Influence of collagen and natural casings on the polycyclic aromatic hydrocarbons in traditional dry fermented sausage (Petrovská klobása) from Serbia

Snežana Škaljac, Ljiljana Petrović, Marija Jokanović, Tatjana Tasić, Maja Ivić, Vladimir Tomović, Predrag Ikonić, Branislav Šojić, Natalija Đžinić, and Biljana Škrbić

Abstract
The aim of this study was to determine the content of polycyclic aromatic hydrocarbons—PAHs (acenaphthylene, anthracene, fluorene, phenanthrene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene)—from Environmental Protection Agency list (United States Environmental Protection Agency) in traditional dry fermented sausage (Petrovská klobása) stuffed in collagen (C) and natural casings (N). Benzo[a]pyrene as well as PAH4 (benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, and chrysene) were not detected in all examined samples. Results obtained in this study indicated that at the end of drying, as well as at the end of storage period, total content of 13 US-EPA PAH was significantly \( (P < 0.05) \) lower in sausages with collagen casing (56.2 and 73.6 \( \mu \)g/kg, respectively) than in sausages with natural casing (137.1 and 206.2 \( \mu \)g/kg, respectively).

Introduction

Smoking is considered to be one of the oldest technologies used for conservation of meat sausages. During smoking, phenolic substances, which show antioxidant and antimicrobial properties, are generated. Besides that, phenolic substances have considerable importance in organoleptic properties of smoked meat products. Nowadays, smoking technology is because of these sensory active compounds mainly used for aromatization of meat products with recognizable organoleptic profile, widely demanded on the market.\(^1,2\) However, in process of smoking, undesirable carcinogenic substances, such as polycyclic aromatic hydrocarbons (PAHs), are produced as well.\(^3,4\)

The PAHs include a large group of organic compounds which consist of two or more fused aromatic rings. They represent a class of organic pollutants that are carcinogenic, mutagenic, and teratogenic.\(^5–7\) On the basis of the occurrence and carcinogenicity, United States Environmental Protection Agency (US-EPA) set 16 PAHs as priority pollutants. These compounds are naphthalene-Naph, acenaphthylene-Acy, acenaphthene-Ace, anthracene-Ant, fluorene-Fln, phenanthrene-Phe, fluoranthene-Flt, pyrene-Pyr, benz[a]anthracene-BaA, benzo[b]fluoranthene-BbF, benzo[k]fluoranthene-BkF, benzo[a]pyrene-BaP, chrysene-CHR, indeno[1,2,3-cd]pyrene-IcP, dibenz[a,h]anthracene-DhA, and benzo[ghi]perylene-BgP.\(^8\) Maximum contents of BaP and PAH4 (BaA, BbF, BaP, and CHR) in smoked meat products are set by European Commission Regulation (2 and 12 \( \mu \)g/kg, respectively).\(^9\)

There is a number of factors which are related to the smoking process and which affect the composition of smoke, and the concentration of PAH uptake in the products: technology of smoking, exposure of the edible parts to the smoke, the type of wood, and the combustion...
temperature as the most critical one.\textsuperscript{[4,10–12]} Also, important role in deposition and penetration of smoke components into smoked sausages has barriers i.e., casings (e.g., collagen casings, cellulose-peelable casings, natural, etc.).\textsuperscript{[13–15]} The fact that some reports\textsuperscript{[16–18]} indicated the possibility of higher levels of PAH concentrations in traditionally smoked foods increased interest in their control and quantification in this type of products.

One of the traditional smoked meat products is \textit{Petrovská klobása}, the dry fermented sausage that is produced in the Republic of Serbia. Because of its specific and recognizable quality, this product has been protected with a designation of origin according to the Serbian legislation. Production of these sausages in small-scale households usually implies usage of natural casing (pig large intestines), but collagen casing could be used as well.\textsuperscript{[2,12,19–21]} Thus, the objective of this study was to determine the effects of different casings (natural and collagen) on the content of 13 US-EPA priority PAH (Acy, Ant, Fln, Phe, Pyr, BaA, BbF, BkF, BaP, CHR, IcP, DhA, and BgP) in traditional dry fermented sausage—\textit{Petrovská klobása}.

