

Proteolysis and texture profile of traditional dry-fermented sausage as affected by primary processing method

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Abstract: In this study, proteolysis and texture profile of Petrovska klobasa sausages produced with different raw batter mixing procedures (traditional, manual vs. mechanical) were investigated. Physicochemical analyses (pH value, moisture, nitrogen fraction), Labon-a-Chip (LoaC) electrophoresis-based protein analyses and texture profile analyses were performed in order to follow the drying/ripening process. The results obtained showed that manual mixing caused better water holding capacity of meat proteins, and thus, at the end of the drying process and under identical thermo-hygrometric conditions, control sausages had higher moisture content and lower hardness and chewiness values compared to their mechanically mixed counterparts. The increase in non-protein nitrogen and free amino acid nitrogen content and the results of electrophoretic separation of myofibrillar proteins indicated somewhat more intense proteolytic changes in manually-mixed sausages. However, the different mixing procedures did not significantly affect ($p > 0.05$) sensory texture scores of Petrovska klobasa at the end of the drying process.

Keywords: dry-fermented sausage, mixing procedure, proteolysis, texture.

Introduction

Traditional dry fermented sausages have great significance and economic value in all European countries. Such products are highly appreciated by consumers and are considered of high sensory quality. The wide variety of this type of sausage in the European geographic zone is a consequence of variations in raw materials, formulation and manufacturing processes, which arise from different traditional habits in countries and/or regions. Geographical indication often defines the name of the sausage (*Casaburi et al.*, 2007; *Vignolo et al.*, 2010). This is the case for *Petrovska klobasa*, a dry-fermented sausage traditionally manufactured in north-western Serbia (Municipality of Backi Petrovac, Vojvodina province). Due to its production characteristics (smoking, plus long drying and ripening phases) and sensory attributes (aromatic and spicy-hot taste, dark red colour and hard consistency), it has been protected with a designation of origin (PDO) under Serbian legislation. In general, processing implies five well-defined phases: meat mincing, mixing with seasonings, stuffing, smoking and drying/ripening. Presently, this sausage is produced in the traditional manner according to original recipes, without the use of chemical

additives or microbial starters. It is produced in micro-processing plants within village households during winter, and undergoes slow drying and ripening processes (*Petrovic et al.*, 2006; *Ikonic et al.*, 2013; 2016). An important part of the production process is mixing the raw material (minced meat and back fat) with seasonings, and in traditional practice, this is performed manually. This introduces a portion of the indigenous microbiota into the sausage batter. In order to meet market demands and produce greater quantities of standard quality *Petrovska klobasa*, it is necessary to apply more mechanisation and efficient control and management of thermo-hygrometric conditions during the drying/ripening phase than occurs in traditional conditions. Implementation of mechanisation implies the discharge of manual and the introduction of mechanical mixing for the sausage batter (*Marcos et al.*, 2016).

Proteolysis is one of the main biochemical phenomena which occurs during the ripening of fermented sausages, and is influenced by both muscle and microbial enzymes. Proteolysis results in the formation of several low molecular weight components, i.e. peptides, amino acids, aldehydes, organic acids and amines, which influence and define the final texture characteristics and aroma of

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the product (Dalmis and Soyer, 2008; Roseiro et al., 2008; Spaziani et al., 2009). Regarding meat products, quality is strongly affected by their texture characteristics. The textural profile of dry-fermented sausages throughout the manufacturing process is usually determined by consecutive instrumental measurements, since sensory analysis can be applied on the final product (Szczesniak, 2002; Gonzalez-Fernandez et al., 2006; Barbut, 2007).

Having in mind that any changes in production processes can affect the final quality of dry-fermented sausages, the aim of this study was to evaluate the effect of mixing procedure (manual vs. mechanical) on physicochemical traits, proteolysis and texture characteristics of *Petrovska klobasa*.

Materials and methods

Preparation of *Petrovska klobasa*

Petrovska klobasa sausages were manufactured from a mixture of lean minced pork (80%) and back fat (20%) obtained from carcasses of large white cross breed pigs. The minced meat and fat mixture ($\varnothing \approx 10$ mm) was divided in two parts and the same amounts of seasonings (red hot paprika powder, salt, raw garlic paste, caraway and sucrose) were added. One part was mixed manually (control – C) and the other was mixed mechanically (experimental – E) (Fig. 1). Both parts were mixed until homogenous batter was obtained. After mixing, batters were immediately stuffed into collagen casings (500 mm long and 55 mm in diameter) and sausages were entirely processed in a traditional drying/ripening room during 60 days, until the required moisture content ($<35\%$) was achieved (Serbia, 2015). The environmental conditions in this

traditional room were highly dependent on outdoor climate conditions and were measured regularly during the processing. Average temperature and relative humidity during 60 days of processing were 8.3°C and 79.3% , respectively.

