

# Effect of Ripening Conditions and Storage Time on Oxidative and Sensory Stability of *Petrovská Klobása* Sausage

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**Abstract**—The influence of ripening conditions (traditional and industrial) on oxidative and sensory stability of dry fermented sausage (*Petrovská klobása*), during 7 months of storage, was investigated. During the storage period the content of free fatty acids was significantly higher ( $P < 0.05$ ), while the content of malondialdehyde was significantly lower in the sausage subjected to traditional conditions of drying. At the end of the storage period, content of hexanal in the sausage subjected to traditional conditions of ripening ( $1.67 \mu\text{g/g}$ ) was significantly lower ( $P < 0.05$ ) in comparison with this content in the sausage subjected to industrial conditions of ripening ( $4.94 \mu\text{g/g}$ ). Traditional conditions of ripening at lower temperatures have led to better sensory properties of odor and taste of traditional dry fermented sausage, *Petrovská klobása* after 2 and 7 months of storage.

**Keywords**—Lipid oxidation, *Petrovská klobása*, sensory stability, storage time.

## I. INTRODUCTION

IN many European countries, the demand for traditional food products has increased. Moreover, food and gastronomy form an inherent link with tourism in Europe, with a renewed interest of consumers in typical and regional food [1]. *Petrovská klobása* is a traditional dry fermented sausage produced in Backi Petrovac (the province of Vojvodina, Serbia) exclusively from pork meat and fat with the addition of red hot paprika powder, salt, garlic, caraway and sugar. Red hot paprika has a dominant role in the formation of aroma of the *Petrovská klobása*. This product is also characterized by a dark-red color and firm consistency [2]. Fermented sausages are products that contain a high percentage of fat. Fat is responsible for numerous properties of the fermented sausages. From a physiological aspect, fat is an important source of energy as well as of essential fatty acids and liposoluble vitamins [3]. Products formed during lipolysis and lipid oxidation have an important role in the formation of

odor, taste and texture of the final product. However, fermented sausages also show some negative properties as a consequence of high content of animal fat [4]. Lipolysis is the first step in the process of auto-oxidation of free fatty acids [5]. Moreover, the oxidative degradation of lipids of meat and meat products involves the oxidation of unsaturated fatty acids, especially polyunsaturated fatty acids and cholesterol [6]. Polyunsaturated fatty acids having three or more double bonds are primarily tied to phospholipids and are important for the development of the characteristic flavor state of food. The free radicals formed in lipid oxidation ( $R\bullet$ ) react with oxygen producing peroxy radicals ( $ROO\bullet$ ). In this initial process  $ROO\bullet$  react with several RH resulting in lipid hydroperoxides ( $ROOH$ ), which are the main primary products of oxidation [7], [8]. Moreover, during secondary oxidation changes in free fatty acids, compounds such as aldehydes, ketones, carboxylic acids are being created. Aldehydes are the main products formed during the lipid oxidation. Even in small amounts aldehydes disturb the favorable sensory properties of food [9], [10]. Also, many aldehydes formed during the smoking process or from lipid peroxides are carcinogenic and can cause diseases of the digestive tract [11]. Propanal and hexanal are the most commonly used indicators of lipid oxidation in food due to their higher oxidative stability in detection compared to unsaturated aldehydes [12]. Propanal is a typical product of the n-3 oxidation and hexanal is a product of oxidative degradation of n-6 polyunsaturated fatty acids [13].

*Petrovská klobása* is a traditional product that is currently being transferred from small-scale production to industrial. At this point, preservation of product during distribution and storage phases is very important. Thus, the aim of this study was to comparatively examine the effect of ripening methods (in the traditional and industrial conditions) and storage time on oxidative and sensory stability of traditional dry fermented sausage, *Petrovská klobása*.