\section*{Materials and methods}

\subsection*{Sausage preparation}

The sausages were produced from minced lean pork meat, pork fat, and seasonings (homemade red hot paprika powder, salt, caraway, crushed garlic, and sugar) in traditional manner.\textsuperscript{[12]} One half of the seasoned batter was stuffed in natural—\textit{N} (pig large intestines; 400–500 mm long; 45–55 mm in diameter) and the other in collagen—\textit{C} (500 mm long; 55 mm in diameter) casings. After the resting day, the sausages were smoked in traditional conditions (direct cold smoking methods). According to Serbian legislation, the required smoke temperature to achieve cold smoking conditions should be below 25°C.\textsuperscript{[22]} Smoke was produced by the combustion of sweet cherry wood, and the sausages came in direct contact with it, with maximum distance of 3 m between fire and the sausages. The temperature and humidity in the smokehouse were not controlled but were under the influence of outdoor conditions, i.e., temperature was from 1.8 to 15.6°C (average 8.69°C) and relative humidity was from 54.4% to 95.5% (average 74.4%). The full smoking process lasted about 10 days (7 × 5 h of smoking; with pauses on the first, third, and seventh day of production). The smoking process was followed by drying and the ripening processes which were conducted in order to reach moisture content in sausages lower than 35% (for about 60 days). After drying process, all sausage samples were stored under controlled conditions (temperature 10°C and relative humidity 75%) in industrial chamber, till the 270th day. Samples for the analyses were taken at 0 day of production (control—0), at the end of drying (N1 and C1), and the end of storage period (N2 and C2). All determinations were made in three samples.

\subsection*{PAH determination}

In this study, concentrations of four PAHs isomeric groups were determined; the low molecular weight PAHs with two or three benzene rings (Acy, Fln, Phe, and Ant), and the high molecular PAH with four (Pyr, BaA, CHR, BbF, and BkF as markers of the other four benzene ring-PAH of toxicological significance), five (BaP, IcP, and DhA), and six rings (while only BgP was determined as marker of other six benzenic ring-PAH). A standard mixture of 13 EPA PAH (Acy, Fln, Phe, Ant, Pyr, BaA, CHR, BbF, BkF, BaP, IcP, DhA, and BgP) in acetone (0.5 mg/ml) was purchased from AccuStandard Inc. (New Haven, USA). The analysis of 13 EPA PAHs was carried out on a GC 6890N gas chromatograph coupled to a MS 5975 mass spectrometer (Agilent, Palo Alto, CA, USA). PAH extraction method and chromatographic conditions were performed according to Škaljac et al.\textsuperscript{[12]} Briefly, 6 g of samples was mixed with water, acetonitrile, and MgSO\textsubscript{4} and NaCl in centrifuge tube. The tube was then centrifuged at 1056g for 15 min. Supernatant was transferred to a smaller tube containing MgSO\textsubscript{4}, PSA, and C18 (Restek Q373) and again centrifuged for 15 min at 1056g. Obtained supernatant was transferred to a glass vial, evaporated to dryness in a stream of nitrogen, reconstituted in acetone, and analyzed on Agilent HP-5 MS column. Sample was injected
on 250°C and the following temperature program was applied: isothermal at 70°C for 2 min, at 25°C/min to 150°C, at 3°C/min to 200°C, and at 8°C/min to 280°C. As a carrier gas, helium with a flow of 2.6 mL/min was used. The identification of PAHs was performed by mass spectrometry with quadrupole mass filter, working in the electron impact-positive ion mode.