Samples

Samples of sausage types C and E were taken during processing on days 0, 2, 6, 9, 15, 30 and 60. On each sampling occasion, three sausages of each type were taken for physicochemical and texture profile analyses while the rest of the samples were prepared and stored at -20°C for electrophoretic separation of proteins. Sensory analysis was performed after 60 days of processing, at the end of the drying process.

Physicochemical analysis

The pH of sausages was measured using the portable pH meter Testo 205 (Testo AG, USA) equipped with a combined penetration tip with temperature probe. Moisture content was quantified according to the recommended ISO standard (ISO, 1997). The non-protein nitrogen (NPN) and free amino acid nitrogen (FAAN) were determined according to the methods described by Ikonc et al. (2013). The nitrogen fractions content were expressed as g/100 g dry matter (dm) of sausage.

Electrophoretic separation of proteins – Loac method

The extraction and separation of myofibrillar proteins was performed as described by Ikonc et al. (2013), using Agilent 2100 bioanalyzer (Agilent



Figure 1. Traditional, manual (C) and mechanical (E) mixing of *Petrovska klobasa*

Technologies, Santa Clara, CA) in combination with the Protein 230 Plus LabChip Kit and the dedicated 2100 expert software.

Texture Profile Analysis (TPA)

Texture analysis was performed as described by Bourne (1978), at room temperature, using TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, UK) equipped with a standard cylindrical plate of 75 mm in diameter. The samples (cylinders) 2 cm thick and 2.54 cm in diameter, after discarding the external layer of the sausage, were compressed twice to 50% of their original thickness at a constant test speed of 1 mm s⁻¹. The following parameters were determined: hardness (g), springiness, cohesiveness and chewiness (g). Hardness was defined by peak force during the first compression cycle. Adhesiveness was obtained from the negative force area under the curve obtained between cycles. Springiness was defined as the rate at which a deformed sample goes back to its un-deformed condition after the deforming force is removed. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Finally, chewiness was obtained by multiplying hardness, cohesiveness and springiness. Measurements were carried out six times for each sausage sample.

Sensory analysis

The sensory analysis was performed by an 8-member panel with previous experience in testing dry-fermented sausages. The casing was removed and the sausages were cut into slices of

approximately 3 mm thickness and served at room temperature on white plastic dishes. Panel tasters were asked to score samples by using a 0 (worst) to 5 (best) scale for texture attributes.

Statistical analysis

The effects of drying time and mixing procedure on the variables studied were analysed by Factorial ANOVA (Statistica 13.2, Dell Inc., 2016). Duncan's post hoc test was performed for comparison of mean values. Differences were considered significant at $p < 0.05$.

Results and Discussion

Changes in pH and moisture content during the drying period (Table 1) showed the prevailing tendencies observed in this type of product. Intensive pH decline was registered in both C and E sausages, reaching the isoelectric point (pI) of actomyosin (≈ 5.0) after 15 days of smoking/drying phase. In the next 15 days, pH continued to fall, achieving minimal values (C – 4.94, E – 4.99). Further on, slight pH increases in both sausages occurred, due to proteolysis processes, i.e. liberation of peptides, amino acids, ammonia and amines (Kaban and Kaya, 2009; Spaziani *et al.*, 2009). Similarly, moisture content in sausages decreased significantly ($p < 0.05$) during the drying period, reaching values lower than 35%, required by national legislation for dry-fermented sausages (Serbia, 2015), after 60 days. Somewhat lower moisture content was registered in E sausages, indicating the slightly faster drying process of sausages produced by mechanically mixing the batter. Based on the results of pH and moisture content, it can be concluded that at 60

Table 1. Changes in pH and moisture content (%) of *Petrovská klobása* sausages during the drying process

