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Original scientific paper

Concentration of arsenic and heavy metals in snail tissues

Hava Mahmutovic[1], Radmila Markovic[2], Jelena Janjic[2], Natasa Glamoclija[2], Branislav Baltic[3], Nenad Katanic[4], Jelena Ciric[2]*

A b s t r a c t: The aim of this study was to determine arsenic and heavy metal concentrations (lead, mercury, copper, cadmium, zinc, iron and manganese) in snail tissues (foot and digestive gland) obtained from snail farms in Serbia (near urban areas). Snail samples were analysed using atomic absorption spectrophotometry. A total of 730 individual snail samples were included in this study. Snails were packed into plastic bags and transported to the laboratory at the Faculty of Veterinary Medicine, University of Belgrade. The levels of arsenic and mercury in the examined snail tissues were below the detection limit of the analytical method. Concentrations of heavy metals were higher in digestive gland tissues than in foot tissues.

Keywords: *elements, foot, digestive gland, monitoring, environmental pollution.*

Introduction

The city of Belgrade and the nearby region situated on two rivers (Sava and Danube) is one of the most economically developed regions in Serbia. Rivers play an important role in the economic and social development in this region. Rapid development of industrialisation and urbanisation in recent decades has resulted in significant negative impacts on ecosystems. Heavy metal concentrations have rapidly grown in the urban environments from these anthropogenic sources (*Milanov et al*., 2016; *Janjic et al*., 2015; *Ivanovic et al*., 2016; *Jovanovic et al*., 2017; *Ciric et al*., 2018). In spite of that, heavy metals are natural substances, so are also considered as environmental contaminants (*Gawad*, 2018). In general, they not degrade but accumulate throughout the trophic chain (*Gupta and Singh*, 2011). When the body accumulates heavy metals and does not metabolise them, they can be toxic (*Gawad*, 2018). Copper, manganese, zinc and iron are essential metals for many organisms. Cadmium and lead are non-essential metals, and their toxic effect can be relatively high in comparison to other metals (*Zhiyou et al*., 2016).

Land snails (*Helix pomatia*) accumulate heavy metals, and this property can be utilised for heavy metal monitoring in urban environments. Snails are a very suitable tool for diagnostics of regions contaminated with different heavy metals. Many studies (*Beeby and Richmond*, 2003; *Notten et al*., 2005; *Dallinger et al*., 2005; *Nica et al*., 2012) showed that land snails can accumulate high levels of copper, zinc, cadmium and lead in their foot and digestive gland tissues. The land snails have the potential to be used in environmental monitoring as model invertebrates for Cu, Zn, Cd and Pb accumulation in terrestrial ecosystems (*Gomot-De Vaufleury*, 2000).

Previous studies in Serbia showed accumulation of heavy metals in fish tissues from different locations (*Jaric et al*., 2011; *Subotic et al*., 2013; *Janjic et al*., 2015; *Milanov et al*., 2016; *Ivanovic et al*., 2016; *Jovanovic et al*., 2017). However, data on concentrations of different heavy metals in snail tissues are very limited in Serbia. This study investigated the concentration of arsenic and heavy metals (lead, mercury, copper, cadmium, zinc, iron and manganese) in snail tissues (foot and digestive gland) taken from snail farms in Serbia.

Materials and methods

Individuals of the adult land snail, *Helix pomatia* (35.1±1.1 mm in shell diameter and 19.6±1.00 g in body weight) were collected during 2017 from five different farms (F1, F2, F3, F4, F5) located near

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Belgrade city, Serbia. F1, F2, F3 and F4 were located near traffic roads, and F5 was near the Danube River. Altogether, 730 individual snails were randomly collected (146 from each farm). Animals were packed into plastic bags and transported to the laboratory for heavy metal determinations, performed at the Faculty of Veterinary Medicine, University of Belgrade. The animals were washed with deionised water, steam treated (at 95–100°C for 5 min), and the digestive glands and foot were rapidly dissected out. Half of the sample size of these tissues was used for heavy metal determination.

The concentrations of heavy metals were measured in the snail tissues. The digestive gland and foot samples were dried at 60°C for at least 48 h in Petri dishes. Half a gram of each dried sample was transferred into a 50 mL Erlenmeyer flask and 10 mL of concentrated nitric acid (65 % HNO_3, Merck, Germany) was added. The flask was heated on an electric plate at low temperature until digestion was completed. When the digested solution became clear, the solution was evaporated to dryness. The sample was diluted with 10 mL of deionised water (18.2 MΩ cm^{-1}) and filtered through a Whatman filter (Sigma-Aldrich, USA). Heavy metals were measured in triplicate by atomic absorption spectrophotometry (GBC 932 plus atomic absorption spectrometer, GBC Scientific Equipment, USA). All reagents and chemicals were of the highest purity grade available from Merck or Sigma-Aldrich, Germany.

The statistical analysis was performed using the GraphPad Prisma version 7.00 software. Means were compared for significance of differences by the Student's t-test at the probability of 0.05. The samples were analysed in triplicate for each farm.

Results and discussion

Heavy metal concentrations in the different snail tissues (foot and digestive gland) of *H. pomatia* collected at the five snail farms are given in Table 1. These elements were chosen due to their frequency in environmental pollution (due to urban area, industrial area, traffic road etc.). Also, previous studies have shown that land snails are capable

Table 1. Heavy metal concentrations (μg g^{-1}) from snail (*Helix pomatia*) tissues from five different farms, F1-F5 (N=30; mean±standard deviation)

Heavy metal	F1	F2	F3	F4	F5
	Foot				
Cd	0.19±0.01^{x}	0.17±0.03^{x}	0.22±0.02^{x}	0.13±0.01^{x}	0.11±0.02^{x}
Hg			BLD		
Pb			BLD		
As			BLD		
Cu	12.46±1.86^{a}	26.58±3.67^{b}	21.60±3.52^{b}	9.74±1.44^{ax}	21.01±3.91^{bx}
Zn	11.49±1.64^{x}	12.83±1.58^{x}	12.51±1.26^{x}	12.11±1.12^{x}	13.09±1.65^{x}
Fe	10.25±1.33^{ax}	12.30±2.50^{bx}	10.66±2.69^{ax}	9.77±0.62^{ax}	10.34±1.78^{ax}
Mn	2.42±0.22^{ax}	1.69±0.11^{bx}	1.65±0.17^{bx}	2.05±0.19^{ax}	1.80±0.11^{bx}
	Digestive gland				
Cd	0.66±0.03^{ay}	1.14±0.10^{by}	1.25±0.08^{by}	0.90±0.02^{ay}	0.92±0.08^{ay}
Hg			BLD		
Pb			BLD		
As			BLD		
Cu	11.68±0.65^{a}	26.62±1.97^{b}	23.33±1.64	19.00±1.98^{y}	11.41±1.01^{ay}
Zn	35.42±4.53^{y}	67.23±5.90^{ay}	46.06±3.15^{y}	29.78±1.28^{by}	30.49±2.92^{by}
Fe	77.66±8.57^{y}	130.67±9.43^{ay}	86.76±6.54^{y}	49.18±3.84^{by}	49.64±5.57^{by}
Mn	104.43±10.61^{y}	296.08±39.68^{ay}	172.99±10.75^{y}	79.48±4.49^{by}	76.65±7.92^{by}

Legend: BLD - below the limit of detection; Within a row, means with different lower-case superscript letters (^{a,b}) differ significantly (p<0.05); Within a column, means with different lower-case superscript letters (^{x,y}) per heavy metal (p<0.05) differ significantly.

of accumulating high levels of different heavy metals (*Beeby and Richmond*, 2003; *Notten et al*., 2005; *Dallinger et al*., 2005; *Nica et al*., 2012). Cadmium concentrations in foot tissues ranged between 0.11±0.02 µg g^{-1} (F5) and 0.22±0.02 µg g^{-1} (F3). In digestive gland tissues, Cd concentrations ranged between 0.66±0.03 µg g^{-1} (F1) and 1.25±0.08 µg g^{-1} (F3). The farm location had a significant influence on heavy metal concentrations in the digestive gland tissues ($p<0.05$). Similar, highly significant differences were found when comparing the higher Cd concentrations in snail digestive glands with the lower Cd concentrations in snail foot tissues (Table 1). *Massadeh et al*. (2016) showed that snail tissues are very good indicators for Cd accumulation. Similar results were presented in a study by *Ciric et al*. (2018). Our study suggests that variances in Cd level are indicative of environmental exposure differences between the farms (data not shown). The concentrations of Mg, Pb and As were below the limit of detection in the examined snail tissues.

Copper concentrations in snail foot tissues ranged between 9.74±1.44 µg g^{-1} (F4) and 26.58±3.67 µg g^{-1} (F2) (Table 1). The highest Cu levels were measured in digestive gland tissues from F2, and foot tissue levels from this farm were similarly high. In fact, the Cu concentrations from F2 were significantly higher compared to F1 and F4 ($p<0.05$) (Table 1). Also, significant differences in Cu levels were found between foot and digestive gland tissues from both F4 and F5. Copper is an essential element for snails (*Yap and Noorhaidah*, 2012). Similar results were presented in studies by *Coeurdassier et al*. (2007), *Massadeh et al*. (2016) and *Ciric et al*. (2018).

Zinc concentrations in snail foot tissues ranged between 11.49±1.64 µg g^{-1} (F1) and 13.09±1.65 µg g^{-1} (F5). The concentration of Zn in foot tissues was significantly lower compared to digestive gland tissues, from all examined farms. *Nica et al*. (2012) and *Ciric et al*. (2018) found the heavy metal concentration in different snail tissues depends on the farm and investigated snail tissue. These authors also showed that digestive gland tissues accumulated higher amounts of heavy metals compared to other snail tissues. Similar results were determined in our study.

Iron concentrations in snail foot tissues ranged between 9.77±0.62 µg g^{-1} (F4) and 12.30±2.50 µg g^{-1} (F2), and in snail digestive gland tissues were between 49.18±3.84 µg g^{-1} (F4) and 130.67±9.43 µg g^{-1} (F2). The results obtained show that Fe concentrations were significantly ($p<0.05$) higher in digestive gland tissues compared to foot tissues. Higher concentrations of Fe are probably due to an environmental pollution or other anthropogenic sources. According to *Gomot and Pihan* (1997), *Gomot de Vaufleury and Pihan* (2000) and *Ciric et al*. (2018), snails are target organisms that could be utilised for measuring environmental pollution with industrial waste.

Manganese concentrations in snail foot tissues ranged from 1.65±0.17 µg g^{-1} (F3) to 2.42±0.22 µg g^{-1} (F1). In snail digestive gland tissues, Mn concentrations ranged from 76.65±7.92 µg g^{-1} (F5) to 296.08±39.68 µg g^{-1} (F2). Significant differences were observed between tissues (foot and digestive gland tissues) and between the farms (Table 1). The levels of Mn reported in this study are similar to results of *Sivaperumal et al*. (2007) and *Iwegbue et al*. (2008).

Conclusions

Based on the present study, snails are a very good species for use in biomonitoring of various environmental pollutants. In particular, *Helix pomatia* accumulated high amounts of Fe and Mn (digestive gland tissues).

Koncentracija arsena i teških metala u tkivu puževa

Hava Mahmutović, Radmila Marković, Jelena Janjić, Nataša Glamočlija, Branislav Baltić, Nenad Katanić, Jelena Ciric

A p s t r a k t: Cilj ovog ispitivanja bio je utvrđivanje koncentracije arsena i teških metala (olova, žive, bakra, kadmijuma, cinka, gvožđa i mangana) u tkivima puževa (stopalo i digestivni trakt) poreklom sa odabranih farmi u Srbiji (u blizini urbanih područja). Uzorci puževa su analizirani pomoću atomskog apsorpcionog spektrofotometra. U ovu studiju uključeno je ukupno 730 pojedinačnih uzoraka puževa. Uzorci su upakovani u plastične kese, dopremljeni u laboratoriju Veterinarskog fakulteta, Univerziteta u Beogradu. Nivo arsena i žive u ispitivanim uzorcima puževa bio su ispod granice detekcije analitičke metode. Koncentracija teških metala bila je veća u digestivnom tkivu u poređenju sa koncentracijom teških metala u stopalu puževa.

***Ključne reči:** elementi, stopala, digestivni trakt, monitoring, zagađenje životne sredine.*

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Original scientific paper

Effect of incorporating blackthorn fruit (*Prunus spinosa L.*) extract in natural casing on quality of Kranjska sausage

Snjezana Mandic[1], Danica Savanovic[1], Ana Velemir[1], Vesna Kalaba[2], Jovo Savanovic[3], Vanja Jokanovic[1]*

A b s t r a c t: This study aimed to evaluate the effectiveness of natural casing treatment with ethanol or aqueous extract of the blackthorn fruits (Prunus spinosa L.) on the quality of vacuum packed Kranjska sausages. Three experimental groups of sausages were produced. Firstly, a conventional sausage was filled in a natural casing; secondly, sausage was filled in a natural casing that was previously submerged in ethanol extract of blackthorn, and; thirdly, sausage was filled in a natural casing that was previously submerged in aqueous extract of blackthorn. The sausages were produced in industrial conditions, stuffed into the pretreated natural casings, vacuum packaged and stored at 4°C for 60 days. There were no significant differences (p>0.05) in chemical composition or in sensory quality between the different sausages. This study showed that extract of blackthorn fruits (Prunus spinosa L.) incorporated into natural casing before the filling operation reduced the number of lactic acid bacteria on the outside surface of vacuum packed Kranjska sausages stored 60 days at 4°C. The sausages with treated casings did not have much better oxidative stability during storage, likely because the herbal extracts did not diffuse into the filling, and were present in amounts too small to significantly affect decreases of the acid and peroxide numbers, or increases in thiobarbituric acid reactive substance values.

Keywords: *Kranjska sausage, blackthorn fruits extract, natural casing.*

Introduction

Kranjska sausage is a coarsely chopped cooked sausage made from a mixture of pork and beef meat, firm fatty tissue, connective tissue and additional ingredients. Kranjska sausage is stuffed into natural or adequate artificial casings, followed by pasteurisation and smoking as a heat treatment. In addition to achieving the necessary shelf-life, during thermal processing, sausages also achieve a specific colour, aroma and consistency.

Cooked sausages have relatively short shelf-life and are maintained at temperatures from 0 to 4ºC. The manner of packaging can significantly affect the product's shelf-life, microbiological status and the sensory properties of the product. Better (longer) shelf-life of cooked sausages is achieved by vacuum packing and packing in modified atmosphere. Vacuum packing can contribute to prolonged shelf-life, slower chemical changes, and maintenance of desirable sensory properties in product (*Lukic et al*., 2013; *Sojic et al*., 2015).

Some psychrotrophic micro-aerobic bacteria that produce organic acids with an unpleasant odour can cause problems in vacuum packaged products (*Baltic et al*., 2012; *Nychas and Skandamis*, 2005). Further, lipid and pigment oxidation are the two main causes of quality deterioration, limiting the quality and acceptability of meat and meat products. Oxidation processes lead to colour changes, off-odour development, the production of potentially toxic compounds and modification of nutritional characteristics (*Popova et al*., 2009; *Haile et al*., 2013; *Kralova*, 2016). The rate of oxidation processes can be controlled or minimised by addition of natural or synthetic antioxidants (*Grujic et al*., 2009; *Movahed et al., 2012; Savanovic et al*., 2014a; *Savanovic et al*., 2014b). Increasing consumer awareness and health consciousness, however, has resulted in pressure to avoid utilising synthetic additives, which necessitates the use of natural additives to extend shelf-life and improve product safety (*Descalzo and Sancho*, 2008; *Wojciak et al*., 2011; *Velasco and Williams*, 2011; *Savanovic et al*., 2014a). The exploration

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and application of natural products that have antioxidant and antibacterial activities in meat products could be desirable and very useful to extend their storage shelf-life and perhaps aid in prevention of foodborne diseases (*Hromis et al*., 2014; *Kumar and Tanwar*, 2011; *Abdel-Salam et al*., 2014). Sources of natural antioxidants usually are spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits and vegetables (*Mata et al*., 2007; *Karabacak and Bozkurt, 2008*; *Opara and Al-Ani*, 2010; *Maciel et al*., 2011; *Ozvural et al*., 2016). Amongst those, blackthorn fruits (*Prunus spinosa* L.) are rich in components with antioxidant properties (*Sikora et al*., 2013; *Radovanovic et al*., 2013; *Marchelak et al*., 2017).

The use of edible coatings and films to preserve food quality has intensified recently (*Ozvural et al*., 2016; *Hromis et al*., 2013). These coatings act as a barrier to the transit of oxygen and water, thereby slowing oxidation reactions and retaining moisture (*Tyburcy et al*., 2010; *Shon et al*., 2011). This is the main mechanism by which coatings enhance quality and prolong storage life. Furthermore, adding plant extracts and some natural biopreservatives gives the coatings antimicrobial and antioxidant properties (*Krkic et al*., 2012; *Adzaly et al*., 2015; *Raesi et al*., 2016; *Krol et al*., 2017). The significance of the casings in the process of sausage production is complex, beginning with the filling of the sausages, then participating in volumetric, structural and chemical changes that occur within the sausage during the production process and ending with the consumption of sausage. In addition, all casings function as a microbiological barrier that protects sausages during production, storage and distribution. The aim of this study was to investigate the effect of submerging natural casing in herbal extracts (*Prunus spinosa L.*) on the quality and shelf-life of vacuum packed Kranjska sausage.

Materials and methods

Sausage formulation and processing

Cooked sausages (Kranjska sausage) were used as a model-product in the study. Sausages were produced in industrial conditions according to the producer's specification. The following ingredients were used: pork meat, beef meat, firm fatty tissue, water, salt, starch, spices and preservative E 250. Prepared sausage batter was filled into natural pork casings, diameter 32–34 mm (DERMA DD., Varazdin, Croatia). Three experimental groups of sausages were produced. Firstly, a conventional sausage was filled in a natural casing (Sausage 1). Secondly, sausage was filled in a natural casing that was previously submerged (for 2 hours) in ethanol extract of blackthorn (Sausage 2). Thirdly, sausage was filled in a natural casing that was previously submerged (for 2 hours) in aqueous extract of blackthorn (Sausage 3). For the preparation of extracts, blackthorn fruits were washed and the petioles and the seeds removed. For ethanol extract, 40 g blackthorn fruits were homogenised with 160 mL 80% ethanol, centrifuged for 10 minutes at 1000 rpm, then treated in an ultrasonic bath for 30 minutes followed by 30 minutes on a magnetic stirrer. The mixture was filtered and the obtained filtrate was evaporated to a dry residue, first on a vacuum evaporator and then in a dry steriliser at 50°C. For aqueous extract, instead of 80% ethanol, distilled water was used. The other stages in the preparation of the aqueous extract were the same as for the preparation of ethanol extract. The prepared dry extracts were diluted to 100 mg ml^{-1} concentration using the solvent used for extraction.

After filling, the sausages were thermally processed until the temperature reached 72°C in the centre, and after heat treatment, they were chilled and vacuum packed. Sausages were stored in the dark at 4°C until analysis, i.e., under usual storage conditions for this kind of product. Analyses of sausages were carried out at different times, during 60 days of storage. The average chemical composition of the tested sausages was determined at the beginning of the storage period. Determination of the peroxide number, acid number, thiobarbituric acid reactive substance (TBARS) value, pH, instrumental hardness measurement and instrumental colour measurement was carried out after 1, 15, 30, 45, 60 days of storage. Sensory analysis of selected quality indicators of all three sausage groups was carried out after 15, 45 and 60 days.

Chemical analysis

Water content (drying at 105°C to constant mass), fat content (according to the Soxhlet method) and protein content (according to the Kjeldahl method) were determined according to *AOAC procedures* (2006). The standard method (*ISO 3496*, 1994) was used to determine the hydroxyproline content. Collagen content was calculated by multiplying hydroxyproline content (%) by a factor of 8 and the proportion of collagen in meat proteins was further calculated as follows: content (quantity) of the collagen in proteins (%) = collagen content (%) × 100 / total protein content (%). Total ash or mineral matter in sausages was determined by the method of dry incineration (*ISO 936*, 1998). The content of NaCl was determined according to Mohr method. The content of nitrite (expressed as $NaNO_2$) was determined according to the reference method (*ISO 2918,* 1975). The content of phosphates (expressed as P_2O_5) was determined by the spectrophotometric method (*ISO 13730*, 1996). The peroxide number was determined according to the method of *ISO 3960* (2001). To determine the acid number, the sausages were subjected to lipid extraction and then further analysed according to the method of *ISO 660* (2000). TBARS value was determined according to the method of *Tarladgis et al*. (1960) and *Botsoglou* (1994). All measurements were made in triplicate.

pH measurement

The pH value was determined according to the reference method *ISO 2917* (2004). Measurements were made using a digital pH meter with a combined electrode (HANNA HI 93161) for the direct measurement of pH in meat and meat products. Before and during the pH reading, the pH meter was calibrated using standard buffer solution (pH buffer calibration was 7.02 and 4.00 to 20°C). The result is expressed as the arithmetic mean of three measurements.

Instrumental hardness measurement

The sausage hardness was determined mechanically by a universal texture meter, the Texture Analyzer TA.XT plus (Stable Micro Systems), which measures the shear force needed to cut the meat. Warner-Blatzler shear force was used with the HDP/BSK knife cutting blade. The load cell was 25 kg, the speed was 4.00 mm s^{-1}, and the distance was 20.00 mm. The test samples were prepared by cutting rectangular forms from the sausage (1×1 cm, length 5 cm) on which the measurement was performed. The instrument measures the force (kg) needed to move the knife cell a certain distance (mm) into the sausage tissue. The device thus stimulates the chewing process. The mean of ten measurements was recorded.

Instrumental colour measurement

Instrumental colour measurement was performed using a spectrophotometer CM-2600d (Konica Minolta Sensing Inc., Japan), with 8 mm port size, illuminant D65 and a 10° standard observer, and after standardisation of the instrument with respect to the white calibration plate. Colour parameters, expressed as CIE L*, a* and b* values, were determined as indicators of lightness, redness and yellowness, respectively. The measurements were performed on the outside surface and on the cut surface immediately after cutting the sausage. The mean of 30 measurements was recorded for each colour parameter.

Descriptive sensory analysis

A descriptive sensory analysis scoring method was used to evaluate sausage quality. Before sensory evaluation, the sausages were cooked at 80°C for 5 min. The cooked and cold sausages were served with their casings. In initial preparation for the sausage sensory evaluation, coefficient of significance (Cs) was determined for each selected sensory attribute parameter (the sum of these is 20). Appropriate Cs were multiplied by the score given after sensory evaluation of each selected attribute (in a scale from 5 for very good quality, to 1 for very bad, unacceptable quality). Addition of all results of evaluated sensory attributes multiplied by Cs, produced the overall score, expressed as percentage of maximum possible product quality, or 100% for the best quality. For these cooked sausages the most important sensory attributes were evaluated: outside appearance and/or casing (Cs=3), cut appearance (Cs=3), cut colour (Cs=4), smell, aroma and flavour (Cs=6), and consistency Cs=4. Scoring forms with description of the sensory attributes and possible defects for each quality level were provided for the assessors (*Savanovic and Grujic*, 2008; *ISO 13299*, 2003; *ISO 4121*, 2003).

Microbiological analyses

In the examined sausages, the number of lactic acid bacteria, isolated on Plate Count Agar (PCA), was determined according to the method in Bosnia and Herzegovina (*BAS EN ISO 4833*-1, 2014; *BAS EN ISO 4833*-2, 2014). The number of lactic acid bacteria was determined on the outside surface of the sausages.

Statistical analysis

The results of this study are presented as mean values and their standard deviations. One factor analysis of variance (ANOVA) was performed using the IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, United States). Where significant differences ($p<0.05$) were detected, Tukey's post hoc test was used to compare treatment means and create statistically homogeneous groups.

Results and discussion

The quality of cooked sausages is defined by the meat protein content, with regard to total protein content and the relative content of the connective tissue protein in total proteins. Additional indicators of sausage quality are water content and fat content. The chemical composition of the Kranjska sausage at the beginning of storage is shown in Table 1. As can been seen, there were no significant differences ($p>0.05$) in chemical composition between the different sausages. The results show the chemical parameters of the examined sausages, analysed immediately after production, were in accordance with the appropriate legal regulations (*Official Gazette,* 2013; *Official Gazette,* 2015; *Official Gazette,* 2018), valid in Bosnia and Herzegovina where production was realised.

Sensory evaluation of all prepared sausage groups are presented in Figure 1. There was no statistically significant difference ($p>0.05$) between the standard (control) sausage and the sausage in casings previously treated with aqueous or ethanol extract of blackthorn fruits. A uniform quality of the examined sausages was observed in all sensory evaluation on all days (15, 45 and 60 days of storage). Also, the use of casings that were previously treated with aqueous or ethanol extract of blackthorn fruits enabled maintenance of the original food integrity and had no influence on the sensory properties of the examined sausages. During the storage period there was a slight deterioration of the sensory quality of all examined sausage groups (Figure 1).