Besides the demonstrated linearity of the method, as a part of quality assurance for the analytical measurement of PAHs in the smoked meat, the limits of detection (LOD) and the recovery experiments as well as recovery experiments were carried out. LOD were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3, evaluated on the basis of noise obtained with the analysis of the blank samples. The LOD of the PAHs was 0.6 μg/kg for BgP; 0.5 μg/kg for DhA; 0.4 μg/kg for Ant, Pyr, IcP; and 0.3 μg/kg for all other PAH. The PAH recoveries were calculated as differences in PAHs content in spiked and unspiked samples relative to the spiked level. The recoveries were from 62.3% to 101.0% for the PAH components. Detection limits (LOD) and recovery method meet the criteria set by the Regulation European Commission.[23]

**Determination of nutritional quality**

Content of moisture, fat, and ash in Petrovská klobása samples was determined according to methods recommended by the International Organization for Standardization,[24–26] respectively.

**Statistical analysis**

Results are presented as mean value $(N = 3) \pm$ standard deviation. The value was analyzed statistically with one-way ANOVA. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using Statistical software version 12.[27]

**Results and discussion**

Tables 1 and 2 present results of the content of 13 EPA priority PAHs, as well as nutritional quality characteristics of Petrovská klobása determined in raw sausage mixture, at the end of drying and at the end of storage period, are presented. The PAHs determined in raw sausage mixture before smoking (0 day of production) were Acy (2.6 μg/kg; i.e., 5.6 μg/kg dry matter), Fln (2.1 μg/kg; i.e., 4.6 μg/kg dry matter), Phe (4.9 μg/kg; i.e., 10.7 μg/kg dry matter), while other investigated PAHs were below the limit of detection. These findings were consistent with the results of Roseiro, Gomes, and Santos[28], who reported that in raw sausage mixture, over 99% of the total PAH were light PAH (Naph, Ace, Ant, Fln, Phe). Contents of Phe and Fln were lower, and content of Ant was higher than that found by Roseiro et al.[28] (46.54 μg/kg dry matter, 53.53 μg/kg dry matter, nd, respectively) in raw sausage mixture of Portuguese traditional sausage, while the contents of these compounds were similar with the ones determined in meat sausages by Mottier, Parisod, and Turesky[29] (6.95, 0.98, 0.76 μg/kg, respectively). On the other hand, Martorell et al.[30] reported lower value of Σ16 US-EPA PAHs (1.25 μg/kg), comparing to values obtained in present study.

The PAHs determined in sausages at the end of drying period (60th day of production) were Acy, Ant, Fln, Phe, and Pyr, while other investigated PAHs were below the limit of detection (Table 1). At the end of the drying period, the PAH contents in sausage samples with natural and collagen casings were higher than those determined in raw sausage mixture. The most abundant PAH in sausage samples was Phe, which was in accordance with the results for Spanish, Portuguese, and Italian smoked sausages.[1,28,31–33] Content of Phe was 19.1 μg/kg for C1 and 39.9 μg/kg for N1, with significant difference $(P < 0.05)$ between different types of casings. Also, contents of Acy, Fln, Ant, and Pyr were significantly $(P < 0.05)$ lower in sausages with collagen casing (10.1, 11.5, 12.8, 2.7 μg/kg, respectively) than in the sausages with natural casing (34.9, 38.0, 16.0, 8.3 μg/kg, respectively). Although the sausages with natural casing had significantly $(P < 0.05)$ lower content of moisture (N1—32.5%; C1—33.9%) and higher $(P < 0.05)$ content of total fat (N1—34.3%; C1—32.7%) than sausages with collagen casing, the observed differences in the
The content of PAH compounds couldn’t be attributed to this fact. Results of the 13 US-EPA PAH (Table 1) calculated on the dry matter basis show a big difference between sausages with natural (N1 203.1 µg/kg dry matter) and collagen (C1 85.0 µg/kg dry matter) casing.