## II. MATERIALS AND METHODS

### A. Material

Sausages were produced in the winter period by using traditional manufacturing technology. Stuffing for the experimental sausages was made from chilled lean pork and fat in relation 85:15. Pork and firm fat tissue were ground to pieces the size of 10mm, and then the following ingredients were added: 2.50% red hot paprika powder, 1.80% salt, 0.20%

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raw garlic paste, 0.20% caraway and 0.15% crystal sugar. Starter cultures were not added so the sausages were subjected to spontaneous fermentation. Stuffing was hand-mixed, using a specific technique of tipping over and squashing for 10 minutes. The made stuffing was then filled into collagen casings (diameter of 55mm). The sausages were subjected to straining during 24h. Subsequently sausages were smoked in chamber in the traditional way for 12 days with breaks. The atmospheric conditions during smoking were:  $t = 5-10^{\circ}\text{C}$ ,  $\text{RH} = 75-85\%$ . After the smoking process, sausages were divided into 2 groups (T and I). Sausages of the T group were subjected to uncontrolled drying and ripening in traditional conditions ( $t = 0-10^{\circ}\text{C}$ ,  $\text{RH} = 95-80\%$ ) until achieving the moisture content of 35% (90 days). Sausages of I group were subjected to controlled drying conditions ( $t = 8-10^{\circ}\text{C}$ ,  $\text{RH} = 90-75\%$ ) until achieving the moisture content of 35.0% (60 days). After the drying process, both groups of sausages were stored,  $t = 10^{\circ}\text{C}$ ,  $\text{RH} = 75\%$ , for 7 months.

### B. Methods

#### 1. Acid Number

Acid number (mg KOH/g lipid), was quantified according *SRPS ISO* [14].

#### 2. TBARS Determination

TBARS (2-thiobarbituric acid reactive substances) test was performed using to the method of Bostoglou et al. [15], with modifications. Total volume of TCA was added to the sample and extraction was performed in ultrasonic bath XUB 12 (Grant Instruments, Cambridge, UK) [8]. Spectrophotometer Jenway 6300 (Jenway, Felsted, United Kingdom) was used. TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample.

#### 3. Aldehydes Determination

Static headspace gas chromatographic (SHS-GC) analyses were performed on Agilent 7890A GC System (Agilent Technologies, Santa Clara, California, USA) equipped with a capillary split/split less inlet, total electronic pneumatic control of gas flow, headspace auto sampler and FID. Static headspace (SHS) sampling was performed with the headspace sampler, CombiPAL System (CTC Analytics, Zwingen, Switzerland). A 2.5mL HS syringe for CombiPAL was used, for the injection of 2.0mL of vapor phase from the 10mL headspace vials. Chromatographic conditions and aldehydes standard preparation is performed according to Mandić et al. [16]. Homogenized sample was accurately weighed (2.00g) into 10mL screwcapped headspace vial.

#### 4. Sensory Evaluation of Odor and Taste

A panel consisting of seven trained members of different ages performed sensory evaluation of odor and taste. The casing was removed; the sausages were cut into slices of approximately 4mm thickness and served at room temperature on white plastic dishes. Three slices were served from each batch. Water and unsalted toasts were provided to cleanse the palate between samples. Evaluation was performed according

to quantitative descriptive analysis (QDA), using a scale from 0 to 5, with a sensitivity threshold of 0.25 points [17]. Each mark means distinctive quality level, described as follows: 5 – extraordinary, typical, optimal quality; 4 – observable deviations or insignificant quality defects; 3 – drawbacks and defects of quality; 2 – distinct to very distinct drawbacks and defects of quality; 1 – fully changed, atypical properties, product unacceptable; 0 – visible mechanical or microbiological contamination, atypical product.

### 5. Statistical Analysis

Statistical analysis was carried out using STATISTICA 8.0 (StatSoft, Inc., Tulsa, OK, USA). All data were presented as mean value with their standard deviation indicated (mean  $\pm$  SD). Variance analysis (ANOVA) was performed, with a confidence interval of 95% ( $P < 0.05$ ). Means were compared by t-test and Duncan's multiple range test.

