Influence of Different Smoking Procedures on Polycyclic Aromatic Hydrocarbons Formation in Traditional Dry Sausage Hercegovačka kobasica

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Abstract: The concentrations of 16 polycyclic aromatic hydrocarbons (PAH) in smoked dry sausage Hercegovačka kobasica were investigated. The sausages were stuffed in two different casings (collagen and natural) and smoked in traditional and industrial smokehouses. The highest concentration of PAH 16 were detected in sausages in natural casings smoked in the traditional manner. The samples smoked in the industrial chamber stuffed in collagen casing showed the lowest PAH 16 content. The content of PAH 4 in sausage smoked in the traditional way and stuffed in natural casing averaged 24.46 µg/kg, which is more the double of maximum prescribed concentration of 12 µg/kg. The concentration of cancerogenic benzo[a]pyrene averaged 7.79 µg/kg in sausage stuffed in natural casing and smoked in the traditional way, which is almost four times the legislative prescribed values (2 µg/kg). Sausage smoked in the traditional manner and stuffed in collagen casing showed lower values for PAH 4 (13.88 µg/kg) and benzo[a]pyrene (4.97 µg/kg), but these values were also above the legislative prescribed values.

Keywords: smoking with open fire; PAH; industrial smoking

1. Introduction

Hercegovačka kobasica is a dry sausage produced in Herzegovina, a part of Bosnia and Herzegovina. It is a pork meat product with paprika, hot paprika, and garlic added as spices, stuffed traditionally into pig colon. Today’s consumers want to return to the original and traditional products that are produced by small, family businesses and usually sold at the city markets. The specific taste and aroma are a result of the traditional production that includes smoking with an open fire. This can, however, lead to the formation of potential polycyclic aromatic hydrocarbon (PAH) accumulation [1–3]. This process is conditioned by the duration of smoking, temperature of wood combustion, type of wood, moisture of wood, and casing type [4–7]. Wood combustion results with different volatiles that end up in the air and can accumulate in the smoked meat products. The smoking process serves as one of the food preservation methods and includes the penetration of volatile compounds through the casing (natural or collagen) into the stuffing. This results in meat products with a characteristic aroma, color, and taste, which are very appealing to the consumers [8]. Incomplete wood combustion usually results in the formation of different PAHs, some of which are denoted as hazardous to human health when found in food. PAHs contain one or more condensed benzene rings, while some display carcinogenic and mutagenic properties [9–11].
PAHs are soluble in organic solvents, solids at room temperature, and lipophilic. This is why they can be found in meat and meat products which have been exposed to smoking [12].

The US Environmental Protection Agency (EPA) designated 16 polycyclic aromatic hydrocarbons [naphthalene (NA), acenaphthylene (ACL), acenaphthene (AC), fluorene (FL), anthracene (AN), phenanthrene (PHE), fluoranthene (FA), benzo[a]anthracene (BaA), pyrene (PY), chrysene (CHR), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBahA), benzo[ghi]perylene (BghiP) and indeno[1,2,3-cd]pyrene (IP)] (PAH16) as priority environmental pollutant [13]. As a reference for the determination of PAHs in food, the European Food Safety Authority (EFSA) concluded that the concentrations of BaP, as well as the sum of the concentrations of four PAHs BaP, BaA, BbFA, and CHR (PAH4) [14], would show the practical value of food safety. European legislation (European Commission (EU) Regulation no. 835/2011 [15] allows the 2 µg/kg as the maximum permissible concentration of BaP in meat products; the sum PAH4 concentrations should not exceed 12 µg/kg. Bosnia and Herzegovina’s legislation leans toward these values [16].

The sausage were produced from pig meat, pig fat, red hot paprika powder, red sweet paprika powder, and garlic stuffed into natural (pig colon) and artificial (collagen) casings. The intention of this paper was to examine the effect of industrial and traditional smoking procedure and to compare the effect of casing on PAH concentrations in Hercegovačka kobasica.