The content of PAH increased during storage because the PAH from casings penetrate into the sausages, and their concentration is stabilized after some time.\(^{4,6,12}\) On the contrary, during storage, a decrease in PAH content is expectable, caused by light decomposition and interaction with other components of the formulation.\(^{34,35}\) Thus, in this study, PAH contents at the end of storage period were determined in order to examine safety of Petrovská klobása during the shelf life.

The PAHs determined in both analyzed sausage groups at the end of storage period (270th day of production) were Acy, Ant, Fln, Phe, and Pyr. Other investigated PAHs were below the limit of detection (Table 1). At the end of storage period, contents of PAH were significantly (\(P < 0.05\)) higher in both groups of sausages compared with their counterparts at the end of drying process (Table 1). This phenomenon was mainly caused by further drying of sausages, which resulted in lower content of moisture and higher content of total fat in samples from both examined groups. The content of moisture at the end of storage period ranged from 16.8% (C2) to 17.2% (N2), while the content of total fat was in the range from 42.6% (N2) to 44.9% (C2) (Table 2). Further, the results of the 13 US-EPA PAH calculated on the dry matter basis (Table 1) confirmed that there was no additional contamination by PAH compounds in the sausages with collagen casings during storage period (C1—85.0 µg/kg dry matter; C2—88.5 µg/kg dry matter).

### Table 1. Content of polycyclic aromatic hydrocarbons (µg/kg) in dry fermented sausage Petrovská klobása.

| Polycyclic aromatic hydrocarbons (µg/kg) | 0 day of production | End of drying period | End of storage period |
|-----------------------------------------|---------------------|----------------------|-----------------------|
|                                         | O       | N1  | C1  | N2  | C2  |
| Acenaphthylen                            | 2.6±0.4 | 34.9±3.4 | 10.1±0.8 | 42.9±1.4 | 22.3±1.3 |
| Anthracene                               | <0.4    | 16.0±1.4 | 12.8±1.1 | 51.4±1.8 | 6.0±0.8  |
| Fluorene                                 | 2.1±0.3 | 38.0±0.8 | 11.5±0.1 | 48.0±4.0 | 16.2±1.7 |
| Phenanthrene                              | 4.9±0.5 | 39.9±0.5 | 19.1±1.8 | 57.8±5.8 | 22.5±1.6 |
| Pyrene                                   | <0.4    | 8.3±1.1 | 2.7±1.2  | 6.1±0.2  | 6.6±0.7  |
| Benz[a]anthracene                        | <0.3    | <0.3 | <0.3 | <0.3 |
| Benzo[b]fluoranthene                     | <0.3    | <0.3 | <0.3 | <0.3 |
| Benzo[k]fluoranthene                     | <0.3    | <0.3 | <0.3 | <0.3 |
| Benzo[a]pyrene                           | <0.3    | <0.3 | <0.3 | <0.3 |
| Chrysene                                 | <0.3    | <0.3 | <0.3 | <0.3 |
| Indeno[1,2,3-cd]pyrene                   | <0.4    | <0.4 | <0.4 | <0.4 |
| Dibenzo[a,h]anthracene                   | <0.5    | <0.5 | <0.5 | <0.5 |
| Benzo[ghi]perylenel                      | <0.6    | <0.6 | <0.6 | <0.6 |
| ΣEU PAH\(^{a}\)                          | nd      | nd  | nd  | nd  |
| Σ6 IARC PAH\(^{b}\)                      | nd      | nd  | nd  | nd  |
| Σ7 US-EPA PAH\(^{c}\)                    | nd      | nd  | nd  | nd  |
| Σ13 US-EPA PAH (µg/kg dry matter)        | 21.0    | 203.1 | 85.0 | 249.0 | 88.5 |

nd: Content of PAH < LOD detected; only concentrations of PAH > LOD were used for statistical analysis.

In the same row, different letters mean that values are significantly different (\(P < 0.05\)).

Results are expressed as means ± standard deviations (\(N = 3\)).