Changes in the pH of tested sausages during storage are shown in Table 2. According to obtained pH values, significant differences were detected among the treatments ($p<0.05$). According to *Vukovic* (2006), cooked sausages have pH values in the range of 6.0–6.5, which is in accordance with this study. At the beginning of the study, the pH of the control sausage 1 was 6.29, sausage 2 (filled in casings previously treated with ethanol extract of blackthorn fruits) pH was 6.28, and sausage 3 (filled in casings previously treated with aqueous extract of blackthorn fruits) pH was 6.31. The pH of all the treatments decreased during storage ($p<0.05$). The decrease in pH can be due to the accumulation of metabolites by lactic acid bacteria (*Lukic et al*., 2013; *Baltic et al*., 2012). The highest pH during storage was measured in control sausages. Similar results for pH of the control have been demonstrated by other investigators in vacuum packaged sausages (*Zhang et al*., 2017; *Slima et al*., 2017). In the current study, after 60 days of storage, the pH of sausages filled in casings previously treated with ethanol or aqueous extract of blackthorn fruits were lower (5.66 for sausage 2 and 5.69 for sausage 3) than the pH of control sausage (5.80). Changes in pH of vacuum packed product during storage might result from the production of lactic acid through

Table 1. The average chemical composition of the Kranjska sausages at the beginning of the storage period *($X_{sr} \pm SD$)*

Parameter	Sausage 1	Sausage 2	Sausage 3
Moisture (%)	56.01 ± 0.85^{A}	54.05 ± 0.34^{AB}	52.49 ± 0.36^{B}
Ash (%)	3.32±0.04	3.28±0.04	3.24±0.04
Fat (%)	17.85 ± 0.29^{A}	19.21 ± 0.08^{AB}	20.98 ± 0.24^{B}
Proteins (%)	19.13±0.44	19.28±0.03	20.09±0.28
NaCl (%)	2.51±0.06	2.57±0.02	2.59±0.02
Total phosphates (%)	0.40±0.01	0.42±0.00	0.41±0.01
Nitrites (mg/kg)	84.20±0.57	81.68±0.98	81.42±1.48
Hydroxyproline (%)	0.22±0.03	0.21±0.06	0.28±0.02
Collagen (%)	1.74±0.03	1.65±0.46	2.22±0.13
Relative collagen content in meat proteins (%)	9.1±0.03	8.6±0.46	11.1±0.13

Legend: Data are expressed as mean ± standard deviation; ^{A,B} Means in the same row with different superscript letters are different ($p<0.05$).

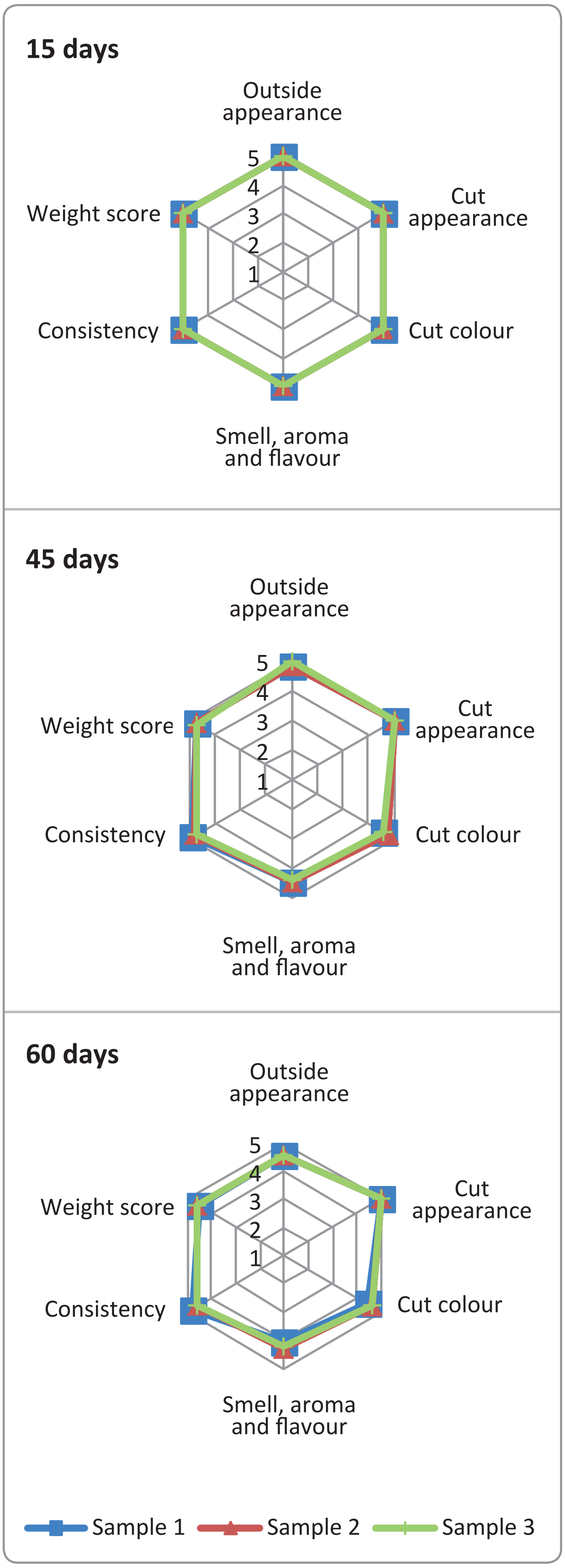

Figure 1. Sensory analysis results of selected quality indicators of all three sausage groups during the storage period

lactic acid bacteria (LAB) metabolism and carbonic acid formation through dissolution of CO_2 into meat aqueous phase (*Gupta and Sharma*, 2016; *Sharma et al*., 2017).

Average values of instrumental hardness measurement, i.e, the cut-off force of the cross section, of the sausages during the storage period are shown in Table 2. There were no large deviations in the obtained results, with all values ranging between 0.32 to 0.52 kg. Also, there was no proper sequence in the obtained results, which is probably due to the presence of different amounts of solid fatty tissue in different parts of the sausages and because of the crumbling of the stuffing. Sausage hardness depends primarily on the composition or type and quantity of meat and fat tissue, as well as the diameter of the sausage (*Sang-Keun et al*., 2016). Sausages with lower fat content are less succulent, have a firmer consistency, and the surface is uneven and wrinkled (*Mendoza et al*., 2001). The highest cut-off force was measured at 45 days (0.52 kg) in sausage 2 in the casing previously dipped in ethanol extract of blackthorn fruits, while the smallest force (0.32 kg) was measured in the same sausages at the beginning of the study. During storage, significant hardness changes were recorded in sausage 2 ($p<0.05$), while in sausages 1 and 3, there were no significant changes ($p>0.05$) in hardness values during 60 days of storage.

Average acid and peroxide numbers and TBARS values of sausages during storage are shown in Table 3. The acid number is an indication of the onset of hydrolytic lipid degradation in the meat, and its increase during meat storage is a common occurrence. The value of the acid number is related to the content of water in the meat, which contributes to hydrolysis reactions (*Naz et al*., 2005). The acid numbers were significantly different ($p<0.05$) between the tested sausages during 30 days of storage. The lowest results were obtained at the beginning of the test, 2.68 mg KOH/g in control sausage, 2.63 mg KOH/g in the sausages that were filled in casings previously treated with ethanol extract of blackthorn fruits and 2.37 mg KOH/g in the sausages that were filled in casings previously treated with aqueous extract of blackthorn fruits. During the storage, in all sausages, significant increases ($p<0.05$) in the acid numbers were recorded. The highest acid numbers were obtained at the end of storage, when the average acid number of control sausages was 3.67 mg KOH/g, and acid numbers were 3.75 mgKOH/g for sausage 2 and 3.57 mgKOH/g for sausage 3 (Table 3). An increase in the acid number

Table 2. Average pH and instrumental hardness values for three sausage groups during storage

Storage period (days)	pH values			Hardness (kg)		
	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3
1	6.29±0.02^{aA}	6.28±0.01^{aAB}	6.31±0.03^{aB}	0.41±0.15	0.32±0.07^{a}	0.45±0.15
15	6.26±0.11^{aA}	6.03±0.02^{cB}	6.01±0.01^{bB}	0.39±0.11	0.40±0.16^{ab}	0.44±0.13
30	6.22±0.02^{aA}	6.10±0.03^{bcC}	6.16±0.01^{cB}	0.38±0.10	0.43±0.14^{ab}	0.36±0.12
45	6.06±0.11^{bC}	5.71±0.09^{dA}	5.89±0.08^{dB}	0.40±0.09^{A}	0.52±0.14^{bB}	0.40±0.09^{A}
60	5.80±0.08^{cA}	5.66±0.02^{dB}	5.69±0.02^{eB}	0.38±0.09	0.38±0.08^{a}	0.40±0.15

Legend: Data are expressed as mean ± standard deviation; ^{a,b} Means in the same column with different superscript letters are different ($p<0.05$); ^{A,B} Means in the same test and same row with different superscript letters are different ($p<0.05$).

was observed in the work of *Lukic et al*. (2013) in samples of fresh beef during storage.

The peroxide number indicates the level of primary oxidation of fatty acids and it shows the amount of hydroperoxide as the primary product of autoxidation processes. Peroxide number is mainly related to the meat pH, as when this is closer to pH 7, the conditions for oxidation are more favourable (*Xie and Wang*, 2007). Average peroxide numbers for all three sausage groups are shown in Table 3. At the beginning of the study, all three sausages had the same peroxide number (0.06 mmol kg^{-1}). During the storage, a significant increase ($p<0.05$) in the peroxide number was observed in all sausages. A more drastic increase in the peroxide number, in all sausages, was determined in the final phase of the storage, i.e., in the period from 45 to 60 days. At the end of the study, the peroxide number in control sausage (1) was 0.35 mmol kg^{-1}, the sausages filled in casings previously treated with ethanol extract of blackthorn fruits (2) had a somewhat higher number of 0.39 mmol kg^{-1}, and in sausages with the use of aqueous extract of the blackthorn fruits (3) peroxide number was 0.28 mmol kg^{-1} (Table 3).

The TBARS value is widely used to evaluate secondary lipid oxidation products in meat and meat products. Malonaldehyde (MDA), the major degradation product of lipid peroxides, was used as a marker to determine the degree of lipid peroxidation. The results of the TBARS test expressed as MDA content (mg kg^{-1}) are shown in Table 3. The lowest results were observed on the day 1 (0.07 mg kg^{-1} for sausage 1, 0.11 mg kg^{-1} for sausages 2 and 3). It was remarkable that TBARS values of all the treatments

Table 3. Average values of acid and peroxide numbers and TBARS values of sausage during storage

Storage period (days)	Acid number (mg KOH g^{-1})			Peroxide number (mmol kg^{-1})			MDA (mg kg^{-1})		
	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3
1	2.68 ±0.06^{aA}	2.63 ±0.62^{aA}	2.37 ±0.58^{aB}	0.06 ±0.05^{a}	0.06 ±0.05^{a}	0.06 ±0.02^{a}	0.07 ±0.01^{aA}	0.11 ±0.01^{aB}	0.11 ±0.01^{aB}
15	2.86 ±0.12^{aA}	2.60 ±0.04^{aB}	2.44 ±0.02^{aC}	0.10 ±0.05^{bA}	0.13 ±0.09^{bB}	0.15 ±0.00^{bC}	0.08 ±0.01^{aA}	0.12 ±0.01^{aB}	0.11 ±0.00^{aB}
30	2.85 ±0.16^{aA}	2.56 ±0.00^{aB}	2.56 ±0.10^{aB}	0.13 ±0.05^{cA}	0.15 ±0.08^{cB}	0.16 ±0.20^{bC}	0.18 ±0.01^{b}	0.20 ±0.000^{b}	0.19 ±0.00^{b}
45	3.59 ±0.11^{b}	3.75 ±0.17^{b}	3.50 ±0.22^{b}	0.15 ±0.04^{dA}	0.15 ±0.12^{cA}	0.22 ±0.09^{cB}	0.21 ±0.04^{b}	0.20 ±0.05^{b}	0.20 ± 0.01^{c}
60	3.67 ±0.19^{b}	3.75 ±0.04^{b}	3.57 ±0.05^{b}	0.35 ±0.04^{eA}	0.39 ±0.06^{dB}	0.28 ±0.02^{dC}	0.22 ±0.02^{b}	0.21 ±0.01^{b}	0.21 ±0.00^{c}

Legend: Data are expressed as mean ± standard deviation; ^{a,b} Means in the same column with different superscript letters are different ($p<0.05$); ^{A,B} Means in the same test and same row with different superscript letters are different ($p<0.05$).

Table 4. Instrumental colour measurements on the surface of the examined sausages during storage

Storage period (days)	L*			a*			b*		
	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3
1	56.46 ±1.0^{6}	56.58 ±0.9^{5ab}	55.73 ±1.0^{4a}	14.97 ±0.6^{4}	14.81 ±0.8^{2}	14.49 ±1.1^{0}	21.26 ±1.5^{4aA}	20.12 ±1.2^{7AB}	19.50 ±1.8^{6B}
15	56.70 ±1.2^{4}	56.11 ±1.0^{7a}	57.03 ±1.1^{0bc}	14.53 ±0.6^{4}	15.06 ±0.5^{5}	14.69 ±0.6^{9}	19.79 ±1.2^{7b}	20.37 ±1.2^{9}	20.25 ±1.6^{4}
30	55.97 ±0.8^{4}	56.74 ±1.8^{4ab}	56.16 ±1.4^{5ab}	14.73 ±0.5^{1}	14.57 ±1.0^{5}	15.23 ±0.9^{8}	20.45 ±0.8^{6ab}	19.69 ±1.48	20.34 ±1.3^{5}
45	56.12 ±0.9^{1}	56.90 ±1.7^{1ab}	56.49 ±1.1^{3ab}	15.02 ±0.7^{0}	15.02 ±1.0^{5}	14.87 ±0.6^{5}	20.67 ±1.1^{9ab}	19.75 ±1.8^{3}	20.34 ±1.42
60	57.01 ±1.0^{0}	57.78 ±1.1^{2b}	57.85 ±1.3^{8c}	14.46 ±0.7^{0}	14.27 ±0.6^{2}	14.39 ±1.0^{3}	19.98 ±1.3^{9ab}	19.25 ±1.8^{4}	20.31 ±1.5^{6}

Legend: Data are expressed as mean ± standard deviation; ^{a,b} Means in the same column with different superscript letters are different (p<0.05); ^{A,B} Means in the same light parameter and row with different superscript letters are different (p<0.05)

increased due to lipid oxidation throughout storage (p<0.05). The highest results (0.22 mg kg^{-1}) were obtained at the end of the storage in control sausage. The content of MDA in control sausages was somewhat higher than in the other two sausage groups (0.21 mg kg^{-1}). Lipid oxidation in meat during storage is a natural process and these results are in agreement with other authors (*Ozvural et al*., 2016, *Krol et a*l., 2017; *Kouziunis et al*., 2017).

One of the most important quality attributes of meat and meat products is colour, since it influences consumer acceptability. Colour stability during storage is very important quality attribute of meat products. Meat colour depends on the concentration and redox state of haeme pigments in meat (*Wojciak et al*., 2011; *Savanovic et al*., 2014b). Values for colour parameters L* (lightness), a* (redness) and b*(yellowness) on the surface of the examined sausages during storage are shown in Table 4. The values for parameter L* did not differ significantly between the sausages (p>0.05). During storage, parameter L* values for sausages 2 and 3 increased (p<0.05) from 56.58 to 57.78 and from 55.73 to 57.85, respectively. Values for parameter a* on the surface of the examined sausages did not significantly change (p>0.05) during the 60 days of storage,

Table 5. Instrumental colour measurements on the cut surface of the examined sausages during storage

Storage period (days)	L*			a*			b*		
	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3	Sausage 1	Sausage 2	Sausage 3
1	65.75 ±2.9^{2}	65.65 ±2.3^{0}	65.80 ±1.6^{0}	9.81 ±1.62	9.33 ±1.34	9.59 ±0.84	10.08 ±0.6^{4ab}	9.71 ±1.02	10.47 ±0.9^{3}
15	64.25 ±4.3^{2}	68.09 ±2.6^{9}	65.17 ±4.0^{9}	10.26 ±2.4^{3}	8.18 ±1.82	10.04 ±2.4^{2}	9.90 ±0.91^{a}	10.29 ±0.8^{2}	10.66 ±0.8^{2}
30	65.35 ±1.3^{5}	65.86 ±2.4^{9}	65.32 ±1.2^{6}	9.74 ±1.05	9.13 ±1.32	9.80 ±0.93	10.48 ±1.2^{8ab}	10.07 ±1.14	10.51 ±1.0^{0}
45	63.20 ±1.4^{5A}	66.03 ±3.0^{9AB}	67.02 ±1.3^{4B}	10.98 ±1.0^{1A}	9.38 ±2.00^{AB}	8.95 ±1.12^{B}	10.38 ±0.8^{1ab}	10.28 ±1.1^{8}	10.63 ±0.55
60	65.99 ±2.2^{2}	67.54 ±2.3^{3}	68.67 ±3.0^{2}	10.17 ±1.2^{4}	9.13 ±1.14	8.31 ±1.80	11.07 ±0.5^{7bA}	10.41 ±0.9^{3AB}	9.70 ±1.18^{B}

Legend: Data are expressed as mean ± standard deviation; ^{a,b} Means in the same column with different superscript letters are different (p<0.05); ^{A,B} Means in the same light parameter and row with different superscript letters are different (p<0.05).

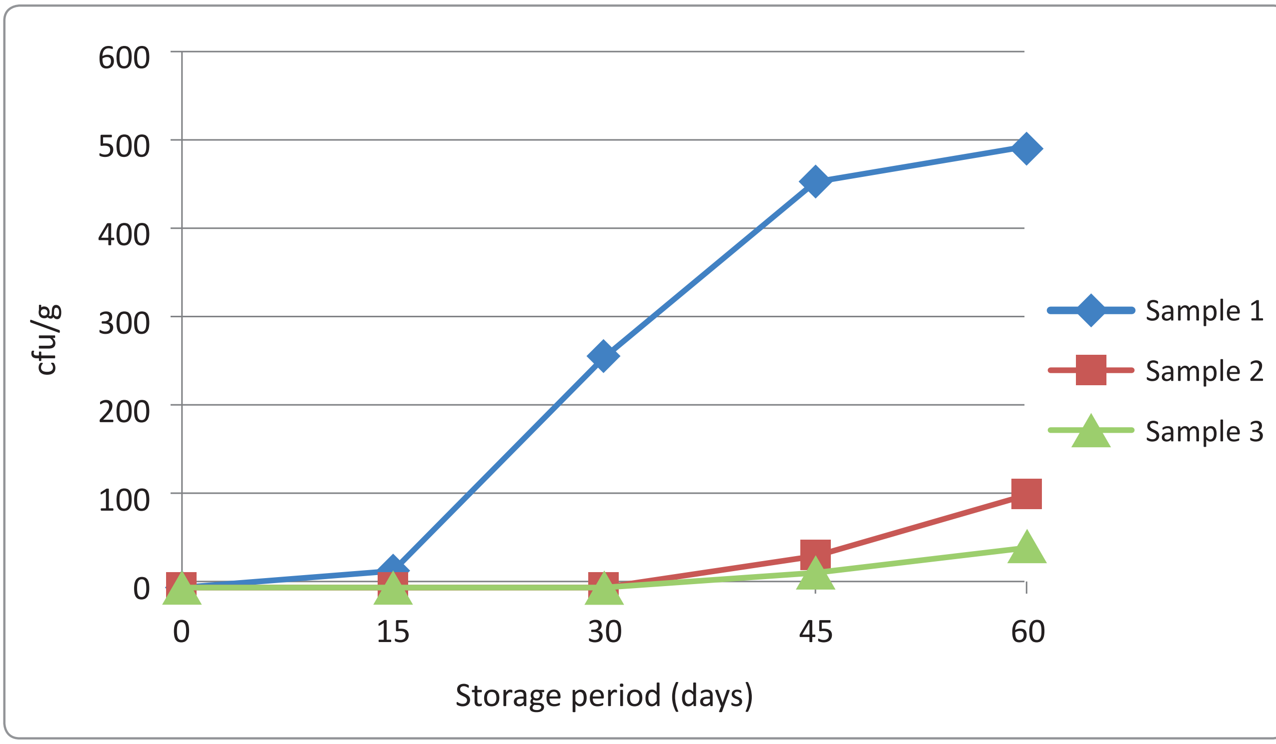

Figure 2. Number of lactic acid bacteria on the outside surface of sausages during storage

and values for parameter b* decreased ($p<0.05$) only in the control sausage. Redness (a*) is often used as an indicator of meat and meat product colour stability, and it is an important indicator of colour changes during storage (*Rubio et al*., 2008). *Hromis et al.* (2013) reported that the lightness (L*) of the sausage surface increased with a chitosan-caraway film coating application, while the redness (a*) and yellowness (b*) did not change, and the coated sausages showed a better colour stability than the sausage core through the storage time. The results of the instrumental colour measurement on the cut surface of the examined sausages during storage are shown in Table 5. The parameters of L* and a* did not change significantly during storage, while the only significant change in parameter b* values was observed in control sausages during the storage (b* increased from 10.08 to 11.07).

The number of lactic acid bacteria on the outside surface of the sausages gradually increased until the end of the study (Figure 2). After 60 days of storage, the number of lactic acid bacteria in the control sausage was the highest, when it amounted to 497 cfu g^{-1}. Incorporation of the blackthorn fruit (*Prunus spinosa L.)* extract in natural casing had an antimicrobial effect, because sausages 2 and 3 had lower numbers of lactic acid bacteria during 60 days of storage. At the end of the storage, sausage 2 (filled in casings previously treated with ethanol extract of blackthorn fruits) had 106 cfu g^{-1} and in sausage 3 (filled in casings previously treated with aqueous extract of blackthorn fruits) the number of lactic acid bacteria was 46 cfu g^{-1}. A large number of lactic acid bacteria can cause spoilage of vacuum packed fresh meat (*Baltic et al*., 2012). Therefore, treatment of natural casings with blackthorn fruit (*Prunus spinosa L.)* extract combined with low storage temperatures has potential for controlling the growth of lactic acid bacteria in vacuum packed sausages.

Conclusion

The results suggest that treating natural casings with aqueous or ethanol extract of the blackthorn fruits (*Prunus spinosa* L.) had positive impact on the quality and likely on the shelf-life of vacuum packed Kranjska sausage.

Use of blackthorn fruit (*Prunus spinosa L.*) extract in the sausages had no significant effect on the change of chemical composition and sensory characteristics, during the storage. Kranjska sausage filled in casings previously treated with aqueous or ethanol extract of blackthorn fruits had a smaller number of lactic acid bacteria on their outside surfaces during 60 days' storage in vacuum packs.

On the basis of the obtained results, it can be concluded that blackthorn fruit (*Prunus spinosa L.*) extract likely did not diffuse into the filling, and the amount was too small to affect the reduction of acid or peroxide numbers or increases of TBARS values. Probably, blackthorn fruits (*Prunus spinosa L.*) extract would have a much more effective antioxidant action if added to the sausage filling, where its effect would be more pronounced.

Uticaj tretmana prirodnih omotača ekstraktom plodova trnjine (*Prunus spinosa L.*) na kvalitet Kranjske kobasice

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A p s t r a k t: Cilj ovog istraživanja je bio da se ispita uticaj tretmana prirodnog omotača etanolnim i vodenim ekstraktom plodova trnjine (Prunus spinosa L.) na kvalitet vakuum pakovane Kranjske kobasice. Izrađene su tri eksperimentalne grupe uzoraka kobasica. Prva grupa bila je konvencionalna kobasica punjena u prirodni omotač, druga grupa bila je punjena u prirodni omotač koji je prethodno potopljen u etanolni ekstrakt trnjine, a treća grupa je bila punjena u prirodni omotač koji je prethodno potopljen u vodeni ekstrakt trnjine. Kobasice su proizvedene u industrijskim uslovima, punjene u prethodno pripremljene prirodne omotače, vakuum pakovane i skladištene na 4°C, tokom 60 dana. Nije bilo značajnih razlika (p> 0.05) u hemijskom sastavu i senzornom kvalitetu između različitih uzoraka kobasica. Ova istraživanja su pokazala da ekstrakt plodova tnjine (Prunus spinosa L.) kojim je tretiran prirodni omotač pre operacije punjenja utiče na smanjenje broja mlečnokislinskih bakterija na spoljnoj površini vakuum pakovanih Kranjskih kobasica, skladištenih 60 dana na niskim temperaturama. Kobasice sa tretiranim omotačima nisu imale mnogo bolju oksidativnu stabilnost tokom skladištenja, jer biljni ekstrakt verovatno nije difundovao u nadev, njegova količina je bila suviše mala da bi značajno uticala na smanjenje kiselinskog i peroksida broja i TBARS vrednost.

