (8 non-compliant livers: three from Belgrade, two from Podunavlje and one each from Branicevo, Raska and Zlatibor). *Petrovic and Jankovic* (2008) detected Cd in 21% of all examined liver samples and 2% of them were non-compliant samples originating from Kolubara. The authors explained that the Kolubara basin and the thermal power plants located there may be the reason for high Cd concentrations in pheasant livers from this district. The mean Cd levels in fowl liver samples from birds living in a non-ferrous metallurgy area reported by *Szymczyk and Zalewski* (2003) ranged from 0.13 mg kg^{-1} to 0.18 mg kg^{-1}. These Cd concentrations were lower than those measured in pheasant livers from most Serbian districts in the current study. However, the mean Cd concentration of non-compliant livers measured by *Szymczyk and Zalewski* (2003) (1.12 mg kg^{-1}), was higher than that determined in our study (0.837 mg kg^{-1}). *Koréneková et al.* (2008) established a lower mean Cd level in shot pheasant livers (0.037 mg kg^{-1}) than in our current study (0.254 mg kg^{-1}).

Conclusion

In summary, most of the pheasants examined within the Serbian National Residue Monitoring Program during three years (2013–2015) contained Cd in their tissues. Leg muscles had lower Cd concentrations than livers. In 2013, 2014 and 2015, Cd was detected in 43.28%, 65.31% and 83.33% of all pheasant leg muscle samples, respectively. The highest Cd levels in leg muscles were measured in pheasants from Podunavlje, Macva and Zlatibor districts. All leg muscle samples in this study had Cd concentrations below the permitted MRL. However, the livers of 15 of the pheasants analysed had Cd levels which exceeded the permitted MRL (MRL=0.500 mg kg^{-1}). The number of non-compliant pheasant liver samples increased over the years. The lowest mean Cd level in pheasant livers was measured in birds from Morava district (0.035 mg kg^{-1}), while the highest level was in birds from Branicevo district (0.574 mg kg^{-1}). According to the results from this study and having in mind the small number of samples collected from some districts, it was impossible to conclude which districts likely had the highest environmental Cd pollution. On the other hand, considering the fact that pheasants are suitable bioindicators of environmental pollution, it could be concluded that environmental Cd pollution is increasing. Therefore, levels of Cd should be followed by continued monitoring of pheasant tissues.

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Original scientific paper

Microbiological safety and quality of salmon: health benefits and risk

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A b s t r a c t: A total of 703 samples were tested over 1-year period. Listeria monocytogenes was isolated from 12.4% and 2.3% of fish and environmental swabs, respectively. The ratio of n-6/n-3 which is between 1:1 and 4:1, as more desirable parameter of the lipid quality for nutritive benefit e.g. reducing the risk of many diseases, in the fresh salmon, cold and hot smoked salmon was close to 1:1 (fresh salmon, 1.28; cold smoked salmon, 0.98; hot smoked salmon, 1.59). The fatty acid composition of smoked salmon products was also expressed as mg 100 g^{-1} which is important from the nutritional point of view. The examined salmon products had a high content of eicosapentaenoic acid and docosahexaenoic acid (742 to 1567 mg 100g^{-1}) and fulfill requirements for their sufficient contents of recommendations in the World. On the other hand, cold smoked salmon can be naturally contaminated with low numbers of L. monocytogenes. This could represent a serious hazard for susceptible individuals or "YOPI" (young, old, pregnant or immunocompromised individuals).

Keywords: *salmon, fatty acids, Listeria monocytogenes, quality, safety.*

Introduction

Fish plays an important role in the human diet, and there is an observed increase in the consumption of fish per capita in Europe (*Novoslavskij at al.,* 2016). In 2013, global per capita consumption of fish was estimated at 19.7 kg, with fish accounting for about 17 percent of the global intake of animal proteins and 6.6 percent of all proteins consumed (*FAO*, 2016). The beneficial effects to humans of consuming fish, particularly oily fish such as salmon, herring and mackerel with a high content of the *n-3* highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA; 20:5*n-3*) and docosahexaenoic acid (DHA; 22:6*n-3*), have been well documented (*British Nutrition Foundation Task Force*, 1992; *British Nutrition Foundation*, 1993; *International Society for the Study of Fatty Acids and Lipids*, 1994; *Krauss et al.,* 2000). Due to the benefits of *n-3* polyunsaturated fatty acids (PUFAs) with regard to human health, particularly the suppression of many diseases, human consumption of fish is increasing worldwide. In contrast, clinical studies indicated that high amounts of *n-6* PUFAs and high *n-6*/*n-3* ratios promote the pathogenesis of many diseases. An optimal *n-6*/*n-3* ratio, which is between 1:1 and 4:1, is more desirable for reducing the risk of many diseases (*Simopoulos*, 2002). Marine ingredients were the only sources of EPA and DHA in Norwegian salmon feed in 2012, and since fish meal and fish oil are limited resources, both the retention of EPA and DHA and the utilization of these from trimmings and by-products are important aspects (*Ytrestøyl et al*., 2015). The shortage of fish oil and the resulting increase of plant oils with a high content of *n-6* fatty acids (FAs) in salmon diets have increased the *n-6*/*n-3* ratio in salmon fillets during the last decade which raises concerns both for fish health and for the beneficial health effects of salmon for the consumer, and it is, therefore, important to optimize the retention of EPA and DHA in commercial salmon farming (*Ytrestøyl et al*., 2015). The FA composition of fish can expressed as mg 100g^{-1} wet fillets, which importantly, shows the nutritional value of fish for human consumption (*Karakatsouli,* 2012).

Salmon is also high in vitamin E and possess high antioxidant properties (*Sallam,* 2007). The product is rich in proteins, low in carbohydrates (*Chitlapilly-Dass*, 2011) and reduction of the risk of coronary heart disease (CHD) mortality and stroke are identified as the main health benefits of *n-3* PUFAs from cold smoked salmon. On the

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other hand, the high lipid content in fish may protect microorganisms from thermal treatment or freezing (*Serio et al*., 2011). Cold smoked salmon is a raw, ready to eat food and, therefore, poses a risk to human health if it is contaminated with pathogens along the food chain (*Garrido et al.*, 2008). Namely, several listeriosis outbreaks have been associated with smoked fish, including cold smoked rainbow trout (*Ericsson et al*., 1997; *Miettinen et al.*, 1999) and cold smoked salmon (*Garrido et al.*, 2008).

The objective of the present study was to examine the presence and numbers of the foodborne pathogen *Listeria monocytogenes* in salmon. The FA composition of salmon products, important from the nutritional point of view, was also determined and was expressed as mg $100g^{-1}$.

Materials and methods

Samples

A total of 703 samples of salmon were examined over 1-year period. The samples included fresh, hot and cold smoked salmon (with or without herbs) as well as samples from the salmon processing environment (surfaces and drains). The samples, each consisting of 5 sample units coming from a production batch, upon reaching the laboratory, were kept refrigerated and analyzed within 2 h. Environmental swabs were collected according to the standard reference method (*SRPS ISO*, 2010a).

Isolation, identification and enumeration of L. monocytogenes

Isolation, identification, and enumeration of *L. monocytogenes* were performed following the standard method (*SRPS ISO*, 2010b), according to the Serbian regulation on conditions of food hygiene during production, processing and transport (*Serbia*, 2010). Biochemical identification of the *Listeria* isolates was performed using the culture method in combination with commercially available API *Listeria* identification system (bioMérieux, France). API tests were performed according to the manufacturer's instructions.

Enzyme-linked fluorescent assay

The procedure of screening *L. monocytogenes* in salmon and environmental samples by compact automated mini Vidas (*L. monocytogenes* Xpress) utilised an enrichment step, as prescribed by the VIDAS LMX producer (bioMérieux). At the end of an assay, the results were analyzed automatically by the instrument, which generated a test value for each sample. This value was then compared to internal references (thresholds) and each result was interpreted as positive or negative. All positive results obtained were confirmed by the culture method (*SRPS ISO*, 2010b), or by using chromogenic agar (Agar Listeria according to Ottaviani and Agosti, Oxoid, Basingstoke).

Capillary GC analysis of FAs

Total lipids for FAs determination were extracted from six of the salmon products (3 brands, 2 batches, analyzed in duplicate) by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) as reported by *Spiric* et al. (2010). Fatty acid methyl esters (FAMEs) in the extracted lipids were prepared by transesterification using 0.25 M TMSH (trimethylsulfonium hydroxide) in methanol (*ISO*, 2000). FAMEs were determined by GC Shimadzu 2010 (Kyoto, Japan) equipped with a split/splitless injector, fused silica cyanopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA) and flame ionization detector. The injection volume was 1 μL in the split ratio of 1:50. Nitrogen was used as carrier gas at a flow rate of 1.33 mL min^{-1}. The injector and detector temperatures were 250°C and 280°C, respectively. The column oven temperature was programmed starting at 125°C to a final temperature of 230°C. Total analysis time was 50.5 min. Chromatographic peaks in the samples were identified by comparing relative retention times to FAME peaks in the Supelco 37 Component FAME mix standard. Percentages of total FAs were converted to amounts of FA per 100 g of fish products, according to *Exler* et al. (1975).

Statistical analysis

The obtained data for the FA composition of salmon are reported as mean values ± the standard deviations. Analysis of variance (ANOVA) was performed using the Tukey-Kramer HSD test to analyze the data at the 5% level of significance.

Results and Discussion

Microbiological methods and enzyme-linked fluorescent assay

The overview of analyzed samples and occurrence of *L. monocytogenes* in the selected salmon processing line is presented in Table 1.Table 1. Overview of analyzed samples and occurrence of *L. monocytogenes* in a selected fish salmon processing line.

Table 1. Overview of analyzed samples and occurrence of *L. monocytogenes* in a selected salmon processing line

Group	Sample type	Number of samples	Number (%)positive for *L. monocytogenes*
Salmon and salmon products	Fresh, hot and cold smoked salmon (with or without herbs)	218	27 (12.4%)
Environmental samples	Swabs	485	11 (2.3%)
Total		703	38 (5.4%)

Table 2. Mean total lipid content (%) and fatty acid composition (% of total FAs and mg $100g^{-1}$) of six selected salmon products

	Fresh salmon		Cold smoked salmon		Hot smoked salmon with herbs	
	(% of total FA)	(mg or g $100g^{-1}$)	(% of total FA)	(mg or g $100g^{-1}$)	(% of total FA)	(mg or g $100g^{-1}$)
Total lipid[A]		18.27±0.05^{a}		14.33±0.03^{c}		17.25±0.05^{b}
Fatty acids[B]						
C14:0	2.50±0.01^{b}	457^{y}	2.14±0.03^{c}	307^{z}	2.74±0.02^{a}	470^{x}
C15:0	0.17±0.01^{a}	31^{x}	0.16±0.01^{a}	23^{y}	0.18±0.01^{a}	30^{x}
C16:0	9.50±0.02^{c}	1733^{y}	9.93±0.05^{b}	1423^{z}	10.98±0.02^{a}	1900^{x}
C16:1*n-7*	3.60±0.03^{a}	657^{x}	2.78±0.03^{c}	398^{z}	3.41±0.02^{b}	590^{y}
C17:0	0.10±0.01	18.24	Nd	nd	0.10±0.01	20
C18:0	2.29±0.02^{a}	418^{x}	2.30±0.03^{a}	330^{y}	2.30±0.03^{a}	400^{x}
C18:1*n-9*	38.05±0.10^{c}	6941^{y}	48.57±0.04^{a}	6960^{y}	44.57±0.05^{b}	7706^{x}
C18:2*n-6*	13.67±0.77^{a}	2493^{x}	13.77±0.03^{a}	1973^{y}	12.40±0.02^{a}	2140^{y}
C18:3*n-6*	0.09±0.01^{a}	17.33^{x}	nd	nd	0.10±0.01^{a}	20^{x}
C18:3*n-3*	4.28±0.03^{b}	782^{x}	4.70±0.02^{a}	674^{z}	4.20±0.03^{b}	730^{y}
C20:0	0.20±0.01^{a}	37.39^{y}	0.27±0.02^{a}	39^{y}	0.27±0.02^{a}	50^{x}
C20:1	4.21±0.01^{b}	768^{y}	4.07±0.01^{c}	583^{z}	5.10±0.10^{a}	880^{x}
C20:2*n-6*	1.14±0.02^{a}	209^{x}	0.93±0.01^{b}	133^{y}	1.03±0.01^{ab}	180^{x}
C20:3*n-6*	0.33±0.01^{b}	61^{y}	0.55±0.01^{a}	79^{x}	0.34±0.01^{b}	60^{y}
C20:3*n-3*	3.63±0.01^{b}	662^{y}	3.51±0.02^{c}	503^{z}	4.71±0.03^{a}	810^{x}
C20:5*n-3*	3.38±0.03^{a}	617^{x}	2.13±0.02^{c}	305^{z}	2.40±0.02^{b}	410^{y}
C22:5*n-3*	2.11±0.06^{a}	386^{x}	1.21±0.02^{c}	173^{z}	1.55±0.02^{b}	270^{y}
C22:6*n-3*	5.21±0.02^{a}	950^{x}	3.05±0.02^{c}	437^{z}	3.62±0.02^{b}	630^{y}
SFA	14.78±0.04^{b}	2696^{y}	14.79±0.02^{b}	2119^{z}	16.57±0.02^{a}	2860^{x}
MUFA	51.21±0.29^{c}	9341^{x}	55.41±0.02^{a}	7940^{z}	53.08±0.03^{b}	9180^{y}
PUFA	33.23±0.01^{a}	6061^{x}	29.81±0.07^{c}	4272^{z}	30.35±0.05^{b}	5250^{y}
n-3	18.73±0.25^{a}	3416^{x}	14.58±0.02^{c}	2089^{z}	16.48±0.06^{b}	2850^{y}
n-6	14.65±0.02^{b}	2673^{x}	15.24±0.03^{a}	2190^{z}	13.87±0.03^{c}	2400^{y}
n-3/n-6	1.28±0.03^{a}		0.98±0.03^{b}		1.19±0.04^{a}	
n-6/n-3	0.78±02^{c}		1.04±0.02^{a}		0.84±0.03^{b}	

[A] Total lipids are expressed as%; [B] Fatty acids are expressed as% of total FA and mg $100g^{-1}$; All values are reported as mean±SD; nd = not detected; $^{a, b, c, x, y, z}$; Values in the same row with the same letter are not significantly different (P≥0.05).