\(^{a}\)BaA, BbF, BaP, and CHR; \(^{b}\)BaA, BbF, BkF, BaP, IcP, and DhA; \(^{c}\)BaA, BbF, BkF, BaP, CHR, IcP, and DhA.

### Table 2. The content of moisture, total fat, and total ash (%) in dry fermented sausage Petrovská klobása.

| Characteristics of nutritional quality (%) | 0 day of production | End of drying period | End of storage period |
|-------------------------------------------|---------------------|----------------------|-----------------------|
|                                           | O       | N1  | C1  | N2  | C2  |
| Moisture                                  | 54.3±0.1 | 32.5±0.2 | 33.9±0.2 | 17.2±0.2 | 16.8±0.1 |
| Total fat                                 | 23.4±0.5 | 34.3±0.7 | 32.7±0.3 | 42.6±0.1 | 44.9±0.1 |
| Total ash                                 | 2.5±0.1  | 3.9±0.1  | 3.8±0.1  | 4.9±0.2  | 4.9±0.2  |

In the same row, different letters means that values are significantly different (\(P < 0.05\)).

Results are expressed as means ± standard deviations (\(N = 3\)).
On the other hand, during storage period, there was an increase of the 13 US-EPA PAHs in the sausages with natural casings (N1—203.1 μg/kg dry matter; N2—249.0 μg/kg dry matter). This difference could be attributed to physical characteristics of these types of casings, considering that storage conditions of both groups of sausages were the same.

At the end of storage period, contents of Acy, Ant, Fln, and Phe were significantly (P < 0.05) lower in sausages with collagen casing (22.3, 6.0, 16.2, 22.5 μg/kg, respectively) than in the sausages with natural casing (42.9, 51.4, 48.0, 57.8 μg/kg, respectively). García-Falcón and Simal-Gándara[13] investigated PAH contents in sausages with tripe and collagen casings and suggested that the collagen-based casing behaved as a better barrier to PAH. A number of studies have recently continued this research.[14,15] Gomes et al.[14] studied the effect of casing type, smoking procedures, and fat content on the PAH content in Portuguese dried fermented sausages. Results of these authors indicated significant difference in PAH content regarding the type of casing, showing that the use of synthetic casings instead of natural ones contributed reduction of PAH content in smoked sausages. Results of our study were consistent with these findings, suggesting that the collagen casing can help reducing the PAH levels in dry fermented sausages. This effect is probably caused by differences in the physical properties of these casings.[36] Average pore diameter (599.8 nm) and high porosity (66.8%) of natural casings allow the fat to flow through the casing and cover its external surface, making it moist and sticky. These casing's characteristics combined with product's wrinkled surface provide that soot particles are easily captured and adhered to sausage surface. On the contrary, markedly lower average pore diameter (48.2 nm) and porosity (16.6%) of synthetic casings do not allow the fat to flow through the casing. In that way, the surface of synthetic casing is nonsticky, it is dry and smooth, and showing no affinity for soot particles.[36] Therefore, the higher content of PAH determined in sausages stuffed in natural casings could be the consequence of higher penetration of PAH, accumulated at the surface of the product.

The values for PAHs with three and four rings determined in Petrovská klobása with natural and collagens casings, at the end of drying as well as at the end of storage period, were lower than those reported for Portuguese smoked sausages,[14,28,33] higher than those obtained for Spanish smoked sausages,[1] and similar with Italian smoked sausages.[32]

The BaP as well as PAH4 (BaA, BbF, BaP, and CHR) maximum contents of which in smoked meat products are set by European Commission Regulation (2 and 12 μg/kg, respectively),[9] were below the limit of detection in all examined samples of Petrovská klobása (Table 1).

Further, United States Environmental Protection Agency has classified seven PAHs (7 US-EPA PAH) (BaA, BbF, BkF, BaP, CHR, IcP, and DhA) as a suitable indicator for the occurrence of PAHs in food.[37] On the other hand, the International Agency for Research on Cancer established the BaA, BaP, BbF, BkF, IcP, and DhA (6 IARC PAH) as possible human carcinogens.[38] As it can be seen from the results (Table 1), the 6 IARC PAH as well as 7 US-EPA PAH were not detected in analyzed sausages, at the end of drying nor at the end of storage period.

