

INFLUENCE OF TRADITIONAL SMOKING ON CONTENT POLYCYCLIC AROMATIC HYDROCARBONS IN DRY FERMENTED SAUSAGES WITH COLLAGEN AND NATURAL CASINGS

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Abstract – The concentrations of 13 polycyclic aromatic hydrocarbons from Environmental Protection Agency list (EPA PAHs) were examined in two groups of fermented dry sausages (*Petrovska klobása*) smoked on traditional way. The sausage mixture from the first group of samples was stuffed in the natural casings (N), while the second was stuffed in collagen casings (C). Analyses of PAHs were determined using gas chromatography with mass spectrometry. Content of pyrene, anthracene, acenaphthylene, fluorene and phenanthrene was significantly ($P<0.05$) lower in sausage with collagen casings (2.7; 12.8; 10.1; 11.5; 19.1, $\mu\text{g}/\text{kg}$ respectively) than in the sausage with natural casings (8.3; 16.0; 34.9; 38.0; 39.9 $\mu\text{g}/\text{kg}$ respectively). Other examined PAHs were below the limit of detection in all the samples. According to the results the maximum acceptable content established by the EU of $5\mu\text{g}/\text{kg}$ for BaP in smoked meat products was not exceeded in any samples of *Petrovska klobása*. Results obtained in this study indicated that total content of 13 EPA PAHs was significantly ($P<0.05$) lower in sausage with collagen casings ($56.2\mu\text{g}/\text{kg}$) than in the sausage with natural casings ($137.1\mu\text{g}/\text{kg}$).

Key Words – *Petrovska klobása*, Traditional smoking, US-EPA PAHs

I. INTRODUCTION

Petrovska klobása, which is traditionally produced in Bački Petrovac, is fermented dry sausage that is highly valued product in Serbia and in neighboring countries. It is produced in the traditional way without additives and starter cultures [1, 2]. This product is protected in Serbia with designation of origin (PDO) according to Serbian legislation because of its specific and distinctive quality [3]. Smoking is one of the oldest processing methods applied in food preservation by the action of

antimicrobial constituents such as phenols. It is still widely used in Serbian traditional dry fermented sausages production (*Petrovska klobása*) to obtain peculiar sensorial characteristics due to phenol derivatives, carbonyls, organic acids. Wood smoke contains a large number of polycyclic aromatic hydrocarbons (PAH) and their alkylated derivatives [4]. They may be regarded as potentially genotoxic and carcinogenic to humans [5] due to a metabolic activation in mammalian cell to dioepoxides causing errors in DNA replication and mutation, which initiates the carcinogenic process [6, 7].

The United States Environmental Protection Agency (7US-EPA PAHs) has classified seven PAHs (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene and indeno[1,2,3-c,d] pyrene) as Group B2, probable human carcinogens [7]. In turn, the International Agency for Research on Cancer (6IARC PAHs) established that benzo[a]anthracene and benzo[a]pyrene are probable human carcinogens, while benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene and indeno[1,2,3-c,d] pyrene are possible human carcinogens [8]. Among PAHs, the benzo[a]pyrene (BaP) is used as a marker for the occurrence and effect of carcinogenic PAHs in food. According to EU legislation, a limit of $5.0\mu\text{g}/\text{kg}$ for smoked meat products has been agreed [9].

A number of factors related to the smoking process affect the composition of smoke as well as the PAHs uptake in the products, with the combustion temperature is critical [10]. Also, deposition and penetration of smoke components into smoked food depends on presence of barriers

such as casings (e.g. natural, collagen casings, cellulose-peelable casings) [11, 12].

Thus, the main objective of this study was to determine the content of 13 EPA priority PAHs (acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benzo[a]anthracene, chrysene, benzo [b] fluoranthene, benzo [k] flouranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo [g,h,i]perylene, and indeno[1,2,3-cd]pyrene) in dry fermented sausage *Petrovská klobása* smoked in the traditional (direct smoking) way. The specific aim was to verify can the collagen and natural sausages casings affect the levels of PAHs in the *Petrovská klobása*.