L. monocytogenes was isolated from 12.4% and 2.3% of salmon and environmental samples, respectively. All screening-positive samples were also tested by the reference culture method (see above). The enzyme-linked fluorescent assay, mini Vidas, reported the relative fluorescent value of the sample, the relative fluorescent value of the standard (RFVs), and test value, which is a quotient of the sample value and standard value. For the positive samples, RFVs ranged from 10624 to 12243. *L. monocytogenes* was isolated from all samples which were positive according to mini VIDAS.

FA expressed as weight percent of the total FAs

The lipid composition (expressed as% and FA composition (expressed as the percentage of the total FAs and in mg $100g^{-1}$) of the salmon products are shown in Table 2.Table 2: Mean total lipid content (%) and fatty acid composition (% of total FAs and mg $100g^{-1}$) of six selected salmon products

The most predominant FAs in salmon were oleic (C18:1*n-9*), linoleic (C18:2*n-6*) and palmitic acids (C16:0). No significant differences were observed in the saturated fatty acid (SFAs) content between the fresh and cold smoked salmon ($p\geq0.05$), while the hot smoked salmon with herbs contained a significantly higher content of SFA ($p\leq0.05$). The content of monounsaturated fatty acids (MUFAs) was significantly higher in the cold smoked salmon than in the fresh salmon, but was the highest in the hot smoked salmon with herbs ($p\leq0.05$). The PUFA content was significantly higher in fresh salmon than in hot smoked or cold smoked salmon ($p\leq0.05$). Of the *n-6* series FAs among the PUFAs, higher levels were measured in fresh and cold smoked salmon, while hot smoked salmon had the lowest levels. Of the *n-3* series FAs, higher levels were detected in fresh and hot smoked salmon, with the lowest level in cold smoked salmon. The content of linolenic acid (C18:3*n-3*), which is the precursor of EPA (C20:5*n-3*) and DHA (C22:6*n-3*), was high in fresh salmon, cold smoked salmon and hot smoked salmon 4.28%, 4.70% and 4.20%, respectively. Fresh salmon contained significantly higher amounts of EPA, DPA (C22:5*n-3*) and DHA compared to cold smoked salmon and hot smoked salmon with herbs ($p\leq0.05$). No significant differences were observed in the *n-3*/*n-6* ratio in the fresh salmon (1.28), cold smoked salmon (0.98) and hot smoked salmon (1.59). However, a significantly higher n-3/n-6 ratio was observed in the salmon than in freshwater fish such as carp (*Ljubojevic et al*., 2013; *Trbovic et al*., 2013).

FAs expressed as FA amounts (mg 100 g^{-1} of product)

The FAs in the salmon were also expressed as amounts of FA per 100 g of product. These data are more useful to obtain the absolute quantities of *n-3* and *n-6* PUFA consumed per specific fish serving. Our research showed that cold smoked salmon and hot smoked salmon with herbs contained significantly lower ($p\leq0.05$) amounts of *n-6* PUFA than fresh salmon (2190 mg $100g^{-1}$, 2400 mg $100g^{-1}$ and 2673 mg $100g^{-1}$, respectively). The amounts of n-3 PUFA were the higher in the fresh salmon than in hot smoked salmon with herbs and cold smoked salmon (2673 mg $100g^{-1}$, 2400 mg $100g^{-1}$ and 2190 mg $100g^{-1}$, respectively) ($p\leq0.05$). The quantities of EPA were the highest in the fresh salmon (617 mg $100g^{-1}$) compared to the hot smoked salmon with herbs and cold smoked salmon (410 mg $100g^{-1}$ and 305 mg $100g^{-1}$, respectively). DHA content was also the highest in the fresh salmon (950 mg $100g^{-1}$) compared to hot smoked salmon with herbs and cold smoked salmon (630 mg $100g^{-1}$, 437 mg $100g^{-1}$, respectively). Thus, intake of EPA plus DHA through consumption of 100 g of fresh salmon was 1567 mg, through consumption of cold smoked salmon intake was 742 mg and through consumption of hot smoked salmon with herbs was 1040 mg.

The main factors for bacterial contamination of seafood are contamination of the raw material, from the environment and from the processing, and bacterial growth conditions (temperature, water activity, pH, microbial interactions, etc.) (*Løvdal*, 2015). Because *L. monocytogenes* is a significant food safety concern, the prevalence of *L. monocytogenes* in smoked fish products in Europe has been extensively studied. The overall rate we determined is consistent with studies (with reported incidence levels between 5% and 35%) in individual EU member states. In our study, *L. monocytogenes* was isolated from 12.4% and 2.3% of fish and environmental swabs, respectively (Table 1). All *L. monocytogenes* positive salmon samples showed a contamination level below 100 CFU g^{-1}. This is comparable with data provided by *Uyttendaele et al.* (2009). The product with the highest prevalence of *L. monocytogenes* was smoked salmon. This high prevalence could be due to the low smoking temperature involved during the cold-salmon processing, as these conditions would be ideal for the proliferation of *L. monocytogenes* if the raw salmon harbored the pathogen or acquired it from the processing environment (*Chitlapilly-Dass,* 2011). Despite considerable efforts to improve the safety

of vacuum-packed smoked fish products, listeriosis outbreaks linked to the presence of *L. monocytogenes* at infective levels in these seafood commodities have been well documented, highlighting the need for technological interventions (novel alternative technologies such as irradiation and high pressure processing) to address the food safety risk posed by the presence of this pathogen in the final products (*Tocmo et al*., 2014).

The second part of the microbiological aspect of our study was detection of *L. monocytogenes* in environmental swabs from the salmon processing line (surfaces and drains). Out of 485 environmental samples analyzed, 11 (2.3%) samples were *L. monocytogenes* positive. Environmental swabs from the surface of slicing and trimming tables, slicing machines, fish filleting and trimming knives, belt glazer, and work table were positive for *L. monocytogenes*.

Similar data for EPA and DHA to than obtained in our study was published for frozen salmon slices, cold smoked salmon and hot smoked salmon (*Lerfall et al.,* 2016). Relatively high *n-3* PUFA levels and lower *n-6* PUFA levels provided a very favorable *n*-3/*n*-6 ratio in the other studies too, e.g. the cold smoked salmon of conventional reared salmon (*Lerfall et al.,* 2016; *Ytrestøyl, et al*., 2015). The optimal n-6/n-3 ratio is between 1:1 and 4:1 (*Simopoulos*, 2002), a more desirable parameter of the lipid quality for nutritive benefit e.g. reducing the risk of many diseases, and was close to 1:1 in fresh salmon, cold and hot smoked salmon studied. The data expressed as mg $100g^{-1}$ of products are more important from the nutritional point of view and are more useful obtaining the absolute quantities of *n-3* and *n-6* PUFAs consumed per specific fish serving (*Karakatsouli,* 2012). With respect to the cardiovascular diseases, prospective epidemiological and dietary intervention studies indicate that consumption of oily fish or dietary *n-3* long-chain PUFAs (equivalent to a range of 250 to 500 mg of EPA plus DHA daily) decreases the risk of mortality from CHD and sudden cardiac death. An intake of 250 mg of EPA plus DHA per day appears to be sufficient for primary prevention in healthy subjects (*EFSA*, 2010). Among the most notable recommendations, the French Food Safety Agency have determined that for their population, EPA and DHA intakes should be at least 250 mg each (*FAO/WHO,* 2013). Therefore, the salmon products studied, having a high content of EPA and DHA (742–1567 mg $100g^{-1}$), fulfill these requirements.

Conclusion

Relatively high n-3 PUFA levels and lower n-6 PUFA levels in salmon products in the current study provided a very favorable n-6/n-3 ratio, as was shown in other published studies. In the fresh salmon, cold and hot smoked salmon, the n-6/n-3 ratio was close to 1 in our study (fresh salmon, 1.28; cold smoked salmon, 0.98; hot smoked salmon, 1.59). The FA composition of salmon, when expressed as mg $100g^{-1}$ wet provides important data, as it demonstrates the nutritional value of the consumed fish. Intake of 250 mg of EPA plus DHA per day is recommended for primary prevention of CHD and sudden cardiac death in healthy subjects. The salmon products examined had a high content of EPA and DHA (742 to 1567 mg $100g^{-1}$) and so would fulfill EFSA dietary recommendations from this point of view. Also, although the salmon studied was quite often contaminated with Listeria monocytogenes, the levels of this pathogen should not represent a hazard to healthy individuals and can be considered as natural, low level contamination.

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Review paper

Production characteristics and safety parameters of Sremska ham

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A b s t r a c t: Sremska ham is a traditional dry-cured meat product manufactured by salting, smoking and drying pork hams in a mainly natural environment. The traditional production process of cured ham in Srem region (Vojvodina, Serbia) is standardized and adjusted to good manufacturing and good hygiene practice procedures. Quality parameters for Sremska ham are well defined, and include mass, ripening time, flavour etc., in order to attain product with standardized and well recognized quality. Sremska ham is produced from pork meat obtained from healthy, well rested, late maturing animals, mass 120 to 150kg. Hams are dry salted and the process itself lasts for 4 to 6 weeks depending on the ham mass. Meat selection based on pH is of great importance in order to achieve proper salt diffusion in ham. Salt diffusion occurs in the desired manner only if the pH of the meat is less than 6.0. Biohazards which can occur during the production of Sremska ham are Clostridium botulinum spores, if conditions for their germination and toxin production exist, mycotoxin producing moulds, and infestation by insect larvae. Those hazards can be eliminated or reduced by maintaining a low temperature during the critical production stages and ensuring sufficient salt content to inhibit C. botulinum growth. Moulds and insects are controlled by maintaining appropriate storage conditions. The manufacturing process of Sremska ham should be based on HACCP principles, while preserving the traditionality of the process.

Keywords: *Sremska ham, traditional meat product, food safety.*

Introduction

Southern Slavic countries have a long tradition in production of dried meat made from pork, beef or ovine meat. In the Serbian language, besides the term "sunka" which means ham, the terms "prsut" and "prsuta", derived from the Italian word "prosciutto," which also means ham, are often used. Although cured meat production is similar across Europe, there are differences in production process that affect final product characteristics and quality.

The final product can vary in mass, appearance, flavour, texture and salt content, depending on ham mass, type of cut, quantity of added salt and salting duration, smoking intensity, drying and ripening time (*Zlender*, 1986; *Careri et al*., 1993; *Buscailhon et al*., 1994; *Shivazappa et al*., 2002, *Vukovic et al.*, 2005b).

Sremska ham is a traditional dry-cured meat product manufactured by salting, smoking and drying of pork hams in a mainly natural environment. It has been produced in the Srem region for centuries, although other parts of Vojvodina are well known for similar products. Sremska ham production starts at the end of autumn or beginning of winter, when low temperatures ensure product safety, and finishes at the end of summer, when higher environmental temperatures contribute to flavour development (*Vukovic*, 2005b).

Cured meat products have certain distinct characteristics depending on the region they are produced in and equipment used for production (*Petrovic et al.,* 2011). Sremska ham has a unique flavour due to the complexity of the production process, but on the other hand, this complexity can pose a risk for food safety, because of the many potential hazards which can occur.

Sremska ham production process

The traditional cured ham production process in the Srem region is standardised and adjusted to good manufacturing and good hygienic practice procedures. The quality parameters of Sremska ham are well defined with regard to mass, ripening time,

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flavour etc., in order to attain product with standardized and well recognized quality (*Vukovic et al.*, 2005a).

Raw material selection

Sremska ham is produced from pork meat obtained from healthy, well rested, matured animals, mass 120 to 150 kg. Meat should be selected from older animals, because their meat contains less water, has a firm texture and more intense colour than meat from young pigs.

Slaughter and carcass processing is performed in the usual way. The ham is cut from the half carcass with a cross cut between the last lumbar and the first sacral vertebrae. The other cut is made at the knuckle. The pelvic bones are usually separated from the ham, in this case producing "short hams", with a mass around 8 kg.

"Long hams" are obtained by cutting ham together with the pelvic region, sometimes with last two vertebrae, and they weigh more than 12 kg. Part of the skin is then removed from the inner ham so salt can more easily diffuse into the deeper tissue. Excess fat is afterwards trimmed and the ham is shaped. The ham is then left for 18 to 24 hours in a cold room (up to 5°C) (*Vukovic*, 2006).

Salting

The hams are dry salted, and the process itself lasts for four to six weeks, depending on the mass of the ham. Meat selection based on pH is of great importance in order to achieve proper salt diffusion in Sremska ham. Salt diffusion occurs in the desired manner only if the meat pH is less than 6.0. Connective tissue membranes are, in that case, ruptured as a result of lactic acid accumulation during postmortem glycolysis and the meat structure becomes open. Special attention must be paid to the red muscles of the ham (*m. gracilis, m. quadriceps femoris* and *m. adductor*), where pH values higher than 6.0 can be expected, but which could result in slow salt diffusion (*Teodorovic et al.*, 2015).

In order to accelerate salt diffusion, it is necessary to loosen up the knee joint by manually flexing it a few times. This procedure allows salt to penetrate the meat near the knee joint, which is especially sensitive to spoilage. It is important to squeeze out blood from the larger blood vessels by pressing ham from the knuckles to the head of the hip bone, because any remaining blood can act as a medium for undesirable bacterial growth (*Vukovic et al.*, 2005a).

Table salt is used for dry salting. Salt is thoroughly rubbed into the ham, especially in any cavities or hollows if they occur and near the bones and blood vessels, as these are the areas where spoilage typically begins. Hams are then stacked one on another, and are restacked every few days. This increases the pressure in the hams, so salt diffusion is faster and all excess water can drain. Salting lasts for four weeks, and hams are then hung on poles so that the salt can diffuse evenly through the tissues during the next eight weeks.

The process of salting and salt diffusion must take place at temperatures less than 5°C, to prevent microbial spoilage and especially because of the risk of potential germination of *Clostridium botulinum* spores (*Vukovic et al*, 2005b; *Teodorovic et al.*, 2015). From the viewpoint of quality and acceptability for consumers, the salt content in Sremska ham does not need to be greater than 6%. However, from the aspect of microbiological stability and to safeguard consumer health, in particular preventing the formation of botulinum neurotoxin (*Vukovic et al.*, 1999; 2005b) which can form in dry-cured ham, at least 4.5% salt is required. This requirement is met by salting ham with 3 to 4% salt, which is less salt than is used in the traditional process, but which results in a product which is more acceptable to consumers, while at the same time remaining microbiologically safe (*Leistner et al.*, 1983; *Vukovic et al.*, 2005b). When the salt diffusion is completed, and the hams become microbiologically stable, they are transferred to higher temperature maturation areas, first at 25 to 30°C and then at 12 to 14°C, where enzymes that participate in the maturation of the product are activated.