## III. RESULTS AND DISCUSSION

Free fatty acid content at the end of the drying process were within the range from 7.11mg KOH/g of lipids for the sausage I to 14.62 mg KOH/g of lipids for the sausage T (Table I). The obtained results are in agreement with the results Muguerza et al. [18], for similar products in the type of fermented sausages. During the storage time, in both sausages, free fatty acid content was significantly increasing ( $P < 0.05$ ). The increase in free fatty acids content is probably the result of endogenous enzymes activity as well as the activity of enzymes of microorganisms [5]. Moreover, lipolytic changes are followed by oxidative changes that compounds such as unsaturated fatty acids and cholesterol are easily subjected to [18]. Malondialdehyde is a typical degradation product formed during lipid oxidation of polyunsaturated fatty acids [17]. At the end of the drying process in sausages produced in traditional (T) and industrial (I) conditions of ripening, the content of malondialdehyde was 0.79 and 1.25 $\mu\text{g/g}$ , respectively (Table I). Unlike the sausage I, in the sausage T, malondialdehyde content was reduced after 2 months of storage period. Malondialdehyde values after 7 months of storage period were 0.16 $\mu\text{g/g}$  for sausage T and 0.93 $\mu\text{g/g}$  for sausage I, and were significantly lower ( $P < 0.05$ ) compared to the determined value of malondialdehyde in sausages of T and I groups after 2 months of storage. Reduction of malondialdehyde values during storage was probably the result of interaction of malondialdehyde with compounds such as sugars, nitrites, amino acids [17]. Furthermore, malondialdehyde values at the end of the drying process as well as during the entire period of storage in the tested sausages were significantly lower ( $P < 0.05$ ) in sausage subjected to traditional conditions of drying and ripening. The content of malondialdehyde was negatively correlated with the free fatty acid content in both sausages at the end of the drying process as well as during the entire period of storage. Berger et al. [19] suggested that lower values of free fatty acids content may be a result of intense oxidative changes. Obtained results shown that oxidation of free fatty acids in the traditional dry fermented sausage *Petrovská klobása* during

prolonged storage time (7 months) was slower when sausages are processed under conditions of drying at lower temperatures.

Obtained results shown that oxidation of free fatty acids in the traditional dry fermented sausage *Petrovská klobása* during prolonged storage period (7 months) was slower when

sausages are processed under conditions of ripening at lower temperatures. Table I shows aldehyde content at the end of the drying process and during 2 and 7 months of storage. Aldehydes are bearers of a wide range of fragrances and flavors in food [10].

TABLE I  
CHANGE IN LIPID OXIDATION PARAMETERS IN *PETROVSKÁ KLOBÁSA* SAUSAGE DURING STORAGE PERIOD

Lipid oxidation parameters	Sausage	End of drying	2 months	7 months
Acid number (mg KOH/g lipid)	T	14.62 <sup>xc</sup> ±0.01	26.23 <sup>xb</sup> ±0.23	36.78 <sup>xa</sup> ±1.85
	I	7.11 <sup>yc</sup> ±0.01	15.25 <sup>yb</sup> ±1.45	30.38 <sup>ya</sup> ±2.44
TBARS (mg MDA/kg)	T	0.79 <sup>xb</sup> ±0.02	0.36 <sup>yb</sup> ±0.01	0.16 <sup>xc</sup> ±0.02
	I	1.25 <sup>xb</sup> ±0.01	1.63 <sup>xa</sup> ±0.02	0.93 <sup>xc</sup> ±0.02
Aldehydes (μg/g)				
Propanal	T	1.86 <sup>b</sup> ±0.53	0.94 <sup>xb</sup> ±0.01	32.59 <sup>a</sup> ±1.57
	I	2.68 <sup>b</sup> ±0.26	4.80 <sup>xb</sup> ±0.10	44.32 <sup>a</sup> ±10.67
Hexanal	T	0.12 <sup>b</sup> ±0.08	0.05 <sup>yb</sup> ±0.00	1.67 <sup>ya</sup> ±0.07
	I	0.05 <sup>b</sup> ±0.00	0.22 <sup>xb</sup> ±0.01	4.94 <sup>xa</sup> ±1.29

xy The values of the same column significantly differ with 95% probability ( $P<0.05$ )

abc The values of the same row significantly differ with 95% probability ( $P<0.05$ )

Propanal was the most dominant aldehyde at the end of the drying process as well as during storage period in both groups of sausages. It is in agreement with literature data [20]. After 2 months of storage, the content of propanal ranged in an interval from 0.94μg/g in the sausage T to 4.80μg/g in the sausage I. After that, the content of propanal was significantly increased ( $P<0.05$ ) in both groups of sausages. The content of hexanal in the tested sausages of T and I groups, after 2 months of storage was 0.05μg/g and 0.22μg/g, respectively. The obtained results of hexanal content are very similar to values that were found in fermented sausages by Josquin et al. [20].