Parameter	Sausage type	Time (days)							DT	MP	DT × MP
		0	2	6	9	15	30	60			
pH	C	5.74 ^{fg}	5.75 ^{fg}	5.67 ^f	5.26 ^d	5.06 ^{bc}	4.94 ^a	5.00 ^{ab}	*	ns	*
		±0.04	±0.02	±0.10	±0.15	±0.06	±0.02	±0.03			
	E	5.81 ^g	5.72 ^f	5.53 ^e	5.10 ^c	5.01 ^{abc}	4.99 ^{ab}	5.06 ^{bc}			
		±0.02	±0.02	±0.04	±0.06	±0.03	±0.02	±0.02			
Moisture content	C	54.3 ^j	54.1 ^j	53.1 ⁱ	50.2 ^f	48.6 ^e	41.1 ^c	33.9 ^b	*	*	*
		±0.04	±0.06	±0.08	±0.32	±0.18	±0.05	±0.23			
	E	55.6 ^l	54.8 ^k	51.7 ^h	50.6 ^g	48.2 ^d	41.2 ^c	32.3 ^a			
		±0.01	±0.02	±0.26	±0.02	±0.47	±0.31	±0.10			

Legend: Results are given as mean value ± standard deviation; ^{a–l} Means with different superscript letters are different ($p < 0.05$); C – control, prepared from manually mixed batter; E – experimental, prepared from mechanically mixed batter; DT – drying time effect; MP – mixing procedure effect; ns – not significant; * $p < 0.05$

Table 2. Evolution of NPN and FAAN content (expressed as g/100 g dm) in *Petrovská klobása* sausages during the drying process

Parameter	Sausage type	Time (day)							DT	MP	DT×MP
		0	2	6	9	15	30	60			
NPN	C	0.58 ^a ±0.01	0.62 ^c ±0.01	0.65 ^d ±0.01	0.66 ^d ±0.01	0.73 ^f ±0.01	0.82 ^h ±0.01	0.97 ⁱ ±0.01			
	E	0.61 ^b ±0.00	0.62 ^c ±0.00	0.69 ^e ±0.00	0.69 ^e ±0.01	0.78 ^g ±0.01	0.89 ⁱ ±0.00	0.99 ^k ±0.00	*	*	*
FAAN	C	0.20 ^b ±0.01	0.22 ^c ±0.01	0.20 ^{ab} ±0.00	0.22 ^c ±0.00	0.29 ^d ±0.00	0.33 ^f ±0.00	0.38 ^h ±0.00			
	E	0.20 ^b ±0.01	0.20 ^b ±0.01	0.19 ^a ±0.00	0.23 ^c ±0.00	0.31 ^e ±0.01	0.35 ^g ±0.01	0.37 ^h ±0.00	*	ns	*

Legend: Results are given as mean value ± standard deviation; ^{a-k} Means with different superscript letters are different (p<0.05); C – control, prepared from manually mixed batter; E – experimental, prepared from mechanically mixed batter; NPN – non-protein nitrogen; FAAN – free amino acids nitrogen; DT – drying time effect; MP mixing procedure effect; ns – not significant; * p<0.05

days, the ripening process in both *Petrovská klobása* sausages was not completely finished due to low pH values (C – pH 5.00, E – pH 5.06), although the drying process was considered finished. According to the Code of Practice for this traditional sausage (Petrovic et al., 2006), the pH value of product ready for consumption has to be higher than 5.4.

NPN and FAAN content increased significantly (p<0.05) during processing in both analysed sausage types (Table 2), being approximately 65% and 88% higher in dried products compared to raw sausage batter, respectively. Nevertheless, the growth of these nitrogen fractions was slightly higher in C sausages, indicating more intensive proteolysis in manually

mixed sausages, even though the differences in FAAN were not significant (p>0.05). Hence, the results obtained showed that degradation of proteins during drying/ripening of fermented sausages, i.e. liberation of polypeptides, peptides, amino acids, aldehydes, amines etc., was followed by an increase in NPN and FAAN content, as was previously reported by a number of authors (Casaburi et al., 2007; Dalmiş and Soyer, 2008; Kaban and Kaya, 2009; Casquete et al., 2011).

Fig. 1 shows the Loac gel-like electrophoretograms of myofibrillar proteins for control (C) and experimental (E) sausages. Generally, important changes in qualitative and quantitative composition of