***Ključne reči:** Kranjska kobasica, ekstrakt trnjine, prirodni omotač.*

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Effects of different hydrocolloids on the texture profile of chicken meat emulsions

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A b s t r a c t: The aim of the study was to produce chicken breast meat emulsions with the addition of different hydrocolloids that would have comparable, or better, textural properties to those produced with phosphates (E 450-452). We prepared 10 emulsions from mechanically separated chicken breast meat (as three experimental repetitions) with the additions of: phosphates (control; 0.7%), and three different levels of one each of carrageenan, xanthan (these additives were used at 0.5%, 0.8% and 1% levels) or potato starch (1%, 1.5%, 2%). Instrumental measurements of colour (CIE L^*, a^*, b^*) and texture (texture profile analysis, stress relaxation tests) were performed, along with evaluation of the sensory attributes (descriptive analysis). These chicken breast meat emulsions with different hydrocolloids significantly differed in their instrumentally measured colour values and most texture parameters (hardness, cohesiveness, gumminess, chewiness, resilience, F_0, Y_{30}), and in some of the sensory attributes (colour, firmness, aroma). The increases in carrageenan and potato starch additions affected some of the measured colour values and the sensory attributes, although the measured texture parameters were not affected. The increases in xanthan addition showed changes in the colour, texture and sensory profiles. Those chicken breast meat emulsions with phosphate (0.7%), carrageenan (0.5%, 0.8%) and potato starch (2.0%) were the most similar in colour, texture and aroma. Those with potato starch showed non-significant trends for improved attributes compared to the control group, due to their intense aroma.

Keywords: *meat emulsions, phosphate, hydrocolloids, texture parameters, sensory properties.*

Introduction

Over the last 30 years or so, the search has gone on for solutions to replace the addition of phosphates to meat (e.g., E 338-452), one of the most widely used additives in the meat industry (*Feiner*, 2006). Phosphates have very wide applications in the meat industry, as they can improve water binding, and in connection with the salts included, they can stabilise the texture of meat products. As a consequence, phosphates can increase the solubility of proteins, act as chelators, prevent the oxidation (and rancidity) of lipids, and inhibit the growth of certain microorganisms (*Feiner*, 2006; *Fonseca et al.,* 2011).

Accordingly, the addition of phosphates to fresh or processed meat and sausages increases the water holding capacity, and thus, the amount of water in the product. This will directly reduce production costs for the product. Therefore, due to the possibility of misrepresenting a meat product by inclusion of high levels of water, producers are limited by law in terms of the addition of phosphates to meat products (*European Union*, 2008). Also, excessive uptake of phosphate by the body can lead to deterioration of human health (*Ellam and Chico*, 2011). Furthermore, meat products with high levels of added phosphates can show deterioration of some of the sensory attributes, accompanied in particular by an unpleasant soap-like and astringent flavour, as well as a tougher and more rubbery texture (*Sebranek*, 2009).

For these reasons, phosphates are often substituted by, or used in combination with, carrageenan (*Barbut and Mittal*, 1992; *Trius and Sebranek*, 1996; *Pietrasik and Duda*, 2000; *Amako and Xiong*, 2001; *Pietrasik*, 2003; *Ayadi et al.,* 2009; *Cierach et al.,* 2009; *Chun et al.,* 2014; *Gao et al.,* 2016), xanthan (*Palaniraj and Jayaraman*, 2011), alginate, casein or sodium caseinate, gelatin, guar gum, carob gum and arabic gum, hydrolysed plant proteins, starch (*Liu et al*., 2008; *Inguglia et al*., 2017), carboxymethyl cellulose, glucomannans, xyloglucan, white melon or yellow mustard (*Sinapis alba* L.), and other such substitutes (*BeMiller in Huber*, 2011; *Brewer*, 2012; *Tamsen et al.,* 2018). Indeed, from the health and sensory point of view, phosphate substitutes continue to be sought intensively, with positive

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results mainly seen for the use of hydrocolloids and modified starches.

The aim of the present study was to produce meat emulsions by addition of different substitutes for phosphates, to achieve similar, or even better, textural properties compared to the equivalent meat emulsion produced using phosphates. For this purpose, we prepared 10 groups of chicken breast meat emulsions that included the phosphate mixture (control) plus three different concentrations of three different hydrocolloids: carrageenan, xanthan and potato starch.

Materials and Methods

Materials

Mechanically deboned chicken breast meat was obtained from Pivka Perutninarstvo (Pivka Poultry; Kal, Slovenia), which was stored at −2.3°C and used 30 h after slaughter. According to the nutrition declaration of the producer, 100 g of the chicken breast meat contained mean levels of 14.07 g protein, 20.12 g fat, 63.99 g water, and 1.65 g collagen.

The further materials used included: sunflower oil (Cekin brand; Tovarna olja Gea, Slovenska Bistrica, Slovenia); nitrite salt (0.6% Na nitrite; Prava Aroma, Zrkovci, Slovenia); seasoning mixture for special salami (Etol, Skofja vas, Slovenia); phosphate mixture (Aroma Universal K; Prava Aroma, Zrkovci, Slovenia); carrageenan (E 407a and NaCl; Aubygel RPI 1010; Cargill, Minneapolis, USA); dextrose (dextrose monohydrate; CDex 02044; Cargill, Minneapolis, USA); sodium erythorbate (E 316; RFI Food Ingredients, Düsseldorf, Germany); xanthan (E 415; Jungbunzlauer, Wulzeshofe, Austria); and potato starch (CGel 30002; Cargill, Minneapolis, USA).

The phosphate mixture had a composition of dextrose, phosphates (E 450, E 451) and sodium erythorbate (E 316), at a weight ratio of 355: 300: 45, respectively.

Preparation of chicken breast meat emulsion

The chicken breast meat emulsions were produced with addition of phosphates (control), carrageenan (E 407a), xanthan (E 415) or potato starch. The control emulsion was made from 75% mechanically deboned chicken breast meat, 5% sunflower oil, 20% ice, 1.5% nitrite salt, 0.7% phosphate mixture, and 0.3% seasoning mixture. Another nine groups of meat emulsions were produced based on these raw materials and additives, whereby instead of the phosphate mixture, the different hydrocolloids were added to provide three different levels, low, medium and high, as detailed in Table 1. As the

Table 1. Extra additions to the chicken breast meat emulsions for the different experimental groups. All samples included 75% mechanically deboned chicken breast meat, 5.0% sunflower oil, 20.0% ice, 1.5% nitrite salt, and 0.3% seasoning mixture.

Group	Extra addition (%)				
	Phosphate mixture	Dextrose + Na-erythorbate	Carrageenan	Xanthan	Potato starch
Control (phosphate)	0.70	–	–	–	–
Carrageenan	–	0.40	0.5	–	–
	–	0.40	0.8	–	–
	–	0.40	1.0	–	–
Xanthan	–	0.40	–	0.5	–
	–	0.40	–	0.8	–
	–	0.40	–	1.0	–
Potato starch	–	0.40	–	–	1.0
	–	0.40	–	–	1.5
	–	0.40	–	–	2.0

phosphate mixture included dextrose and sodium erythorbate as well as the phosphates, the same levels of dextrose and sodium erythorbate were added to the remaining nine experimental groups.

Mechanically deboned chicken meat, nitrite salt, phosphate mixture (control group) or each level of hydrocolloid with dextrose and sodium erythorbate, and half of the ice were homogenised (Stephan UMC 5 electronic; Stephan Nahrungsmittel und Verfahrenstechnik, Hameln, Germany) at 2400 rpm until an internal temperature of 6°C was reached. Then the sunflower oil, seasoning mixture, and the other half of the ice were added, with further homogenisation at 2400 rpm until an internal temperature of 11.9°C was reached. The emulsions formed were used to fill plastic casings (diameter, 4 cm; length, 10 cm). All of the samples were simultaneously thermally treated in a cooking chamber (Fessmann GmbH und Co KG, Winnenden, Germany) until the final temperature of 72°C, then cooled and stored at 4°C until the sensory and physico-chemical analyses. The experiment was performed as three repetitions.

Chemical composition analysis

The chemical compositions of the chicken breast meat emulsions were determined using a meat analyser (Food Scan Meat Analyser; Foss, Hilleroed, Denmark). On the basis of the near-infrared absorption technique, the meat analyser provided information on the content of water, protein, fat and salt in these meat products. Nitrite was determined by the AOAC Official Method 973.31 for nitrite in cured meats (*AOAC*, 1997). The data obtained are expressed as the means of two parallel determinations per sample, where the samples originated from experimental groups of the first experimental repetition, as control, 0.5%, 0.8% and 1.0% carrageenan, 0.5%, 0.8% and 1.0% xanthan, and 1.0%, 1.5% and 2.0% potato starch.

Colour analysis

A chromometer (Minolta CR-400; Konica Minolta Optics, Inc., Osaka, Japan; illuminant C, 0° viewing angle) was used to determine the *Commission Internationale de l'Eclairage* (CIE; International Commission on Illumination) L^* (lightness), a^* (±, red to green) and b^* (±, yellow to blue) values for the surface of a 1-cm slice of each chicken breast meat emulsion. A white ceramic tile with the specifications of Y=93.8, x=0.3134, y=0.3208 was used to standardise the colorimeter. The CIE L^*, a^* and b^* colour values are given as the means of four measured at different locations on the slice surface.

Texture profile analysis and stress relaxation test

The textural parameters were measured using a texture analyser (TA.XT Plus; Stable Micro Systems Ltd., Surrey, UK) with a permissible load of 50 kg. Texture profile analysis and stress relaxation tests were carried out on the chicken breast meat emulsions (without the plastic casings). Sample preparation for the texture profile analysis and stress relaxation tests were based on *Morales et al.* (2007). The samples were cut into 3-cm-high cylinders. The diameters of the cylinders ranged from 3.9 mm to 4.0 mm. For the contact attachment, a 100-mm cylindrical probe (P100) was used. For the texture profile analysis, the samples were compressed twice to 50% of their original length (with 5 s between these compression cycles) and at a crosshead speed of 5 mm s^{-1}, with analyses repeated as four determinations. The force *versus* time curves were recorded, and the following parameters were calculated: hardness, adhesiveness, cohesiveness, gumminess, springiness, chewiness and resilience. For the stress relaxation tests, the samples were compressed to 50% of their original length at a crosshead speed of 1 mm s^{-1}. The force decay, or relaxation, *versus* time after compression was recorded, and the relaxation curves were normalised according to the level of force decay $Y(t)$ defined in Equation (1):

$$Y(t)=(F_0-F(t))/F_0 \qquad (1)$$

where F_0 (kg) is the initial force, and $F(t)$ is the decaying force recorded after t s of relaxation (*Moralles et al*., 2006).

Sensory analysis

To evaluate the sensory qualities of these chicken breast meat emulsions, a panel of four qualified and experienced panellists in the field of meat products was appointed (*Gasperlin et al.,* 2014). The evaluations were carried out under defined, precisely prescribed, and controlled and reproducible operating conditions. This included: arrangement of the laboratory, samples and accessories, and organisation of the assessment (*ISO 8589*, 2007).

The samples were taken out of the refrigerator and left at room temperature so that the temperature of the slices during analysis was ~15°C. For the sensory evaluation, 1-cm-thick slices of the samples were prepared, placed on white ceramic plates, and given to the panellists. The plates were equipped with a number, thus ensuring the anonymity of the samples. To neutralise the taste, the panel used the central dough of white bread. Sensory analysis was carried out in three sessions (one for each experimental repetition), organised on three consecutive days.

On the basis of preliminary tasting for the purpose of the evaluation, the panel decided in favour of, and applied, an analytical-descriptive test (*Golob et al.,* 2005). The analysis was performed by scoring the sensory attributes on a structured scale from 1 to 7 points, where a higher score indicated greater expression of a given property. The exception here was saltiness, which was evaluated by scoring on a structured scale of 1 to 4 to 7 (1-4-7). Here, a score of 4 points was considered optimal, with scores of 4.5 or higher indicating greater expression of saltiness, and those of 3.5 or lower indicating insufficient expression of saltiness. These sensory profiles of the emulsion samples were assessed using five descriptors that were grouped into four blocks: appearance, texture, smell and aroma.

Statistical analysis

The data were tested for normal distributions using the UNIVARIATE procedure (SAS/STAT, USA). The differences according to additive in different levels were analysed through a general linear model procedure and Duncan tests, with a 0.05 level of significance. In evaluating these data, the impact of the experimental repetitions was also taken into account, as this effect was significant for most of these analysed parameters ($p \leq 0.05$), and was thus included in the statistical model.

Results and Discussion

The basic chemical parameters were determined in parallel for the four main experimental groups (i.e., control, carrageenan, xanthan, potato starch), as produced in the first repetition. The mean contents of 100 g of the chicken breast meat emulsions were 10.52±0.16 g protein, 19.28±0.68 g fat, 66.14±0.34 g water, and 0.88±0.10 g salt, with residual nitrite at 0.112±0.003 g. The homogeneity of the experimental groups was confirmed on the basis of the corresponding standard deviations.

The instrumentally measured a^* value (i.e., shade of red colour) on the surface of the chicken breast meat emulsions was the colour parameter that varied the most across the samples (coefficient of variability, 4.4%). The colours of these emulsions were significantly affected by the type and level of hydrocolloid additions. As all of these experimental groups of the chicken breast meat emulsions contained the same levels of nitrite salt (1.5%), these differences in colour were due to the type and quantity of each of the hydrocolloids added.

The data given in Table 2 for the L^* value show that this was lower (i.e., darker emulsions) for the addition of carrageenan (0.8%, 1.0%) and starch (all levels), compared to the control and the other groups (i.e., 0.5% carrageenan; xanthan, all levels). *Mittal and Barbut* (1994) reported that xanthan addition to frankfurters increased the L^* value. Generally, the colour parameters of the 0.5% carrageenan and 0.5% and 1.0% xanthan groups remained close to the control group.

Texture profile analysis (*Bourne*, 1978) and stress relaxation tests (*Pons and Fiszman*, 1996) are the methods that are most frequently used for evaluation of the textural properties of foods. The rheological behaviour of foods, including the texture, can be studied using several instrumental methods (e.g., compression, torsion, tension, stress). At present, the most commonly used are Warner-Bratzler tests and texture profile analysis, which are based on measurements of sample compression. Texture profile analysis parameters have already been obtained for meat products in many previous studies (e.g., *de Ávila et al.,* 2014). Furthermore, the effects of the types and levels of the additives used in the present

Table 2. Effects of the different levels of the hydrocolloids on the instrumentally measured colour parameters of the surfaces of slices of the chicken breast meat emulsions (n=120).

Group	Level (%)	Colour parameter L^*	a^*	b^*
Control	0.7	71.17^{ba}	13.72^{b}	11.85^{c}
Carrageenan	0.5	69.77^{c}	14.09^{a}	12.25^{a}
	0.8	69.86^{c}	13.83^{ba}	12.25^{a}
	1.0	70.97^{b}	13.19^{dc}	11.89^{c}
Xanthan	0.5	71.67^{a}	13.29^{c}	12.18^{ba}
	0.8	71.70^{a}	12.47^{e}	12.24^{a}
	1.0	71.18^{ba}	13.04^{d}	12.31^{a}
Potato starch	1.0	69.59^{c}	14.04^{a}	11.95^{c}
	1.5	69.90^{c}	14.05^{a}	12.03^{bc}
	2.0	70.56^{b}	13.42^{c}	12.03^{bc}
SE	–	0.23	0.09	0.06
p_A	–	≤0.001	≤0.001	≤0.001

Legend: n, number of observations in experiment; p_A, statistical probability of addition effect; means with different superscript letters within columns differ significantly ($p \leq 0.05$; significance of differences between groups)

Table 3. Effects of the different levels of the hydrocolloids on the instrumentally measured texture profile analysis parameters of the chicken breast meat emulsions (n=120).

Group	Level (%)	Texture profile analysis parameter						
		Hardness (N)	Adhesiveness (N s)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)	Resilience
Control		124.1^{ba}	−1.88	0.90	0.71^{a}	87.55^{a}	78.89^{a}	0.39^{a}
Carrageenan	0.5	130.1^{a}	−3.47	0.86	0.65^{ba}	84.14^{a}	72.45^{ba}	0.33^{b}
	0.8	131.0^{a}	−2.07	0.87	0.61^{ba}	78.33^{abc}	68.36^{bdac}	0.30^{b}
	1.0	126.8^{a}	−1.92	0.88	0.64^{ba}	80.47^{ab}	70.76^{bdac}	0.31^{b}
Xanthan	0.5	108.4^{bc}	−2.12	0.87	0.61^{ba}	66.15^{dc}	58.17^{dc}	0.32^{b}
	0.8	117.5^{ba}	−1.70	0.89	0.56^{b}	64.24^{d}	57.55^{d}	0.29^{b}
	1.0	66.3^{d}	−1.87	0.86	0.42^{c}	27.38^{e}	23.47^{e}	0.18^{c}
Potato starch	1.0	120.9^{ba}	−2.66	0.89	0.68^{a}	81.47^{ba}	72.86^{ba}	0.34^{b}
	1.5	104.6^{bc}	−1.81	0.91	0.73^{a}	75.86^{abc}	69.08^{bac}	0.36^{b}
	2.0	114.8^{ba}	−1.53	0.88	0.69^{a}	79.98^{ba}	71.51^{bac}	0.33^{b}
SE		19.0	1.57	0.09	0.11	15.68	14.97	0.06
p_A		≤0.001	0.093	0.844	≤0.001	≤0.001	≤0.001	≤0.001

Legend: n, number of observations in experiment; p_A, statistical probability of addition effect; means with different superscript letters within columns differ significantly (p≤0.05; significance of differences between groups)

study were significantly different (p≤0.001) across the texture profile analyses (Table 3), including for hardness, cohesiveness, gumminess, chewiness and resilience. However, the adhesiveness and springiness of the chicken breast meat emulsions were not significantly affected by the different hydrocolloid additions (p>0.05).

When *Marchetti et al*. (2013) added carrageenan and xanthan into meat emulsions that contained oil, higher levels of aggregates were formed and the matrix was more interconnected. This was reflected in their higher cohesiveness compared to the emulsions produced solely with bacon, without the addition of these hydrocolloids. In the present study, however, compared to the control, none of the additions of carrageenan or potato starch (all levels for both) affected the cohesiveness. However, the cohesiveness of our emulsions was significantly lower for the additions of 0.8% and 1.0% xanthan. *Marchetti et al*. (2013) also reported that the addition of carrageenan or xanthan into meat emulsions with oil produced products with similar hardness compared to their control with solely bacon. They stated that the filling matrix was enhanced due to the gelatin capacity of these biopolymers. In the case of carrageenan and starch addition into meat emulsions, the gumminess and chewiness did not vary significantly compared to the control, but these properties were generally reduced by addition of xanthan.

The stress relaxation test takes into account the viscoelastic nature of a sample. Although there are no data on the use of the stress relaxation tests on poultry emulsions, the test has been used satisfactorily for other products, such as gels (*Peleg and Pollak*, 1982), fish (*Herrero et al.,* 2004) and hot dogs (*Skinner and Rao*, 1986). Most foods are biologically active or physically unstable, and their mechanical properties can change significantly over a very short period of time, which can greatly limit the use of tests such as the stress relaxation test. Indeed, this test applies a certain force for a long time during the analysis; e.g., for 30 s to 90 s (*Peleg and Pollak*, 1982; *Purkayastha and Peleg*, 1986). From the data for the present study given in Table 4, it can be seen that all of the carrageenan additions increased F_0 (i.e., the initial pressing force), and generally, the xanthan additions decreased F_0 compared to the control. This is in agreement with the findings from other studies (*Bater et al.,* 1992; *Mittal and Barbut*, 1994; *Hsu and Chung*, 2001). The F_0 of those chicken breast meat emulsions with potato starch were comparable (1.0%, 1.5%) or higher (2.0%) than the control.

The quantitative descriptive analysis of the sensory attributes of these 10 experimental chicken breast meat emulsion groups indicated that the phosphate (control) and the type and level of the added hydrocolloids affected the perceived colour, firmness and aroma. On the contrary, all of these groups of emulsions were estimated to have similar juiciness and smell, and appropriate saltiness (Table 5).

The panellists estimated that compared to the control group, the chicken breast meat emulsions with the low level of added carrageenan (0.5%) and all levels of potato starch were the most similar for colour, while 0.8% and 1.0% carrageenan and all levels of xanthan were less successful (Table 5). As is known from the literature, the addition of carrageenan, xanthan and starch affects the texture, and therefore, the finding that perceived firmness of our chicken breast meat emulsions with the various additions showed differences was not surprising. However, although increasing the level of carrageenan did not change the texture, the panellists described the effects of the increased levels of xanthan as providing a softer and more fragile emulsion; indeed, for 1.0% xanthan, these effects even became unacceptable. In contrast, 2.0% potato starch made the texture of the emulsion firmer.

Table 4. Effects of the different levels of the hydrocolloids on the instrumentally measured texture parameters in the stress relaxation tests of the chicken breast meat emulsions (n=120).

Group	**Level**	**Stress relaxation test parameter**	
	(%)	F_0	Y_{30}
Control	0.7	95.91[cd]	0.33[a]
Carrageenan	0.5	104.82[b]	0.37[b]
	0.8	100.11[bc]	0.36[ab]
	1.0	119.56[a]	0.36[ab]
Xanthan	0.5	103.31[bc]	0.37[b]
	0.8	88.08[ef]	0.37[b]
	1.0	47.63[g]	0.70[c]
Potato starch	1.0	92.36[de]	0.36[ab]
	1.5	102.78[bc]	0.36[ab]
	2.0	84.49[f]	0.36[ab]
SE		4.87	0. 02
p_A		<0.0001	<0.0001

Legend: n, number of observations in experiment; F_0, initial force; Y_{30}, force decay level after 30 s; p_A, statistical probability of addition effect; means with different superscript letters within columns differ significantly ($p \leq 0.05$; significance of differences between groups)

Increases in the hardness of meat products have been reported previously when carrageenan was added, also at 0.2% and 0.5% (*Barbut and Mittal,* 1992; *Xiong et al*., 1999; *Hsu and Chung*, 2001; *Ayadi et al*., 2009). These studies are, thus, in good agreement with the findings in the present study, both in terms of the sensory assessments and the instrumentally measured hardness of the emulsions. The addition of xanthan to food depends on the density and the desired consistency of the food. In the present study, the panellists described the texture of xanthan emulsions as soft, plastic and fragile. It can be assumed here that xanthan was used at too high a level (i.e., 0.8%, 1.0%), as addition of 0.2% to 0.5% xanthan have usually been used for the production of meat emulsions (*Palaniraj and Jayaraman,* 2011).

The conclusions reached by the panellists about the aroma of these chicken breast meat emulsions were interesting. The aroma of the control group was assessed as worse than optimum due to a perceived soap-like aroma. The aroma of these emulsions with added starch at all levels was evaluated as the best, although this did not reach statistical significance. Comparable, but slightly worse (although also not statistically significant), aromas were assessed for those emulsions with added carrageenan and xanthan at all levels, which is not surprising, as carrageenan leaves an unusual off-aroma in the mouth, which was described by the panellists as bitter. These assessments are in agreement with findings of *Ayadi et al*. (2009), who also reported the addition of carrageenan does not significantly affect the aroma of sausages. In the available literature, there is a report that the addition of xanthan can also have negative effects on the sensory acceptability of sausages (*Barbut and Mittal*, 1992).

Of note here, both of the instrumental texture parameter tests provided satisfactory data, as the correlation analysis of the parameters obtained demonstrated the suitability of both of these methods (Table 6). Overall, however, the stress relaxation test might be more suitable for measurement of the texture of meat emulsions with various additions of hydrocolloids, such as those used in our study.

Multivariate analysis

Linear discriminant analysis was performed to define these experimental groups of chicken breast meat emulsions on the basis of the parameters that differed the most or those that contributed most to the

Table 5. Effects of the different levels of the hydrocolloids on the sensory attributes of the chicken breast meat emulsions (n=120).