II. MATERIALS AND METHODS

A. Material

The materials used in this study were samples of the dry fermented sausages (*Petrovská klobása*). The basic formulation of sausage mixture (*Petrovská klobása*) was: lean pork meat (85%) and solid fat pork tissue of the back and neck (15%). Other ingredients added per kg of pork meat and fat were: red hot spice pepper powder, salt, garlic, cumin, sugar, and then the mixture of sausages were mixed manually. Sausage mixture was immediately stuffed in natural casings – N (pig colon 400-500 mm long and 45-55 mm in diameter) collagen casings - C (500 mm long and about 55 mm in diameter). Production the mixture of sausages and smoking were realised in a registered craft object in Bački Petrovac. Smoking was done on traditional way. In the smokehouse the smoke was produced by the combustion of sweet cherry wood, with the distance of 3 m between fire and sausages, which came in direct contact with the smoke (direct smoking). 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 16.6°C, and the humidity was from 54.4 to 95.5%. The full smoking process lasted about 10 days with pause. Samples for the analyses were taken at the end of drying process at 60th day. Samples were homogenized and stored in dark at – 20 °C until analyzed. All determinations were made in three samples from each batch in duplicate.

B. Method

The samples were extracted and cleaned up using the QuEChERS method (quick easy cheap effective rugged and safe): 6 g of ground homogenized sample was placed in a 50 ml centrifuge tube, 12 ml of water and 15 ml of acetonitrile was added and the tube was vigorously shaken, followed by the addition of salts (6 g of MgSO₄ and 1.5 g of NaCl) and agitation for another 5 minutes. The tube was then centrifuged at 3000 rpm for 15 minutes (Eppendorf 5430 centrifuge); 8 ml of supernatant was transferred to a 15 ml tube containing 900 mg of MgSO₄, 150 mg of PSA and 150mg of C18 (Restek Q373). The tube was shaken for 5 minutes and then centrifuged for 15 min at 3000 rpm. 6 ml of supernatant was transferred to a glass vial, evaporated to dryness in a stream of nitrogen, reconstituted in 0.2 ml of acetone and analyzed.

The analysis of PAHs was carried out on a GC 6890N gas chromatograph coupled to a MS 5975 mass spectrometer (Agilent, Palo Alto, CA, USA). The separation of PAHs was performed on Agilent HP-5 MS column (30 m x 0,25 mm x 0,25 µm), injection temperature was 250 °C, injection volume 5 µL (splitless). Helium with a flow of 2.6 mL/min was used as carrier gas. The following temperature programme 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. The identification of PAHs was performed by mass spectrometry with quadruple mass filter, working in the electron impact (EI) positive ion mode. The temperatures of the source and the transfer line were 230°C and 280 °C, respectively. Temperature of the quadruple was 150 °C. Acquired data were analyzed using Extracted Ion Chromatogram (EIC) from the total ion chromatogram (TIC) in SIM and SCAN mode.

Detection limits of the PAHs were 0.6µg/g for benzo[g,h,i]perylene, 0.5µg/g for dibenzo[a,h]anthracene, 0.4µg/g for anthracene, pyrene, chrysene and indeno[1,2,3-cd]pyrene and 0.3µg/g for all other PAHs.

One way, Post-hoc (Duncan test) was performed using the software package Statistica 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA. Differences were considered significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

Content of thirteen EPA polycyclic aromatic hydrocarbons in traditional dry fermented sausage *Petrovská klobása* was determined using GS-MS method for analysis. The results of content 13 US-EPA priority PAHs in dry fermented sausage with *Petrovská klobása* with natural pig colon (N) and collagen (C) at the end of drying period are given in Table 1.

Content of pyrene, anthracene, acenaphthylene, fluorene and phenanthrene was significantly ($P < 0.05$) lower in sausage with collagen casings (2.7; 12.8; 10.1; 11.5; 19.1, respectively) than in the sausage with natural casings (8.3; 16.0; 34.9; 38.0; 39.9, respectively). Our results are in accordance with the results reported by other authors for sausages with natural and collagen casings [11, 12].