Ripening

At this stage of the process, a stable colour and flavour are formed, and the product becomes microbiologically and chemically stable. Before smoking and drying, hams are first rinsed in warm water, which rehydrates the dried skin, as well as removes any mucus and excess salt from the surface. Afterwards, Sremska ham is subjected to cold smoking. This phase takes place under controlled conditions in climate chambers at temperatures from 12 to 25°C. In the case of traditionally smoked ham, classic smokehouses at ambient temperature during the cold, winter months are used. During the first stage of ripening, products are dried at temperatures of 10 to 15°C and relative humidity up to 80%. The second period of maturation occurs at a higher temperature of 16 to 18°C, which contributes to

the enzymatic changes which lead to the formation of desirable aroma and texture in the ham. As a result of proteolysis, a softer texture is obtained and the ham is easier to chew (*Vukovic et al.*, 2005b). Studies on Sremska ham (*Vukovic et al*., 2005b) show the use of such traditional manufacturing processes is justified; in this case, the hams are dried for three months at 15°C and relative air humidity of 75%, followed by a ripening process which lasts another six months.

During ripening, the accumulation of aromatic substances occurs, and these are formed as a result of proteolysis, which takes place under the influence of tissue enzymes. The proteolysis index (PI) is an indicator of the level of maturity of the ham, and indicates the non-protein nitrogen and total nitrogen ratio, expressed in percentages. The optimal PI for dry meat products ranges from 26 to 30% (*Careri et al*, 1993), and for Sremska ham, the PI ranges from 25.5 to 26.6% after a 12 month ripening period. However, during such long-term aging, the fleshy part of the ham (which is not covered with skin) can become over-dried, so producers often put ham that is not matured enough on the market. Hams can be expediently vacuum packaged, which prevents over-drying while allowing enzyme activity to continue, and thus, facilitates the normal ripening process and the formation of acceptable flavour and texture of the product (*Vasilev et al*. 2007a, 2007b).

Quality parameters of Sremska ham

During the production of Sremska ham, weight loss as a result of drying is usually 29 to 39%, being higher if the product is made from lighter hams. The water activity (a_w) ranges from 0.89 to 0.92, which is sufficient to ensure the microbiological stability of the ham. The pH of finished ham is high as a result of proteolysis during the ripening process, and ranges from 5.70 to 5.90 at the end of drying, up to 6.45 to 6.65 at the end of ripening. The meaty part of the product contains 59.8 to 61.9% water, 4.2 to 6.3% fat, 4.9 to 6.3% salt, and 25.8 to 27.2% meat proteins. It is interesting that the degree of oxidation of fat in Sremska ham is low, whereby thiobarbituric acid reactive substances (TBARS) values are up to 0.30 mg of malondialdehyde kg^{-1} (*Vukovic et al.*, 2005a). When ripening occurs in vacuum packaging, the oxidation parameters are even lower and reach only 0.1 mg of malondialdehyde kg^{-1} (*Vukovic et al.*, 2005a, *Vasilev et al.*, 2007a). Sremska ham and other meat products must meet Serbian regulatory requirements that define the quality of this product group: the surface is dry and clean; external appearance, cut appearance, smell, taste, consistency and texture are characteristic for the type of meat and mature product; the appearance, colour, smell and taste of the product originate from smoke, the shape must be characteristic, with a neatly processed border; the colour of the meaty part in cross section should be characteristic and stable; the fatty tissue should be white, while the surface can be yellowish; the ratio between the water content and protein content in the meaty part of the product has to be a maximum of 2.5:1 (Serbia, 2015). According to research conducted by *Vukovic et al.* (2005a), Sremska ham contains an average of 61% water and 26.5% proteins, so the protein to water ratio in the product is 2.26:1, which meets the required limit.

Factors of importance for Sremska ham safety

The food safety system in the Republic of Serbia is based on the principles regulated by EU laws (*European Commission*, 2002) and includes: responsibilities of food and feed business operators**;** traceability of food, and; risk analysis. The main laws, which are the pillar of the whole food safety system in the Republic of Serbia, and which are in accordance with European measures are the Law on Veterinary Matters (*Serbia*, 2010), and the Law on Food Safety (*Serbia*, 2009) and the Law on Animal Welfare (*Serbia*, 2009).

Within the Law on Food Safety (*Serbia*, 2009), there is a legal basis for adoption of bylaws that include: hygiene conditions applied to primary products provided to consumers in small amounts, and; deviations from general and specific conditions applied to small food producers, having in mind that these deviations may be prescribed in the case of traditional method application in some phases of food production and distribution, as well as in cases in which food businesses are situated in areas with special geographic limitations (*Karabasil et al.*, 2015).

Important factors for food safety are based on the principles of the Hazard Analysis and Critical Control Points (HACCP) system. HACCP is an integrated control system applied to the food production chain, with the goal of avoiding potential chemical, physical and microbiological hazards which can endanger consumers' health. The principles of HACCP are described by Codex Alimentarius (*Anon*, 1993).

Identification of potential hazards

The traditional process of obtaining dry ham in natural environments is very difficult to keep under control, and because of this, it could potentially endanger consumer health. In natural ambient conditions, climatic factors are variable. A special problem is to provide the optimal temperature for the salting and salt diffusion phases of production in order to prevent growth of the neurotoxic bacterium *C. botulinum*. In other production stages such as smoking, drying and ripening, products could be contaminated with carcinogenic substances derived from smoke, mycotoxin-producing moulds and insect larvae.

In order to eliminate potential hazards, Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) should be introduced in the production process. Then, the facility will have optimal hygienic and technological requirements for the implementation of HACCP system.

Botulism

Botulism associated with the consumption of dry hams could occur in many European countries. It usually occurs in regions where hams are produced by individual households and are consumed without heat treatment (*Tompkin*, 1980; *Lücke et al.*, 1982; *Lücke and Roberts*, 1993; *Vukovic,* 2000). According to official figures, in eight out of ten registered cases of botulism, dry ham produced by traditional manufacturing techniques was the source of poisoning (*Vukovic et al.*, 1999; *Vukovic et al.,* 2000; *Vukovic,* 2005a; *Vasilev and Vukovic*, 2008.)

The presence of botulinum toxin could be considered as an indicator of bad hygiene practices during pig slaughter, as well as other omissions during the production process. The main problem is an insufficient quantity of salt as a consequence of slow salt diffusion into the depth of the ham, which does not prevent growth of *C. botulinum.* High environmental temperatures during the salt diffusion phase can be a serious problem in traditional production, when *C. botulinum* spores, if present, could germinate and produce toxin. Moreover, *C. botulinum* type B, which is most often found in dry ham, is non proteolytic, so during growth and toxin production, no signs of spoilage occur in the product which would warn consumers of the danger (*Lücke and Roberts*, 1993). Only 0.1 g of food containing this toxin could lead to a fatal outcome (*Peck and Stringer*, 2004).

Implementation of appropriate control measures during animal transport pre-slaughter, slaughter (prevention of contamination), cooling, storage, meat transport and ham production (prevention of spore germination) play a primary role in preventing the occurrence of botulism in ham. In cases of increased stress, *Clostridia* spores, which could be present in the intestines of healthy animals, can break through the natural intestinal defence barrier, be carried by the bloodstream and enter into the meat (*Lücke and Roberts,* 1993; *Vukovic*, 2005a; *Anon*, 2005). When choosing raw material under hygienic conditions, the pH of the meat should be measured, as well as the temperature in the depths of the pork ham. The optimum temperature is between 0 to 4°C, while the pH should be less than 6.0, because of the slower salt diffusion. The salting should last long enough that the a_w drops below 0.96, and the concentration of salt in the water of the product is higher than 5.0% (*Anon*, 2005; *Vukovic*, 2005a; *Vukovic et al.,* 2005b; *Vasilev and Vukovic*, 2008).

Moulds

Moulds are able to grow on hams during the long curing and drying process, despite the high salt content and low temperatures, which are unable to inhibit these organisms. Smoke has a bactericidal and fungicidal effect. However, fungicides in smoke (phenols, aldehydes) are not always present in sufficient quantities, so they do not effectively stop the growth of moulds on the ham surface. Their concentration decreases over time due to evaporation and diffusion into the deep parts of the meat, where aldehydes react with proteins (*Möhler*, 1978). Most of the moulds able to grow on hams are harmless, but some of them can produce mycotoxins, such as *Penicillium* and *Aspergillus* species (*Hofmann*, 1985), and it is necessary to provide conditions to inhibit their growth and mycotoxin production. Mould growth on meat products can be prevented by adopting some of the following measures: (1) good hygiene during production and storage; (2) smoking of products; (3) ensuing the optimal relative air humidity during ripening and storage (<75%); (4) vacuum packaging; (5) coating products with various mixtures (a mixture of pork fat, flour and spices or a creamy mixture of spices containing 30% of garlic), and; (6) using additives for surface treatment of products (*Teodorovic et al.,* 2015).

Insects

Sremska ham provides an optimal environment for the development of many types of arthropods, which can be a problem during storage. Among arthropods of note are the larvae of cheese fly (*Piophila*

casei), but less often larvae of other flies (domestic house fly, stable fly, blow fly), *Aglossa pinguinalis* caterpillars (a butterfly), moth larvae (*Dysmassia parietarella*), larder beetle larvae (*Dermestes lardarius*), red-legged ham beetle (*Necrobius rufipes*), flour mite (*Tyrogliphys farinae*) and other insects. Their presence causes disgust and repulsion, and can cause various conditions in consumers, including intestinal myiasis and allergic reaction to flour mite. The larder beetle larvae, for example, digs channels in the ham, rendering it unusable (*Raseta,* 1994).

Regular and thorough enforcement of several measures can prevent insects from infecting hams. These start from the selection of raw materials which must have the specified characteristics, to conditions in the ripening premises, where all surfaces, must be thoroughly cleaned and treated with insecticides. As preparations of choice, the most commonly used are insecticides based on pyrethroids. In addition to these measures, which are implemented in order to combat pests, the important thing is constant control. This work should be conducted several times a year (*Relic et al.,* 2005).

Critical control points in Sremska ham production

Raw material selection (*CCP*)
Cutting and processing of ham
Weight measurement/classification
Preparing hams for salting
Salt mixture preparation
Salting and salt difusion (CCP)
Washing and dripping
Final processing
Smoking
Drying (CCP)
Ripening

Figure 1. Technological procedure for Sremska ham production

Figure 1 shows the technological process of Sremska ham production with marked critical control points (CCP):

At the first step in the process, animals should be slaughtered in registered establishments, using the two knife technique, with a separate, sterile knife for each ham. The raw material for the production of Sremska ham should be obtained by cutting the chilled pork carcass halves in special rooms where the air temperature is lower than 12°C. The temperature in the depths of the meat should be lower than 5°C (*Lücke and Leistner*, 1979; *Leistner*, 2000, *Vukovic*, 2005b). In order to facilitate a normal salt diffusion process, the meat pH should be less than 6.2 (*Sanabria et al.*, 1997), less than 6.1 (*Buscailhon et al.*, 1994), or less than 5.8 (*Leistner* 2000). At a very low pH, proteolytic changes are the most intense and these could lead to a bitter taste and an unacceptably soft structure of meat (*Schivazappa et al*, 2002). As the pH of the meat directly influences the salt diffusion process, the selection of meat according to pH is a CCP in the production of dry ham. The next CCP is the operation of salting and salt diffusion, because of the risk of developing non proteolytic *C. botulinum* type B. The critical limit is a temperature of 4°C, as temperatures below this prevent spore germination until a sufficient salt content develops in the deepest parts of the ham. Drying lasts for three months and if the temperature rises above 15°C, there is a risk of *C. botulinum* proliferation until the a_w decreases below 0.89 to 0.92, sufficiently low to ensure these bacteria do not grow (*Vukovic et al.*, 2005a). Under such conditions, only moulds are able to grow on the surface of the product, and these can be controlled by the measures described above.

Bacterial hazards in the Sremska ham production process can be eliminated or reduced if the temperature in the critical production stages is less than the minimum temperature of growth for *C. botulinum*, and if the content of salt in the product can inhibit this pathogen. Therefore, the manufacturing process of Sremska ham must be based on HACCP principles including appropriate controls at critical points throughout the process.

Conclusion

Sremska ham is a traditional dry-cured meat product manufactured by salting, smoking and drying of pork hams under natural environmental conditions in the geographical area of Srem. The technological process of production of Sremska ham includes: appropriate selection of raw materials, salting, salt diffusion, smoking, drying and ripening. The final product is microbiologically stable and achieves the required characteristic aroma, consistency and texture.

Typical biohazards in Sremska ham include *C. botulinum* spores, if conditions exist for their germination and toxin production, mycotoxin-producing moulds, and insect larvae infestations. Those

hazards can be eliminated or reduced by the low temperature maintained during the critical production stages and ensuring sufficient salt content to inhibit *C. botulinum* growth. Moulds and insects can be controlled by maintaining appropriate storage conditions. The manufacturing process of Sremska ham should be based on HACCP principles, while preserving the traditionality of the process.

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Microbiological status of minced pork meat in vacuum and modified atmosphere packaging

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A b s t r a c t: The aim of this study was to evaluate the effectiveness of different packaging conditions (vacuum and modified atmosphere) on the microbiological status (total viable count, lactic acid bacteria and Enterobacteriaceae), total volatile basic nitrogen (TVB-N) and pH in minced pork meat. Pork mince was packaged in vacuum, modified atmosphere with 20% O_2, 50% CO_2 and 30% N_2 (MAP 1) or modified atmosphere with 20% O_2, 30% CO_2 and 50% N_2 (MAP 2), refrigerated at 3±1°C and examined on the days 0, 3, 6, 9 and 12 of storage. The average total viable counts and total Enterobacteriaceae counts in vacuum packaged mince were statistically significantly higher ($p<0.01$; $p<0.05$) than in modified atmosphere packaged mince with both combinations of gases, on different days of storage. The largest decrease of total viable count and Enterobacteriaceae count was noted in modified atmosphere packaged mince with the higher concentration (50%) of CO_2.