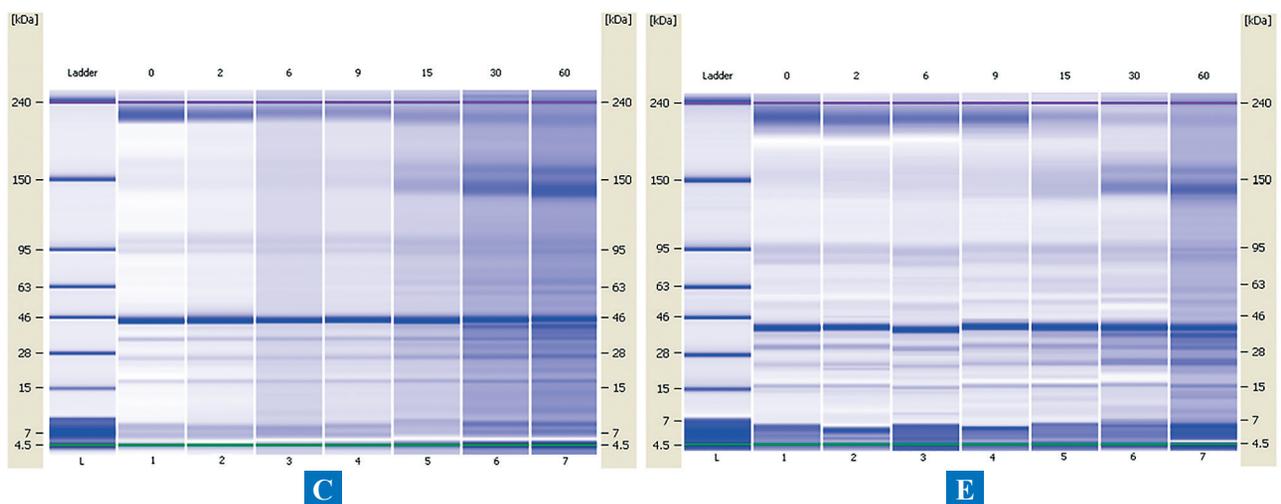


Figure 2. Loac gel-like image of myofibrillar protein extracts during the drying of *Petrovská klobása*: C – control, prepared from manually mixed batter; E – experimental, prepared from mechanically mixed batter (lanes 1–7 correspond to days 0–60). Lane L – Ladder, molecular weight standards in the range of 4.5–240 kDa.

myofibrillar protein extracts were recorded during the drying period. Still, some differences in the intensity of certain protein fractions between the differently processed sausages were obvious. The concentrations of myosin (myosin heavy chain – MHC, ≈ 220 kDa) and actin (≈ 45 kDa), the most important myofibrillar proteins, were significantly reduced during processing compared to their initial levels. This phenomenon was particularly pronounced for MHC, since it was extensively hydrolysed in both analysed sausages (C $\approx 90\%$, E $\approx 94\%$). This result is in agreement with the findings of several authors (Casaburi *et al.*, 2007; Roseiro *et al.*, 2008; Spaziani *et al.*, 2009; Ikonc *et al.*, 2013). However, actin showed greater stability. After 60 days of C and E sausage processing it lost about 55% and 40% of its initial concentration, respectively. Significant actin hydrolysis was previously reported in numerous publications dealing with dry-fermented sausages (Dalmis, & Soyer, 2008; Spaziani *et al.*, 2009). As a consequence of MHC degradation and co-migration of the resulting products, an important increase of protein bands at ≈ 145 and 160 kDa was observed in both sausage types in our study. This phenomenon was more pronounced for manually

mixed sausages. Similar findings related to myosin degradation and formation of polypeptides with molecular weights in the range of 120–150 kDa were previously reported (Martín-Sánchez *et al.*, 2011; Spaziani *et al.*, 2009). Moreover, the intensity of protein bands at ≈ 74 , 66, 41, 34, 31, 25 and 14 kDa increased during the ripening, while five (≈ 60 , 40, 30, 22, 15 kDa) and three (≈ 105 , 40, 22 kDa) newly-formed components were registered in sausage C and E, respectively. The appearance and accumulation of several polypeptides in the range of 14–100 kDa have also been observed by a number of authors (Dalmis & Soyer, 2008; Martín-Sánchez *et al.*, 2011) during processing of dry-fermented sausages. Furthermore, a reduction in the protein band at ≈ 100 kDa (probably α -actinin) was observed throughout the drying period of both sausages, in concordance with findings of Casaburi *et al.* (2007) and Dalmis and Soyer (2008).