Group	Level (%)	Sensory property (intensity)					
		Colour (1–7)	**Firmness (1–7)**	**Juiciness (1–7)**	**Saltiness (1–4–7)**	**Smell (1–7)**	**Aroma (1–7)**
Control	0.7	6.1^{a}	5.1^{b}	6.2	4.0	5.9	5.6^{bac}
Carrageenan	0.5	6.0^{a}	5.5^{ba}	6.0	4.0	6.0	5.6^{bac}
	0.8	5.7^{bc}	5.5^{ba}	6.0	4.0	5.8	5.5^{bc}
	1.0	5.5^{dc}	5.5^{ba}	5.9	4.0	5.7	5.4^{bc}
Xanthan	0.5	5.3^{d}	4.6^{c}	5.9	4.0	5.7	5.5^{bac}
	0.8	5.5^{dc}	4.4^{c}	6.0	4.0	5.8	5.5^{bac}
	1.0	5.5^{dc}	3.4^{d}	6.2	4.0	5.8	5.3^{c}
Potato starch	1.0	6.0^{a}	5.5^{ba}	6.0	4.0	5.8	5.8^{ba}
	1.5	5.8^{ba}	5.2^{b}	6.0	4.0	5.9	5.7^{ba}
	2.0	5.8^{ba}	5.7^{a}	6.1	4.1	5.9	5.8^{a}
SE		0.1	0.2	0.2	0.1	0.1	0.1
p_A		<0.0001	<0.0001	0.812	0.781	0.419	0.041

Legend: n, number of observations in experiment; p_A, statistical probability of addition effect; means with different superscript letters within columns differ significantly ($p \leq 0.05$; significance of differences between groups)

similarities within each defined group (i.e., the addition of phosphate or hydrocolloids). The 16 parameters grouped in the three blocks were included in this analysis, as the instrumentally measured parameters of colour and texture, and the sensory attributes.

This linear discriminant analysis defined the following parameters as being the most discriminating: gumminess, chewiness, firmness, hardness, Y_{30} and a^* value. In all (i.e., 120 samples, 16 variables), nine discriminant functions were obtained. Function 1 explained 52% of the total variance, function 2 explained 23%, function 3 explained 8% and function 4 explained 7%; the other functions together explained 6% of the total variance.

Table 6. Relationships between the instrumental measurements and the sensory evaluations of the texture profiles (Pearson correlation coefficient, r) of the chicken breast meat emulsions (n=120).

Instrumental measure	Parameter	Sensory analysis for firmness	Stress relaxation test	
			F_0	Y_{30}
Texture profile analysis	Hardness	0.42^{**}	0.66^{**}	0.74^{**}
	Adhesiveness	−0.03	−0.01	0.07
	Springiness	0.09	0.14	0.39
	Cohesiveness	0.43^{**}	0.47^{**}	0.66^{**}
	Gumminess	0.53^{**}	0.67^{**}	0.79^{**}
	Chewiness	0.51^{**}	0.61^{**}	0.77^{**}
	Resilience	0.43^{**}	0.53^{**}	0.75^{**}
Stress relaxation test	F_0	0.68^{**}		0.82^{**}
	Y_{30}	0.66^{**}		

Legend: n, number of observations in experiment; * $p \leq 0.05$; ** $p \leq 0.001$; F_0 initial force; Y_{30} force decay level after 30 s

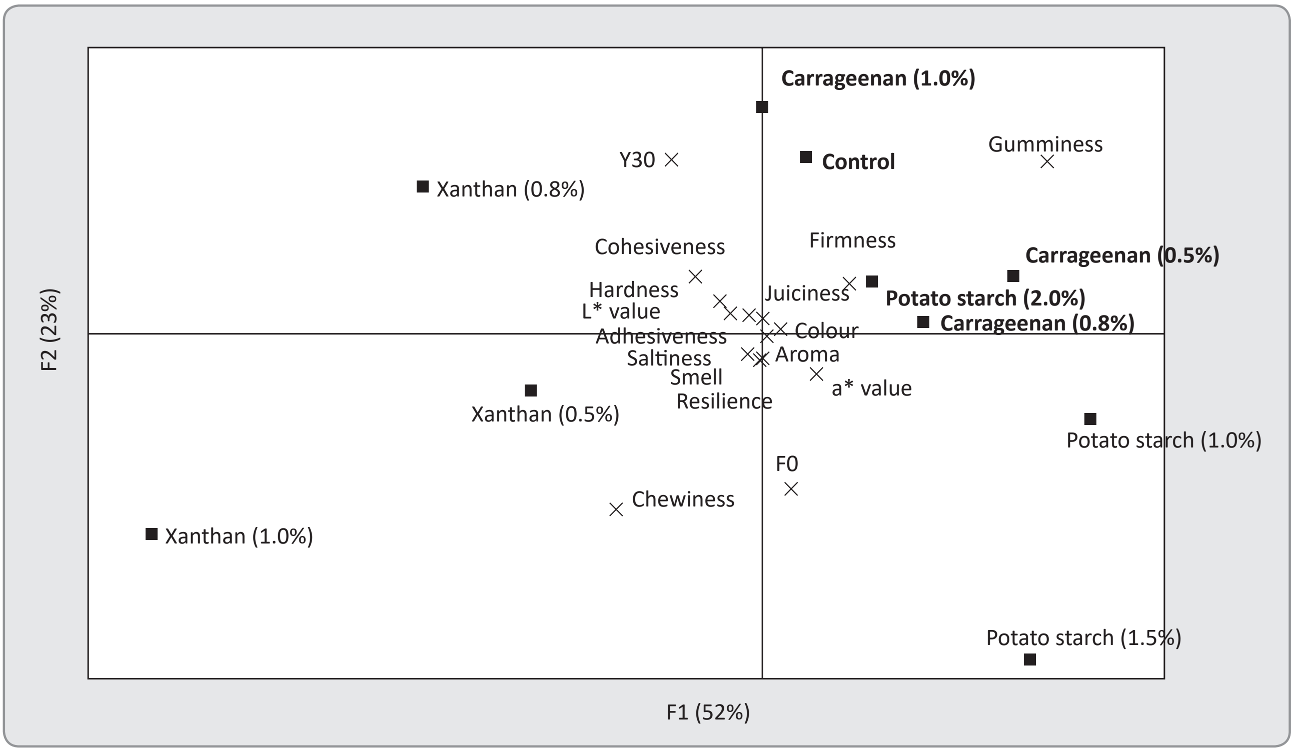

Figure 1. Linear discriminant analysis for the scores for the properties defined by principal component analysis for the 10 groups of chicken breast meat emulsions: additions of phosphate and the hydrocolloids, carrageenan, xanthan and potato starch (■, group centroids).

Figure 1 shows the effects of the attributes of the two main functions. Function 1 clearly distinguished a group of variables that was positioned far from the origin, which included gumminess and perceived firmness. These variables were negatively correlated with chewiness, which function 1 positioned on the opposite side. Function 2 essentially grouped the textural variables, with Y_{30}, cohesiveness and hardness positioned furthest from the origin; function 2 positioned F_0 on the opposite side. Smell, aroma and saltiness were positioned close to each other, thus showing high positive correlations.

This analysis divided the chicken breast meat emulsions into three separate profiles that were clearly noted: the first profile that comprised the control (phosphate) group and the additions of carrageenan (all levels) and potato starch (2.0%); the second profile that comprised the potato starch 1.0% and 1.5% groups; and the third profile that comprised the additions of xanthan (all levels). Specific importance can be given to the first profile that included the control chicken breast meat emulsion along with the emulsions with carrageenan and potato starch (at the highest levels), which were defined on the basis of the 16 parameters discussed that grouped these together close to the control.

Overall, the accuracy of the placement of each sample into its corresponding group was 75%, where 30 observations out of 120 were misplaced (Table 7). Here, three observations from the potato starch 2.0% group were placed with the controls; two observations from the control group were placed with the carrageenan 0.5% group and one with the potato starch 1.0% group. These misplaced samples confirmed the similarities of the low carrageenan (0.5%) and high potato starch (2.0%) groups to the control group, and for the medium xanthan (0.8%) and low potato starch (1.0%) groups (Table 7).

Conclusions

These data support the hypothesis that chicken breast meat emulsions with added phosphates or different hydrocolloids can differ significantly according to their instrumentally measured colour and texture parameters and their sensorial qualities. The differences across these parameters are not always dependent on the type of additive, but are rather the result of the levels of the hydrocolloids used in the emulsions. Overall, it can be said that generally there were no changes in the instrumentally measured textures (with the exception of F_0),

Table 7. Classification matrix for the control and experimental groups of the chicken breast meat emulsions according to the linear discriminant analysis.

Group	Level	Predicted group membership										Total
	(%)	Control	Carrageenan (%)			Xanthan (%)			Potato starch (%)			
			0.5	0.8	1.0	0.5	0.8	1.0	1.0	1.5	2.0	
Control		9	2	0	0	0	0	0	1	0	0	12
Carrageenan	0.5	0	10	1	0	0	0	0	1	0	0	12
	0.8	0	2	9	1	0	0	0	0	0	0	12
	1.0	0	0	0	9	1	1	0	0	0	1	12
Xanthan	0.5	0	0	0	0	10	0	1	0	0	1	12
	0.8	1	0	0	0	0	11	0	0	0	0	12
	1.0	0	0	0	0	0	0	12	0	0	0	12
Potato starch	1.0	0	4	1	0	0	0	0	4	3	0	12
	1.5	0	0	0	0	1	0	0	1	8	2	12
	2.0	3	0	0	1	0	0	0	0	0	8	12

while increased carrageenan and potato starch additions affected the instrumentally measured colour parameters (with the exception of b^* value for carrageenan) and sensory attributes (with the exception of firmness for carrageenan). However, the increasing xanthan additions resulted in changes to both the instrumental and sensory profiles of these chicken breast meat emulsions**.**

Uticaj različitih hidrokoloida na profil teksture mesne emulzije od pilećeg mesa

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*A p s t r a k t: Cilj istraživanja je bio da se proizvedu mesne emulzije od pilećeg belog mesa uz dodatak različitih hidrokoloida koji bi mogli imati iste ili bolje teksturne osobine od emulzija proizvedenih uz upotrebu fosfata (E 450-452). Pripremili smo 10 emulzija od mehanički odvojenog pilećeg belog mesa – meso grudi (kao tri eksperimentalna ponavljanja) sa dodatkom: fosfata (kontrola, 0,7%) i tri različita nivoa karagenana i ksantana (0,5%, 0,8%, 1%) i krompirovog skroba (1%, 1,5%, 2%). Izvršeno je instrumentalno merenje boje (CIE L *, a *, b *) i teksture (analiza profila teksture, test relaksacije stresa), zajedno s procenom senzornih svojstava (deskriptivna analiza). Emulzije od pilećeg belog mesa sa različitim hidrokoloidima značajno su se razlikovale u instrumentalno izmerenim vrednostima za boju, kao i u većini parametara teksture (tvrdoća, kohezivnost, žilavost, lakoća žvakanja, otpornost, F0, I30), i u nekim senzornim osobinama (boja, čvrstoća, ukus). Povećano dodavanje karagenana i krompirovog skroba uticalo je na neke od izmerenih vrednosti za boju i senzorna svojstva, iako izmereni parametri za teksturu nisu bili pod uticajem. Povećano dodavanje ksantana uticalo je na promene u boji, teksturi i senzornim profilima. Mesne emulzije od pilećeg belog mesa sa fosfatom (0,7%), karagenanom (0,5%, 0,8%) i krompirovim skrobom (2,0%) bile su najsličnije u boji, teksturi i ukusu. Emulzije sa krompirovim skrobom pokazale su trendove, koji nisu bili značajni, za poboljšanje osobina u poređenju sa kontrolnom grupom, zbog intenzivne arome.*

Ključne reči: *mesne emulzije, fosfat, hidrokoloidi, parametri teksture, senzorna svojstva.*

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Original scientific paper

Evaluation of the content and safety of nitrite utilisation in meat products in Serbia in the period 2016–2018

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A b s t r a c t: Nitrites are inorganic salts widespread in water, fruits, vegetables, meat and meat products. Application of nitrites in the meat industry is necessary for multiple reasons. They suppress development of some microorganisms in food and are a source of nitrogen oxide that is widely involved in physiological functions of metabolism, food intake and energy balance. On the other hand, nitrites in food can produce nitrosamines that increase the risk of cancer. During regular quality control in 2016–2018, 972 samples of meat products were analysed and verified for compliance with regulations concerning nitrite levels, and the average participation of these meat products in daily intake of nitrite was estimated. The amount of nitrites in the examined meat products was within the permitted limits. The daily intake of nitrite in Serbia from meat and meat products was estimated as being from 0.015–0.020 mg kg^{-1} body weight, which is below the limit values set by EFSA for safe daily intake, i.e. 0.06–0.07 mg kg^{-1} body weight.

Keywords: *nitrites, nitrates, meat products, consumer health, statistical evaluation, acceptable daily intake.*

Introduction

In everyday human nutrition, the presence of nitrates, nitrites and nitrosamines in food is inevitable. These compounds are widespread in water, fruits, vegetables, and meat and meat products. This group of chemicals has multiple, necessary roles in the meat industry. From the bacteriological point of view, nitrite has an inhibitory role in the growth of microorganisms. This is of great importance in preventing the growth of surviving resistant microorganisms after commercial sterilisation processing to manufacture canned meat products (*Codex Alimentarius Commission,* 2016). Due to the antioxidant effect of nitrites, they are also used as preservatives (*Govari and Pexara,* 2015). Nitrates and nitrites are used in cooked and boiled sausages, fermented sausages, cured and smoked meat products etc. (*Ducic et al.,* 2017). The technological significance of their use in meat processing is that they provide an adequate red colour for the meat, give a characteristic aroma to cured meat, and have bacteriostatic and bactericidal effects (*Parthasarathy and Bryan,* 2012).

Regarding human health, the use of nitrites in the meat industry has both positive and negative aspects. A positive effect of nitrites in food is that they participate in the suppression of some microorganisms and so can prevent foodborne diseases. Nitrites are also a source of nitrogen oxide, which has significant participation in the physiology of metabolism, food intake and energy balance. Also on the positive side of the use of nitrates and nitrites are the numerous beneficial effects for metabolic and cardiovascular health (*Bedale et al.,* 2016). On the other hand, the negative effect on human health is the possibility of compounds forming that increase the risk of cancer (*Parvizishad et al.,* 2017). When we consume nitrates and nitrites from fruits and vegetables, numerous protective compounds are also ingested that prevent the negative effects of nitrites on the human body. In meat products, though, these protective compounds are not present, which increases the risk to consumers' health. Therefore, nitrite use is regulated and legally permitted quantities are specified for application in the meat industry.

This research included several tasks and goals. Importantly, the nitrite content in meat products was analysed to assess compliance with legislation. In addition, on the basis of the obtained data, the real contribution of nitrites in meat products to the total daily intake (DI) of nitrites in Serbia was evaluated. Particular attention regarding the impact on consumer health was paid to semi-prepared products and products that are subsequently thermally

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treated, such as minced meat, bacon, cooked sausages and canned meats, and which are used for frying, in pizzas, and in frozen and grilled products.

Materials and methods

All chemicals and standards used in the study were analytical grade. The content of nitrite in meat products was determined using the reference method (*ISO, 1999*).

Meat products and sample preparation

Meat products (n=972) were produced locally or imported. The meats were classified as smoked meats, fermented sausages, cooked sausages, ready-to-eat and fully cooked meat meals, canned meats, cooked sausages, semi-prepared minced meats, bacon, or cured meats, and their contents of nitrites were measured. All products were examined as part of regular quality control of meat products or on request.

Sample preparation for nitrite determination was in accordance with the procedure described in the reference method (*ISO, 1999*).

Statistical analysis

Data was processed using common statistical analytical techniques. MS Office Excel software with Data Analysis Tool Pack was used to compile data and for statistical processing.

Results and discussion

Meat products

The study was conducted on 972 samples of meat products in Serbia: fermented sausages, boiled or cooked sausages, canned, smoked, semi-prepared minced or cured meats, ready-to-eat and fully cooked meat meals and bacon. In all of these products, except for semi-prepared minced meat products, the use of nitrite was regulated (*Official Gazette of RS,* 2015; *Official Gazette of RS,* 2013). Figure 1 shows the number of samples analysed per group of products.

The largest number of samples (314) was in the group of cooked sausages, which accounted for almost one third of the total number of meat products.

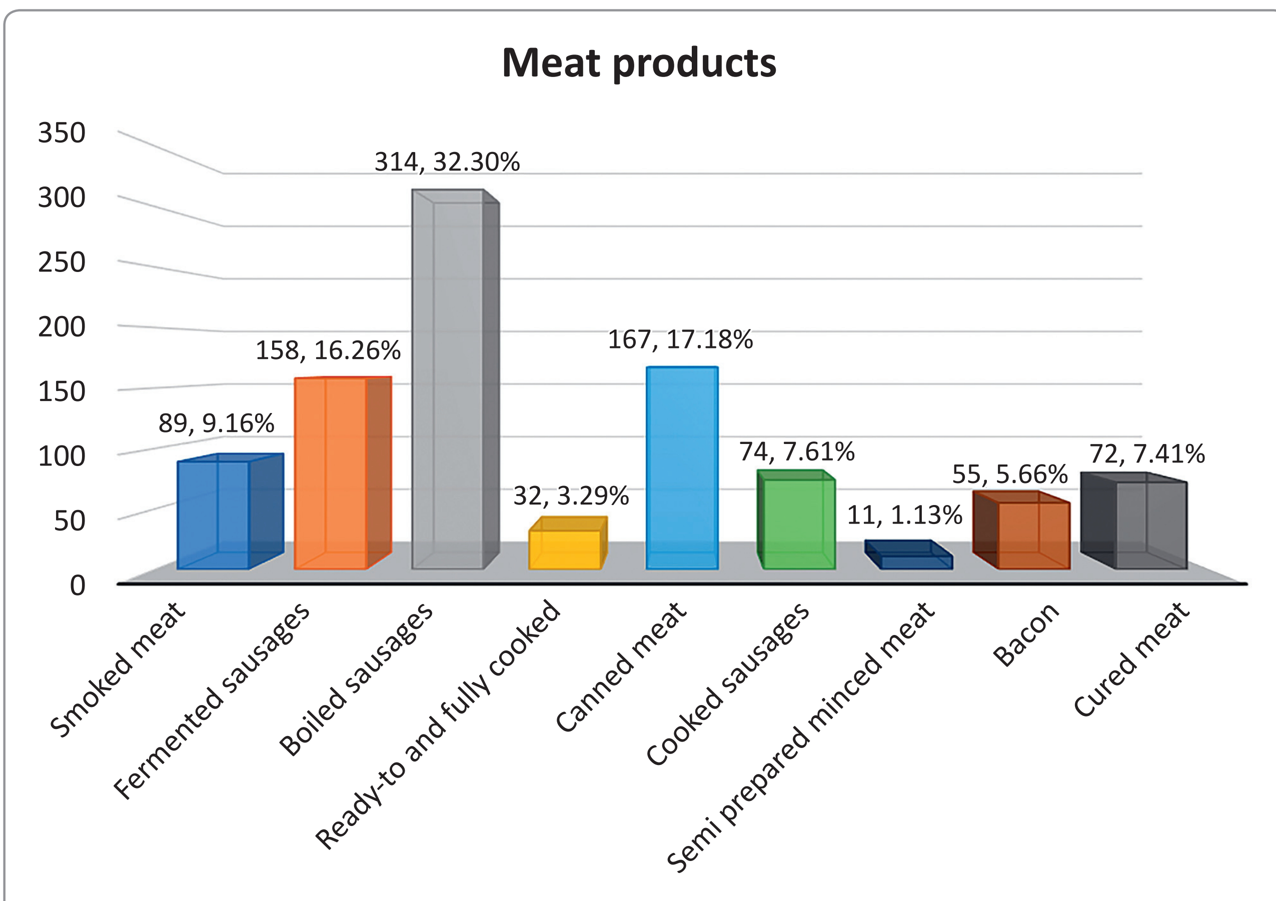

Figure 1. Number and percentage of meat products categorised in the nitrite content survey.

The high number of cooked sausages tested can be explained by the high consumption of cooked sausages, which, of course, directly affects the amounts of locally produced and imported cooked sausages available in Serbia. The second largest groups were fermented sausages and canned meat (16–18%). The third group included smoked meat products, cooked sausages, cured products and bacon (5–10%). Ready-to-eat and fully cooked meat meals and semi-prepared minced meat products accounted for <5% of meat products examined. Generally speaking, the distribution of the meat products tested was in direct proportion to their consumption levels in Serbia. The only exception was semi-prepared minced meat products, because their actual consumption level is far greater than their proportionate participation in this study. Their small number is due to the fact that nitrites are not specifically regulated in these products (*Official Gazette of RS,* 2015), and examination of their nitrite content is not covered by quality control of semi-finished products, but these analyses are performed occasionally and only on request.

Nitrite content

The average, minimum and maximum nitrite contents (mg kg^{-1}) in the meat products are shown in Table 1.

The highest levels of nitrite were found in cooked sausages, canned and smoked meats and bacon. Given the preparation technology of these products, such values were expected. The lowest nitrite levels were determined in fermented sausages, ready-to-eat and fully cooked meals and semi-prepared minced meat products. The reasons for the low content of nitrite are different for each of these three product groups. In fermented sausages, the content of nitrite decreases during the ripening of sausages as a result of the process that takes place in the sausage. In meat meals, the nitrite originates mainly from added meats (sausage, bacon, etc.) that are among the many ingredients in the meals, and, therefore, their contribution to the nitrite content in the meals is low. In the semi-prepared minced meat, it is common to use ordinary salt (NaCl) instead of nitrite salt. The average nitrite content ranged from 10–15 mg kg^{-1} in cooked sausages and cured meat products.

Statistical distributions of nitrite levels within the meat product groups differed. In the canned meats and cooked sausages, nitrite levels were normally distributed, while for other products, the distributions were log-normal or exponential. The distribution of nitrite levels was not examined for semi-prepared minced meat products because the number of tested samples in that group was insufficient for reliable statistical processing. The indicated distribution of test results is related to the stability of the nitrite content in the different meat products and is the consequence of their differing preparation and processing methods. In the case of products with thermal treatment, the decrease of nitrite content is slower than in the case of cured, smoked or fermented products. Reduction of nitrite content is particularly significant in fermented sausages where the main process is nitrite conversion into nitrates in the weak acid environment. Therefore, in fermented sausages, the presence of nitrite becomes latent

Table 1. Nitrite content in the examined categories of meat products

Meat product	No. of samples	Average content (mg kg^{-1})	Minimum content (mg kg^{-1})	Maximum content (mg kg^{-1})	Maximum permitted limit (mg kg^{-1})
Smoked meats	89	24.89	0	120.98	150
Fermented sausages	158	0.65	0	7.54	150
Cooked sausages	314	36.60	0	111.7	150
Ready-to-eat and fully cooked	32	0.48	0	4.34	150
Canned meats	167	35.96	0	87.58	100
Cooked sausages	74	12.49	0	89.59	150
Semi-prepared minced meats	11	0.89	0	9.78	/
Bacon	55	23.95	0	97.42	150
Cured meats	72	10.72	0	127.42	150

because the process is reversible and nitrates can, under certain conditions, be recovered into nitrites (*Zivkovic and Stajic,* 2016). Additionally, nitrite levels were measured in 972 meat samples, which is an indication of the reliability of the results and conclusions obtained by statistical processing.

A graphical representation of the data on the nitrite content of the meat product groups is given in Figure 2.

Compliance with legislation

The content of nitrites in meat products, i.e. quantities added to the products in order that they are safe for use in human nutrition, is closely related to process hygiene. When hygiene of meat production and processing facilities is improved, the possibility of adding less nitrite to meat products is increased, and by extension, the meat/food produced is likely safer. Compliance with legal regulations and information on the real content of nitrites in meat products, among other parameters, indirectly points to the implementation of good manufacturing practice and a Hazard Analysis and Critical Control Point system (*Tomasevic et al.,* 2017).

In the current research, no samples of meat and meat products with nitrite content greater than permitted values (*Official Gazette of RS,* 2013) were detected. In the case of semi-prepared minced meat products, nitrite (9.78 mg kg^{-1}) was detected in one sample. For these products, the quantity of nitrite is not regulated (*Official Gazette of RS,* 2013), and moreover, the current regulation for meat and meat products (*Official Gazette of RS,* 2015) does not allow the presence of nitrites in these products.

Meat product consumption and estimation of nitrite DI in Serbia

The average monthly consumption of different types of food per household in Serbia during 2016 is shown in Figure 3 (*Bulletin, Household budget survey,* 2016).

The data show that in Serbia, monthly, meat and meat products are consumed more than all foodstuffs listed, almost twice as much as milk, cheese and eggs or bread and cereals. The data illustrate the importance of the intake of nutrients, but also additives, salts and other ingredients, through meat and meat products.