Keywords: *Lactic acid bacteria, Enterobacteriaceae, total viable count, minced pork meat.*

Introduction

Modern meat packaging techniques are intended to maintain microbial and sensory quality of the product (*Seydim et al.*, 2006). The use of modified atmosphere packaging (MAP) to extend the shelf life of meat has been recognized for many years (*Martínez et al.*, 2005). It is well known that the composition of different gases in modified atmosphere systems can be an effective means to inhibit growth of aerobic spoilage organisms and some food-borne pathogens (*Ivanovic et al.*, 2015; *Ivanovic et al.*, 2014). Oxygen and carbon dioxide are common in gas mixtures used for MAP of raw meat. The presence of O_2 is very important in the storage of raw meats, as it maintains the meat pigment myoglobin in its oxygenated form, oxymyoglobin, which gives raw meat its bright red colour. The CO_2 is responsible for the bacteriostatic effect in modified atmospheres. This bacteriostatic effect is influenced by the concentration of CO_2, the age and load of the initial bacterial population, the storage temperature and the type of meat being packaged (*Martínez et al.*, 2005). Increased levels of CO_2 (20 to 40%) in refrigerated storage have been shown to inhibit microbial populations, and especially the growth of gram negative bacteria, by increasing their lag phase (*McMillin*, 2008).

Nitrogen is an inert gas and no chemical interaction with substances with which it comes to contact. N_2 is used to prevent contraction of coatings for packaging, prevent lipid oxidation and prevent insect attack, etc. (*Ivanovic*, 2014).

In addition to the microbiological status of minced pork meat, pH is also an important parameter used to define pork quality. It is well known that changes in some meat quality traits can affect many other meat quality attributes and pork quality overall (*Dokmanovic et al.*, 2015).

The aim of this study was to examine the microbiological status of minced pork meat in vacuum and MAP with two different levels of CO_2, and to examine the changes in total volatile basic N_2 (TVB-N) and pH in the mince, during storage at 3±1°C over a period of 12 days.

Materials and Methods

Preparation of meat, packaging materials and storage

Raw pork meat was obtained from a slaughterhouse and minced in a sterile grinder (Meat Grinder TB-300E, Thunderbird Food Machinery,

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Dallas, USA). The minced pork meat was collected (in 100±5 g amounts) in sterile plastic bags. A packaging machine, VARIOVAC (Variovac Primus, Zarrentin, Germany), was used to package the minced meat in a foil (oriented polyamide/ethylene vinyl alcohol/polyethylene dynapack foil; Polimoon, Kristiansand, Norway) with low permeability to gas. The degree of permeability of this foil was O_2: 3.2 cm^3 m^{-2} day^{-1}; N_2: 1 cm^3 m^{-2} day^{-1}; CO_2: 14cm^3 m^{-2} day^{-1} at 23°C; and water vapour: 15 g m^{-2} day^{-1} at 38°C. The minced meat was packaged in vacuum or in the modified atmospheres MAP 1 or MAP 2. MAP 1 consisted of 20% O_2, 50% CO_2 and 30% N_2; MAP 2 consisted of 20% O_2, 30% CO_2 and 50% N_2. The packaged minced pork meat was stored at 3±1°C for 12 days. Six packs subjected to each packaging method were analysed on each of days 0, 3, 6, 9 and 12 of storage. The mince pH was measured with a pH meter (TESTO 205; Lenzkirch, Germany), and every three days during storage, the TVB-N was examined according to *Goulas and Kontominas* (2007).

Microbiological and chemical analysis

From each analysed pack of minced meat, 10 g was transferred to a Stomacher bag (Stomacher 400 Classic Bags, Belgrade, Serbia, VICOR), 90 mL of maximum recovery diluent (Merck, Germany) was added and the content was homogenized for 1 min with a stomacher blender (Stomacher 400 Circulator, Seward, UK). Microbial determinations were performed according to the following analytical methods: *ISO* (2008), total viable count; *ISO* (1998b), lactic acid bacteria count, and; *ISO* (2009), *Enterobacteriaceae* count. Chemical analyses were performed according to the following analytical methods: *ISO* (1998a), moisture content; *ISO* (1992b), lipid content; *ISO* (1992a), protein content, and; *ISO* (1999), total ash.

Statistical analysis

Statistical analysis of the results was conducted using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and standard deviation (SD). One-way ANOVA and post hoc Tukey's test were performed to assess the significance of differences among various groups. Values of $p<0.05$ and $p<0.01$ were considered significant.

Results and Discussion

The moisture content of raw minced pork meat at the beginning of study was 70.31±0.22%, the lipid content was 18.68±0.32%, protein content was 18.68±0.32% and total ash content was 1.01±0.03%.

The total viable counts in minced pork meat during 12 days of storage are presented in Table 1. At the beginning of the study, the total viable count in the minced meat was 8.10±0.16 log CFU g^{-1}. The average total viable count varied from 7.66±0.14 log CFU g^{-1} (vacuum) to 7.84±0.23 log CFU g^{-1} (MAP 1) on day 3 of storage. On day 3, there was no statistically significant difference ($p>0.05$) between the average total viable counts in the mince subjected to the three packaging regimes. Comparing the storage days, a statistically significant difference in the total viable count was found on days 6 and 9 of storage. The average total viable count in vacuum packaged mince was significantly higher ($p<0.01$) than the average total viable count in both MAP 1 and MAP 2 on day 12 of storage.

Analysis of the minced meat on days 0 and 12 showed that there was a statistically significant difference ($p<0.01$; $p<0.05$) in total viable counts under the same packaging conditions (Figure 1). The statistically significant differences were found in vacuum ($p<0.05$) and MAP 1 ($p<0.01$).

Kakouri and Nychas (1994) and *Bell et al.* (1995) found that mesophilic aerobic bacteria needed

Table 1. Total viable count (log CFU g^{-1}) in minced raw pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	8.10±0.16	7.74±0.29	7.77^{A}±0.08	8.42^{AB}±0.05	8.41^{AB}±0.09
MAP 1	8.10±0.16	7.84±0.23	8.16^{AB}±0.16	8.57^{AC}±0.05	7.66^{AC}±0.07
MAP 2	8.10±0.16	7.66±0.14	7.70^{B}±0.08	8.03^{BC}±0.10	8.15^{BC}±0.11

Legend: $^{A, B, C}$ Within a column, means with a common superscript letter are significantly different, $p< 0.01$

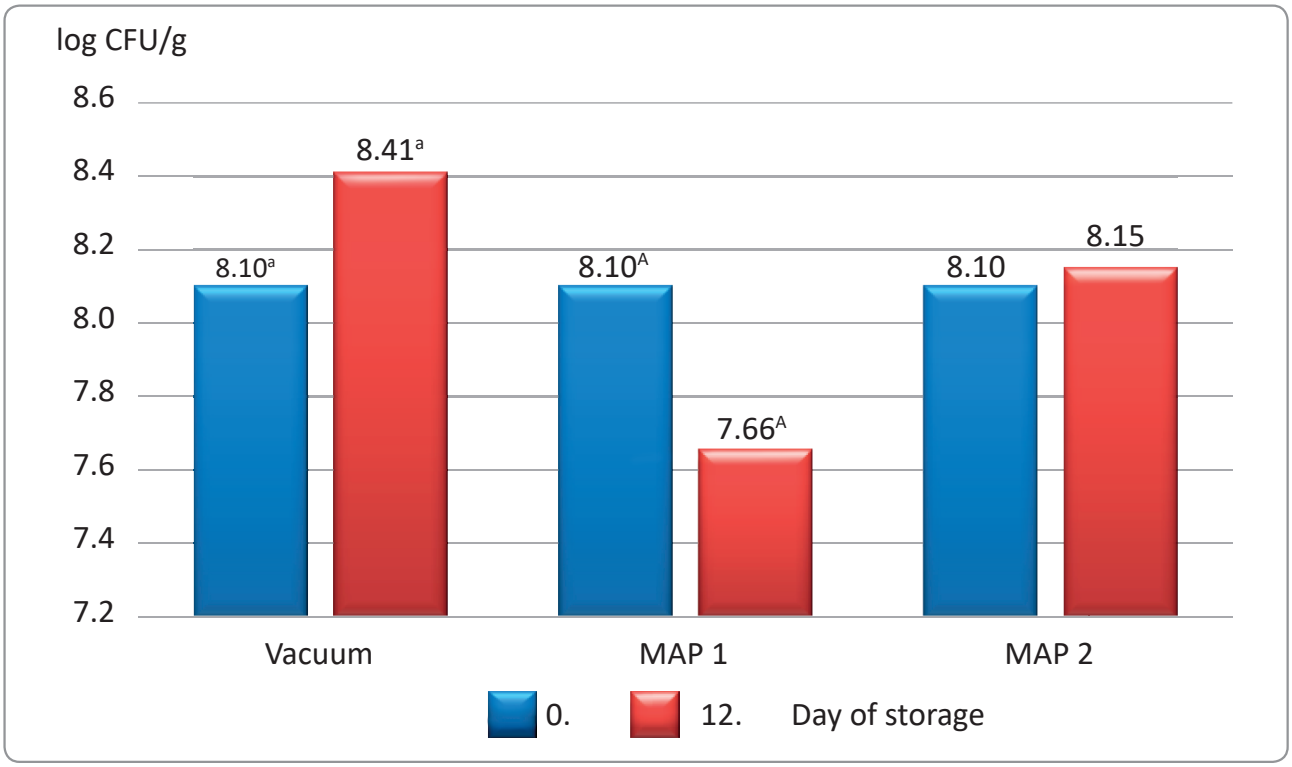

Figure 1. Total viable count at the beginning and at the end of the study ($^{a}p<0.05$; $^{A}p<0.01$)

approximately 10 days to achieve these counts (7.7 to 8.4 log CFU g^{-1}) in the same atmosphere in pork meat. *Zeitoun et al.* (1994) detected decreased total aerobic bacteria packaged in MAP with 90% CO_2 and 10% O_2. According to Table 1, total aerobic bacteria in our study grew slower under MAP 2. An explanation for this situation could be the high concentration of CO_2 which has bacteriostatic effects (*Arashisar et al.*, 2004). It is known that antimicrobial effect of CO_2 increases depending on the solubility, which is increased by the low water temperature, and CO_2 prolongs lag phase of bacterial growth and increases generation time (*Arashisar et al.*, 2004).

During the storage period, the average total *Enterobacteriaceae* count varied from 7.16±0.06 log CFU g^{-1} (day 0) to 7.72±0.05 log CFU g^{-1} (day 12) in vacuum packaged minced meat, up to 7.60±0.08 log CFU g^{-1} in MAP 1 mince and up to 7.66±0.05 log CFU g^{-1} in MAP 2 mince (Table 2). A significant difference ($p<0.01$) was found on day 6 between the average total *Enterobacteriaceae* count in vacuum and MAP mince.

The average total *Enterobacteriaceae* count on day 0 in vacuum was significantly lower ($p<0.01$) than the average total *Enterobacteriaceae* count in vacuum packaged mince at the end of study. Also, similar results were found in MAP 1 and MAP 2 mince (Figure 2).

The presence of *Enterobacteriaceae* in meat and meat products is determined in order to assess the general hygienic status of meat. Some

Table 2. Counts of *Enterobacteriaceae* (log CFU g^{-1}) in minced raw pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	7.16±0.06	7.17±0.26	7.63^{AB}±0.05	7.62±0.04	7.72±0.05
MAP 1	7.16±0.06	7.10±0.07	7.62^{A}±0.05	7.65±0.04	7.60±0.08
MAP 2	7.15±0.05	7.16±0.06	7.46^{B}±0.04	7.62±0.05	7.66±0.05

Legend: $^{A, B}$ Within a column, means with a common superscript letter are significantly different, $p<0.01$

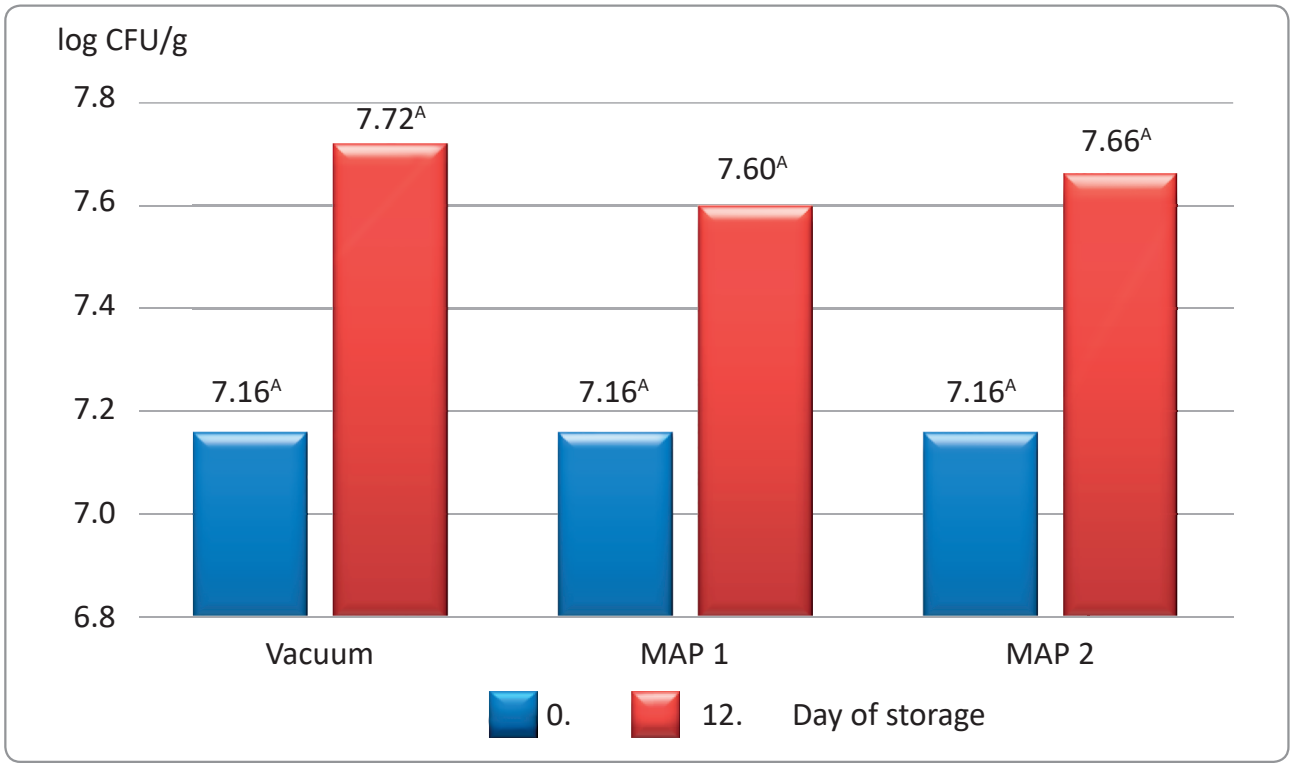

Figure 2. *Enterobacteriaceae* at the beginning and at the end of the study (^{A} p< 0.01)

authors recommend that the average number of *Enterobacteriaceae* can be used as criteria in assessing the sustainability of meat (*Ivanovic et al.*, 2014; *Djordjevic et al.*, 2016). Some of the *Enterobacteriaceae* are of interest to public health while others have commercial importance because of their ability to cause deterioration of meat and meat products during storage at refrigeration temperatures (*Ivanovic et al.*, 2014). CO_2 causes damage to bacterial cell membranes and causes changes in its functions, including the effect on the absorption processes and transport of nutrients through the membrane. This gas also penetrates into the bacterial cells, which leads to decreases in intracellular pH (acidification), and causes direct changes in the proteins' physico-chemical properties (*Siverstvik et al.* 2002; *Goulas and Kontominas*, 2007; *Cornforth and Hunt*, 2008). The average total *Enterobacteriaceae* count in our vacuum packaged mince was higher than the average total *Enterobacteriaceae* count in mince in both types of MAP from day 6 of storage. *Djordjevic et al*. (2016) found that the largest decrease in average total *Enterobacteriaceae* count was observed in minced meat packed in modified atmosphere with 50% CO_2. On day 12 of storage, significantly lower average total *Enterobacteriaceae* counts were measured in mince from MAP with 50% CO_2 than in mince from MAP with 30% CO_2 (*Djordjevic et al.*, 2016).