Results of texture profile analysis are shown in Table 3. Hardness and chewiness of both types of sausages, as expected, increased progressively during processing. From day 9, hardness and chewiness values for E sausages were somewhat higher than those for C sausages. This phenomenon

Table 3. Evolution of texture profile of *Petrovská klobása* sausages throughout the drying process

Parameter	Sausage type	Time (day)						DT	MP	DT \times MP
		2	6	9	15	30	60			
Hardness (g)	C	306 ^a	499 ^{abc}	624 ^{bc}	923 ^d	1562 ^e	2611 ^g	*	*	*
		± 44.9	± 68.8	± 164	± 178	± 175	± 157			
	E	298 ^a	452 ^{ab}	718 ^{cd}	923 ^d	1794 ^f	3098 ^h			
		± 40.2	± 76.5	± 227	± 127	± 208	± 432			
Springiness	C	0.39 ^{abcd}	0.36 ^{afg}	0.39 ^{abcd}	0.42 ^{de}	0.39 ^{abcd}	0.34 ^f	*	ns	ns
		± 0.04	± 0.04	± 0.02	± 0.02	± 0.03	± 0.01			
	E	0.38 ^{abc}	0.38 ^{abg}	0.44 ^e	0.41 ^{bcde}	0.42 ^{cde}	0.34 ^{fg}			
		± 0.05	± 0.03	± 0.04	± 0.02	± 0.03	± 0.02			
Cohesiveness	C	0.40 ^a	0.35 ^{bc}	0.38 ^{ac}	0.40 ^a	0.40 ^a	0.33 ^b	*	*	*
		± 0.03	± 0.03	± 0.03	± 0.02	± 0.03	± 0.03			
	E	0.40 ^a	0.35 ^{bc}	0.46 ^d	0.46 ^d	0.40 ^a	0.33 ^b			
		± 0.03	± 0.02	± 0.06	± 0.02	± 0.02	± 0.04			
Chewiness (g)	C	48.4 ^a	63.2 ^a	91.4 ^{ae}	156 ^{bc}	205 ^c	292 ^d	*	*	ns
		± 11.1	± 13.0	± 24.2	± 32.2	± 100	± 39.5			
	E	45.1 ^a	59.4 ^a	142 ^{bc}	171 ^{bc}	304 ^d	343 ^d			
		± 9.33	± 10.3	± 38.3	± 32.3	± 47.3	± 75.3			

Legend: Results are given as mean value \pm standard deviation; ^{a–h} Means with different superscript letters are different ($p < 0.05$); C – control, prepared from manually mixed batter; E – experimental, prepared from mechanically mixed batter; DT – drying time effect; MP – mixing procedure effect; ns – not significant; * $p < 0.05$

could be due to the faster pH decrease of E sausages (5.10) toward the isoelectric point of protein (≈ 5.0). Decline of pH is followed by the solubilisation of myofibrillar proteins which aggregate to form a gel with an ordered protein network, and so contribute to sausage firmness (Gonzalez-Fernandez et al., 2006; Spaziani et al., 2009). At the end of drying process, hardness values were 2611g and 3098 g for C and E sausages, respectively ($p < 0.05$). Chewiness values at the end of processing reached values of 292 g and 343 g for C and E sausages, respectively. Since the texture characteristics, are influenced by the drying process as well as the fermentation process (i.e. the pH decline), differences in hardness and chewiness values at the end of the drying process could be due to the difference in moisture content between sausages. Significant negative correlations ($p < 0.05$) between pH and moisture content on one hand and hardness and chewiness on the other were registered by Gonzalez-Fernandez et al., (2006), Bozkurt and Bayram (2006) and Casaburi et al., (2007). In our study on day 9, springiness, and on days 9 and 15, cohesiveness, was higher for E sausages than for C sausages. Lack of variability in cohesiveness was previously explained by Spaziani et al. (2009) as due to small difference in pH between samples when pH was close to the isoelectric point of proteins.

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The results of sensory analysis did not reveal any significant difference ($p < 0.05$) in texture between sausages at the end of the drying process. The observed differences in the instrumental analysis of texture were not detected in the sensorial analysis of *Petrovská klobása*. Hence, both C and E sausages showed relatively high scores for texture, being 3.92 and 3.95, respectively.

Conclusions

The influence of different mixing procedure (manual vs. mechanical) was analysed during the *Petrovská klobása* drying period. Most probably, manual mixing induced better water holding capacity of proteins, thus resulting in higher moisture content and lower hardness and chewiness values in C sausages at the end of the drying process. According to evolution of nitrogen fraction content and the results of LoaC electrophoretic separation of myofibrillar proteins, it can be concluded that manually mixed sausages underwent slightly more intense proteolytic changes. Nevertheless, different mixing procedures did not significantly affect the sensory attributes of texture. Further research will be related to transfer and adjustment of traditional technology to industrial conditions in order to achieve *Petrovská klobása* of optimal and standard quality.

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