Generally, results of this study show that at the end of drying, and specially at the end of storage period, total content of 13 US-EPA PAHs was significantly (P < 0.05) higher in sausages stuffed in natural casing (137.1 and 206.2 μg/kg, respectively) than in those stuffed in collagen casing (56.2 and 73.6 μg/kg, respectively). The results obtained in this study (Table 1) indicated safety of Petrovská klobása from PAH content point of view and showed that casing type is a variable that has an important effect on their content in smoked sausages. Thus, casings should be considered in sausages manufacturing in order to obtain healthier products.

**Conclusion**

The Acy, Ant, Fln, Phe, and Pyr were determined in both sausages with natural and collagen casings, while contents of BaA, BbF, BkF, BaP, CHR, IcP, DhA, and BgP were below the limit of detection. The overall results indicated that the total content of 13 US-EPA PAHs was significantly (P < 0.05)
lower in sausages with collagen casing than in sausages with natural ones in both examined periods. The BaP and PAH4 (BaA, BbF, BaP, and CHR) were below limit of detection in all examined samples of Petrovská klobása with natural, as well as with collagen casings.

Funding

This research was financially supported by the Ministry of Education, Science and Technology, Republic of Serbia, project TR31032 and ON172050.

References

1. Lorenzo, J. M.; Purrinos, L.; García-Falcón, M. C.; Franco, D. Polycyclic Aromatc Hydrocarbons (Pahs) in Two Spanish Traditional Smoked Sausage Varieties: “Androlla” and “Botillo”. Meat Science 2010, 86, 660–664. DOI: 10.1016/j.meatsci.2010.05.032.

2. Ikonicić, P.; Jokanović, M.; Petrović, L.; Tasić, T.; Škaljac, S.; Šojić, N.; Tomović, V.; Tomić, J.; Danilović, B.; et al. Effect of Starter Culture Addition and Processing Method on Proteolysis and Texture Profile of Traditional Dry-Fermented Sausage Petrovská Klobása. International Journal of Food Properties 2016, 19(9), 1924–1937. DOI: 10.1080/10942912.2015.1089280.

3. Babaoglu, A. S.; Karakaya, M.; Ož, F. Formation of Polycyclic Aromatic Hydrocarbons in Beef and Lamb Kokorec: Effects of Different Animal Fats. International Journal of Food Properties 2017, 20(9), 1960–1970. DOI: 10.1080/10942912.2016.1225761.

4. Ledesma, E.; Rendueles, M.; Díaz, M. Contamination of Meat Products during Smoking by Polycyclic Aromatic Hydrocarbons: Processes and Prevention. Food Control 2016, 60, 64–87. DOI: 10.1016/j.foodcont.2015.07.016.

5. Janoszka, B.; Warzecha, L.; Blaszczzyk, U.; Bodzek, D. Organic Compounds Formed in Thermally Treated High-Protein Food. Part I: Polycyclic Aromatic Hydrocarbons. Acta Chromatica 2004, 14, 115–128.

6. Šimko, P.; Factors Affecting Elimination of Polycyclic Aromatic Hydrocarbons in Smoked Meat Foods and Liquid Smoke Flavourings. Molecular Nutrition & Food Research 2005, 49, 637–647. DOI: 10.1002/mnfr.200400091.

7. Yoon, E.; Park, K.; Lee, H.; Yang, J. H.; Lee, C. Estimation of Excess Cancer Risk on Time-Weighted Lifetime Daily Intake of PAHs from Food Ingestion. Human and Ecological Risk Assessment 2007, 13, 669–680. DOI: 10.1080/10807030701226871.

8. US EPA. Office of the Federal Registration (OFR). Appendix A: Priority Pollutants: Public Data Release, Office of Environmental Information United States Environmental Protection Agency - Federal Registration: Washington, DC, 1982; 47, 52309.