Table 3. Counts of lactic acid bacteria in raw minced pork meat during 12 days storage (log CFU g^{-1}) in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	5.78±0.06	5.80±0.08	5.94^{a}±0.04	6.01^{AB}±0.06	6.31^{A}±0.06
MAP 1	5.78±0.06	5.81±0.03	5.82^{aA}±0.05	6.31^{AC}±0.03	6.34^{B}±0.04
MAP 2	5.78±0.06	5.82±0.05	5.97^{A}±0.07	5.91^{BC}±0.05	6.13^{AB}±0.04

Legend: $^{A, B, C}$ Within a column, means with a common superscript letter are significantly different, p<0.01
^{a} Within a column, means with a common superscript letter are significantly different, p<0.05

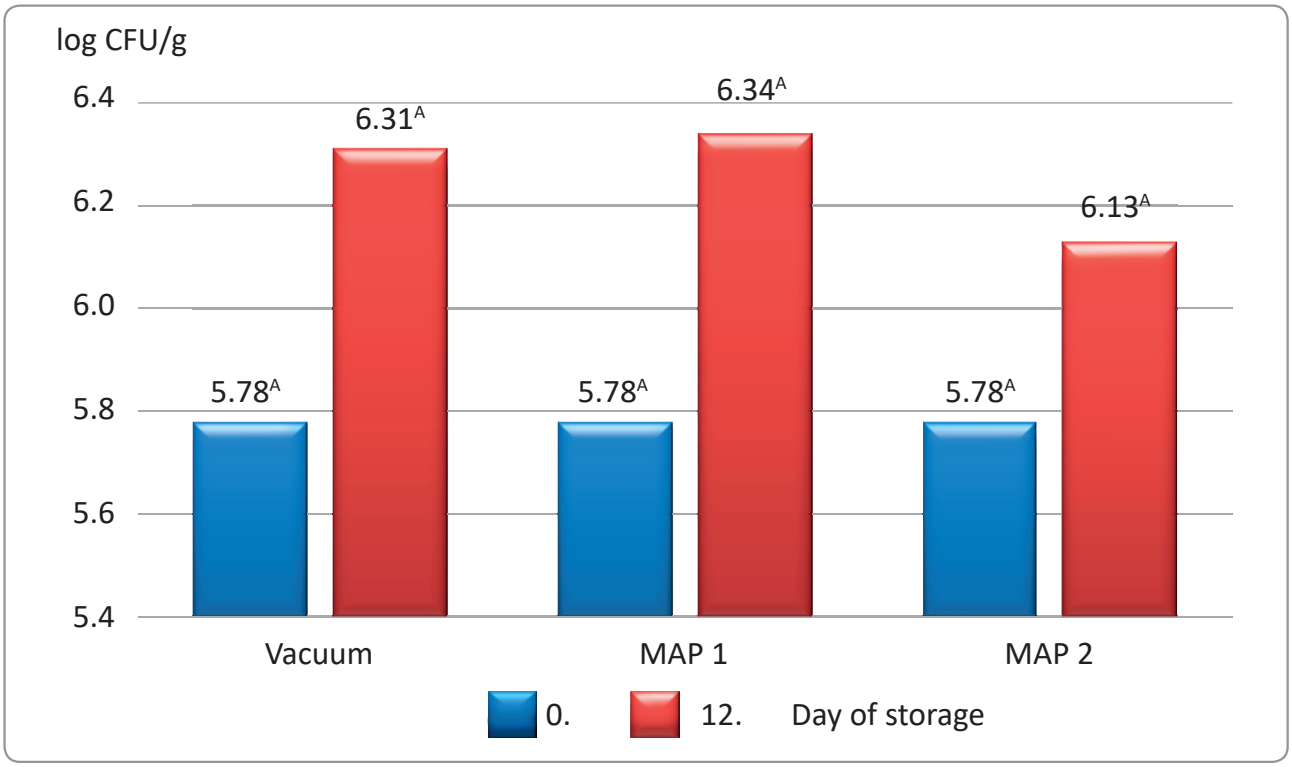

Figure 3. Lactic acid bacteria at the beginning and at the end of the study (^{A} $p<0.01$)

The average lactic acid bacteria count in minced raw meat was 5.78±0.06 log CFU g^{-1} at the beginning of the study (Table 3). On days 6, 9 and 12, statistically significant differences ($p<0.05$; $p<0.01$) were found between the average lactic acid bacteria counts. During the storage period, the average lactic acid bacteria count increased from day 0 to 6.31±0.06 log CFU g^{-1} (day 12) in vacuum packaged minced meat, up to 6.34±0.04 log CFU g^{-1} in MAP 1 mince and up to 6.13±0.04 log CFU g^{-1} in MAP 2 mince.

Significant differences ($p<0.01$) were found in lactic acid bacteria numbers in the mince on day 0 and day 12 in vacuum, MAP 1 and MAP 2 (Figure 3).

Lactic acid bacteria are considered to be specific spoilage organisms that contribute to meat spoilage stored under packaging conditions in which the concentration of CO_2 is increased (*Nychas and Skandamis*, 2005). *Lactobacillus* spp., *Leuconostoc* spp. and *Carnobacterium* spp. are among the most frequently encountered genera on vacuum or modified atmosphere packaged meat and play an important role in the spoilage of refrigerated raw meat (*Parente et al.,* 2001; *Nychas and Skandamis*, 2005). *Daly and Acton* (2004) reported that fresh 92% lean ground beef in high O_2 packaging atmospheres initially had a mean count of 3.72 log CFU g^{-1} of lactic acid bacteria with outgrowth to 5.4 log CFU g^{-1} in 9 days at 4.4°C, similar to the outgrowth found in the current study for minced pork meat in the same time period.

The TVB-N values increased in the mince during storage in vacuum packaging (9.83±0.27 mg N $100g^{-1}$ and 38.79±1.65 mg N $100g^{-1}$ on days 0

Table 4. TVB-N values (mg N $100g^{-1}$) in raw minced pork during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	9.83±0.27	13.41±0.47	21.18±0.70	30.47±0.77	38.79±1.65
MAP 1	9.83±0.27	13.25±0.63	20.54±0.86	29.91±0.71	37.36±1.06
MAP 2	9.83±0.27	13.44±0.25	20.70±0.95	30.31±0.68	37.77±1.48

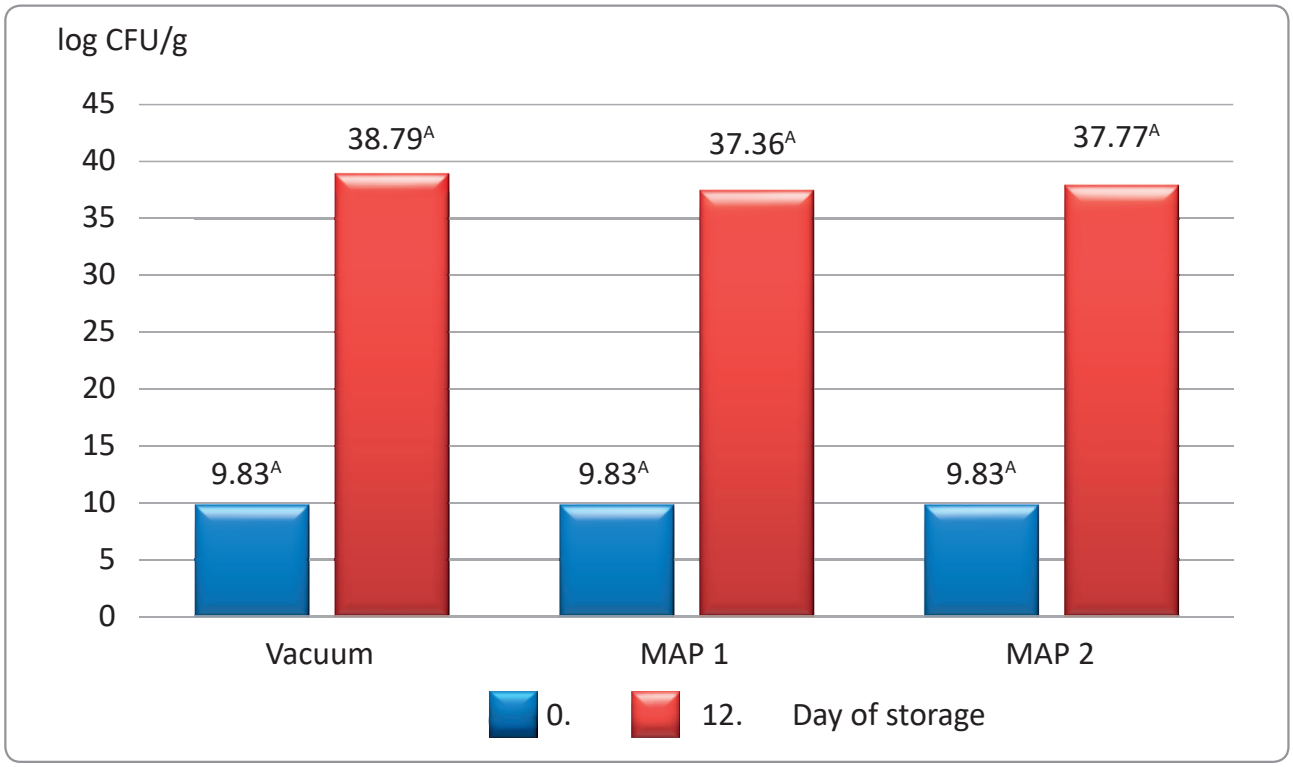

Figure 4. TVB-N values at the beginning and at the end of the study ($^{A-}$ $p<0.01$)

and 12, respectively; Table 4). In MAP 1, TVB-N values increased during storage time (9.83±0.27 mg N 100g^{-1} and 37.36±1.06 mg N 100g^{-1} on days 0 and 12, respectively; Table 4). TVB-N in MAP 2 was 9.83±0.27 mg N 100g^{-1} and 37.77±1.48 mg N 100g^{-1} on day 0 and 12, respectively. No significant differences in TVB-N values were detected between vacuum, MAP 1 and MAP 2 stored pork minced meat during the storage period. However, although differences were not significant, TVB-N values in pork were numerically highest in vacuum packaging on the end of the study (day 12).

Storage time was a significant factor for TVB-N value increases, regardless of the packaging effect (Figure 4). Significant differences ($p<0.01$) were found in vacuum, MAP 1 and MAP 2 at the beginning and at the end of the study (Figure 4).

The TVB-N value is used as a chemical indicator for assessing the freshness of pork (*Ivanovic et al.*, 2015). The TVB-N value measures the concentrations of compounds responsible for the occurrence of unpleasant smell and taste of meat (ammonia, dimethylamine, trimethylamine, amines). The prescribed limit value for TVB-N content of pork meat is 30 mg N 100g^{-1} (*Connell*, 1990). In this study, TVB-N values on day 12 were above the prescribed limit. TVB-N values for pork meat in vacuum packages on days 9 and 12 of storage were very high (>30 mg N 100g^{-1}) as compared to beef (*Brewer and Wu*, 1993), poultry (*Ulu*, 2004), and rabbit meat (*Fernandez-Espla and O'Neill*, 1993).