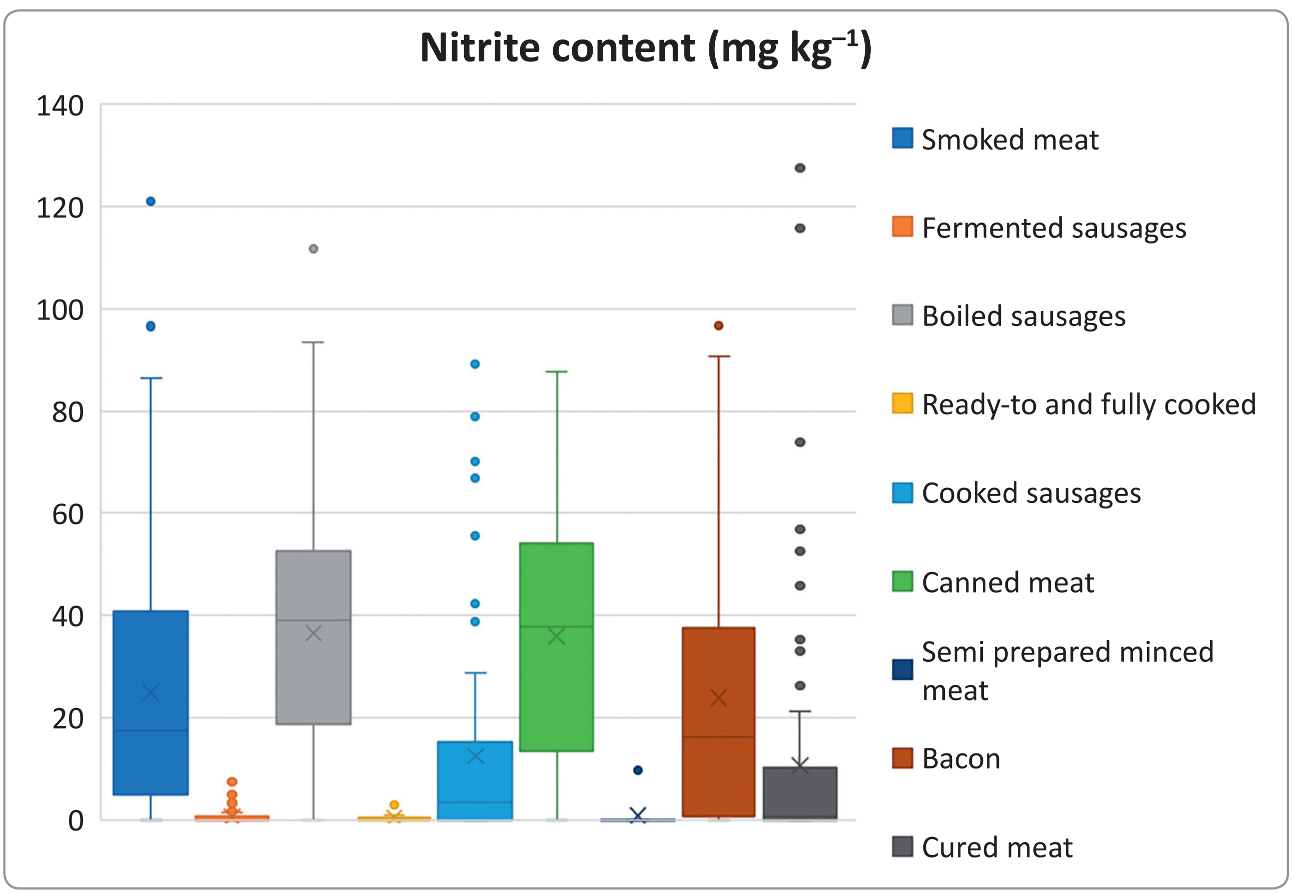

Figure 2. Nitrite content (mg kg^{-1}) in the examined meat products

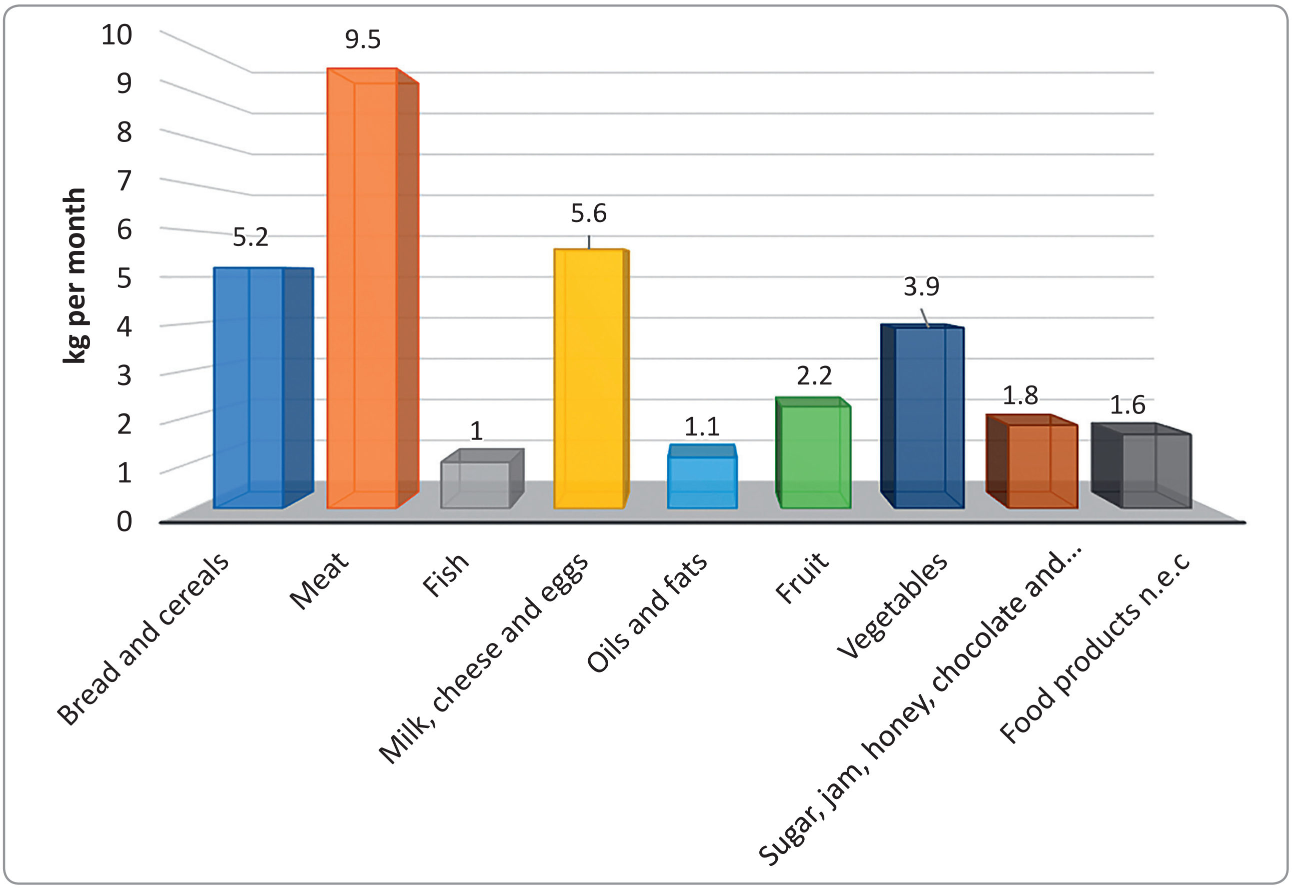

Figure 3. Average monthly food consumption (kg per month) per household in Serbia

On an annual basis, the consumption of meat products per household is almost equal to the consumption of pork and poultry meat, which are the most important categories of meat consumed in Serbia (Figure 4). The average DI of nitrite in Serbia via meat products can be roughly estimated using data on nitrite content in meat products as well as data on consumption of dried meat and meat products. The calculation used the average number of household members for 2016 of 2.88 inhabitants per household (*Bulletin, Household budget survey,* 2016):

$$DI = (0.092 \cdot 24.89\ mg\ kg^{-1} + 0.163 \cdot 0.65\ mg\ kg^{-1} + 0.323 \cdot 36.60\ mg\ kg^{-1} + 0.033 \cdot 0.48\ mg\ kg^{-1} + 0.172 \cdot 35.96\ mg\ kg^{-1} + 0.076 \cdot 12.49\ mg\ kg^{-1} + 0.057 \cdot 23.95\ mg\ kg^{-1} + 0.074 \cdot 10.72\ mg\ kg^{-1}) \cdot (39.1\ kg\ year^{-1} + 15.6\ kg\ year^{-1}) : (365\ days\ year^{-1} \cdot 2.88\ inhabitants\ per\ household) = 1.22\ mg\ nitrite\ daily\ per\ capita.$$

There is no data on the average body weight (b.w.) of the Serbian population in recent literature. If it is assumed that the average body weight is between 60 and 80 kg, DIs of 0.015–0.020 mg kg^{-1} b.w. are obtained. These values are far below the acceptable daily intake (ADI) of 3.7 mg kg^{-1} b.w. according to EFSA recommendations, and below the safe level of DI of 0.07 mg kg^{-1} b.w. (*EFSA,* 2017). However, these nitrite consumption levels originating from meat and meat products contribute about 1/4 to 1/3 of the safe level of nitrite DI, especially when a more conservative ADI value of 0.06 mg kg^{-1} b.w. (*EFSA,* 2017) is considered. It should be noted that the calculated intake levels are only part of the DI of nitrite, and that other foods contribute to a higher intake, especially leafy vegetables, etc.

Health risks

As a result of the development of the meat industry and its need to adapt to consumers by offering attractive products with longer shelf lives, new tastes, and so on, many food additives have been used, and one of most significant roles belongs to nitrites. A series of studies was conducted to determine possible positive or negative effects of nitrite on human health (*Sindelar and Milkowski,* 2012). In addition to inducing antimicrobial effects, preventing the oxidation of lipids and achieving the appropriate aroma and colour in meat products, nitrites also have effects on the human body. Numerous studies highlight

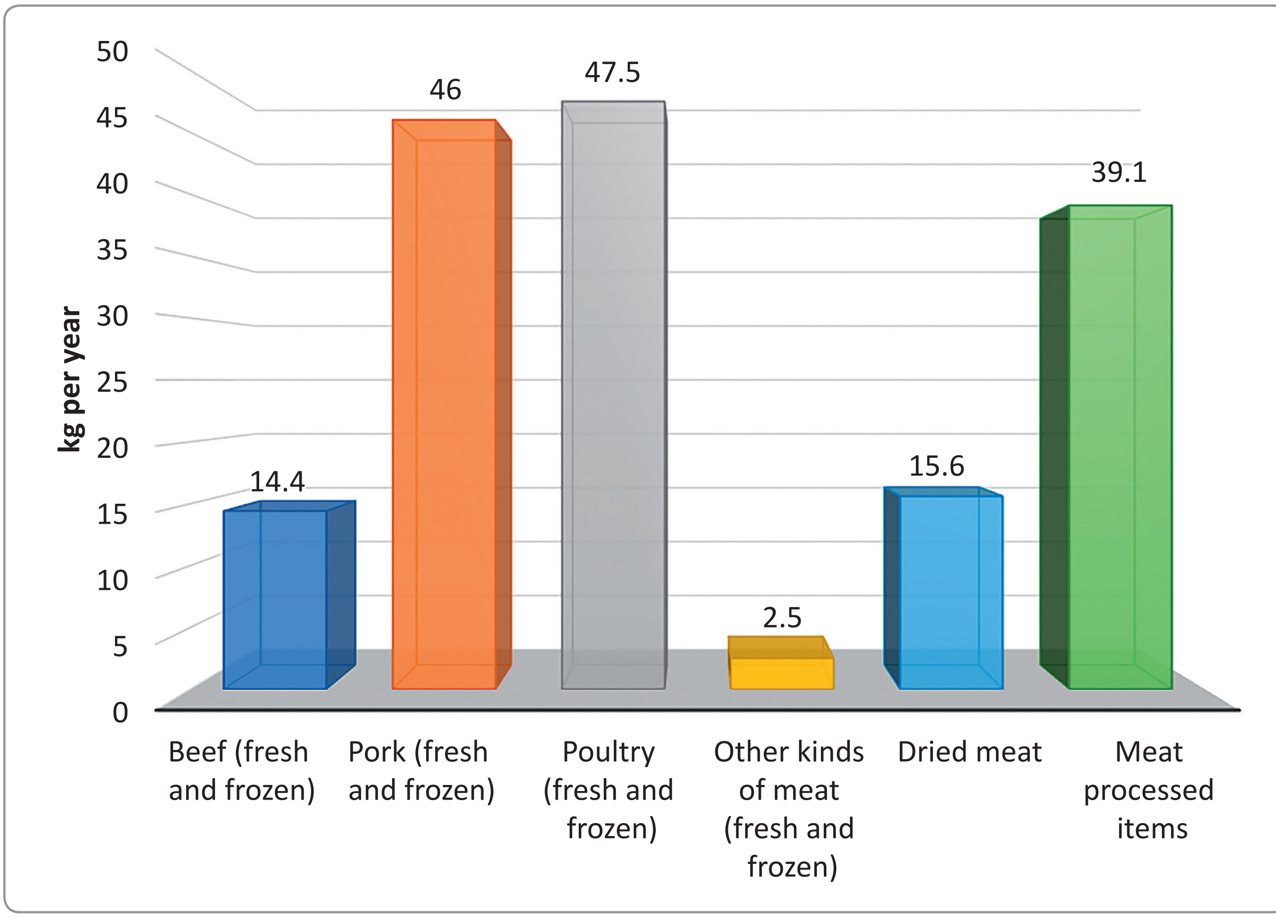

Figure 4. Average annual meat and meat products consumption (kg) per household in Serbia

the beneficial contribution to the physiological functions of metabolism, food intake and energy balance. However, other studies indicate use of nitrites and their involvement in a number of metabolic processes result in the formation of carcinogenic compounds (*Lammarino et al.,* 2013). Nitrosamines, carcinogenic compounds resulting from the use of added nitrates and nitrites, can also be formed from naturally occurring nitrates and nitrites in food. It is, therefore, necessary to add them to meat products in as little as possible amounts that are still technologically effective (*Codex Alimentarius Commission,* 2018). Large amounts of these compounds in the body contribute to the development of cancer in gastrointestinal tract organs, the most commonly affected being colon, rectum, liver, thyroid and stomach (*Kobayashi,* 2018). The substances themselves do not directly affect the development of cancer, but they promote its proliferation and so are classified as risk factors.

Health risks increase if nitrite content in meat products exceeds the permitted limits. Studies have shown that use of nitrates and nitrites in appropriate doses may prevent or slow down the onset of some diseases (*Bryan and Ivy,* 2015). Risks, though, are particularly increased in people with hypertension, coronary, heart disease, obesity or metabolic disorders (*Ghasemi and Jeddi,* 2017). The current study in Serbia on meat and meat products sampled between 2016 and 2018 found the amount of nitrite in these foods was under the allowed limits (except for one sample). In addition, important information from a health perspective is that the DI of nitrite derived from meat products is relatively low, below the recommended ADI (*EFSA,* 2017).

However, particular care must be taken when drawing conclusions, since consideration should also be given to the specificity of the chemical behaviour of nitrite in certain groups of meat products, the technological process of production and any subsequent thermal treatment of these products. Under appropriate conditions, nitrosamines, which are carcinogenic, can occur at elevated temperatures. Also, nitrite alone does not directly affect the production of cancer, but it promotes the proliferation and malignant transformation of tumour cells (*Pengyan et al.,* 2018).

The presence of added nitrites in semi-prepared minced meat products was, in comparison with the other tested products, perhaps the greatest risk to consumer safety. In Serbia, traditional minced meat products prepared by grilling or barbecuing at high temperatures are one of the most frequently consumed fast foods. Nitrites are added to these products to maintain a fresh meat colour. On several occasions, unauthorised addition of metabisulphite for microbiological preservation was established in these products (*Koricanac et al.,* 2017). The probability of reactions involving nitrites and the formation of compounds potentially harmful to consumer health increases with the combined action of metabisulphites that influence the reduction of pH and thermal treatment at high temperatures. However, an insufficient number of these products has been analysed to allow a clear conclusion on the level of consumer vulnerability. A more extensive study to determine the content of nitrites, as well as bisulphite, expressed as SO_2 content, would show the real state of the presence of these compounds. This would be a good basis for assessing the potential risks to the health of consumers in Serbia, as well as for regulating the unauthorised use of these additives.

Conclusion

The nitrite content was measured in 972 samples of meat products in Serbia. The representation of the product groups in our complete population of analysed products reflected their demand and consumption in Serbia. Among our product groups, the highest consumption levels were of thermally treated products such as cooked sausages and canned meats, suggesting that cheaper products with prolonged shelf lives are the most common choice of consumers.

We found the consumption of nitrite via meat products in Serbia is below the safe level. This conclusion, however, should be taken with reserve because, in the absence of official statistical data related to the biometric parameters of the population in Serbia, likely DIs of nitrite were calculated within the boundaries we determined. The results of the assessment of DI are, therefore, indicative, not final. Meat and meat products are not the only source of nitrites, but consumption of these products is widespread among the Serbian population, and therefore, the contribution meat makes to consumer nitrite intake must be taken into consideration. The addition of nitrites to semi-prepared minced meat products, which are the most common form of fast food besides pizza, is a potential risk factor. It is necessary, on the one hand, to increase public awareness of the health risk of nitrite/nitrate use, and on the other hand, to conduct suitable controls on meat products, including on the popular semi-prepared minced meat products that are not covered by regular quality control or legislation (*Koricanac et al., 2017; Janjic et al.,* 2015).

Evaluacija sadržaja i bezbednosti upotrebe nitrita u proizvodima od mesa u Srbiji u periodu 2016–2018

Aleksandar Bajčić, Radivoj Petronijević, Nenad Katanić, Dejana Trbović, Nikola Betić, Aleksandra Nikolić, Lazar Milojević

A p s t r a k t: Nitriti su neorganske soli široko rasprostranjene u vodi, voću, povrću, mesu i proizvodima od mesa. Njihova primena u industriji mesa je višestruka i neophodna. Pozitivno utiču na suzbijanje razvoja određenih mikroorganizama u hrani i predstavljaju rezervoar azot oksida koji ima široku primenu u fiziološkoj funkciji metabolizma, unosa hrane i energetskog bilansa. Sa druge strane, mogući su uzročnici nastanka kancerogenih nitrozamina koji povećavaju rizik od nastanka raka. Ispitano je 972 uzorka proizvoda od mesa u toku redovne provere kvaliteta u periodu od 2016-2018 godine, proverena usaglašenost sa zakonskom regulativom i procenjeno prosečno učešće proizvoda od mesa u dnevnom unosu nitrita. Sadržaj nitrita u proizvodima od mesa u kojima je sadržaj regulisan je bio u granicama dozvoljenog. Procenjeno je da se dnevni unos nitrita u Srbiji kreće u granicama od 0.015–0.020 mg kg^{-1} telesne mase, što je ispod graničnih vrenosti koje je postavila EFSA za nivo bezbednog dnevnog unosa od 0.06– .07 mg kg^{-1} telesne mase.

Ključne reči: *nitriti, nitrati, proizvodi od mesa, zdravlje potrošača, statistička procena, prihvatljivi dnevni unos.*

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Reducing sodium chloride content by partial replacement with potassium chloride or ammonium chloride in pork stew

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A b s t r a c t: The goal of this study was to investigate the influence of reducing sodium chloride content in pork stew by partial replacement of sodium chloride with potassium chloride or ammonium chloride, with a target to achieve optimal salty taste. The trial consisted of five groups. In the control pork stew, only sodium chloride was added. In group 1, one third of sodium chloride was replaced with potassium chloride; in group 2, one half of the sodium chloride was replaced with potassium chloride; in group 3, sodium chloride was reduced by half and one quarter of ammonium chloride was added compared to the control stew, and in group 4, sodium chloride was reduced by 62.5%, and the same weight of ammonium chloride as sodium chloride was added. Sensory evaluation was performed by ten trained assessors using numeric scales. Evaluations of colour acceptability and consistency were without statistical differences (P≥0.05). The most expressed saltiness was evaluated in group 1 due to it having the largest amount of added sodium chloride. Ammonium chloride added in the pork stew in group 3 intensified saltiness of the product, even though the sodium chloride level was a half that of the control group. Taste acceptability was directly correlated with saltiness acceptability and evaluated as better in group 2 and group 4 stews. Statistically significant differences in taste acceptability were established between group 2 and group 3 stews (P≤0.01), between control and group 2 stews (P≤0.05), and between group 3 and group 4 stews (P≤0.05).

Keywords: *pork stew, sodium chloride reduction, potassium chloride, ammonium chloride.*

Introduction

Sodium chloride (NaCl) is the primary, most common food additive that gives a salty taste and prolongs shelf life of food. Taste perception of food is unitary and composed of anatomically independent sensory systems that emphasise the role of general taste perception, and it is associated with the food flavour as well as with trigeminal sensations (*Rolls et al., 2010*). Salt contributes to food consistency, masks metallic tastes and off notes and rounds out overall impressions of food (*Gillette, 1985*). Although salt is very important in food, dietary sodium intake in almost all cases is above the recommended level, and it is the most common cause of essential hypertension in humans and consequential cardiovascular disorders (*Weinberger et al.*, 2001).

Dietary sodium intake can be reduced in several ways such as a sodium restricted diet, sodium reduction by stealth, use of salt substitutes and other approaches (*Liem et al., 2011*). The most common way to reduce sodium content in food is to use NaCl substitutes such as other chloride salts, particularly potassium chloride (*Guàrdia et al.,* 2006*)*. Diet salts, containing mixtures of sodium chloride and potassium chloride are already available on the market.

Due to the aforementioned problems, foods, especially meat products, are often a subject of investigation to reduce their sodium content. There are many scientific studies about sodium reduction in pasteurised and sterilised meat products such as in cooked sausages and in meat products that are not thermally treated such as dry fermented sausages and dry meats. Nowadays, ready-to-eat food and prepared meals have become an important choice for people due to busy lifestyles and lack of time. Beside restaurants, the meat industry, for these purposes, produces various, mainly canned, prepared meals containing meat and vegetables.

The need to reduce sodium in meat products and generally in food will be an aim of the food industry in the future; fast food chains will also have to address this issue, even if people think the amount of salt consumed *via* fast food is not so large (*Moran*

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et al., 2017). Nonetheless, use of salt replacers presents a difficult problem because of degradation of desirable sensory characteristics, including texture and, of course, salty taste (*Kamenik et al.,* 2017*; Kang et al.,* 2014).

The goal of this study was to investigate the influence of reducing the sodium chloride content in pork stew by partial replacement of sodium chloride with potassium chloride or ammonium chloride, with a target to achieve optimal salty taste.

Materials and Methods

The trial consisted of five sample groups of pork stew with the compositions presented in Table 1. In the control group of pork stew, only sodium chloride was added. In group 1, one third of the sodium chloride was replaced with potassium chloride, while in group 2, one half of the sodium chloride was replaced with potassium chloride. In group 3, sodium chloride was reduced by half and one quarter of ammonium chloride in the relation to control group was added, and in group 4, sodium chloride was reduced by 62.5% of the amount added into control stew, and an equal weight of ammonium chloride as sodium chloride was added.

Meal preparation

Minced onion was fried in the sunflower oil for 20 minutes, and after that red pepper, salts and pork (pork shoulder, category II), cut into pieces were added, as well as water. Stew was cooked for 80 minutes.

Sensory evaluation

Sensory evaluation was performed by ten trained panellists (*SRPS ISO 8586*, 2015) using numeric scales. Colour acceptability, consistency, saltiness acceptability and taste acceptability were evaluated with a 1–5 point scale, where 1 was the least acceptable and 5 was the most acceptable attribute. Saltiness intensity was evaluated with a 1–5 point scale, wherein 5 was the most expressed attribute and 1 was the least expressed attribute. Preparation and presentation of the stew samples to the assessors (number, coding and randomisation) as well as the fitting out of the serving area (isolation of panellists, lighting conditions) were performed according to *Baltic and Karabasil* (2011). The final ranking was performed according to the sum of all sensory evaluation results, where the best scored stew was ranked in 1st place and the worst in 5th place.

Statistical evaluation

The results obtained were statistically evaluated using Microsoft Excel 2010 and are presented as mean±SD. Statistical differences between means of the examined parameters were determined at the levels of 0.05 and 0.01 by Student's t-test.

Results and discussion

Results of sensory evaluation of colour acceptability, consistency, saltiness acceptability, saltiness intensity and taste acceptability of pork stew are presented in Table 2.

Colour acceptability and consistency were evaluated with very high scores and were very similar between the examined groups of stews, without statistical differences ($P \geq 0.05$). Evaluations for colour acceptability ranged from 4.65 for group 3 to 4.95 for group 2 stew. Evaluations for consistency were also high and were in the range from 4.70 (groups 3 and 4) to 4.80 (group 2). Partially replacing chloride salt with other salts, potassium chloride (groups 1 and 2) or ammonium chloride (groups 3 and 4) had no negative effect on these sensory parameters (colour or consistency).