9. European Commission Regulation No. 835/2011 of 19 August 2011 Amending Regulation (EC) No. 1881/2006 as Regards Maximum Levels for Polycyclic Aromatic Hydrocarbons in Foodstuffs. Official Journal of the European Union 2011, 215, 4–8.

10. Dueđahl-Olesen, L.; White, S.; Binderup, M. L. Polycyclic Aromatic Hydrocarbons (PAH) in Danish Smoked Fish and Meat Products. Polycyclic Aromatic Compounds 2006, 26, 163–184. DOI: 10.1016/j.footfood.2006.070527.

11. Hitzel, A.; Pöhlmann, M.; Schwägele, F.; Speer, K.; Jira, W. Polycyclic Aromatic Hydrocarbons (PAH) and Phenolic Substances in Meat Products Smoked with Different Types of Wood and Smoking Spices. Food Chemistry 2013, 139, 955–962. DOI: 10.1016/j.foodchem.2013.02.011.

12. Škaljac, S.; Petrović, L.; Tasić, T.; Ikonicić, P.; Jokanović, M.; Tomović, V.; Džinić, N.; Šojić, B.; Tjapkin, A.; Škrbić, B. Influence of Smoking in Traditional and Industrial Conditions on Polycyclic Aromatic Hydrocarbons Content in Dry Fermented Sausages (Petrovská Klobása) from Serbia. Food Control 2014, 40(1), 12–18. DOI: 10.1016/j.foodcont.2013.11.024.

13. García-Falcón, M. S.; Simal-Gándara, J. Polycyclic Aromatic Hydrocarbons in Smoke from Different Woods and Their Transfer during Traditional Smoking into Chorizo Sausages with Collagen and Tripe Casings. Food Additives and Contaminants 2005, 22, 1–8. DOI: 10.1080/0265203040023119.

14. Gomes, A.; Santos, C.; Almeida, J.; Elias, M.; Roseiro, L. C. Effect of Fat Content, Casing Type and Smoking Procedures on PAH Contents of Portuguese Traditional Dry Fermented Sausages. Food and Chemical Toxicology 2013, 58, 369–374. DOI: 10.1016/j.fct.2013.05.015.

15. Pöhlmann, M.; Hitzel, A.; Schwägele, F.; Speer, K.; Jira, W. Polycyclic Aromatic Hydrocarbons and Phenolic Substances in Smoked Frankfurter-Type Sausages Depending on Type of Casing and Fat Content. Food Control 2013, 31, 136–144. DOI: 10.1016/j.foodcont.2012.09.030.

16. CAC/RCP 68/2009 Codex Alimentarius Commission (CAC). Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes. http://www.codexalimentarius.org/download/standards/11257/CXP_068e.pdf, 2009, (accessed July 8, 2015).
17. European Commission Regulation No 1327/2014 of 12 December 2014 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Polycyclic Aromatic Hydrocarbons (Pahs) in Traditionally Smoked Meat and Meat Products and Traditionally Smoked Fish and Fishery Products. Official Journal of the European Union 2014, 358, 13–14.

18. European Commission, Health and Consumer Protection Directorate-General, Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Risk to Human. Health of Polycyclic Aromatic Hydrocarbons-Occurrence in Food, Brussels, Belgium. SCF/CS/CNTM/PAH/29 CAC/RCP 68/2009 2002.

19. Ikonić, P.; Petrović, L.; Tasić, T.; Đinić, N.; Jokanović, M.; Tomović, V. Physicochemical, Biochemical and Sensory Properties for the Characterization of Petrovská Klobása (Traditional Fermented Sausage). Acta Periodica Technologica 2010, 41, 19–31. DOI: 10.2298/AP1041019I.