Table 5. The pH of raw minced pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	6.04±0.01	6.02AB±0.01	5.97A±0.02	5.90AB±0.01	5.79AB±0.01
MAP 1	6.04±0.01	5.92A±0.01	5.95B±0.03	5.97A±0.01	5.94A±0.01
MAP 2	6.04±0.01	5.90B±0.01	5.83AB±0.02	5.97B±0.01	5.92B±0.01

Legend: $^{A, B}$ Within a column, means with a common superscript letter are significantly different, $p<0.01$

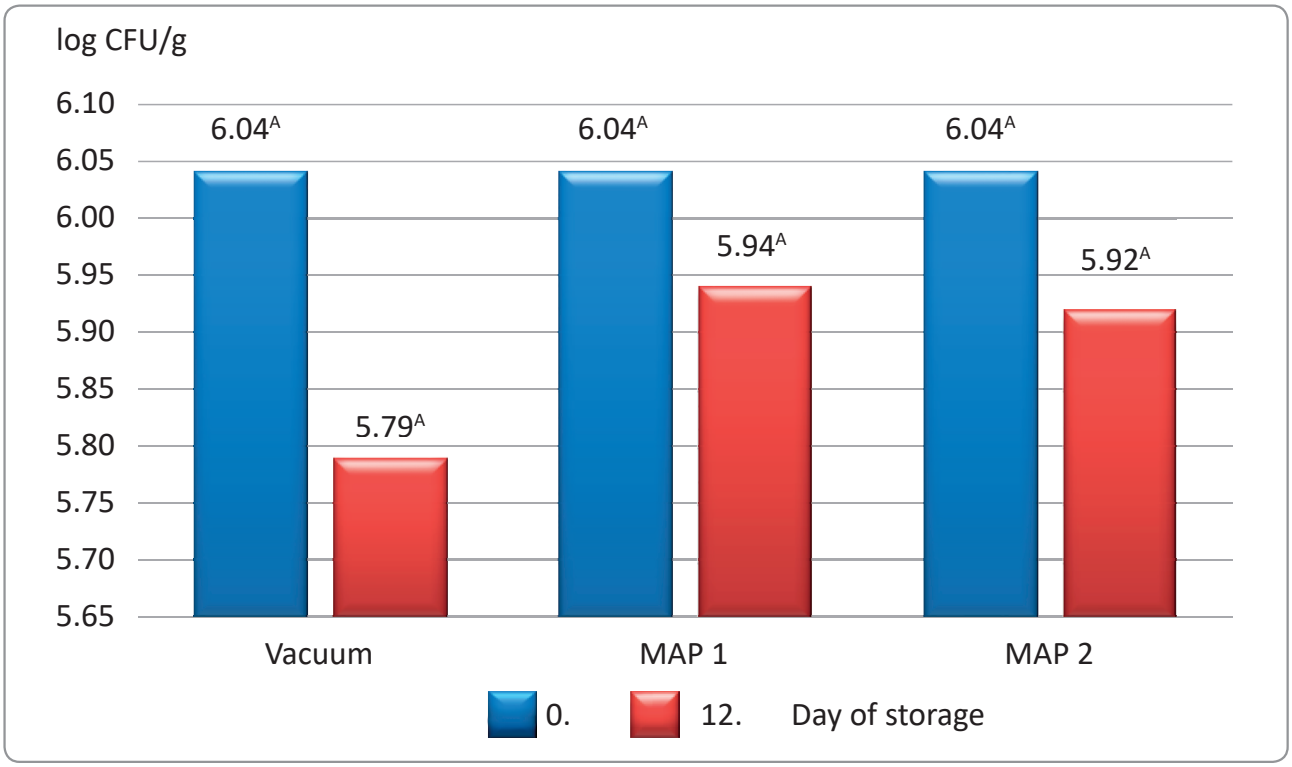

Figure 5. The pH values at the beginning and at the end of the study (^{A} $p<0.01$)

The initial pH of the minced pork was 6.04±0.01 in all packaging types and decreased during the storage period (pH values were 5.79±0.01 in vacuum, 5.94±0.01 in MAP 1 and 5.92±0.01 in MAP 2 on day 12; Table 5). No significant differences occurred in pH between mince packed in vacuum, MAP 1 and MAP 2 during the first three days, but significant differences in pH were detected on days 3, 6, 9 and 12 of storage ($p<0.01$).

At the beginning of the study, the average pH of vacuum, MAP 1 and MAP 2 mince was statistically lower than the average pH of the same groups of minced meat at the end of the study (Figure 5).

In minced meat packaged in MAP, a slight decrease of average pH was noticed. In vacuum packaged minced meat, a slight decrease in average pH value occurred, which can be explained by the absence of CO_2. Similar results were reported by *Nissen et al*. (2000), *Juncher et al*. (2001), *Masniyom et al*. (2002), *Arashisar et al*. (2004), *Martínez et al*. (2005), *Yilmaz and Demirci* (2010), *Djordjevic et al*. (2016) and *Ivanovic et al*. (2015)

Conclusions

This study has shown that MAP with a high concentration of CO_2 is the better choice for packaging fresh pork meat than vacuum and packaging with a lower concentration of CO_2. The microbiological status (total viable count, *Enterobacteriaceae* and lactic acid bacteria) of raw minced pork meat packaged in MAP was acceptable compared to the vacuum samples. TVB-N value was acceptable until the day 12 of the storage period.

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Original scientific paper

Sensory evaluation of cold-smoked trout packaged in vacuum and modified atmosphere

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A b s t r a c t: The aim of the study was to investigate the influence of different packaging methods on the sensory properties of cold smoked trout fillets stored at 3°C during six weeks. Cold smoked trout fillets were vacuumed packaged or packaged in one of two modified atmospheres with a gas ratio of 50% CO_2:50% N_2 or 90% CO_2:10% N_2. Before and after storage, fillets underwent sensory analysis for colour intensity, odour and taste of smoke intensity, tenderness and overall acceptability (on days 0, 7, 14, 21, 28, 35 and 42 of storage). Colour intensity, odour and taste of smoke intensity and overall acceptability of all examined groups of cold smoked trout fillets decreased during storage, while tenderness of the fish fillets remained virtually unchanged. Cold-smoked trout fillets packaged in the modified atmospheres had higher average sensory scores compared to vacuum packaged cold-smoked trout fillets.

Keywords: *trout, smoking, packaging, storage, sensory properties.*

Introduction

Proper nutrition has primary importance in good life quality. For this reason, fish meat, due to its nutritional value, occupies an important place in the human diet. Throughout human history, fish meat has been a significant food source, important for survival and human development. Fish has always been appreciated in coastal areas, especially those with deficient conditions for agricultural development. Fish as a food, in addition to the favourable content of proteins, minerals and vitamins, seems particularly attractive to the consumer because it is a very rich source of essential fatty acids, which play a role in the prevention of many human diseases (*Cvrtila and Kozacinski,* 2006; *Baltic et al.,* 2010).

Food preservation, and therefore smoking, as one of the oldest methods of meat preservation, dates back to prehistoric times, while today, the process of fish smoking in developed countries is applied primarily to improve fish sensory characteristics and conveniently market an assortment of fish meat and products (*Vasiliadou et al.,* 2005*; Foucat et al.,* 2008*; Gomez-Guillen et al.,* 2009). In accordance with the standard for smoked fish, smoke-flavoured fish and smoked dried fish *(Codex Alimentarius,* 2013*)*, cold-smoked fish is produced by treating fish with smoke using time/temperature combinations that will not cause significant coagulation of the proteins in the fish meat, but will cause some reduction of the water activity. Salting, packaging and storage processes are also defined in this standard. In the literature, there are numerous data relating to the factors important for the smoked fish quality. Special attention is dedicated to the shelf life of smoked fish (bacteriological status, physical, chemical, sensory properties), particularly with regard to the packaging method, which aims to extend the shelf life of smoked fish (*Salama and Ibrahim,* 2012; *Loncina et al,* 2013*; Daramola et al.,* 2014*; Rizo et al.,* 2015). The finished product is usually vacuum packaged and refrigerated or frozen and consumed as a ready-to-eat food. The shelf life of cold-smoked fish is three to six weeks at temperatures below 5°C. The consumption of smoked fish has grown significantly in the last decade in many European countries (*Cardinal et al.,* 2001; *Foucat et al.,* 2008). In Serbia, the most commonly consumed smoked fish is cold-smoked trout (*Baltic et al.,* 2006).

Appearance, texture, colour, odour and perhaps taste of fish meat and fish products are analysed at purchase/consumption using human senses (*Olafsdottir et al.,* 2005). ISO standards in the field

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of sensory analysis, adopted in the past 20 years, have contributed to the objectification of sensory analysis (*Baltic and Karabasil,* 2010). Sensory properties of cold-smoked fish depend on numerous factors (nutrition, primary treatment, the brine composition, condition and quality of smoke, packaging method) (*Franco et al.,* 2010*; Ghouaiel et al.,* 2013*; Ameko et al.,* 2016).

The aim of this study was to investigate the influence of different packaging methods on the sensory properties of cold-smoked trout stored at 3°C during 42 days.

Material and methods

Cold-smoked trout samples preparations

Salmon trout (*Oncorhynchus mykiss*) weighed about 1 kg and originated from the Bocac Fish Farm, owned by Tropic, Banja Luka, Republic of Srpska. All salmon trout were grown and fed under the same conditions. The live fish were transported from the fish farm to the processing plant in specialized vehicles with liquid oxygen under controlled temperature conditions. Trout were placed in outdoor open pools that had the conditions necessary for fish survival (a constant water flow, adequate temperature), and were not fed. Trout were primarily processed (stunning, bleeding, evisceration) in the manner usual for this industrial plant. After primary processing, the spine was removed by cutting parallel to it, whereby the left and right halves were obtained. Trout halves were rinsed with water and immersed in barrels with brine (wet salting) with a 9% salt concentration. Rosemary (*Quantum satis*), was added to the brine according to the principles of good manufacturing practice. Fish were brined for 24 hours at 4°C. After brining, fish were drained for one hour at 20°C and then smoked for eight hours in an automated smoke chamber at 28°C. Smoke was produced by combustion of beech sawdust in a generator separated from the smoking chamber. After smoking, fish were cooled at 2°C for ten hours, then residual parts of the ribs were removed and fish halves were sliced from the medial carcass side, into thin fillets, up to 0.5 cm thick, each weighing about 75 g.

Cold-smoked trout fillet packaging

Cold-smoked trout fillets were split into three groups. The first group was vacuum packaged (group I), the second was packaged in a modified atmosphere with 50% CO_2 and 50% N_2 (group II), and the third was packaged in a modified atmosphere with 90% CO_2 and 10% N_2 (group III).

Modified atmosphere packaging was conducted using a Multivac machine (Multivac C350, D-87787 Wolfertschwenden, Germany). The packaging foil was OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene, UPM-Kymmene, Walki Films, Finland) with low gas permeability (degree of permeability to oxygen 5 $cm^3\ m^{-2}\ day^{-1}$ at 23°C, nitrogen 1 $cm^3\ m^{-2}\ day^{-1}$ at 23°C, carbon dioxide $cm^3\ m^{-2}\ day^{-1}$ at 23°C and water vapour 15 $g\ m^{-2}\ day^{-1}$ at 38°C). Packages were filled with commercial gas mixtures (Messer Tehnogas, Serbia). The ratio of gas:fish in the packages was 2:1.

After packaging, all three groups of fish fillets were stored at 3°C for six weeks.

Selection of assessors and sensory evaluation

Samples for sensory analyses were taken on day 0 and on days 7, 14, 21, 28, 35 and 42 of storage. Before evaluation, fish fillets were kept at room temperature for 20 min. Fourteen assessors participated in sensory evaluation and were selected according to Serbian/ISO standard (*SRPS*, 2015; *SRPS* 2012a). The evaluation was conducted in a sensory laboratory designed according to Serbian/ISO standard (*SRPS*, 2012b), using quantitative descriptive analysis (*SRPS*, 2001). Four parameters, colour intensity, odour and taste of smoke intensity, tenderness and overall acceptability, were evaluated. The highest score was 5, and the lowest 1, with exception of the tenderness intensity evaluation, where the optimal score was 3.5 (score 5 was for the firmest meat and score 1 the most tender meat).

Chemical composition

The basic chemical composition was determined in fish fillets on day 0. Chemical analyses to determine protein, water, fat and mineral content were conducted according to standard methods (*AOAC,* 1990*)*.

Statistical analysis

Statistical analyses of the results were conducted using the software GraphPad Prism version 6.00 (GraphPad Software, San Diego, California USA, www.graphpad.com). The results were expressed as mean ± SD and are reported in tables and figures. The effects of different packaging treatments were appraised during storage period by one-factor analysis of variance – ANOVA – with Tukey's multiple

comparison test at 99% or 95% confidence levels (differences were considered significant if $p<0.01$ or $p<0.05$).

Results and Discussion

Basic chemical composition of cold-smoked trout fillets

The basic chemical composition of our cold-smoked trout fillets was similar to that reported for this type of fish (*Kilibarda*, 2009). The average water content in the cold smoked trout fillets at the beginning of the study was 71.00±2.43%, the average protein content 8.21±1.03%, the average fat content 2.08±0.46%, the average ash content 4.38±0.92%. The average sodium chloride content was 3.27±0.72. The sodium chloride content of smoked salmon in Norway is 2 to 4% (*Albarracin et al.,* 2011*; Almli and Hersleth,* 2013).

Colour intensity of cold-smoked trout fillets during storage

On day 0, colour intensity, odour and taste of smoke intensity and overall acceptability scores were 5, while tenderness scores were 3.5 for all groups of trout fillets.

The colour intensity of all groups of cold-smoked trout fillets decreased during storage (Table 1; Figure 1). However, the colour intensity of groups I and III drastically decreased. In contrast, *Kolodziejska et al.* (2002) and *Leroi et al.* (2001) showed that colour intensity of vacuum packaged cold-smoked fish did not significantly change during three weeks of storage at 4°C. Likewise, the colour intensity of smoked salmon packaged in vacuum and modified atmosphere stored for 35 days did not change (*Bugueno et al.,* 2003).