Saltiness was the least acceptable in group 3 pork stew (2.90) and was statistically significantly

Table 1. Composition of pork stew

Group	Onion, g	Sunflower oil, ml	Meat, g	Water, ml	Sodium chloride, g	Potassium chloride, g	Ammonium chloride, g
Control	500	50	500	500	16.00	–	–
1	500	50	500	500	10.66	5.33	–
2	500	50	500	500	8.00	8.00	–
3	500	50	500	500	8.00	–	4.00
4	500	50	500	500	6.00	–	6.00

Table 2. Sensory evaluation of pork stew, Mean±SD, n = 10

	Colour acceptability	Consistency	Saltiness acceptability	Saltiness intensity	Taste acceptability
Control	4.75±0.40	4.75±0.40	3.10±0.86 ^{a}	4.60±0.66 ^{x}	3.60±0.54^{a}
1	4.80±0.40	4.75±0.40	3.85±0.81	2.70±1.19 ^{y}	3.75±0.72
2	4.95±0.15	4.80±0.40	4.35±0.90 $^{b, x}$	2.10±0.70 $^{a, y}$	4.50±0.92^{b,x}
3	4.65±0.55	4.70±0.51	2.90±1.04 ^{y}	3.30±1.00 $^{b, y}$	2.95±1.11^{c,y}
4	4.75±0.40	4.70±0.46	3.65±1.03	2.30±0.46 $^{a, y}$	4.20±0.68^{d}

Legend: $^{(a,b,c,d)}$ Values (mean±SD) with different superscript letters are significantly different (P≤0.05);
$^{(x,y)}$ Values (mean±SD) with different superscript letters are significantly different (P≤0.01)

greater (P≤0.01) compared to saltiness of the group 2 stew (4.35). Also saltiness acceptability of group 2 stew was evaluated as better (P≤0.05) than the control stew (3.10). The most expressed saltiness (intensity) was evaluated in the control stew (4.60) due to it having the largest amount of added sodium chloride, and it was significantly higher than those from other stew groups (P≤0.01). The lowest intensity of salty taste was determined in group 2 stew (2.10), in which sodium chloride was reduced by half, and in the group 4 stew (2.30), in which sodium chloride was reduced by 62.5% and ammonium chloride was added. Ammonium chloride added to the group 3 pork stew intensified the saltiness of the product, even though the sodium chloride content was half that of the control stew. Saltiness intensity was evaluated as higher in group 3 stew than in stews 2 and 4 (P≤0.05).

Taste acceptability was directly correlated with saltiness acceptability and evaluated as being greater (i.e., more acceptable) in group 2 stews (4.50) and group 4 stews (4.20) than in stews from other groups. Statistically significant differences were established for this attribute between group 2 and group 3 stews (P≤0.01), between control and the group 2 stews (P≤0.05) and between group 3 and group 4 stews (P≤0.05).

The prepared pork stews were ranked in the following order: 1st place – group 2; 2nd place – group 4; 3rd place – group 1; 4th place – group 3; and 5th place – the control group.

Conclusion

Colour and consistency of the pork stews were not influenced by reducing sodium chloride content and adding potassium chloride or ammonium chloride.

Saltiness was the most expressed in the control group stew due to it having the largest amount of added sodium chloride, and this parameter was significantly higher than in other groups (P≤0.01). More intense saltiness of the group 3 stew, even though sodium chloride was reduced by half compared to the control stew, could be due to ammonium chloride having a synergistic effect on the saltiness of sodium chloride.

Taste acceptability was directly correlated with saltiness acceptability and evaluated more favourably in the group 2 and group 4 stews. Statistically significant differences were established for this attribute between group 2 and group 3 stews (P≤0.01), between control and the group 2 stews (P≤0.05) and between group 3 and group 4 stews (P≤0.05).

The results obtained showed that pork stew presents a good matrix for sodium chloride/sodium content reduction. All samples of pork stew were sensorially acceptable, even those in which sodium chloride was reduced by half and in which sodium was reduced by 62.5% in the relation to the control stew. The great significance of these results is achieving a better sodium:potassium ratio in this product.

Mogućnost redukcije sadržaja natrijum hlorida u svinjskom paprikašu parcijalnom supstitucijom kalijum hloridom i amonijum hloridom

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A p s t r a k t: Cilj rada bio je da se ispita uticaj smanjenja sadržaja natrijum hlorida u svinjskom paprikašu, delimičnom supstitucijom natrijum hlorida kalijum hloridom i amonijum hloridom u svrhu postizanja optimalne slanosti. U ogledu je formirano pet grupa. U kontrolnoj grupi uzoraka dodat je samo natrijum hlorid. U grupi 1, trećina natrijum hlorida zamenjena je kalijum hloridom, dok je u grupi 2, polovina natrijum hlorida zamenjena kalijum hloridom. U grupi 3, natrijum hlorid je upola smanjen, a četvrtina amonijum hlorida je dodata, u odnosu na količinu soli u kontrolnoj grupi, dok je u četvrtoj grupi natrijum hlorid smanjen za 62,5%, a amonijum hlorid dodat u istoj količini kao natrijum hlorid. Senzorska ocena bila je izvedena od strane deset obučenih ocenjivača korišćenjem numeričkih skala. Ocene za prihvatljivost boje i konzistenciju bile su bez statistički značajnih razlika ($P \geq 0.05$). Slanost je bila najizraženija u uzorcima prve grupe usled najveće količine dodate soli. Amonijum hlorid dodat u uzorcima treće grupe pojačao je slanost proizvoda, iako je sadržaj natrijum hlorida upola smanjen u odnosu na kontrolnu grupu. Prihvatljivost ukusa bila je u direktnoj korelaciji sa slanošću i bila je bolje ocenjena u uzorcima iz druge i četvrte grupe. Statistički značajne razlike utvrđene su između uzoraka druge i treće grupe ($P \leq 0.01$), između uzoraka kontrolne i druge grupe ($P \leq 0.05$) i između uzoraka treće i četvrte grupe ($P \leq 0.05$).

Ključne reči: *svinjski paprika, redukcija natrijum hlorida, kalijum hlorid, amonijum hlorid.*

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Partial replacement of sodium chloride with potassium chloride or ammonium chloride in a prepared meal – cooked peas with pork burger

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A b s t r a c t: The aim of this study was to examine the influence of reducing the sodium content in a prepared meal – cooked peas with pork burger – by partial replacement of sodium chloride with potassium chloride or ammonium chloride, with the target of achieving the same salty taste with less sodium content. In the control group of both cooked peas and pork burgers, only sodium chloride was added. In group 1, one third of sodium chloride was replaced with potassium chloride, while in group 2, one half of the sodium chloride was replaced with potassium chloride. In group 3, one third of the sodium chloride was replaced with ammonium chloride and in group 4, sodium chloride was reduced by one half, and one quarter of ammonium chloride was added compared to the control group. Saltiness was less acceptable in the cooked peas from the control group and from group 3 and can be connected with the too intense salty taste of both these pea groups and the appearance of a bitter taste in cooked peas from group 3. In the pork burgers, intensity of saltiness was higher in the control and group 3 burgers than in group 1 (P≤0.05) or in group 2 and group 4 (P≤0.01) products. The taste of cooked peas from group 3 was not acceptable due to their having the most intense saltiness. In the case of burgers, the taste was acceptable for products from all examined groups, and the only statistical difference was between the taste acceptability of control and group 3 burgers (P≤0.01).

Keywords: *cooked peas, pork burgers, sodium chloride reduction, potassium chloride, ammonium chloride.*

Introduction

Prepared and ready-to-eat meals have become an important choice for modern people with respect to their fast lifestyles, particularly in developed countries. Nowadays, meat products and prepared meat meals are often the subject of scientific examination to reduce their sodium chloride content, with the aim of achieving lower sodium content and a better sodium:potassium ratio in the food. Daily dietary sodium intake mainly exceeds the level recommended by World Health Organization, and that can cause several debilitative health effects, primarily linked to essential hypertension and consequent cardiovascular disorders. It has been estimated that 62% of stroke and 49% of coronary heart disease is caused by high blood pressure (*Brown et al.*, 2009).

Sodium chloride has an important role in meat products and in home-prepared meals, and it is the prototypical stimulus for salty taste (*Dotsch et al.*, 2009). Sodium chloride improves the sensory properties of food by increasing saltiness, decreasing bitterness and increasing sweetness and other congruent flavour effects (*Keast and Breslin*, 2003). Saltiness perception is a very complex system and can be explained by the unique sodium-specific transduction mechanism involving epithelial sodium channels (ENaCs) on the taste receptor cells (*Chandrashekar et al.*, 2010). Identification of saltiness occurs when the concentration of sodium is high enough not only to activate the taste receptors, but also produce electrical impulses, which are carried via sensory neurons to the brain where they are decoded, after which the taste quality is identified (*Liem et al., 2011*).

Dietary sodium intake can be reduced in several ways such as a sodium restricted diet, sodium reduction by stealth, use of salt substitutes and other approaches (*Liem et al.*, 2011). Use of other salts has the aim of partial replacement of sodium chloride and can be used to reduce the sodium content

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of meat products, but this is also linked with an appearance of bitter and metallic tastes in the products. The most common sodium chloride replacer is potassium chloride, followed by magnesium and calcium salts and ascorbates (*Ruusunen and Puolanne*, 2005). There are several studies on sodium reduction in cooked sausages, dry fermented sausages and dry meat. However, the meat industry is an important producer of ready-to-eat meals prepared or cooked in advance, with no further cooking or preparation required before being eaten.

There are not many literature data on the use of sodium chloride substitutes in prepared meat meals. Some authors examined the partial replacement of sodium chloride with potassium chloride in meat burgers (*Lilic et al*., 2015), as well as the use of potassium chloride and ammonium chloride as partial replacers of sodium chloride in the prepared meat meal, meatballs in tomato sauce (*Lilic et al*., 2017). Some others (*Murray and Shackelford*, 1991) found that ammonium chloride combined with autolysed yeast could be a suitable replacer of sodium chloride in some meat products. Commercial use of sodium chloride replacers is still restricted, but presently, potassium chloride and ammonium chloride are both recognized as safe.

The aim of this study was to examine the influence of reducing the sodium content in a prepared meal – cooked peas with pork burger – by partial replacement of sodium chloride with potassium chloride or ammonium chloride, with the target of achieving the same salty taste with a lower sodium content.

Materials and Methods

The trial consisted of five groups of samples, the compositions of which are presented in Tables 1 and 2. In the control group of both cooked peas and pork burgers, only sodium chloride was added. In group 1, one third of sodium chloride was replaced with potassium chloride, while in group 2, one half of the sodium chloride was replaced with potassium chloride. In group 3, one third of the sodium chloride was replaced with ammonium chloride and in group 4, sodium chloride was reduced by a half and replaced with the same amount of ammonium chloride.

Meat preparation

Minced onion was fried in sunflower oil and red pepper was added. After that, frozen green peas, salt, sucrose and water were added. The peas were cooked for one hour.

Burgers were prepared from minced pork leg meat (grind plate 3 mm) purchased from a local market. Meat was well mixed with the ingredients in Table 2 to achieve optimal consistency to form into round shapes and roasted after that.

Sensory evaluation

Sensory evaluation was performed by ten trained panellists (*SRPS ISO 8586*, 2015) using numeric scales. Each sensory characteristic was evaluated for both ingredients of the complete meal, cooked peas and pork burgers. Colour acceptability, consistency, saltiness acceptability and taste acceptability were evaluated with a 1–5 point scale, where 1 was the least acceptable and 5 was the most acceptable. Intensity of saltiness was evaluated with a 1–5 point scale, where 5 was the most expressed attribute and 1 was the least expressed attribute. Preparation and presentation of the samples to the assessors (number, coding and randomization) as well as the fitting out of the serving area (isolation of panellists, lighting conditions) were performed according to *Baltic and Karabasil* (2011). The final ranking was done according to the sum of all sensory evaluation results where the best scored meal was ranked highest and the worst ranked lowest.

Table 1. Composition of cooked peas, g or ml.

Group	Minced onion, g	Sunflower oil, ml	Red pepper, g	Frozen peas, g	Sodium chloride, g	Sucrose, g	Water, ml	Potassium chloride, g	Ammonium chloride, g
Control	70	20	5	450	20.50	30	450	–	–
1	70	20	5	450	13.66	30	450	6.83	–
2	70	20	5	450	10.25	30	450	10.25	–
3	70	20	5	450	13.66	30	450	–	6.83
4	70	20	5	450	10.25	30	450	–	5.13

Table 2. Composition of burgers, g.

Group	Pork, g	Sodium chloride, g	Potassium chloride, g	Ammonium chloride, g
Control	500	10.00	–	–
1	500	6.70	3.30	–
2	500	5.00	5.00	–
3	500	6.70	–	3.30
4	500	5.00	–	2.50

Statistical evaluation

The results obtained were statistically evaluated using Microsoft Excel 2010 and are presented as mean±SD. Statistical differences between means of the examined parameters were determined on the level 0.05 and 0.01 by Student's t-test.

Results and discussion

The results of sensory evaluation of colour and consistency of cooked peas and pork burgers are presented in Table 3.

Colour acceptability was evaluated similarly for cooked peas (range 4.30±0.68 to 4.55±0.42) and for pork burgers (range 4.00±0.89 to 4.40±0.62). Also, evaluations of consistency were similar, without statistical differences between means ($P \geq 0.05$). For cooked peas, consistency was in the range 4.15±0.71 to 4.45±0.65 and for burgers, it was in the range 4.20±0.71 to 4.35±0.59. Reducing the sodium chloride and adding other salts did not affect these two sensory characteristics. The absence of any negative influence on product colour by the sodium chloride replacers we used was explained previously for meat burgers with added potassium chloride (*Lilic et al*., 2015) as well as for dry pork meat in which sodium chloride was partially replaced with potassium chloride and ammonium chloride (*Lilic et al*., 2016). Also, the use of potassium chloride and ammonium chloride as partial replacers of sodium chloride did not affect the colour of meatballs in tomato sauce (*Lilic et al*., 2017).

Sensory evaluation of saltiness acceptability, taste acceptability and saltiness intensity is presented in Table 4.

Saltiness acceptability was similarly evaluated in the cooked peas from groups 1, 2 and 4 (4.00±0.97, 3.65±1.10 and 4.05±0.79, respectively) without statistical differences ($P \geq 0.05$). Saltiness of control cooked peas scored lower, i.e., less favourably (2.60±1.22), and was statistically different from all other groups ($P \leq 0.01$), including from the low-scoring group 3 peas (1.45±0.47). The

Table 3. Sensory evaluation (mean±SD; n = 10) of colour acceptability and consistency of cooked peas and burgers.

Group	Cooked peas		Burgers	
	Colour acceptability	**Consistency**	**Colour acceptability**	**Consistency**
Control	4.55±0.42	4.25±0.60	4.40±0.62	4.35±0.67
1	4.55±0.42	4.30±0.60	4.35±0.78	4.35±0.59
2	4.40±0.62	4.25±0.60	4.15±0.78	4.20±0.71
3	4.30±0.68	4.15±0.71	4.00±0.89	4.25±0.0.72
4	4.50±0.50	4.45±0.65	4.30±0.78	4.35±0.63

Table 4. Sensory evaluation (mean±SD; n = 10) of saltiness acceptability, saltiness intensity and taste acceptability of cooked peas and burgers.

Group	Cooked peas			Burgers		
	Saltiness acceptability	**Saltiness intensity**	**Taste acceptability**	**Saltiness acceptability**	**Saltiness intensity**	**Taste acceptability**
Control	$2.60 \pm 1.22^{a,x}$	$4.30 \pm 0.64^{a,x}$	3.10 ± 1.22^{x}	3.85 ± 1.03	$3.95 \pm 1.06^{a,x}$	4.25 ± 0.84^{x}
1	$4.00 \pm 0.97^{b,y}$	3.00 ± 0.97^{b}	3.95 ± 0.88^{x}	3.85 ± 0.90	3.30 ± 0.78^{a}	4.00 ± 0.97
2	3.65 ± 1.10^{y}	$2.65 \pm 0.84^{y,c}$	3.75 ± 0.87^{x}	3.55 ± 1.01	2.80 ± 1.47^{b}	3.70 ± 0.87
3	$1.45 \pm 0.47^{c,y,z}$	$4.15 \pm 1.23^{a,x}$	1.55 ± 0.47^{y}	3.10 ± 0.97	3.80 ± 1.47^{x}	3.30 ± 1.12^{y}
4	4.05 ± 0.79^{y}	$1.60 \pm 1.20^{z,c}$	4.00 ± 0.87^{x}	3.80 ± 0.90	$2.10 \pm 1.22^{b,y}$	3.65 ± 1.03

Legend: $^{(a,b,c)}$ Values (mean±SD) with different superscript letters are significantly different (P≤0.05)
$^{(x,y,z,q)}$ Values (mean±SD) with different superscript letters are significantly different (P≤0.01)

less acceptable saltiness of groups 2 and 3 cooked peas was connected with the too intense salty taste and with the appearance of a bitter taste in group 3 cooked peas. The saltiness of all roasted pork burgers was acceptable and there were no statistical differences between means (P≥0.05).

Intensity of saltiness was the highest in cooked peas from the control group and group 3, and these values were significantly different from groups 2 and 4 cooked peas (P≤0.01) and from group 1 peas (P≤0.05). Saltiness was the most highly expressed in control cooked peas (4.30±0.64), due to this group containing the largest amount of added sodium chloride, and in group 3 cooked peas (4.15±1.23). The saltiness intensity of this latter group, even with its low sodium chloride content, was likely because the ammonium chloride added in a relatively large amount probably acted as an enhancer of salty taste. In the pork burgers, intensity of saltiness was significantly higher in the control and group 3 products than in burgers from group 1 (P≤0.05) and in those from groups 2 and 4 (P≤0.01). This order of saltiness intensity was according to amount of added sodium chloride in the burgers. Similarly to cooked peas from group 3, saltiness intensity was high in burgers from group 3 (3.80±1.47).

The taste of cooked peas from all groups was acceptable, except that of group 3 peas (1.55±0.47), which was due to the most intense, unacceptable saltiness in these peas and which was statistically different from other pea groups. Evaluations of taste acceptability of other groups of cooked peas were similar, with no statistical differences (P≥0.05) between them. In the case of burgers, the taste of burgers from all groups was acceptable, and the only statistically significant difference was the more acceptable taste of control burgers than group 3 burgers (P≤0.01).

Complete meals (cooked peas with pork burger) were ranked in the following order according to the sensory evaluations, from most acceptable to least acceptable: group 2 > control > group 1 > group 4 > group 3.

The most common substitute of sodium chloride is potassium chloride, but other chloride salts such as calcium chloride, magnesium chloride, lithium chloride and ammonium chloride are also potential sodium replacers. As with potassium chloride, potential use of these salts is restricted by their unacceptable flavours. There is very little literature data on the use of sodium chloride replacers in prepared meat dishes. Commercial use of sodium chloride substitutes is further restricted to those substances having FDA approval (*Murray and Shackelford*, 1991). For example, lithium chloride is not allowed because of its pharmokinetic impact. Presently, however, potassium chloride and ammonium chloride are both recognized as safe. Ammonium chloride does not belong to the list of recognised food additives, but has a somewhat special status among the nutritionally acceptable mineral salts (*Cepanec et al.*, 2017). Due to the close similarity of ammonium cation with the alkali metal cations sodium and potassium, ammonium chloride exhibits roughly similar properties to those of sodium chloride and potassium chloride. According to *Murray and Shackelford* (1991), ammonium chloride gives a stronger, less acceptable flavour to food when used alone, but in combination with other chloride salts, contributes to a saltier flavour, particularly when combined with potassium chloride. These authors examined a mixture of ammonium chloride and autolysed yeast as a sodium chloride replacer and found this mixture strongly enhanced salt flavour intensity, and, when incorporated into processed meats, aided in solubilisation of proteins and texturisation.

In conclusion, both potassium chloride and ammonium chloride are suitable substituents for sodium chloride in ready-to-eat meat meals, whether added individually or added in different proportions.

Conclusion

Reduction of sodium chloride content and partial replacement with potassium chloride and ammonium chloride did not affect colour acceptability or consistency of either cooked peas or pork burgers.

Saltiness acceptability was similarly evaluated in the cooked peas from groups 1, 2 and 4. The less acceptable saltiness of control and group 3 cooked peas was connected with the too intense salty flavour and bitterness in peas with ammonium chloride. The saltiness of all the roast pork burgers was acceptable. Saltiness intensity was the highest in the control burgers, as these contained the largest amount of added sodium chloride, and in group 3 burgers, probably because ammonium chloride enhanced the salty taste of this product.

The taste of cooked peas from all groups was acceptable, except for group 3 peas, due to them having the most intense, unacceptable saltiness. In the case of burgers, the taste of burgers from all groups was acceptable. The only statistically significant difference in taste acceptability was between the more acceptable taste of control burgers than of the group 3 burgers.

The complete meat meals were ranked according to sensory evaluations, with group 2 meals ranked the highest (ahead of the control meals) and group 3 meals ranked the lowest.

According to the obtained results, shown by sensory characteristics and according to complete meal ranking, it can be concluded that either potassium chloride or ammonium chloride could be suitable partial substituents of sodium chloride in this type of prepared meat meal; potassium chloride would seem particularly suitable.

Parcijalna supstitucija natrijum hlorida kalijum hloridom i amonijum hloridom u pripremljenom jelu sa mesom – grašak sa faširanom šniclom

Mladen Rašeta, Ivana Branković Lazić, Slobodan Lilić, Nenad Katanić, Nenad Parunović, Vladimir Koričanac, Jelena Jovanović

Apstrakt: Cilj rada bio je da se ispita uticaj smanjenja sadržaja natrijuma u pripremljenom jelu – grašak sa faširanom šniclom, delimičnom supstitucijom natrijum hlorida kalijum hloridom i amonijum hloridom u svrhu postizanja slične slanosti sa manjim sadržajem natrijuma. U kontrolnoj grupi uzoraka dodat je samo natrijum hlorid. U grupi 1, trećina natrijum hlorida zamenjena je kalijum hloridom, dok je u grupi 2, polovina natrijum hlorida zamenjena kalijum hloridom. U grupi 3, trećina natrijum hlorida zamenjena je amonijum hloridom, i, u grupi 4, natrijum hlorid je smanjen za polovinu, a četvrtina amonijum hlorida je dodata, u odnosu na količinu soli u kontrolnoj grupi. Slanost je bila manje prihvatljiva u uzorcima kuvanog graška iz kontrolne i treće grupe zbog previše slanog ukusa i pojave gorkog ukusa u grašku iz treće grupe. U faširanim šniclama, intenzitet slanosti bio je viši u uzorcima kontrolne i treće grupe od one u uzorcima iz prve grupe ($P \leq 0.05$) i iz druge i četvrte grupe ($P \leq 0.01$). Ukus kuvanog graška iz treće grupe nije bio prihvatljiv usled najviše izražene slanosti. Ukus faširanih šnicli iz svih ispitivanih grupa bio je prihvatljiv, a jedina statistička razlika utvrđena je između uzoraka kontrolne i treće grupe ($P \leq 0.01$).

Ključne reči: *kuvani grašak, faširane šnicle, redukcija natrijum hlorida, kalijum hlorid, amonijum hlorid.*

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Original scientific paper

Selected physico-chemical properties of Serbian dry fermented sausages in different meat industries

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A b s t r a c t: Selected physico-chemical characteristics of industrially produced Sremska and Sudzuk sausages were studied to complement information about the quality of typical pork and beef dry fermented products in Serbia. Analysis of production processes was carried out at five meat industries. Water, lipid, protein, NaCl, ash and nitrite contents and pH and water activity were determined at the beginning, in the middle and at the end of the processes. The results of chemical components analyses of Sremska were uniform, while Sudzuk sausages showed greater variations. However, both types of products in all examined meat industries conformed with Serbian legislation for dry fermented sausages. The water activity and pH values showed the sausages can be considered as microbiologically stable products during the whole production process.
Keywords: *Sremska, Sudzuk, physico-chemical characteristics, meat industry.*

Introduction

Sremska and Sudzuk are typical Serbian dry fermented sausages (DFS) from pork and beef meat, with a long tradition of production in the country. These sausages are highly regarded among consumers and have potential for export to other countries. In industrial production, the stability and long shelf life of DFS rely on biochemical and physico-chemical changes in fermentation and drying processes. Effects of multiple antimicrobial factors, the so-called "hurdle concept", which includes adding nitrites and acidulants/starter cultures plus lowering water activity (a_w), provides a hostile environment for spoilage and pathogenic microorganisms (*Ducic et al*., 2014). Levels of nitrites, proteins and moisture and the lowest permitted pH value of DFS are prescribed by official regulations (*Official Gazette*, 2018; *Official Gazette* 2015), while other aspects such as a_w, presence and concentration of acidulants or starter cultures, fat content, and level of NaCl are related to inner meat industry standards and principals of Good Manufacturing Practice.

