The influence of smoking in traditional conditions on content of polycyclic aromatic hydrocarbons in Petrovská klobása

S Škaljac1, Lj Petrović1, M Jokanović1, V Tomović1, T Tasić2, M Ivić1, B Šojić1, P Ikonić2 and N Džinić1

1 Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia
2 Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia

Abstract. The aim of this study was to determine the content of 13 polycyclic aromatic hydrocarbons (acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[g,h,i]perylene) from the Environmental Protection Agency list (US-EPA PAH) in traditional dry fermented sausage Petrovská klobása. Sausages were smoked in traditional conditions and samples for analyses were taken on day 0 of production (0), at the end of drying (T1) and at the end of the storage period (T2). The highest total content of 13 US-EPA PAHs was determined in sausages at the end of the storage period (73.5 μg/kg). Phenanthrene was the most abundant of the PAHs in all examined sausage samples (0-4.90 μg/kg; T1-18.0 μg/kg and T2-26.3 μg/kg). Benzo[a]pyrene, with a maximum allowed content in smoked meat products of 2 μg/kg (EC No. 835/2011), was below the limit of detection in all examined samples. Also, PAH4 (the total content of benz[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) with a maximum allowed content in smoked meat products of 12 μg/kg (EC No. 835/2011), was below the limit of detection in all examined sausage samples. According to the results obtained in this study, and in regard to the European regulation on PAHs content, the dry fermented sausage Petrovská klobása, smoked in traditional conditions, was safe for consumers.

1. Introduction

Petrovská klobása is traditional dry fermented sausage from Bački Petrovac in the north of Serbia. It is produced in the traditional manner without additives and starter cultures. This product is protected with designation of origin (PDO) according to Serbian legislation because of its specific and distinctive quality. Its intense red colour is one of the essential features which differentiate it from other products of the same type. This colour is formed thanks to the use of red paprika and a unique smoking process (usage of specific types of wood, a mixture of cherry and apricot) [1, 2].

The traditional smoking process gives a special colour, taste and aroma to Petrovská klobása [2], but incomplete wood combustion during this process can produce considerable amounts of polycyclic aromatic hydrocarbons – PAHs. PAHs are a group of organic compounds, consisting of two or more
condensed aromatic rings, and which may be regarded as potentially genotoxic and carcinogenic to humans [3, 4, 5]. The PAH content in smoked meat products depends on meat products characteristics and factors related to the smoking process such as direct (traditional conditions) or indirect methods (industrial conditions) [6, 7]. Reports which show that concentrations of PAH in traditional smoked meat products can reach high levels have, in recent years, prompted considerable interest in PAH quantification and control [2, 8, 9].

Thus, the aim of this study was to determine the effects of smoking in traditional conditions on the content of 13 PAHs (acenaphthylene - Acy, fluorine - Fln, phenanthrene - Phe, anthracene - Ant, pyrene - Pyr, benz[a]anthracene – BaA, chrysene - CHR, benzo[b]fluoranthene - BbF, benzo[k]fluoranthene - BkF, benzo[a]pyrene - BaP, indeno[1,2,3-cd]pyrene - IcP, dibenz[a,h]anthracene - DhA and benzo[ghi]perylene – BgP) from the Environmental Protection Agency list (US-EPA PAHs) in Petrovská klobása.

2. Materials and Methods

2.1. Sausage production
The materials used in this study were dry fermented sausages (Petrovská klobása). The basic formulation of sausage mixture for Petrovská klobása was lean pork meat (80%) and pork fat (20 %). Other ingredients were added to the pork meat and fat – red hot paprika powder, salt, garlic, caraway, sugar – and then the mixture was mixed mechanically. Raw sausage mixture was stuffed into collagen casings (500 mm long; 55 mm in diameter). After a rest day, sausages were smoked in traditional conditions.

During smoking in traditional conditions, smoke was produced by the combustion of sweet cherry and apricot wood. Sausages came in direct contact with the smoke (direct smoking), with distance of 3 m between fire and sausages. The smoking process lasted for 10 days, with pauses. Temperature and humidity were not controlled, but were influenced by outdoor conditions. 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 smoking process was followed by drying and ripening, which were conducted in order to reach a sausage moisture content lower than 35% (about 60 days). After drying, sausages were stored under controlled conditions (temperature and relative humidity) in industrial chamber, until day 270.

Samples for analyses were taken at day 0 day of production (control – 0), at the end of drying (T1) and at the end of storage (T2). All determinations were made in three samples.

2.2. PAH determination
The analysis of PAH was carried out on a GC 6890N gas chromatograph coupled to a MS 5975 mass spectrometer (Agilent, Palo Alto, CA, USA). Chromatographic conditions and PAH standard preparation were performed according to Škaljac et al. [2].

2.3. Statistical analysis
All data are presented as mean, standard deviation (SD) and range. Data were analysed statistically with one way ANOVA and post-hoc test (DUNCAN’S test). Differences were considered significant at P < 0.05. Statistical analysis was conducted using STATISTICA software version 13.2.