Table 1. Sensory evaluation of colour intensity of packaged cold-smoked trout during storage

Group	Day of Storage					
	7	14	21	28	35	42
I	4.81^{AB}±0.37	4.31^{A}±0.26	4.19^{aA}±0.26	3.25^{A}±0.27	3.31^{A}±0.26	2.19^{A}±0.37
II	3.25^{AC}±0.38	3.50^{AB}±0.27	3.69^{ab}±0.37	3.49^{B}±0.04	3.13^{a}±0.35	3.56^{AB}±0.42
III	4.19^{BC}±0.26	4.06^{B}±0.18	3.19^{Ab}±0.37	2.69^{AB}±0.26	2.56^{Aa}±0.32	2.63^{B}±0.52

Same letter within a column indicates a significance difference $^{A–C}$ $p<0.01$; $^{a, b}$ $p<0.05$

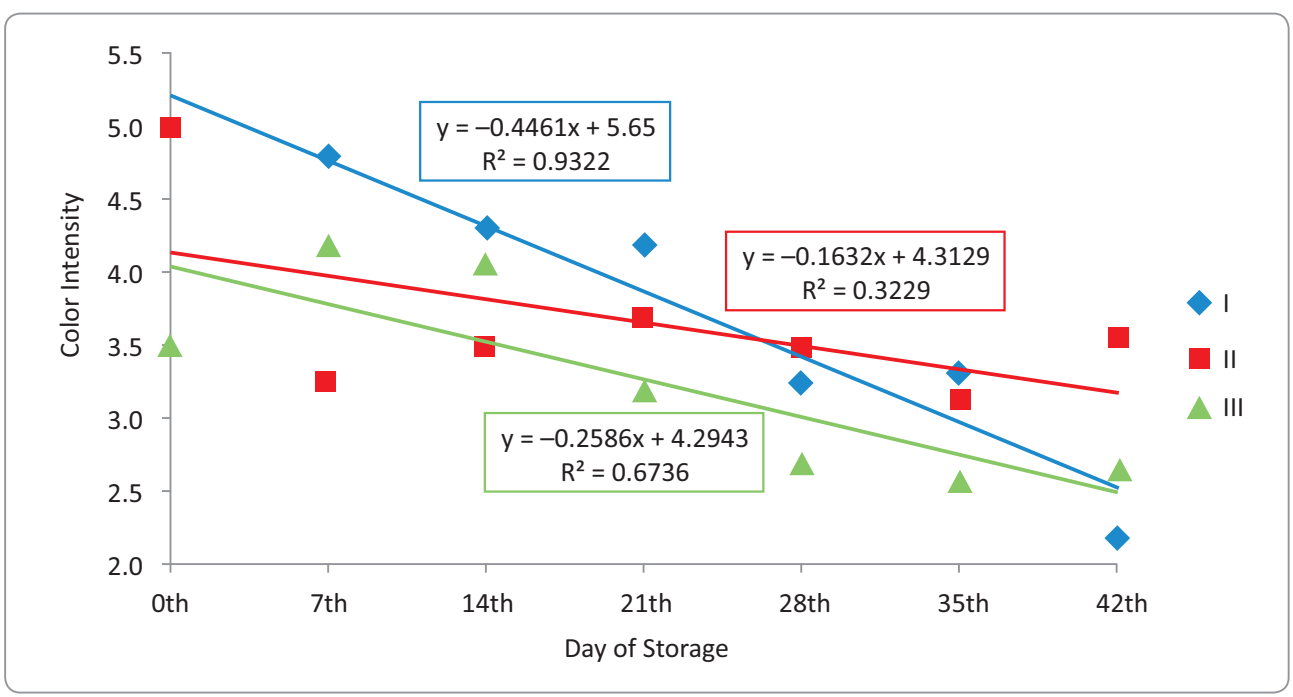

Figure 1. Sensory evaluation of colour intensity of packaged cold-smoked trout during storage

The colour intensity change of group III trout fillets during storage could be attributed to the large proportion of CO_2 in the modified atmosphere packaging. *Masniyom et al.* (2002) examined the sensory properties of fresh sea bass and mackerel packaged in modified atmosphere and also concluded that high CO_2 concentrations caused adverse colour changes. *Choubert and Baccaunaud* (2006) observed that salmon trout had a more intensive colour when it was packaged in a modified atmosphere with a lower content of CO_2 and in combination with N_2, compared to modified atmosphere packaging with higher CO_2 levels.

Numerous studies in the European Union showed that the colour of smoked products (particularly smoked fish) is a primary parameter that leads the consumer to buy a particular type of food (*Espe et al.,* 2004*; Schubring,* 2008). *Johnston et al.* (2000) and *Lakshmanan et al.* (2005) emphasise that undesirable colour, as one of the most important cold-smoked salmon quality parameters, greatly reduces the cost of this product on the market.

Salmon discoloration, as a result of pigment loss, was noted by *Partmann* (1981), when salmon was packaged in a modified atmosphere with 100% CO_2, and by *Barnett et al.* (1982), with salmon, packaged in a modified atmosphere with 90% CO_2. The less intensive colour of trout fillets packaged in modified atmospheres should not result from changes in smoke constituents (although these compounds give smoked fish its characteristic colour), but are likely due to the loss of fish pigments, which give fish its specific, initial colour, as result of CO_2 effects. *Poli et al.* (2006) point out that the reason for decreasing fish colour intensity after packaging in modified atmospheres could be muscle and pigment protein denaturation, as the result of carbonic acid formation. CO_2 dissolving in fish tissues under modified atmosphere packaging leads to acidification and decrease of the meat pH, which causes changes in the fish colour (*Torrieri et al.,* 2006).

Odour and taste of smoke intensity of cold-smoked trout fillets during storage

The odour and taste of smoke intensity of all groups of cold-smoked trout, although high, decreased during storage (Table 2).

The high scores for odour and taste of smoke intensity were not surprising, given the importance this quality has for cold-smoked fish quality (*Jonsdottir et al.,* 2008*; Olafsdottir and Kristbergsson,* 2006). Although odour and taste of smoke intensity scores decreased during the storage period, they remained the highest in group III trout fillets, compared to the other two groups (Figure 2). *Truelstrup Hansen and Huss* (1998) and *Leroi et al.* (1998) in their examination of vacuum packaged cold-smoked trout, also found that during storage, the odour and taste of smoke intensity decreased, and became milder or almost neutral.

Volatile substances that are usually produced by bacteria are the cause of undesirable odours in smoked fish (*Olafsdottir et al.,* 2005*; Dondero et al.,* 2004). These substances include trimethylamine, the volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine, and other low molecular weight substances. The substrates for the formation of these volatile compounds are trimethylamine oxide, sulphur-containing compounds, carbohydrates (ribose and lactate) nucleotides (inosine monophosphate, inosine), and other non-protein nitrogen compounds. Trimethylamine is responsible for the typical sharp "fishy" odour, indicator of spoilage.

An interesting result of the current study is the intense odour and taste of smoke discerned in group III trout fillets during whole storage period. Currently, we are unable to explain this phenomenon, and therefore, it is very interesting as a subject for future research. *Leroi et al.* (1996) produced similar observations during the examination of vacuum packaged cold-smoked salmon, which they attributed to yeast inhibition because of the high CO_2 concentration, and which reduced an undesirable acidic smell. It is also possible that the dissolved CO_2, with consequent decrease in pH, stimulated or accelerated some reactions responsible for forming the odour and

Table 2. Sensory evaluation of odour and taste of smoke intensity of packaged cold-smoked trout during storage

Group	Day of Storage					
	7	14	21	28	35	42
I	$4.00^{aA}\pm0.27$	$4.13^{A}\pm0.26$	$3.31^{AB}\pm0.46$	$3.31^{AB}\pm0.26$	$1.94^{AB}\pm0.50$	$1.31^{aA}\pm0.26$
II	$4.50^{a}\pm0.38$	$3.81^{B}\pm0.46$	$4.38^{A}\pm0.23$	$3.94^{AC}\pm0.18$	$3.31^{AC}\pm0.37$	$1.00^{aB}\pm0.00$
III	$4.75^{A}\pm0.27$	$4.81^{AB}\pm0.26$	$4.63^{B}\pm0.69$	$4.56^{BC}\pm0.18$	$4.31^{BC}\pm0.26$	$3.25^{AB}\pm0.27$

Same letter within a column indicates a significance difference $^{A–C}$ $p<0.01$; ^{a} $p<0.05$

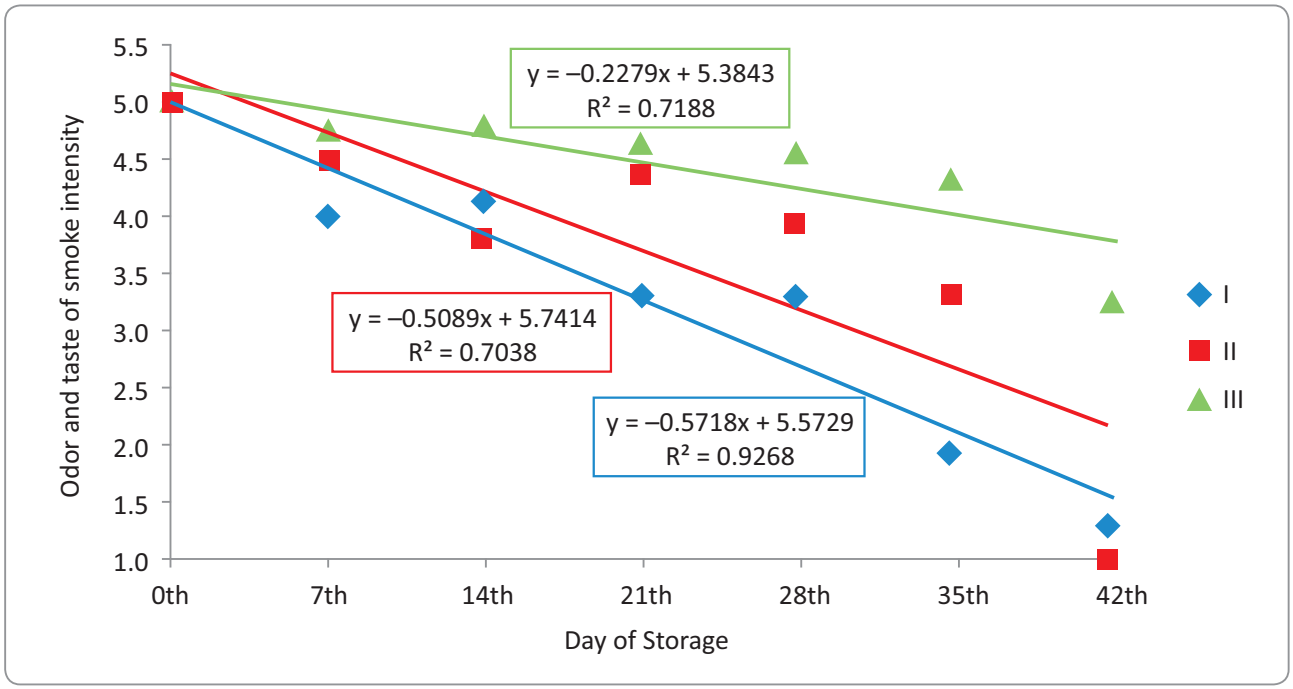

Figure 2. Sensory odour and taste of smoke intensity evaluation of packaged cold-smoked trout during storage

taste of smoke. Phenols are mostly responsible for forming the odour and taste of smoke (*Kostyra and Barylka-Pikielna,* 2006). As well, functional groups of low molecular weight carbonyl compounds (aldehydes, ketones) can affect odour and taste of smoke in smoked products (*Gomez-Guillen et al.,* 2006*; Kostyra and Barylka-Pikielna,* 2006).

Although the high average odour and taste of smoke intensity scores of group III trout fillets in the present study had a positive impact on the overall acceptability of these fillets, it could also have masked, for example, the odour of amine, an initial sign of fish spoilage (*Lyhs,* 2002*; Cardinal et al.,* 2004).

Tenderness evaluation of cold-smoked trout fillets during storage

Average tenderness scores of all groups of cold-smoked trout fillets during the storage period remained essentially unchanged (Table 3; Figure 3).

Cardinal et al. (2004) found a positive correlation between texture and colour intensity of cold-smoked fish (firmer fish had more intense colour) while in the current study, a negative correlation occurred between these two parameters. Also, our group III fish fillets, which had the less intense colour, had a slight decrease of tenderness during storage. The reason for our data disagreeing with literature data could be that colour intensity in the salmon trout we studied depended on smoke, but perhaps also on fish nutrition.

The texture of cold-smoked fish has great importance for product quality (*Lakshmanan et al., 2005*). Autolytic processes play the most important role in changes to fish meat structure during storage, and therefore, for the quality of fish meat. Autolytic degradation can cause undesirable fish softening at the very beginning of storage, even when the microbiota which usually causes spoilage is still not sufficiently developed to do so (*Olafsdottir et al.,*

Table 3. Sensory evaluation of tenderness of packaged cold-smoked trout during storage

Group	Day of Storage					
	7	14	21	28	35	42
I	$3.81^{aA}\pm0.26$	$3.50^{Aa}\pm0.38$	$3.75^{A}\pm0.46$	$3.13^{A}\pm0.23$	$3.75^{ab}\pm0.27$	$4.19^{A}\pm0.26$
II	$4.25^{aB}\pm0.38$	$4.06^{AB}\pm0.18$	$3.25^{a}\pm0.38$	$4.00^{AB}\pm0.27$	$3.38^{a}\pm0.23$	$3.94^{B}\pm0.18$
III	$3.25^{AB}\pm0.27$	$3.00^{aB}\pm0.27$	$2.75^{Aa}\pm0.38$	$3.38^{B}\pm0.23$	$3.31^{b}\pm0.26$	$3.44^{AB}\pm0.32$

Same letter within a column indicates a significance difference $^{A, B}$ $p<0.01$; $^{a, b}$ $p<0.05$

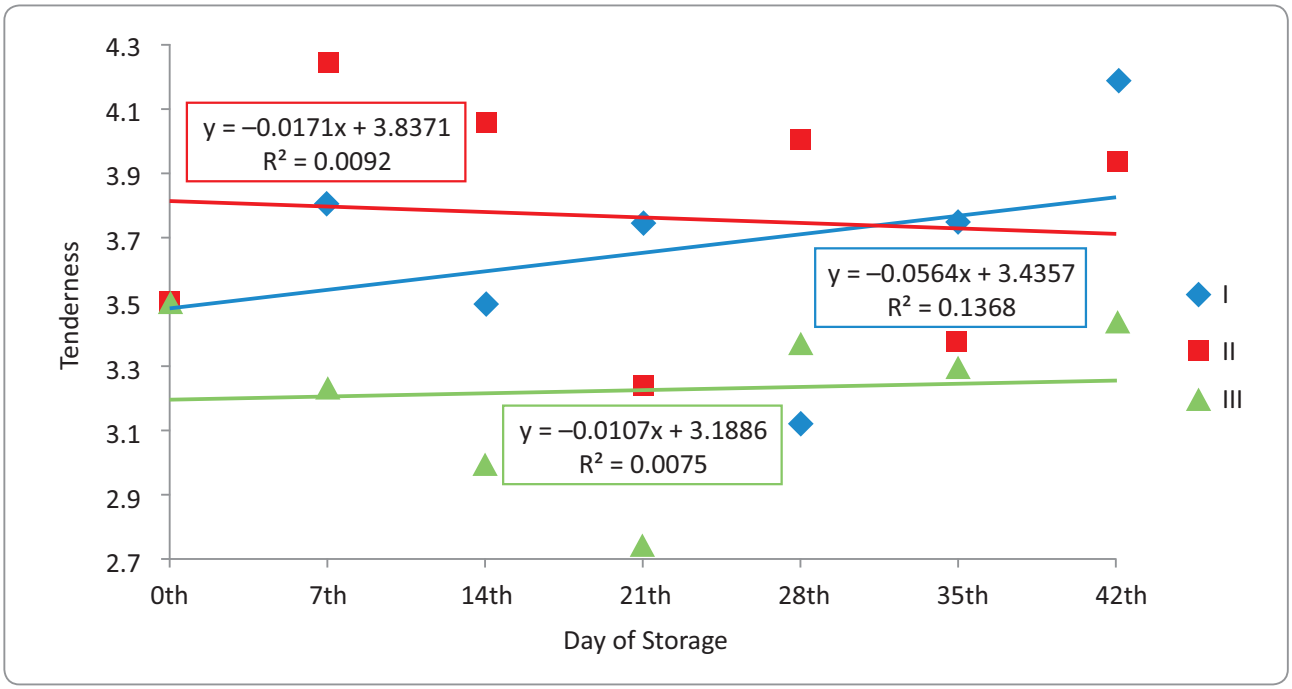

Figure 3. Sensory evaluation of tenderness of packaged cold-smoked trout during storage

2005*; Dondero et al.,* 2004*; Truelstrup Hansen et al.,* 1996).