2. Materials and Methods

2.1. Samples Production and Preparation

Traditional recipe was used for sausage manufacturing. Hercegovačka kobasica was produced from pork meat (75%) and pork fat (25%). The meat and fat is then ground through a grinding plate with holes of 6 to 8 mm in diameter. The ground meat and pork fat is then mixed with salt in the amount of 2%, red paprika in the amount of 1%, hot paprika in the amount of 0.7%, and garlic in the amount of 0.2%. The stuffing is then stuffed into a natural casing (pig’s colon) or a collagen casing (500 mm long; 50 mm in diameter). The two groups were then further divided and subjected to two types of smoking.

The traditional smoking lasted for 30 days. Smoking was scheduled for 2–3 h every day for the first 6 days and every other day for the next 24 days. The sausages were hung three meters from the open fire. The combustion of dry hard wood (beech, hornbeam, and oak) and its sawdust was used to generate smoke. The conditions of production (temperature and relative humidity) were not controlled, but influenced by the atmospheric conditions. The temperature ranged from 3.5 to 11.2 °C (average 6.9 °C) and relative humidity from 61.3 to 90.5% (average 74.2%).

For industrial smoking, the industrial chamber (Mauting, Czech Republic) was employed. The smoke (25.0 °C) was produced using a heating plate on which beech and oak sawdust was placed; this resulted in indirect smoking of the samples. Smoking conditions were monitored and controlled with the average temperature of 18.9 °C and relative humidity of 74.49%. Samples were subjected to smoking for 4 h a day (8 × 30 min) for a total of three days.

Three batches of four groups were prepared and three samples from each group were excluded at the end of smoking in order to conduct the analysis. After the sampling, the casings were peeled from the sausages and stored in the same way as the stuffing; the samples were homogenized and stored in the freezer (−30 °C) in glass jars previously treated with acetone. All the analyses were carried out in triplicate.

2.2. PAHs Determination and Quantification

Extraction was done in the following manner: 3 g of samples were transferred into the centrifuge tube where a mixture of 3 mL of acetonitrile (ACN, Sigma-Aldrich, St. Louis, MO, USA) and 3 mL of water was added. The sample was vortexed for 1 min, followed by the addition of 3 g of anhydrous magnesium sulfate (MgSO4; Merck, Darmstadt, Germany) and 1 g of anhydrous sodium acetate.
The sample was then centrifuged for 5 min at 3000 rpm. 1 mL of upper layer of the acetonitrile extract, along with 150 mg of anhydrous magnesium sulfate, 100 mg of Primary and Secondary Amine (PSA) (Merck, Darmstadt, Germany), and 50 mg of C18 (Merck, Darmstadt, Germany) (Anastassiades, Lehotay, Stajnbaher, & Schenck, n.d.), was then transferred to 5 mL tubes. Centrifugation was applied for 5 min at 3000 rpm to ensure clear and pure extract. At the end, 0.5 mL of the extract was subjected to evaporation under nitrogen gas and reconstituted with hexane, resulting in a sample ready for the analysis on GC-MS (Agilent 7890B/5977A, Santa Clara, CA, USA).

Chromatographic separation of 16 PAHs of interest was performed by gas chromatography in combination with a mass detector (GC-MS), according to Mastanjević et al. (2019) [16]. In short, standard solutions of PAHs were prepared with PAH mix of 16 polycyclic aromatic hydrocarbons (Ultra Scientific, North Kingstown, RI, USA), 500 ± 0.2 µg/mL. Method validation and calibration through matrix blank sample was also performed. Samples were prepared using QuEChERS method (quick, easy, cheap, effective, rugged, and safe preparation) [16]. GC-MS parameters were adjusted as described (Supplementary Material) and reported by Mastanjević et al. 2019 [17]. All analyses were performed in triplicate.

2.3. Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) with significance defined at \( p < 0.05 \) (Fisher’s Least Significant Difference (LSD) test.). Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA) was used for statistical analysis.

3. Results and Discussion

The analysis of stuffing for *Hercegovačka kobasica* production revealed only 2.26 µg/kg for naphthalene and other 15 PAHs were below the quantification threshold. This is in agreement with previous research on similar samples [18,19].