The aim of this study was to investigate selected physico-chemical characteristics in order to complement information on the safety and quality of typical, industrially produced Serbian DFS.

Materials and Methods

Two types of DFS, Sremska and Sudzuk, produced by standard procedures including ripening without starter cultures, were investigated at five commercial meat industries (A, B, C, D, E). In industry A only, both types of sausages were investigated. Sausages were sampled at the start (day 0), at mid-process (day 8) and at the end of the process (day 15). Each sampling included 6 samples of each sausage produced (108 in total). Formulations used for DFS production are presented in Table 1. Regarding raw meat materials: producers A, C and D used semi frozen meat trimmings (−5ºC to −2ºC); B used cooled unfrozen (+4ºC) and frozen meat trimmings (−8ºC), ratio 60:40; E used cooled meat trimmings. Batters for pork sausages and beef sausages were stuffed into natural pork casings and collagen casings, respectively.

Physico-chemical analyses: a_w in sausages was determined using LABSwift-aw set Euro-plug&BAT equipment (Novasina, Switzerland); pH was determined by a hand-held pH meter (Testo 205; Germany). Both methods were according to the manufacturer's instructions.

Chemical analyses: moisture (*ISO 1442*, 1998); lipids (*ISO 1443*, 1992); proteins (*ISO 937*,

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Table 1. Formulations used for fermented sausage production

Ingredient	Sremska			Sudzuk		
	Industry					
	A	B	C	A	D	E
Meat trimmings	65 kg	65 kg	65 kg	60 kg	60 kg	82 kg
Fatty tissue	30 kg	30 kg	30 kg	35 kg	35 kg	15 kg
Spices	2.5 kg Paprika, chillies, coriander, garlic, pepper	2.5 kg Paprika, chillies, coriander, garlic, pepper	0.7 kg Paprika, chillies, coriander, garlic, pepper	2.0 kg Paprika, chillies, onion, pepper, mustard, marjoram, rosemary, basil	0.5 kg Paprika, chillies, coriander, pepper, turmeric	0.4 kg Garlic, pepper, celery
$NaCl:NaNO_2$ mixture (99.5:0.5)	2.5 kg	2.6 kg	2.5 kg	2.4 kg	2.6 kg	2.2 kg
Dextrose	0	0.50 kg	0.35 kg	0	0	0.30 kg
TARI S77*	1.0 kg	1.0 kg	0.6 kg	1.2 kg	1.0 kg	0

*TARI S77 – Glucono-d-lactone (GDL) 42.5%, sugars 42.5%, NaCl 12.5%, sodium ascorbate 2.5%.

1992); NaCl (*ISO 1841–1*, 1999); ash (*ISO 936*, 1998); $NaNO_2$ (*ISO 2918*, 1999).

Statistical analysis: Determination of mean values, standard deviation and analysis of variance (ANOVA) followed by Tukey's test for the significances ($P < 0.05$) of differences between grouped data was conducted by using Statistica Version 12 software (Stat Soft, Inc., Tulsa, USA).

Results and Discussion

Sremska sausages

The pHs of Sremska sausages on the first day of the production processes (Table 2) were in accordance with the results reported by *Kozacinski et al*. (2008) and *Radulovic et al*. (2011) for Sremska at the same stage of production. The exception was the initial pH of sausages from industry B, with values that were considerably lower (5.0) than the other Sremska sausages studied (Table 2). Industry B used unfrozen chilled and frozen meat (see Materials and Methods), and batter prepared from these types of ingredients faster reached fermentation temperature, at which GDL is completely dissolved (21–23°C). During fermentation, the activity of GDL and proliferation of lactic acid bacteria induced significant acidification of the product. Decreasing pH is an important inhibitory factor for constraining potential growth of *Enterobacteriaceae* and other pathogens and undesirable microbiota in sausages at the beginning of the ripening period, when a_w is still high, as is the fermentation temperature. Therefore, on day 8, the pHs of the Sremska sausages were 0.4–0.6 units lower than the pHs at the beginning of production. The pH of the finished products was identical or slightly higher than the pH determined on the middle day of production (Table 2). This slight increase of pH in finished Sremska sausages could be induced by the release of the degradation products of proteins, as well as the decomposition of lactic acid during the remaining drying period (*Salgado et al*., 2006; *Vukovic et al*., 2004). Regarding the level of acidity, all finished Sremska sausages were classified as high-acid fermented sausages, with pH values lower than 5.3 (*Incze*, 2004).

The a_w values of Sremska sausages on the start day were in accordance with the literature data for this type of product (Lücke, 2000). During fermentation and the initial drying period, a_w in sausages from all three industries rapidly reduced, mainly due to the presence of the acidulant, GDL (Table 2). The dissolution of acidulant induced lowering the pH to the isoelectric point of the actin-myosin complex (pH about 5), which leads to changes of the water distribution in the network of these two technologically most important meat proteins. Consequently, the increase in the amount of free, instead of loosely bound water facilitates its removal from the sausages, and the appropriate application of the relative

Table 2. pH and a_w in Sremska and Sudzuk sausages

Type of sausage	Meat industry	pH on day			a_w on day		
		0	8	15	0	8	15
Sremska	A	5.4	4.9	5.0^{A}*	0.95	0.90	0.83^{A}
	B	5.0	4.9	5.0^{A}	0.96	0.87	0.77^{B}
	C	5.5	4.9	5.0^{A}	0.96	0.88	0.84^{A}
Sudzuk	A	5.4	4.7	4.8^{a}	0.97	0.92	0.87^{a}
	D	5.6	5.1	5.2^{b}	0.95	0.89	0.86^{b}
	E	5.7	5.3	5.4^{c}	0.96	0.90	0.88^{a}

* Mean values in the same column with different letters differ significantly (P<0.05) within sausage type.
Statistical significance was analysed for results of the last day of production process.

humidity, temperature and air velocity parameters in the fermentation/drying chambers leads to rapid drying of the product, with a reduced risk of case-hardening (*Incze*, 2004). As a result, the a_w of all Sremska sausages was equal to or less than 0.9 by the middle of the production processes (Day 8) (Table 2). Due largely to the combination of low a_w and pH, sausages become microbiologically stable products without conditions for the growth of most pathogenic microorganisms (*USDA FSIS*, 2011).

Moisture levels in Sremska sausages from all three meat industries on day 0 were similar (Table 3), despite the differences between industries in their use of chilled/frozen meat for sausage batter. The results of chemical analysis show that the reduction in a_w was followed by a decrease in the total amount of water. In the case of producers B and C, Sremska sausages contained below 35% total water on day 8, meaning these half-finished products would already legally fit into the category of DFS

Table 3. Chemical components (mean value±sd*) analysed during production of Sremska sausages

Day	Moisture (%)	Lipids (%)	Proteins (%)	NaCl (%)	Ash (%)	Nitrites (mg kg^{-1})
			Industry A			
0	57.8±1.0	16.3±1.2	22.6±0.1	2,2±0.0	3,4±0.0	136.2±3.1
8	38.3±1.1	32.1±0.9	25.9±0.3	2.6±0.1	3.9±0.1	69.6±1.0
15	$28.8±0.8^{A}$	$39.7±0.8^{B}$	$27.5±0.4^{A}$	$3.8±0.1^{A}$	$4.7±0.1^{A}$	$9.5±0.5^{A}$
			Industry B			
0	55.9±0.7	18.2±0.4	22.5±0.5	2.2±0.1	3.4±0.1	139.3±2.2
8	34.3±0.7	34.4±0.7	26.8±0.5	2.7±0.1	3.9±0.1	70.1±1.8
15	$28.3±0.6^{A}$	$39.5±0.8^{B}$	$27.2±1.0^{AB}$	$3.6±0.1^{B}$	$4.8±0.1^{A}$	$10.3±1.1^{A}$
			Industry C			
0	55.7±0.6	19.2±0.5	21.7±0.4	2.2±0.0	3.4±0.0	139.7±1.2
8	34.2±1.2	35.4±0.6	25.3±0.4	2.7±0.1	3.9±0.0	71.9±0.8
15	$28.9±0.8^{A}$	$40.2±1.1^{B}$	$26.5±0.3^{BC}$	$3.6±0.1^{B}$	$4.8±0.0^{A}$	$10.5±1.1^{A}$

* Mean values in the same column with different letters differ significantly (P < 005)
Statistical significance was analysed for results of the last day of production process

at the half-way point of their production processes (*Official Gazette*, 2015).

The fat content in Sremska sausages at the final stage of production was lower or similar to average values of other industrially produced DFS, which rang from 40% to 50% of the total content (*Olivares et al*., 2010).

The total protein content of Sremska sausages was greater than 20%, so these products from all three meat industries were produced in accordance with the requirements of official regulations (*Official Gazette*, 2015). In addition, the protein contents in our Sremska sausages were in accordance with values reported by *Ferreira et al*. (2007) for Portuguese sausages, but higher than values for different types of commercially produced DFS sampled from the Belgian market (*De Mey et al.,* 2014).

Levels of NaCl and ash increased during fermentation and drying due to reducing water content, so the highest concentrations of these were determined on the last day of production. The values of NaCl in finished Sremska sausages were similar to findings for Greek DFS (*Drosinos et al*., 2005) and to levels of different types of sausages reported by *De Mey et al*. (2014). On the other hand, the final NaCl levels we determined were a little higher than those stated for sausages of Portuguese and Spanish origin (*Fereirra et al*., 2007; *Salgado et al*., 2006).

The initial levels of nitrite in the Sremska sausages, ranging from 136.2 to 139.7 mg kg^{-1}, were in compliance with Serbian legislation (*Official Gazette*, 2018), where the maximum permitted added amount of sodium nitrite is 150 mg kg^{-1}. Expectedly, in finished products concentrations of nitrites in all Sremska sausages were lower (5–15 mg kg^{-1}), but within values recommended for stability during the storage period (*Sindelar and Milkowski*, 2011).

Sudzuk sausages

The pH values of Sudzuk sausages (Table 2) at the start (Table 2; day 0) were slightly higher than findings from study of *Operta et al*. (2012), but markedly lower than pHs reported by *Kozacinski et al*., (2008) for same stage of production. Generally, fermentation led to a drop of pH of the Sudzuk sausages from all three producers, but to varying degrees. The lowest pH was found in the sausages from industry A in the middle of production, probably due to the considerably higher amount of GDL (1.2%) in these products than is usually expected for this additive in fermented sausages (0.5%). On the other hand, the highest pH values were determined in Sudzuk sausages from industry E, where GDL was not added, but only a relatively small quantity of glucose (Table 1). Consequently, the finished products of industries A and D can be classified as

Table 4. Chemical components (mean value±sd*) analysed during production of Sudzuk sausages

Day	Moisture (%)	Lipids (%)	Proteins (%)	NaCl (%)	Ash (%)	Nitrites (mg/kg)
			Industry A			
0	52.5±0.7	24.7±0.6	19.3±0.8	2.2±0.2	3.6±0.2	136.8±3.2
8	37.2±0.4	35.3±0.9	22.8±1.2	3.2±0.2	4.6±0.0	70.5±1.8
15	28.0±0.4^{A}	40.6±0.7^{A}	26.4±1.4^{A}	3.6±0.2^{A}	4.7±0.2^{A}	7.1±0.4^{A}
			Industry D			
0	46.2±0.6	35.1±0.9	15.1±1.1	2.5±0.1	3.6±0.1	128.7±1.9
8	30.6±1.2	44.1±1.7	20.2±0.8	3.9±0.1	5.2±0.1	78.9±1.6
15	23.5±0.7^{B}	50.4±2.5^{B}	21.3±1.4^{B}	4.1±0.2^{B}	5.5±0.2^{B}	9.8±0.4^{B}
			Industry E			
0	56.4±1.1	16.4±1.8	23.1±1.1	2.8±0.1	4.1±0.1	137.3±3.9
8	35.8±0.5	33.9±0.4	25.4±0.5	3.6±0.1	5.0±0.0	71.6±1.6
15	32.0±0.4^{C}	36.5±0.4^{C}	26.2±0.3^{B}	3.9±0.1^{B}	5.3±0.1^{B}	9.2±0.7^{B}

* Mean values in the same column with different letters differ significantly ($P < 0.05$).
Statistical significance was analysed for results of the last day of production process.

high-acid fermented sausages (pH <5.3), while industry E product satisfied the category of low-acid fermented sausages (pH 5.3) (*Incze*, 2004). It should be noted that the pH of Sudzuk sausages from industry A was below 5, a minimum allowed pH value for DFS prescribed by Serbian legislation since 2015.

The initial a_w levels in Sudzuk sausages (Table 2) were in accordance with the literature data for this sausage category and stage of production. During Sudzuk ripening, there was a noticeable decrease in the a_w, which was, however, less pronounced than in Sremska sausages. Differences in a_w decrease are mainly due to variation in size of products. The diameter of the Sudzuk from industries A and D (Ø 38–42 mm) was greater, while those from industry E were the same as the Sremska sausages (Ø 32–36 mm). However, the absence of GDL in Sudzuk from industry E probably had an effect on the less pronounced decrease of a_w compared to Sremska sausages. At the end of the drying process, all Sudzuk sausages had a_w below 0.9, which means they can be categorised as microbiologically stable products with long shelf life.

The results of chemical composition of finished Sudzuk sausages (Table 4) were less uniform than for Sremska sausages. *Operta and Smajic* (2006) also investigated the chemical composition of Sudzuk, but only from one meat industry. Their findings are closest to those from our industry A, although moisture was slightly higher and fat and protein contents lower in our investigation.

The results of initial moisture level in Sudzuk sausages showed great variations, ranging from 46.2% in sausages from industry D to 56.4% in sausages manufactured by industry E. The highest content of water (in sausages from industry E) was likely related to this company's selection of unfrozen, chilled meat for batter preparation. In finished products, water content differed significantly ($P<0.05$) between the different producers. Nevertheless, Sudzuk sausages from all three industries were in accordance with the requirements of Serbian legislation (*Official Gazette*, 2015), since the water content in sausages at the end of the production was less than 35%, while the total protein content was greater than 20%.

The fat content in Sudzuk sausages at the end of the production process also varied significantly ($P<0.05$) between industries, ranging from 36.5% to 50.4%. However, all fat content levels were in the range of average fat content in commercially produced DFS.

The contents of NaCl and ash in Sudzuk sausages after the fermentation and drying processes were similar to or somewhat higher than in finished Sremska sausages, and these parameters were also in the range of values for dry fermented products reported in some other studies (*De Mey et al*., 2014; *Salgado et al*., 2006; *Drosinos et al*., 2005).

The level of added nitrite in sausages on the initial day of production was less than 150 mg kg^{-1}, which is in accordance with the Serbian legislation (*Official Gazette*, 2018). The content of nitrite in all Sudzuk sausages at the end of the production processes varied within the recommended values, i.e. from 5 to 15 mg kg^{-1}.

Concluding remarks

- pHs of sausages at the beginning of processing were in the range of usually expected values (pH 5.5–5.8) for that step of production. The finished products were classified as high-acid sausages (pH<5.3), with an exception of Sudzuk from industry E, which was a low-acid sausage (pH ≥5.3);
- a_w values of all sausages were 0.9 or below starting from mid-process (day 8), which categorised them as microbiologically stable products;
- moisture levels decreased gradually and at the end of process were below 35%, meaning all sausages would be classified as dry fermented products according to official regulation;
- protein contents were higher in Sremska than in Sudzuk sausages, but in both types, protein levels were in accordance with official standards for DFS (≥20%);
- fat contents increased during ripening, reaching values of 40% in Sremska and 50% in Sudzuk, but all were in the range of average fat contents for commercially produced DFS;
- levels of residual nitrite in all sausages at the start (day 0) were lower than the maximum permitted amount (150 mg kg^{-1}); at the end of process (day 15), nitrite levels accorded with recommended levels for long term stability of sausages (5–15 mg kg^{-1});

Odabrane fizičko-hemijske odlike fermentisanih suvih kobasica proizvodenih u različitim industrijama mesa u Srbiji

Miroslav Dučić, Danijela Vranić, Milan Z. Baltić

Apstrakt: Ispitane su fizičko-hemijske odlike industrijski proizvedenih sremske kobasice i sudžuka radi upotpunjavanja saznanja o kvalitetu tipičnih fermentisanih proizvoda od svinjskog i goveđeg mesa u Srbiji. Postupak proizvodnje ispitan je u pet mesnih industrija. Analiziran je sadržaj vode, masti, belančevina, NaCl, pepela, nitrita, kao i pH i aktivnost vode kobasica, na početku, sredini i završetku proizvodnog postupka. Rezultati ispitivanja hemijskog sastava sremske kobasice su ujednačeni, dok su kod sudžuka ustanovljene značajne varijacije. Bez obzira na utvrđene varijacije, u svim industrijama oba tipa proizvoda su u skladu sa važećim propisima o kvalitetu fermentisanih suvih kobasica u Srbiji. Rezultati analize aktivnosti vode i pH vrednosti ukazuju da se ispitane kobasice mogu smatrati mikrobiološki stabilnim proizvodima tokom čitavog postupka proizvodnje.

Ključne reči: *sremska kobasica, sudžuk, fizičko-hemijske odlike, industrija mesa.*

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Measuring Competitiveness in the Meat Industry Market: Are There Any Oligopolies in Serbia?

Vladimir Mitic[1], Natasa Kilibarda[1], Ivana Brdar[1], Marija Kostic[1], Danijela Sarcevic[2], Nedjeljko Karabasil[3], Vule Mizdrakovic[1]*

A b s t r a c t: Production of meat and processing of meat products is a very significant part of the food industry. In order to secure economic growth and development, especially in the meat industry, it is very important to provide free competition for the business entities that operate within it. Therefore, the main purpose of this study was to measure market concentration using the Herfindahl-Hirschman Index (HHI), in order to determine the competition level in the Serbian market. Furthermore, the HHI should provide evidence of any business entities that hold monopolistic or oligopolistic positions on the market. Moreover, another aim of this study was to define to what extent business entities in the Serbian meat industry export products and goods to international markets. The 350 companies that reported the highest revenues during 2013–2017 and which are registered in this sector were studied.

Keywords: *meat industry, perfect competition, Herfindahl-Hirschman index, revenues, market.*

Introduction

According to the Serbian Chamber of Commerce and Industry, the food industry is one of the leading Serbian industries, since it registered a surplus in international trade of €894.1 million in 2016 (*PKS*, 2017). Compared to nearby countries' economies, the Serbian food industry has been in a difficult situation for a very long time, primarily as it does not have covenants with the European Union (EU) or with Central European Free Trade Association (CEFTA) countries.

The meat industry is an important segment of the food industry, which, according to the Chamber of Commerce and Industry of Serbia, employed 89,378 people in 2016 (*PKS*, 2017). In addition, the meat industry enables households and animal husbandry companies to plan production more easily and to be less sensitive to oscillations of supply and demand (*Mirjanic*, 2011). According the Ministry of Agriculture, Forestry and Water Management, average meat consumption was previously 65 kg per capita per year, but today it has fallen to 42 kg of meat per capita per year (*Vlahovic and Puskaric,* 2011; *Dokmanovic et al.,* 2014). Although meat consumption per capita has reduced, it remains a very important part of the consumer basket. We are currently witnessing many debates on the quality of imported food, especially, around the presumption that imported meat and meat products are of lower quality. There is no doubt that developing the meat products and processing sector will both improve the quality of food and increase the confidence of consumers.

Serbian companies engaged in meat production and processing face numerous problems, such as expensive raw materials on the domestic market, constant price and livestock availability fluctuations, and serious competition from EU-based companies subsidized by their own countries. These factors have led to the fact that local meat industry companies are uncompetitive and exposed both on the domestic and world markets. According to the Chamber of Commerce and Industry of Serbia, in the last 15 years, meat exports increased only 2.5-fold, while imports increased approximately 30-fold. With the development of world trade, companies engaged in meat production and processing no longer operate locally, but globally, with many conducting business in several countries and in multiple locations to increase profits. Global meat companies include JBS, Marfrig, Tyson, Cargill, Danish Crown and Nippon Meat Packers (*Belk et al.,* 2014). *Petrovic et al.* (2015) claim that due to the increased global demand for meat and the increase in meat

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consumption per capita, meat production will double by 2020. By becoming better and more efficient businesses, companies from this sector should be able to produce lower-priced products in order to be more competitive, and consequently Serbian consumers would benefit.

Based on these facts, we defined two research questions. Firstly: *What is the level of market competition in the Serbian meat industry*? We will also provide evidence if business entities hold monopolistic or oligopolistic positions on the market. Secondly: *To what extent do meat industry business entities export their products to international markets*?

National Legislation on Market Competition

In order to secure economic growth and development, it is very important to provide free competition for business entities operating within markets. Market structure explains how the market is organised, or how many sellers or buyers are active on the market. Perfect competition exists when there is equilibrium between the quantities of product and services demanded and supplied on a market. However, there are situations where business entities will exploit their competitive advantages, including too few or too many small sellers, a lack of substitutes and the presence of trade barriers (e.g. restricted access to resources, capital intensive operations, legal requirements). Therefore, market structures can indicate the existence of imperfect competition: monopoly (only one seller), monopolistic competition (large number of small sellers) and oligopoly (a few sellers that are relatively large). On those markets, one or a few sellers exploit their position in order to acquire extraordinary returns. Therefore, annual revenues can be used to measure market competition of a specified part of the economy.

Restrictions to free market competition cause increased prices and consequently, decreased quality of products and services. Those effects directly cause damage to consumers and indirectly infringe their basic human rights. Therefore, competition is regulated by law with the aim of enabling business entities to compete on the market by offering high quality products and services at fair prices – in a free and correct manner. The benefits of such an approach are numerous: gaining new consumers and improving their living standard; decreasing the business entity's costs and increasing their business efficiency, and; enabling start up businesses.

The basic legal act regulating and protecting market competition Serbia is the Law on Protection of Competition (*Serbia,* 2009; *Serbia,* 2013). The Law lists three forms of competition infringement, defined as "acts or actions of undertakings that as their purpose or effect have or may have a significant restriction, distortion, or prevention of competition":

1. Restrictive agreements, which as their purpose or effect have a significant restriction, distortion, or prevention of competition. The law allows agreements of minor importance, e.g. "agreements between undertakings whose total market share in the relevant market of products and services on the territory of the Republic of Serbia, do not exceed:
   - 10% of market share, if the parties operate at the same level of production and distribution chain (horizontal agreements);
   - 15% of market share, if the parties operate at the different level of production and distribution chain (vertical agreements)…";
2. Abuse of a dominant position, where "the dominant position holds an undertaking that because of its market power in the relevant market can substantially independently operate in relation to actual or potential competitors, customers, suppliers or consumers". Abuse of a dominant position is relevant for the undertakings whose market share on the defined relevant market is higher than 40%, or who have advantages in accessing the sourcing and distribution markets or who have significant purchasing power, etc. The law also stipulates that abuse of a dominant position exists if a collective dominance is formed. In article 15, it is defined that a dominant position can be held by "two or more legally independent undertakings…if they are economically linked in such a way that in the relevant market they jointly perform or act as one participant".
3. Concentration of undertakings in cases of mergers, acquisitions or joint ventures, where "two or more transactions between the same undertakings were concluded during the period of less than two years…". However, in accordance with article 19 of the law, concentrations are permitted "unless they significantly restrict, distort or prevent competition in the market of the Republic of Serbia or its part…". Decisions on the rights and obligations of undertakings in accordance with the law, as well as monitoring and analysis of competition conditions in individual markets and sectors are in the power of the Commission for the Protection of the Competition of the Republic of Serbia.

Further research in this study will calculate the market share of Serbian meat industry business entities in the market concentration or overall competition. On the basis of this research and the aforementioned law, the situation on the domestic meat market will be determined and qualified as positive or negative accordingly.

Literature Review

Food or meat industries have been a subject of research in many countries. For food markets in European countries, *McCorriston* (2002) and *Wann and Sexton* (1992) concluded that economists need to pay more attention to imperfect competition, because in these markets, oligopolies usually dominate. The same authors argued that the market power of food sellers is growing in many countries. *Lopez et al.* (2002) similarly found that with an increase of market concentration within the food industry, oligopolies' power increases. That leads to the improvements regarding cost efficiency, but it will also increase selling prices of food. Specifically, the authors studied the association between oligopoly and efficiency, as well as their impact on selling prices in 32 food industries. They noted that in 26 (81%) of the food industries, the strength of the oligopoly increased significantly with an increase of market concentration. Likewise, the market power of food sellers is growing in many countries; hence, more attention should be paid to this problem (*McCorriston*, 2002). *Andres* (2008) conducted a study on the market power of oligopolies in the German food industry, including specific details on the German meat market and the European crisis. Prices due to oligopoly were 11% higher for beef and 2.7% higher for pork, compared to prices that would be set in the case of perfect competition (*Anders*, 2008).

