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Original scientific paper

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

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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 ($p<0.05$).

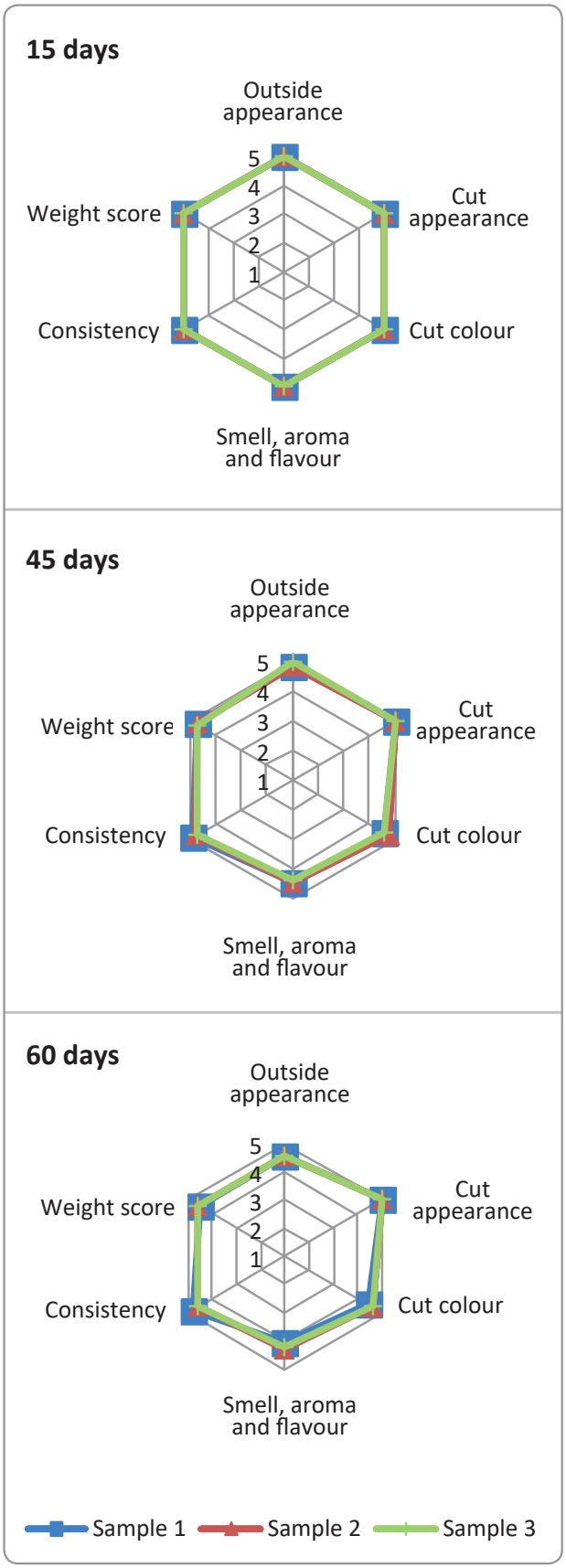

Figure 1. Sensory analysis results of selected quality indicators of all three sausage groups during the storage period

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 ($p<0.05$).

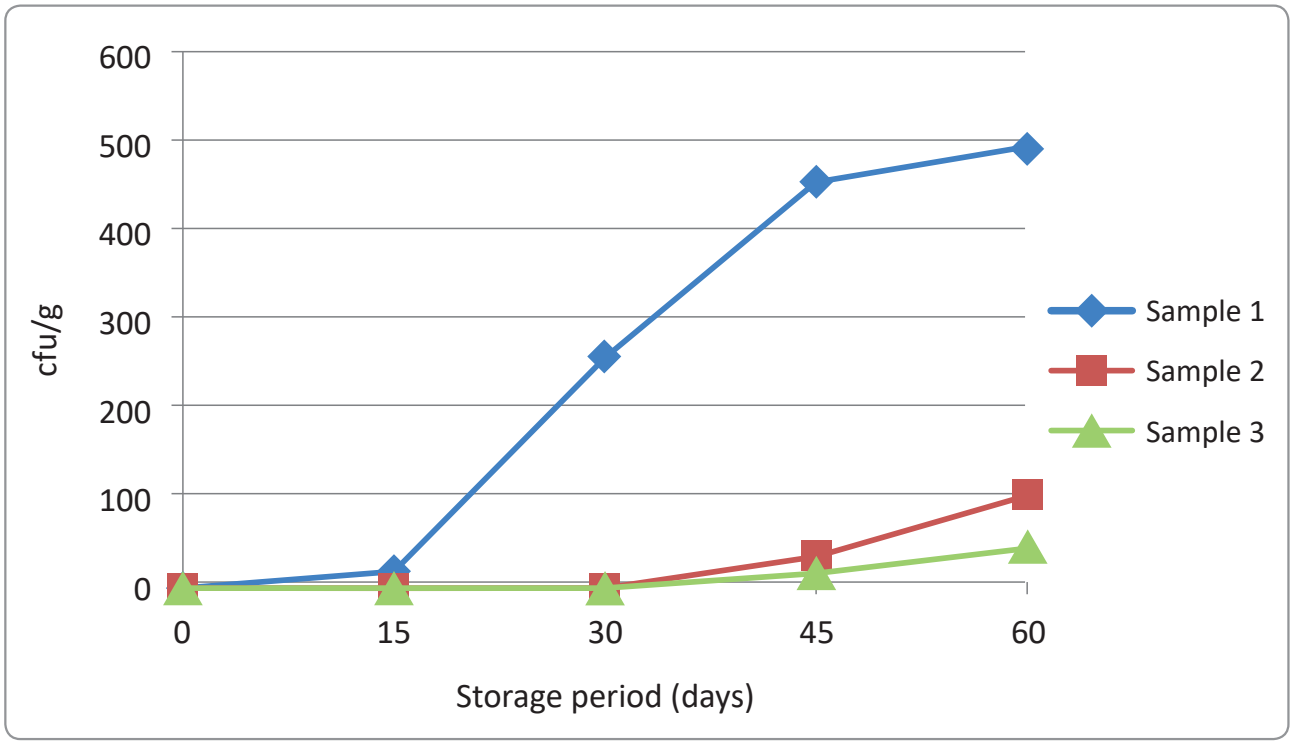

Figure 2. Number of lactic acid bacteria on the outside surface of sausages during storage

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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