One study showed that the reasons for fish meat softening include the manner of handling the raw material prior to slaughter, and the fat composition and storage conditions, which can lead to changes such as disintegration of the extracellular matrix and collagen fractions. This more likely occurs as a result of autolytic processes in fish tissue, rather than being a consequence of microbial activity (*Lund and Nielsen,* 2001). Also, dissolution of CO_2 in the tissues leads to acidification and decreases the pH of cold-smoked fish in modified atmospheres and consequently, changes the meat texture (*Torrieri et al.,* 2006).

The reason the tenderness intensity of group III fish fillets remained almost unchanged during storage could be due to this dissolution of CO_2 in the tissue, decreasing the pH. This would lead to protein changes, meaning the protein had less ability to bind water. Therefore, the consequent water loss resulted in the fish meat becoming firmer or not changing, even if it was affected by autolytic changes from microbiological activity (*Goulas and Kontominas,* 2007b).

Overall acceptability of cold-smoked trout fillets during storage

Average total acceptability scores of the three groups of cold-smoked trout fillets are shown in Table 4, and these decreased during storage. Similar findings were published by *Ibrahim et al.* (2008), *Pantazi et al.* (2008), *Goulas and Kontominas* (2007b)*, Cakli et al.* (2006), *Goulas and Kontominas* (2005), *Dondero et al.* (2004) and *Kolodziejska et al.* (2002). All these authors found that fish, fresh or smoked, packaged in different atmospheres, showed better overall acceptability scores, and thus, had

Table 4. Sensory evaluation of overall acceptability of packaged cold-smoked trout during storage

Group	Day of Storage					
	7	14	21	28	35	42
I	3.94^{AB}±0.18	4.21±0.27	3.00^{AB}±0.38	3.06^{A}±0.32	1.75^{AB}±0.27	1.00±0.00
II	4.44^{AC}±0.18	4.07±0.53	4.13^{Aa}±0.23	3.31^{B}±0.26	3.13^{AC}±0.23	1.00±0.00
III	4.94^{BC}±0.18	4.43±0.35	4.44^{Ba}±0.18	4.00^{AB}±0.27	3.88^{BC}±0.23	1.19±0.26

Same letter within a column indicates a significance difference $^{A–C}$ $p<0.01$; ^{a} $p<0.05$

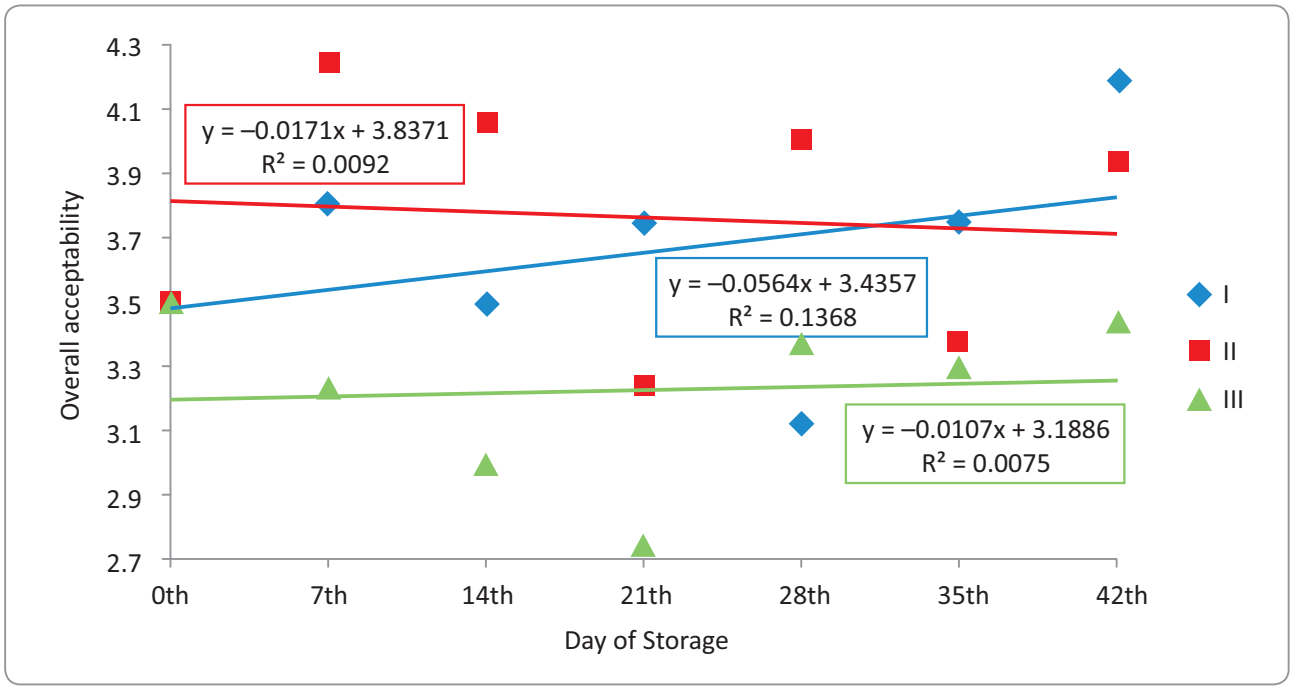

Figure 4. Sensory evaluation of overall acceptability of packaged cold-smoked trout during storage

longer shelf lives, as compared to fish stored in air or under vacuum.

Results of the present study show that significantly the highest average overall acceptability scores were determined for fish fillets packaged in the modified atmosphere with 90% CO_2 and 10% N_2 (group III). Group II fish fillets (modified atmosphere with 50% CO_2 and 50% N_2) had slightly lower overall acceptability scores, and vacuum packaged fish fillets (group I) achieved the lowest score (Figure 4). *Ibrahim et al.* (2008) examined smoked mullet packaged in modified atmosphere and vacuum, and found similar results. One explanation for this could be the fact that food packaged in modified atmosphere retains a more natural appearance than food packaged in a vacuum (*Murcia et al.,* 2003).

Conclusions

Colour intensity, odour and taste of smoke intensity and overall acceptability of cold-smoked trout decreased during storage, while fish fillet tenderness underwent very little change. Cold-smoked trout fillets packaged in modified atmosphere had higher average sensory scores compared to vacuum packaged cold-smoked trout fillets.

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Guidelines for Authors

Meat Technology (ISSN 0494-9846) is a scientific journal that publishes:

Original scientific papers (papers which present previously unpublished results of authors' own investigations using scientific methodology);

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Procedure

Papers are subject to anonymous reviews (two at least), while the decision to accept the paper for publishing is reached by the editor-in-chief, together with subeditors and the members of the editorial board.

Accepted papers are subject to proofreading. The editorial board reserves the right to minor corrections of the manuscript. Where major corrections are necessary, the first author will be notified, and the paper sent for revision, with a set deadline. After all corrections, authors are requested to submit a *Statement by authors* via email to danijelas@inmes.com.

Language

Papers must be written in English (British English spelling). The text should be clear, concise and grammatically correct.

Preparation and submission of manuscripts

The paper should be edited in Microsoft Word software, using Times New Roman font, size 12 pt, line spacing 1.5 and margins of 2 cm. Papers are submitted in electronic form by email: danijelas@inmes.com or institute@inmes.com.

All papers must contain the following (Title, Author line, Affiliation, Corresponding Author, Abstract):

Title In lowercase, bold, font size 14 pt, the title is descriptive of the study.

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Abstract This should contain 150-250 words with a maximum of five key words below, separated by commas (both abstract and key words are in italics, font size 12 pt). The abstract should be typed below the title and author line.

Papers belonging to a category other than original scientific papers can contain sections titled by choice of the author. Original scientific papers should contain the following sections in this order: Introduction, Materials and Methods, Results and Discussion (combined or separate), Conclusion, Acknowledgement (optional) and References. Section titles are typed in lowercase with main words capitalised, font size 12 pt, bold.

Introduction This should contain a clear description of the investigated subject and aim of the research with short citations of the relevant literature (usually not more than 10 years old);

Materials and Methods This section describes materials and methods used and outlines the design of the experiment;

Results and Discussion The results should be processed by statistical methods appropriate to the study; they should be clear and concise using tables, graphs, photographs, illustrations and other. The same result(s) must not be presented in both table and graph. Discussion must be related to the results presented, avoiding repetitions of already stated facts, and using comparison of obtained results and relevant literature data related to similar

groups of products, comparable analytical methods etc.

Numbers in-text and in tables/figures. Use decimal points, not decimal commas. Avoid starting and ending sentences with numbers (re-write the sentence).

Figures are numbered consecutively with cardinal Arabic numerals (Figure 1; Figure 2; Figure 3 etc.). Each figure is referred to in the text using consecutive cardinal Arabic numbers. Figures can be graphs, illustrations, flow diagrams, photographs, maps etc. Figure titles are placed below the figures, centre aligned (in sentence case with the figure number in bold, a full-stop after the figure number, the first word of the title capitalised). Figures and tables are submitted separately, in an appendix.

Tables are numbered consecutively with cardinal Arabic numerals (Table 1; Table 2; Table 3 etc.). Each table is referred to in the text using consecutive cardinal Arabic numerals. Table titles are placed above the tables, centre aligned (in sentence case with the table number in bold, a full-stop after the table number, the first word in the title capitalised). Tables have only three full horizontal lines, one at the top, one at the bottom and one under the column headings. Use superscript letters for table footnotes. Tables should be fully understandable without reference to the text. Figures and tables are submitted separately, in an appendix.

If tables or figures originate from other sources, the author is required to state the source of such data (author, year of publication, journal etc.). Notes should be placed at the bottom of the page containing cited material.

The author should apply the International System of Units (SI system) and current regulation on measuring units and measuring instruments. Symbols for units derived by division are given as negative exponents, e.g. 10 g L^{-1}; 250 V cm^{-2}.

Common abbreviations:

- CFU colony forming units, capitalised, common and so is never spelled out
- kg kilogram, common and so is never spelled out
- L litre, common and so is never spelled out
- Longissimus dorsi (LD) is redundant and so is not used. For the whole muscle, use Longissimus thoracis et lumborum (LTL). The correct terms for the two parts of this muscle are Longissimus thoracis (LT) or Longissimus lumborum (LL).
- mL millilitre, common and so is never spelled out
- µm micrometre, common and so is never spelled out
- mol mole, common and so is never spelled out
- M molar, common and so is never spelled out
- PCR polymerase chain reaction, common and so is never spelled out
- SD standard deviation, capitalised, common and so is never spelled out
- SE standard error, capitalised, common and so is never spelled out
- sp. species (singular), common and so is never spelled out (not capitalised, full-stop)
- spp. species (plural), common and so is never spelled out (not capitalised, full-stop)
- UV ultraviolet, capitalised, common and so is never spelled out
- aw water activity
- h hour(s)
- min minute(s)
- 25°C (no gap after the numeral)
- 20±1°C (no gaps between numbers, sign and unit in-text and in tables/figures)
- $p<0.05$, $p<0.01$ (not italicised, not capitalised, no gaps)
- n=120 (no gaps between the letter, sign and numerals in-text and in tables/figures)
- found in 20.05% of cats…(no gap in-text)

Conclusion This section provides a review of the most important facts obtained during the research.

Acknowledgement This should contain the number of the project i.e. title of the program under which the research was conducted, as well as the name of the institution that funded the project or program. The acknowledgement is written after the conclusion, before the references.

In-text references

Each publication cited in the text must be listed in References. The citations in the text need to be arranged in the following way:

If there is only one author of the cited paper, the author's surname and the year of publication is stated in the brackets (*Thomas and Brooks*, 2008). In case the same author has more publications in the same year, additional letters are added next to the year (*Thomas and Brooks*, 2008a; *Thomas and Brooks*, 2008b).

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If multiple references are cited within the same brackets, citations should be in chronological order, and then in alphabetical order if necessary, e.g. (*ISO*, 1995; *Devine et al*., 2001; *Abayas and Zhao*, 2012). These citations are separated with semi-colons.

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Examples:

Journals:

Givens, D. I., Kliem, K. E. & Gibbs, R. A. (2006). The role of meat as a source of n3 polyunsaturated fatty acids in the human diet. *Meat Science*, *74* (1), 209218.

Books:

Bao, Y. & Fenwick, R. (2004). Phytochemicals in health and disease, CRC Press, Los Angeles, USA.

Books with authored chapters:

Marasas, W. F. O. (1996). Fumonisins: History, worldwide occurrence and impact. In: Fumonisins in food, advances in experimental medicine and biology. Eds. L. S. Jackson, J. W. DeVries & L. B. Bullerman, Plenum Press, New York, pp. 118.

PhD and MSc theses:

Radeka, S. (2005). Grape mash maceration and varietal aroma of Malvazija istarska wine, PhD Thesis, Faculty of Agriculture, University of Zagreb, Croatia.

Laws, regulations, decrees:

Serbia. (2010). Regulation on general and special conditions of hygiene of food at any stage of production, processing and transport. *Official Gazette of the Republic of Serbia*, 72.

European Union. (2013). **Commission regulation (EU) No 1019/2013 of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products. *L 282*, 46–47.**

Symposiums, Congresses:

Harvey, J. (1992). Changing waste protein from a waste disposal problem to a valuable feed protein source: a role for enzymes in processing offal, feathers and dead birds. Alltech's 8th Annual Symposium, Nichdasville, Kentucky, Proceedings, 109−119.

Citations with organisations as authors:

Food and Drug Administration. (1995). Decomposition and histamine-raw frozen tuna and mahi-mahi; canned tuna; and related species; availability of revised compliance policy guide, Federal Registration, *60*, (1), 39754–39756.

European Food Safety Authority. (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal 9* (10), 2393.

Software:

STATISTICA (Data Analysis Software System) (2006). v.7.1., StatSoft, Inc., USA (www.statsoft.com).

Websites:

Technical report on the Food Standards Agency project G010008 (2002). Evaluating the risks associated with using GMOs in human foods, University of Newcastle, UK (http://www.foodsafetynetwork.ca/gmo/gmnewcastlereport.pdf).

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