20. Petrović, L.; Đinić, N.; Tomović, V.; Ikonić, P.; Tasić, T. Code of Practice E Registered Geographical Indication Petrovská Klobása; Intellectual Property Office: Republic of Serbia, Decision No. 9652/06 G-03/06 2007.

21. Tasić, T.; Ikonić, P.; Mandić, A.; Jokanović, M.; Tomović, V.; Savatić, S.; Petrović, L. Biogenic Amines Content in Traditional Dry Fermented Sausage Petrovská Klobása as Possible Indicator of Good Manufacturing Practice. Food Control 2012, 23, 107–112. DOI: 10.1016/j.foodcont.2011.06.019.

22. Serbian Regulation. Rulebook on Quality of Meat Products. Official Gazette of the Republic of Serbia 2015, 94, 1–39.

23. European Commission Regulation No 836/2011 of 19 August 2011 Amending Regulation (EC) No. 333/2007 Laying down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD and Benzo[A]Pyrene in Foodstuffs. Official Journal of the European Union 2011, 215, 9–16.

24. ISO 1442. Meat and Meat Products. Determination of Moisture Content (Reference Method); International Organization for Standardization, Geneva, 1997.

25. ISO 1443. Meat and Meat Products. Determination of Total Fat Content; International Organization for Standardization, Geneva, 1973.

26. ISO 936. Meat and Meat Products. Determination of Total Ash; International Organization for Standardization, Geneva, 1998.

27. StatSoft, Inc. STATISTICA (Data Analysis Software System), Version 12. http://www.statsoft.com/ 2013.

28. Roseiro, L. C.; Gomes, A.; Santos, C. Influence of Processing in the Prevalence of Polycyclic Aromatic Hydrocarbons in a Portuguese Traditional Meat Product. Food and Chemical Toxicology 2011, 49, 1340–1345. DOI: 10.1016/j.fct.2011.03.017.

29. Mottier, P.; Parisod, V.; Turesky, R. J. Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry. Journal of Agricultural and Food Chemistry 2000, 48, 1160–1166. DOI: 10.1021/jf991205y.

30. Martorell, I.; Perelló, G.; Martí-Cid, R.; Castell, V.; Llobet, J. M.; Domingo, J. L. Polycyclic Aromatic Hydrocarbons (PAH) in Foods and Estimated PAH Intake by the Population of Catalonia, Spain: Temporal Trend. Environment International 2010, 36, 424–432. DOI: 10.1016/j.envint.2010.03.003.

31. Lorenzo, J. M.; Purrinos, L.; Bermúdez, R.; Cobas, N.; Figueiredo, M.; García-Falcón, M. C. Polycyclic Aromatic Hydrocarbons (Pahs) in Two Spanish Traditional Smoked Sausage Varieties: “Chorizo Gallego” and “Chorizo De Cebolla”. Meat Science 2011, 89, 105–109. DOI: 10.1016/j.meatsci.2011.03.017.

32. Purcaro, G.; Moret, S.; Conte, L. S. Optimisation of Microwave Assisted Extraction (MAE) for Polycyclic Aromatic Hydrocarbon (PAH) Determination in Smoked Meat. Meat Science 2009, 81, 275–280. DOI: 10.1016/j.meatsci.2008.08.002.

33. Santos, C.; Gomes, A.; Roseiro, L. C. Polycyclic Aromatic Hydrocarbons Incidence in Portuguese Traditional Smoked Meat Products. Food and Chemical Toxicology 2011, 49, 2343–2347. DOI: 10.1016/j.fct.2011.06.036.

34. Bartkiene, E.; Bartkevics, V.; Mozuriene, E.; Krungleviciute, V.; Novoslavskij, A.; Santini, A.; Rozentale, I.; Juodeikiene, G.; Cizeikiene, D. The Impact of Lactic Acid Bacteria with Antimicrobial Properties on Sensory Properties for the Characterization of Petrovská Klobása (Traditional Fermented Sausage). Acta Periodica Technologica 2010, 41, 19–31. DOI: 10.2298/AP1041019I.