Following two months of storage, contents of propanal and hexanal were significantly lower ( $P<0.05$ ) in the sausage produced in traditional conditions of ripening (Table I). Moreover, after 7 months of storage, the contents of propanal and hexanal in both groups of sausages were significantly increased ( $P<0.05$ ). Significant increase in the content of aldehydes during storage period in fermented sausages was determined by Valencia et al. [9] and Ansorena et al. [11]. After 7 months of storage, propanal content ranged in an interval from 32.59μg/g in the traditional, to 44.32μg/g in the sausage produced under industrial conditions of drying and ripening. There was no significant difference ( $P>0.05$ ) between the obtained values of propanal content. Obtained results of propanal content in *Petrovská klobása* were much higher than the values found in fermented sausages by Josquin et al. [20]. On the other hand, the content of hexanal was significantly lower ( $P<0.05$ ) in the traditional than in sausage produced in industrial drying conditions.

At the end of drying process, sensory grades of odor and taste (Table II) for sausage of T and I group did not differ significantly ( $P>0.05$ ). In addition, after 2 and 7 months of storage sausage produced in traditional conditions had a significantly ( $P<0.05$ ) higher grades for odor and taste compared to sausage produced in industrial conditions of

ripening.

TABLE II  
CHANGE IN SENSORY PROPERTIES OF ODOR AND TASTE OF *PETROVSKÁ KLOBÁSA* SAUSAGE DURING STORAGE PERIOD

	Sausage	End of drying	2 months	7 months
Odor and taste	T	4.40a±0.08	4.30ya±0.07	4.10yb±0.02
	I	4.27a±0.09	4.01xb±0.04	3.04xc±0.02

xy The values of the same column significantly differ with 95% probability ( $P<0.05$ )

abc The values of the same row significantly differ with 95% probability ( $P<0.05$ )

#### IV. CONCLUSION

At the end of the storage period TBARS value as well as content of propanal and hexanal was lower in the sausage produced in the traditional condition of ripening. Sensory properties for odor and taste during storage were higher in sausage produced in traditional conditions of ripening.

Obtained results indicate that conditions of slower ripening at lower temperatures result in better oxidative and sensory stability of traditional dry fermented sausage (*Petrovská klobása*) during prolonged storage period (7 months).