The situation in the Serbian meat industry market is conditioned by meat production. As *Maletic and Popovic* (2016) stated, Serbian livestock production is decreasing by 2–3% per year. According to *Aleksic et al.* (2007), the number of cattle was decreasing in the decade prior to 2007 (by about 18%). An additional problem is the small number of slaughterhouses that have EU certificates. As a result, meat production is in constant decline (in Serbia, 69,000 tons were produced, while only 21,000 tons were produced in Vojvodina). *Aleksic et al.* (2007) emphasized that solutions would include the rapid and efficient transformation of cattle production, the consolidation of land holdings, and the creation of specialized farms for production of milk and meat. Since the mid-1980s, pig production in Serbia has decreased, especially in Vojvodina. Poultry production underwent the fastest growth of all livestock production. Serbia produces just over 26 million birds, and numbers of poultry are relatively stable (*Maletic and Popovic*, 2016).

Barkema et al. (2001) researched changes in the United States (US) meat industry and concluded that over time, in order to achieve economies of scale and cost savings, the companies in this industry merged or were acquired. Additionally, they described how large stores bought others, and as they eventually transformed into large retail chains, expanded their business to other cities and countries. Since 1980, the number of slaughterhouses in the US has been steadily decreasing (from 600 to around 170 for cattle and from 500 to around 180 for pigs). Moreover, the number of meat processing companies has declined, while the remaining companies in this sector have increased their market share (*Barkema et al.,* 2001).

Vlahovic et al., (2014) analysed the international pork market in the period from 2008 to 2012. During this period, average global pork production was 9.8 million tons with an annual growth rate of 3.65%, while the annual value of exports was about 27 billion US$. The world's largest pork exporter is the EU, which accounts for 65% of the world's total exports. Additionally, the EU is the second largest producer of pork immediately after China. The largest pork exporting country in the world is Germany, with average annual exports of 1.5 million tons, accounting for 15.6% of total world exports. Next to Germany, the world's largest exporters are the US, Canada, Denmark and Spain, which together account for about 2/3 of total world exports. The world's largest importer is also the EU, accounting for around 50% of total world imports. Germany is the world's largest pork importing country, taking 956,000 tons, which constitutes about 10% of total world imports. In addition, other big importers are Japan, Italy, the Russian Federation and Poland, and with Germany, they account for over 40% of the world's total imports of pork (*Vlahovic et al.,* 2014).

Materials and Methods

Numerous indicators could be used to measure market competition level, including Concentration ratio of leading companies (CRn), Herfindahl-Hirschman index (HHI), Lorenz curve (concentration curve), Gini coefficient and Entropy index *(Mihajlovic et al.,* 2016). HHI has been used the most to measure market competition level and to determine if there are any oligopolies in meat industry markets. HHI has been proven as a good instrument of measuring market competitiveness (market concentration) and was used for this research. HHI is calculated according to:

$$HHI=\sum_{i=1}^{n} X_i^2$$

where, X_i is the market share of *i* company (*Mihajlovic et al.*, 2016). HHI values can range from 0 to 10,000. Low values of this indicator are desirable because they indicate significant competition in the analysed market, i.e. no monopoly. A HHI value of 10,000 means there is a monopoly in the market, i.e. only a seller is present on the market and possesses full market power. A HHI value closer to 0 would represent perfect competition, meaning a large number of companies each with a small market share operates on the market (no single company dominates the market). Perfect competition occurs when enterprises are so small they cannot affect the market price (*Samuelson and Nordhaus*, 2005).

In order to draw a conclusion on the degree of concentration in the Serbian meat industry, the following scale to interpret HHI values was used:

- If the index ranges from 0 to 1,000, the concentration on the market is low;
- If the index ranges from 1,000 to 1,800, the concentration on the market is moderate;
- If the index ranges from 1,800 to 10,000, the concentration on the market is high.

The subjects of this research were the financial statements for the reporting periods between 2013–2017, for companies registered in the meat production and processing. For research purposes, the financial statements of companies from the analysed meat and meat products sector were downloaded from the Serbian Business Registers Agency in order to measure the market concentration, i.e. the level of competition in the market. Altogether, 2,243 companies were engaged in production and processing of meat and meat products in 2017. This survey was conducted on a sub-set of those companies, i.e. the 350 companies in this sector that reported the highest revenues in 2013–2017. We focused on this sub-set of companies because the remainder had extremely low market share and some of them did not show any income in 2017 due to bankruptcy, blockade or suspension of operations as shown in the financial statements.

With the help of HHI, an analysis of the Serbian meat and meat products market was conducted to see if it is closer to perfect competition or operates as an oligopoly. This study can be used for deeper analysis of this sector, as well as for wider competitiveness research on the meat and meat products market in Serbia. Also, in order to respond to the second research question, the total export revenues were calculated, as was the market share for all major meat exporters in Serbia.

Results and Discussion

In order to answer the first research question and determine the competition level in the meat and meat products market in Serbia, the annual total HHI for each reporting year was calculated (Table 1).

It is clear the competition level in the Serbian meat and meat products marked is classified as low, something that could not have been expected. All reporting years were marked by HHIs lower than 1,000, and additionally, values generally decreased over the observed period (Table 1). Having in mind that total revenues increased, we suspect that more entities entered the market, which suggests that barriers to entrance are low. Along with the competition level being low, some entities had higher HHI values than other entities. Table 2 shows the ten top-ranked business entities from 2015 to 2017, while Table 4 in the Appendix shows the values for 2013 and 2014.

Clearly, there are four major contributors to the total HHI in this industry. One of them holds a dominant position with the highest market share

Table 1. Herfindahl-Hirschman index for total revenues from 2013 to 2017

Reporting period	Total revenues in Republic of Serbia dinars	Index
2013	734,734,902	751
2014	754,185,441	649
2015	736,632,818	687
2016	789,069,042	647
2017	909,702,350	554

Table 2. The structure of the ten top-ranked business entities with the highest Herfindahl-Hirschman index for total revenues 2015–2017

Business entity	2015	Business entity	2016	Business entity	2017
I	364	I	324	I	297
II	87	IV	89	IV	66
III	72	III	64	VII	50
IV	58	II	61	II	37
V	19	VII	22	VIII	17
VI	18	V	18	V	17
VII	11	VI	10	XI	11
VIII	11	X	10	IX	11
IX	9	IX	10	VI	9
X	9	XI	9	XII	8

and HHI. However, that percentage of the market is not high enough to consider that the entity holds a monopoly over the market, as is the situation in the Serbian dairy and milk market where only one entity holds almost 40% of the market (*Mihajlovic et al.,* 2016). The other three have similar market shares which could suggest they are working as a group, but since their structure and market share changed over the observed time, that is unlikely. Therefore, in contrast to the US and EU meat industry markets, we can conclude that there are no oligopolies on Serbian meat industry market. Unlike the situation in Serbia, in the US meat market, the decrease in number of entities on the market has led to an increase of individual market share (*Barkema et al.,* 2001). The US market concentration was indicated by the measured HHIs of 1,936 (high concentration) for the beef processing market, while the pork processing market was 1,036 (moderate concentration) (*Barkema et al.,* 2001). Also, in Malaysia the meat industry market concentration was high in the late 1990s to moderate in the 2000s (*Mohamed et al.,* 2015). Finally, in markets in nearby countries and the EU, the situation varies from country to country, but it can be concluded that concentration in the meat production and retail market is relatively moderate (*Einarsson,* 2008). For example, research covering 2008–2013 on Romanian business entities showed that HHI had

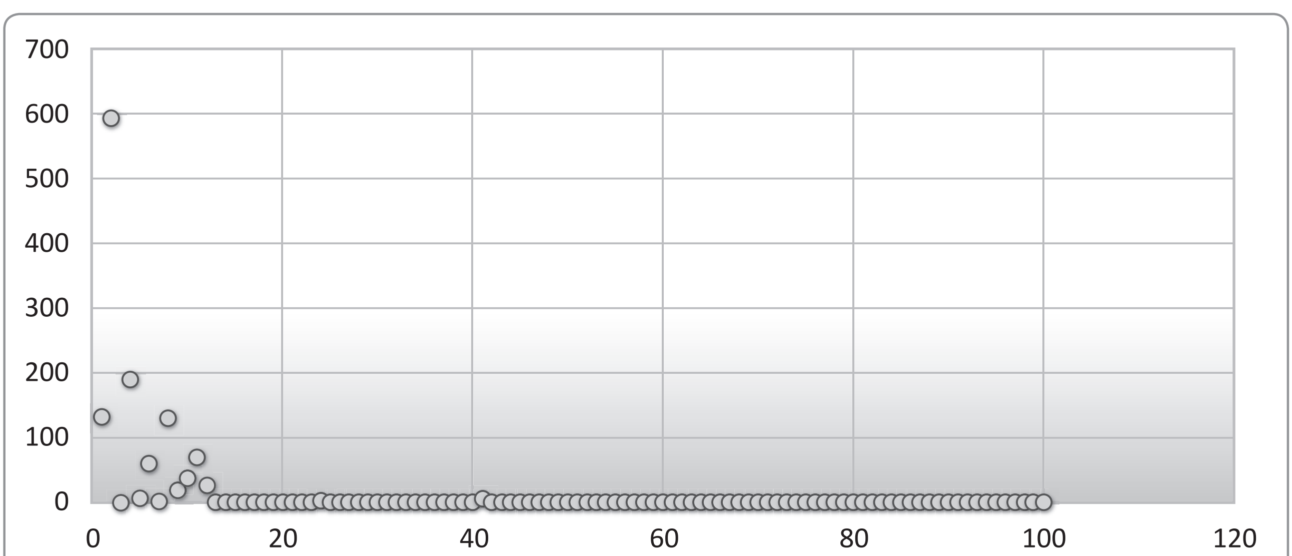

Figure 1. Herfindahl-Hirschman index for total revenue from export in 2017

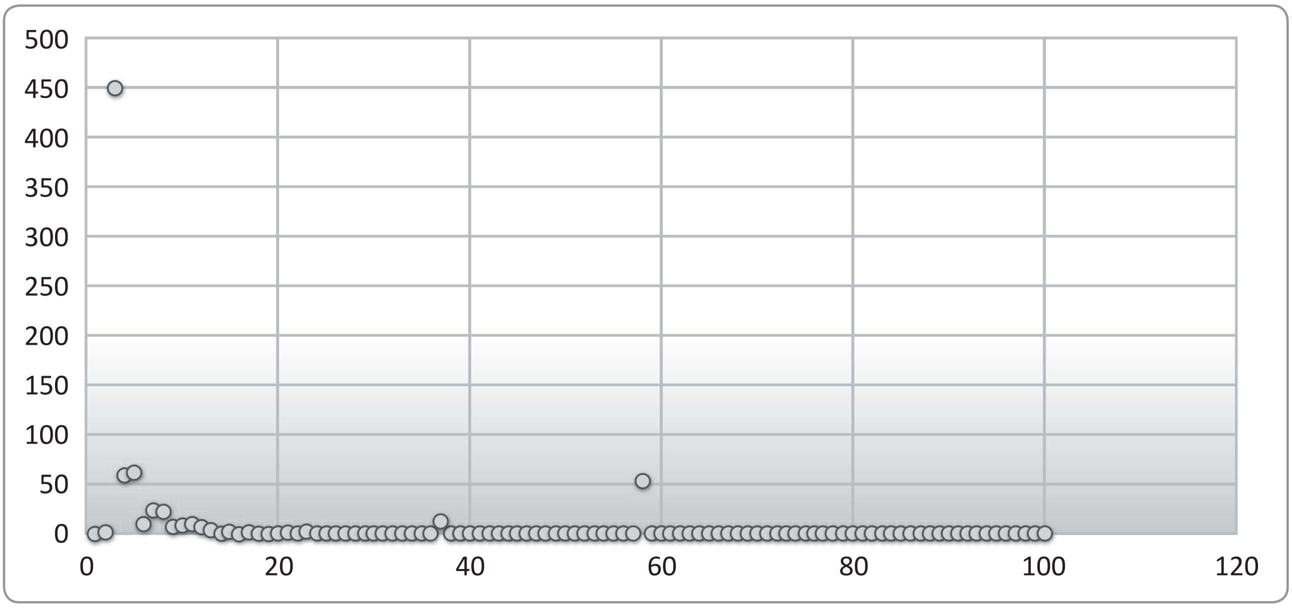

Figure 2. Herfindahl-Hirschman index of total revenues in 2013

a balanced evolution, with relative stability in the sector (*Stanciu et al.,* 2015). Similar results were reported for the pork production sector in the same period (*Popescu,* 2016).

Since it has been hinted that Serbian business entities have to change their business strategy and focus on export, we analysed HHI for total revenues from the meat and meat products export sector in 2017 (Figure 1).

The situation is different when it comes to export. Only 102 Serbian business entities actually export their meat or meat products, which is an extremely low percentage of the total number of entities in this industry. The total HHI for the meat export industry in 2017 was 1,257, more than double the HHI for total revenues (HHI of 554). We conclude the concentration of meat exporters on the market is moderate since the HHI is higher than 1,000. Just as for the meat and meat products industry as a whole, there are four major sellers in the export industry. However, their structure is not the same as for the total revenues.

It is interesting to note that the average percentage of total revenues earned from export by the ten top-ranked exporters is about 13% (Table 3). In addition, the top competitor on the market is not concurrently the main exporter. We concluded that total revenues from export are rather moderate, especially

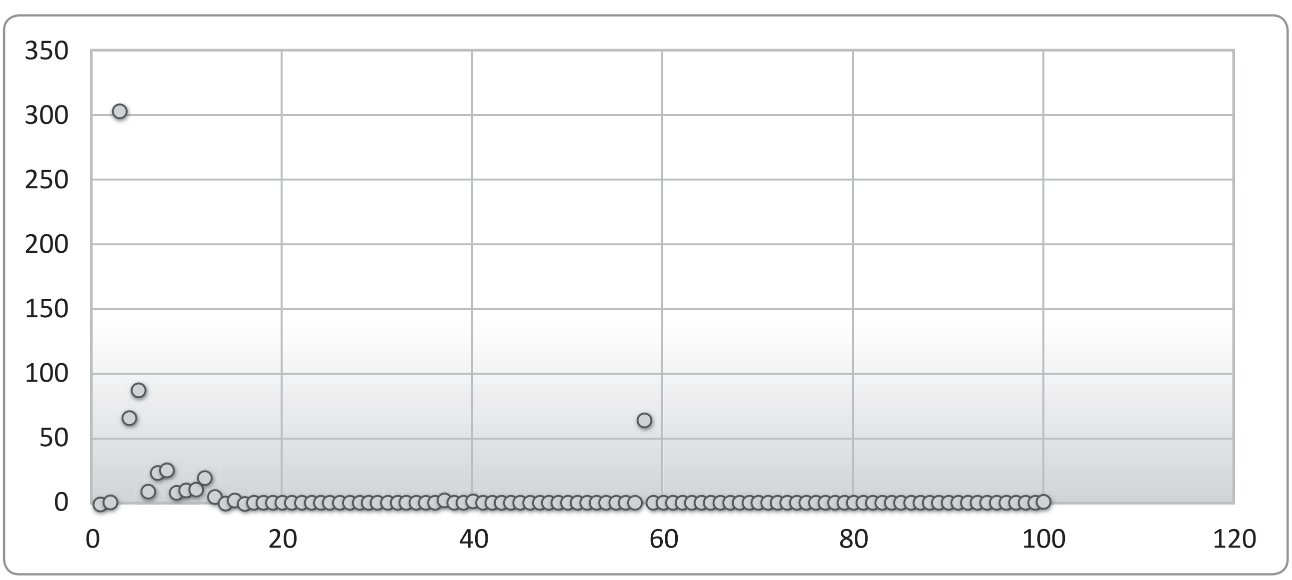

Figure 3. Herfindahl-Hirschman index of total revenues in 2014

Table 3. The structure of the ten top-ranked business entities with the highest Herfindahl-Hirschman index for total revenue from export

Business entity	Total revenue in Republic of Serbia dinars from export	Index	Share of revenue from export in total revenue
VII	18,849,119	592	11%
IX	10,634,779	188	14%
IV	8,904,633	132	13%
XIII	8,782,319	128	15%
XIV	6,386,746	68	16%
VI	5,933,983	59	15%
V	4,665,928	36	15%
X	3,880,177	25	12%
XI	3,143,948	16	11%
XV	1,928,740	6	7%

considering how much world exporters earn from selling meat products. Average global pork production is approximately 10 million tons, while the annual value of exports was near 30 billion US$. As explained previously, the biggest pork exporter is the EU, with more than a half of the world's total exports, but leading exporting countries are Germany, the US, Canada, Denmark and Spain (*Vlahovic et al.,* 2014). Our recommendation for all Serbian business entities is to work on their business strategies, focus their operation activities on fulfilling requirements for export, and increase their market share on the world market.

Conclusions

The aim of this study was to measure the market concentration using the HHI and to determine the degree of competition and the existence of oligopolies in the meat industry in Serbia. By analysing the value of HHI for total revenues in the period from 2013 to 2017, it can be concluded that the market concentration is low. More precisely, although revenues are increasing on average, HHI is generally decreasing from year to year. Therefore, the Serbian meat industry has almost perfect competition. However, a more detailed analysis has shown

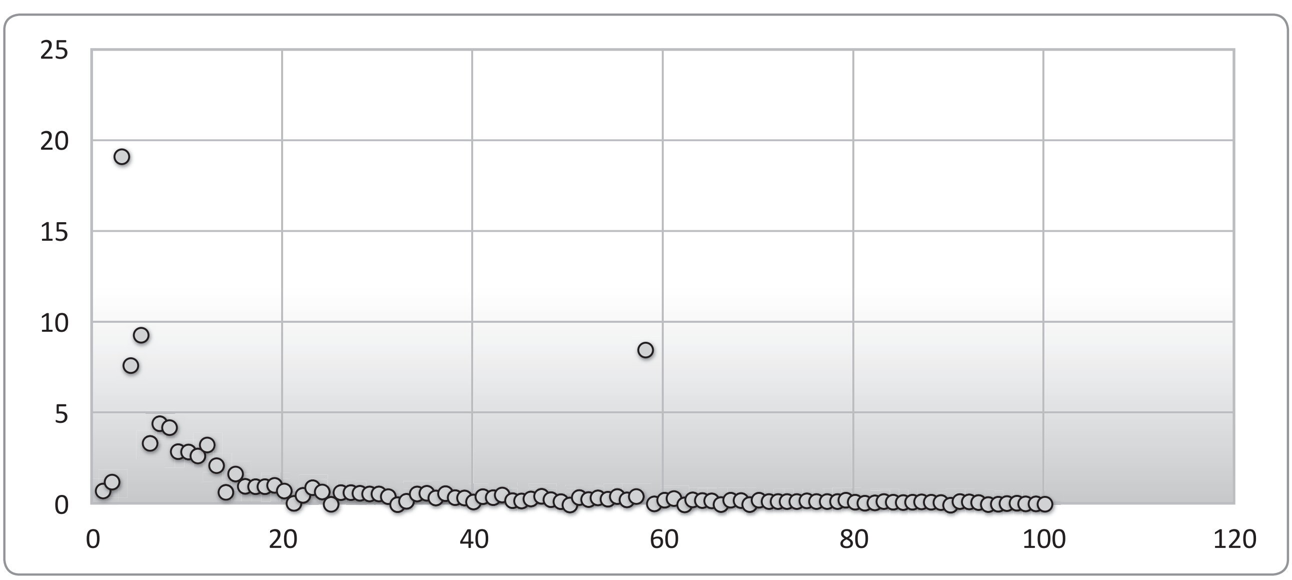

Figure 4. Herfindahl-Hirschman index of total revenues in 2015

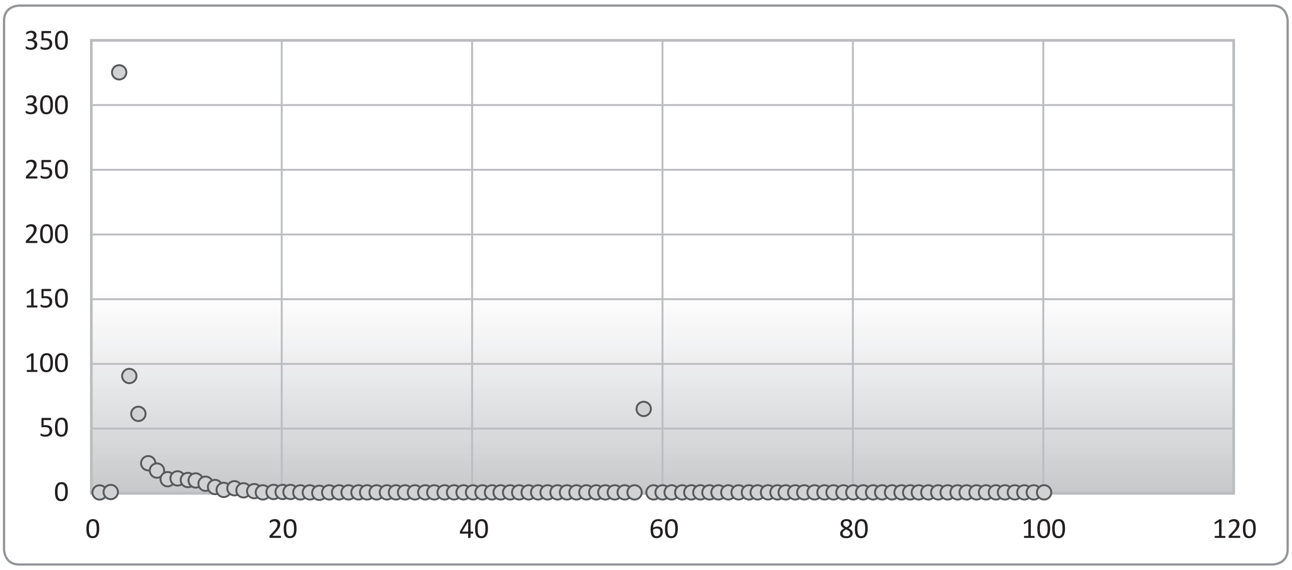

Figure 5. Herfindahl-Hirschman index of total revenues in 2016

that four companies have the largest market share. One of them has the highest revenues in this sector, and it has the largest HHI as well; the HHI for this company constitutes almost half of the total HHI in the entire Serbian meat and meat products industry. This suggests the better positioning of this company and its potential to further increase its market dominance in the future. Also, key results have shown there is no monopoly in the Serbian meat industry, and no company is dominant in the market, while at the same time, there are no oligopolies. When it comes to the export of meat and meat products, the competition is greater, as the annual HHI of 1,257 in 2017 indicates. Less than 10% of all business entities export their products, and that is something that should be changed in the future. In the upcoming period, the recommendation is to compare HHI of meat and other food industries in order to obtain a complete picture and analysis of the Serbian food industry.

Appendix

Table 4. The structure of the ten top-ranked business entities with the highest Herfindahl-Hirschman index for total revenues

Business entity	2013	Business entity	2014
I	450	I	301
II	62	II	87
IV	59	IV	66
III	54	III	64
V	24	VI	25
VI	23	V	24
XVI	13	VIII	19
XI	10	XI	11
VII	10	IX	10
IX	9	VII	10

Merenje konkurentnosti na tržištu industrije mesa – da li u Srbiji postoje oligopoli?

Vladimir Mitić, Nataša Kilibarda, Ivana Brdar, Marija Kostić, Danijela ŠarČević, Nedjeljko Karabasil, Vule Mizdraković

A p s t r a k t: Proizvodnja mesa i mesnih prerađevina je veoma značajan deo prehrambene industrije. Da bi se postigao ekonomski rast i razvoj, posebno u mesnoj industriji, veoma je važno obezbediti slobodnu konkurenciju za privredna društva koji posluju u njoj. Stoga, glavni cilj ovog rada je da se, pomoću Herfindahl-Hirschman indeksa (HHI), izmeri koncentracija tržišta, kao i da se utvrdi nivo konkurencije na srpskom tržištu, ali i da se utvrdi da li postoje subjekti koji na tržištu imaju poziciju monopola ili oligopola. Drugi cilj ovog rada je da se definiše u kojoj meri poslovni subjekti iz srpske mesne industrije izvoze svoje proizvode i robu na međunarodnom tržištu i da li je nivo koncentracije isti. U istraživanju je korišćen uzorak od 350 privrednih društava registrovanih u ovom sektoru, a koja su obelodanila najviše prihode u izveštajnom periodu od 2013. do 2017. godine.

Ključne reči: *mesna industrija, savršena konkurencija, Herfindahl-Hirschman indeks, prihodi, tržište.*

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