The values for PAH with three and four ring determined in *Petrovská sausages* were clearly lower than those reported for Portuguese smoked sausages [13], but these values were higher than those obtained for Spanish smoked sausages [14, 15] and similar with content determined in Italian smoked sausages [16].

The content of the smoke compounds present in the smoked products depends on the nature of the wood (wood species) that burns to generate the smoke and on the temperature of burning [10,17]. The smoking process has been carried out in a traditional smokehouse, in a traditional way, where it is not possible to control the conditions of the process. Because of that content of PAHs in traditional sausages can be very different.

Benzo[a]pyrene content was below the limit of detection in all the samples. The maximum acceptable content established by the EU [9] of $5\mu\text{g}/\text{kg}$ for BaP in smoked meat products was not exceeded in any samples of *Petrovská klobása*.

New scientific opinion adopted by the European Food Safety Authority concluded that BaP alone is not a suitable indicator for the occurrence and toxicity of PAHs in food and that the eight specified PAHs (PAH8) including BaP, BaA, BbF, BkF BgP, CHR, DhA and IcP, for which oral carcinogenicity data are available, and/or a subgroup of these, PAH4, including BaP, BaA, BbF and CHR are more suitable markers [18]. The concentrations of priority PAH8, PAH4, 7US-EPA PAHs and 6IARC PAHs were below the limit of detection in all samples of sausages at the end of drying.

Table 1 Content of polycyclic aromatic hydrocarbons ($\mu\text{g}/\text{kg}$) in dry fermented sausage with natural (N) and collagen (C) casings at end of drying period

Polycyclic aromatic hydrocarbons ($\mu\text{g}/\text{kg}$)		Type of casings	
		Natural N	Collagen C
Acenaphthylene	Acy	34.9 ^b	10.1 ^a
Fluorene	Fln	38.0 ^b	11.5 ^a
Phenanthrene	Phe	39.9 ^b	19.1 ^a
Anthracene	Ant	16.0 ^b	12.8 ^a
Pyrene	Pyr	8.3 ^b	2.7 ^a
Benzo[a]anthracene	BaA	nd	nd
Chrysene	CHR	nd	nd
Benzo[b]fluoranthene	BbF	nd	nd
Benzo[k]fluoranthene	BkF	nd	nd
Benzo[a]pyrene	BaP	nd	nd
Indeno[1,2,3-cd]pyrene	IcP	nd	nd
Dibenzo[a,h]anthracene	DhA	nd	nd
Benzo[g,h,i]perylene	BgP	nd	nd
Σ 13 US-EPA PAHs		137.1 ^b	56.2 ^a

Different letters in the rows ^{a,b} $P < 0.05$;

nd – not detected

The total levels of 13 EPA PAHs was significantly ($P < 0.05$) lower in sausage with collagen casings ($56.2\mu\text{g}/\text{kg}$) than in the sausage with natural casings ($137.1\mu\text{g}/\text{kg}$). This fact suggests that the collagen casings behave as a better barrier to PAHs especial with the low molecular weight.

IV. CONCLUSION

Acenaphthylene, fluorene, anthracene, pyrene and phenanthrene were determined in all analyzed samples, while contents of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1, 2, 3-cd]pyrene

were below the limit of detection in all the samples.

Total content of 13 EPA PAHs was significantly ($P < 0.05$) lower in sausage with collagen casings (56.2 $\mu\text{g}/\text{kg}$) than in the sausage with natural casings (137.1 $\mu\text{g}/\text{kg}$).

According to the results in this study, it could be concluded, that benzo[a]pyrene is not good marker for the total content of 13 EPA PAHs that were examined in this study especial for low molecular weight PAHs, but it is a good marker of 7 US EPA probably carcinogenic PAHs in *Petrovska klobasa* samples.

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