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## REFERENCES

- [1] R. Talon, I. Lebert, A. Lebert, S. Leroy, M. Garriga, T. Aymerich, E.H. Drosinos, E. Zanardi, A. Ianieri, M.J. Fraqueza, L. Patarata, A. Lauková, „Traditional dry fermented sausages produced in small –scale processing units in Mediterranean countries and Slovakia. 1. Microbial ecosystems of processing environments“, *Meat Sci.*, vol. 77, no.4, pp. 570–579, Dec. 2007.
- [2] T. Tasić, P. Ikonić, A. Mandić, M. Jokanović, V. Tomović, S. Savatić, Lj. Petrović, „Biogenic amines content in traditional dry fermented sausage Petrovská klobása as possible indicator of good manufacturing practice“, *Food Cont.*, vol. 23, no. 1, pp. 107–112, Jan. 2012.
- [3] A. Olivares, J. Navarro, M. Flores, „Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage“, *Food Cont.*, vol. 115, no. 4, pp. 1464–1472, Aug. 2009.
- [4] W. Visessanguan, S. Benjakul, S. Riebroy, N. Mongkol Yarchai, W. Tapingkae, „Changes in lipid composition and fatty acid profile of Nham, a Thai fermented pork sausage, during fermentation“, *Food Chem.*, vol. 94, no. 4, pp. 580–588, Mar. 2006.
- [5] L. Zhao, Y. Jin, C. Ma, H. Song, H. Li, Z. Wang, S. Xiao, „Physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices“, *Meat Sci.*, vol. 88, no. 4, pp. 761–766, Aug. 2011.
- [6] E. Hansen, D. Juncher, P. Henckel, A. Karlsson, G. Bertelsen, L.H. Skibsted, „Oxidative stability of chilled pork chops following long term freeze storage“, *Meat Sci.*, vol. 68, no. 2, pp.185–191, Oct. 2004.
- [7] G. Gandemer, „Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products“, *Meat Sci.*, vol. 62, no. 3, pp. 309–321, Nov. 2002.
- [8] A. Mandić, *Antioxidant activities of grape seed extracts from white grape varieties*, Ph. D. Thesis, University of Novi Sad, Novi Sad (Serbia), 2007.
- [9] I. Valencia, D. Ansorena, I. Astiasarán, „Stability of linseed and antioxidants containing dry fermented sausages: A study of the lipid fraction during different storage conditions“, *Meat Sci.*, vol. 73, no.2, pp. 269–277, June 2006.
- [10] J.M. Bruna, E.M. Hierro, L. de la Hoz, D.S. Mottram, M. Fernández, J.A. Juan Ordóñez, „The contribution of *Penicillium aurantiogriseum* to the volatile composition and sensory quality of dry fermented sausages“, *Meat Sci.*, vol. 59, no. 1, pp. 97–107, Sep. 2001.
- [11] D. Ansorena, I. Astisaran, „Effect of storage and packaging on fatty acids composition and oxidation in dry fermented sausages made with added olive oil and antioxidants“, *Meat Sci.*, vol. 67, no. 2, pp. 237–244, June 2004.
- [12] W. Sun, Q. Zhao, H. Zhao, M. Zhao, B. Yang, „Volatile compounds of Cantonese sausage released at different stages of processing and storage“, *Food Chem.*, vol. 121, no. 2, pp. 319–325, July 2010.
- [13] V. Varlet, C. Prost, T. Serot, „Volatile aldehydes in smoked fish: Analysis methods, occurrence and mechanism of formation“, *Food Chem.*, vol. 105, no. 4, pp. 1536–1556, Mar. 2007.
- [14] ISO 660: “Determination of the acid value of the fat (Reference method). In: International Standards Animal and Vegetable Oils and Fats“, *International Organization for Standardization*, Geneva, 1996.
- [15] N.A. Botsoglou, D.J. Fletouris, G.E. Papageorgiou, V.N. Vassilopoulos, A.J. Mantis, A.G. Trakatellis, „Rapid, sensitive and specific thiobarbituric acid method for measurement of lipid peroxidation in animal tissue, food and feedstuff samples“, *J. Agric. Food Chem.*, vol. 42 pp. 1931–1937, 1994.
- [16] A. Mandić, I. Sedej, M. Sakač, A. Mišan, „Static Headspace Gas Chromatographic Method for Aldehydes Determination in Crackers“, *Food Analyt. Meth.*, vol. 6, no. 1, pp. 61–68, 2013.
- [17] B. Šojić, Lj. Petrović, A. Mandić, I. Sedej, N. Džinić, V. Tomović, M. Jokanović, T. Tasić, S. Škaljac, P. Ikonić, „Lipid oxidative changes in traditional dry fermented sausage *Petrovská klobása* during storage“, *Chemical Industry*, submitted to publication.
- [18] G. Muguerza, D. Fista, D. Ansorena, I. Astiasarán, J.G. Bloukas, „Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages“, *Meat Sci.*, vol. 61, no. 4, pp. 397–404, Aug. 2002.
- [19] R.G. Berger, C. Macku, J.B. German, T. Shibamoto, „Isolation and identification of dry salami volatiles“, *J. Food Sci.*, vol. 55, pp. 1239–1242, 1990.
- [20] N. Josquin, J. Linssen, J. Houben, „Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork backfat with pure, pre-emulsified or encapsulated fish oil“, *Meat Sci.*, vol. 90, no. 1, pp. 81–86, Jan. 2012.