3. Results and Discussion
The PAH (μg/kg) contents of Petrovská klobása sausages smoked in traditional conditions are presented in table 1. The PAHs determined in raw sausage mixture before smoking (day 0 of production) were Acy, Fln and Phe, while other investigated PAHs were below the limit of detection. Roseiro et al. [4] reported that in raw sausage mixture, over 99% of the total PAHs were light PAHs (Naph, Ace, Ant, Fln and Phe). These findings were consistent with the results in our study. Roseiro et al. [4] reported higher value of Σ16 US-EPA PAHs (250.33 μg/kg dry matter) in Portuguese raw
sauces, compared to values obtained in our study (Σ13 US-EPA PAHs - 9.57 μg/kg; i.e. 21.7 μg/kg dry matter), but Martorell et al. [10] reported lower value of Σ16 US-EPA PAHs in fresh meat sausages (1.25 μg/kg).

At the end of the drying period (day 60 of production), higher PAH content was determined in the Petrovská klobása compared to those levels determined in raw sausage mixture. The PAH determined in sausages at the end of the drying period were Acy (9.80 μg/kg), Fln (11.1 μg/kg), Phe (18.0 μg/kg), Ant (5.35 μg/kg) and Pyr (2.27 μg/kg), while other investigated PAH were below the limit of detection (table 1). The most abundant PAH in sausage samples was Phe, which was in accordance with the levels in other dry fermented sausages [11]. BaP, as well as PAH4 (BaA, BbF, BaP and CHR), with maximum contents in smoked meat products being set by European Commission Regulation (2 μg/kg and 12 μg/kg, respectively), were below the limit of detection in all examined samples of Petrovská klobása at the end of the drying period (table 1). From the point of view of PAH content, the results obtained in this study confirmed the safety of Petrovská klobása smoked in traditional conditions.

Table 1. Content of 13 US-EPA polycyclic aromatic hydrocarbons (μg/kg) in Petrovská klobása smoked in traditional conditions.

| Polycyclic aromatic hydrocarbons | 0        | T1        | T2        |
|---------------------------------|----------|----------|----------|
| Acenaphthylene Acy              | 2.57a ± 0.35 | 9.80b ± 0.10 | 21.8c ± 1.85 |
| Fluorene Fln                    | 2.10a ± 0.30 | 11.1b ± 0.10 | 13.0c ± 0.65 |
| Phenanthrene Phe                | 4.90a ± 0.50 | 18.0b ± 1.40 | 26.3c ± 2.60 |
| Anthracene Ant                  | nd       | 5.35a ± 0.55 | 12.5b ± 0.40 |
| Pyrene Pyr                      | nd       | 2.27 ± 0.06  | nd        |
| Benz[a]anthracene BaA           | nd       | nd        | nd        |
| Chrysene BbF                    | nd       | nd        | nd        |
| Benzo[b]fluoranthene BkF        | nd       | nd        | nd        |
| Benzo[k]fluoranthene BaP        | nd       | nd        | nd        |
| Benzo[α]pyrene CHR              | nd       | nd        | nd        |
| Indeno[1,2,3-cd]pyrene IcP      | nd       | nd        | nd        |
| Dibenz[a,h]anthracene DhA       | nd       | nd        | nd        |
| Benzo[ghi]peryleneBgP           | nd       | nd        | nd        |
| Σ EU PAH4                       | nd       | nd        | nd        |
| Σ 13 US-EPA PAHs                | 9.57a ±1.15 | 46.52b±0.80 | 73.50c±4.20 |

In the same row, different letters means that values are significantly different (P < 0.05).
nd - not detected
Results are expressed as means ± standard deviations

1BaA, CHR, BbF and BaP;

During storage, the PAH content increases because PAH from casings penetrate into the sausages and because of further drying of sausages [2, 6, 12]. On the other hand, during storage, some decrease in PAH content is also expected, caused by light decomposition and interaction with other components of the formulation [13, 14]. Thus, in this study, PAH contents were also determined in Petrovská klobása sausages at the end of the storage period (day 270 of production). The content of 13 US-EPA PAHs in Petrovská klobása sausages smoked in traditional conditions at the end of the storage period (day 270 of production) are presented in table 1. The PAHs determined in sausage samples were Acy (21.8 μg/kg), Fln (13.0 μg/kg), Phe (26.3 μg/kg) and Ant (12.5 μg/kg). Other investigated PAHs were below the limit of detection. At the end of the storage period, contents of detectable PAHs in sausages were significantly (P<0.05) higher than their contents at the end of the drying process. This phenomenon
was mainly caused by further drying of sausages during the storage period [2]. Results of this study show that at the end of drying and at the end of storage, the total content of $\sum$ US-EPA PAHs was lower compared with content of most other traditional dry fermented sausages [4, 7, 11, 15, 16], but higher compared with Androlla (36.45 $\mu$g/kg) and Botillo (29.39 $\mu$g/kg) dry fermented sausages from Spain [17]. Those authors found that the content of $\sum$ US-EPA PAHs ranged from 36.45 to 2609.81 $\mu$g/kg in traditional dry fermented sausages from Portugal, Spain and Serbia [17]. Results presented in our study show that the process of smoking and drying in traditional conditions proceeded correctly and provided low content of PAH in Petrovská klobása.

4. Conclusion
BaP, as well as PAH4 were below the limit of detection in all examined samples of Petrovská klobása smoked in traditional conditions. According to the results obtained in this study and with regard to the European regulation on PAH content, the dry fermented sausage Petrovská klobása, smoked in traditional conditions was safe for consumers.

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