The concentrations of 16 PAHs in natural and collagen casing used for *Hercegovačka kobasica* production are presented in Table 1. AC and IP were not quantified in any of the sample groups. The highest concentration was quantified for PHE and it from 1181.39 µg/kg in traditional smoking in natural casing, to <limit of quantification (LOQ) in industrial smoking in collagen casing samples. CHR, BbFA, BkFA, and DBahA were detected and quantified only in samples stuffed in natural casings and subjected to traditional smoking. The samples of casings subjected to industrial smoking, both collagen and natural, showed significantly \( (p < 0.05) \) lower PAH concentrations in comparison to casings subjected to traditional smoking. NA was found in higher concentrations in the samples exposed to industrial smoking and, moreover, the natural casings seem to bind NA more efficiently than collagen casing. From the other 15 PAHs, only ACL was quantified in both casings derived from industrial smoking. PHE was found only in natural casing in samples subjected to industrial smoking.

Cancerogenic BaP was quantified in both casings subjected to traditional smoking, with natural at 7.79 µg/kg and collagen at 4.98 µg/kg. For natural casing, this is almost four-fold the legally prescribed concentration (2 µg/kg); it is almost 2.5 times higher than the legally prescribed concentration for collagen. On the other hand, BaP was not quantified in industrial smoked casings.

PAH4 concentration was quantified only in traditionally smoked casings; it amounted to 44.86 µg/kg in natural casing and 21.46 µg/kg in collagen casings. The legally prescribed limit for PAH4 is 12 µg/kg, which makes the analyzed samples abundant with this group of PAHs.

PAH16 amounted to 2028.20 µg/kg in traditional smoked natural casing, while traditional smoked collagen casing showed almost ten-fold lower values (255.14 µg/kg). Casings smoked in the industrial manner showed significantly lower values \( (p < 0.05) \), resulting in 167.47 µg/kg for natural casing and 94.31 µg/kg for collagen casing.

PAH concentrations in *Hercegovačka kobasica* content are presented in Table 2. In the industrial smoked samples, similar as in casings, only NA and ACL were quantified. NA concentration amounted to 113.05 µg/kg in the industrially smoked sausages in natural casing and 44.40 µg/kg in collagen
casing. This is significantly higher than the levels in samples smoked in the traditional manner. Similar as for the casing, the most dominant PAH in traditional smoked samples was PHE, resulting in 79.24 µg/kg in natural casing and 31.59 µg/kg in collagen. AC, CHR, DBahA, and IP were not quantified in any of the samples. BaP amounted to 7.79 µg/kg in traditional smoked sausages in natural casings and 4.98 µg/kg in collagen. In the industrially smoked samples, the BaP content was below the quantification. The BaP content in traditionally smoked sausages was worryingly high; only a few studies of traditionally smoked Estonian and Swedish smoked meat products showed higher BaP concentrations [9,20]. Studies for Spanish, Portuguese, Italian, and Croatian smoked dry sausages reported lower values for BaP [4,5,17,21–25].

Table 1. Polycyclic aromatic hydrocarbons (PAH) contents (µg/kg) in Hercegovačka kobasica casings.

| PAH | Traditional Smoking—Natural Casing | Traditional Smoking—Collagen Casing | Industrial Smoking—Natural Casing | Industrial Smoking—Collagen Casing |
|-----|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| NA  | 54.17 ± 1.03                      | 26.11 ± 1.50                      | 66.23 ± 1.91                     | 51.41 ± 0.80                      |
| ACL | 351.24 ± 5.71                     | 65.77 ± 2.11                      | 84.31 ± 1.94                     | 42.91 ± 0.81                      |
| AC  | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                              |
| FL  | 133.10 ± 2.27                     | 20.33 ± 0.32                      | <LOQ                             | <LOQ                              |
| AN  | 282.05 ± 7.21                     | 15.95 ± 3.29                      | <LOQ                             | <LOQ                              |
| PHE | 1108.39 ± 6.061                   | 79.4 ± 4.06                       | 15.21 ± 1.35                     | <LOQ                              |
| FA  | 233.63 ± 2.04                     | 8.96 ± 0.45                       | <LOQ                             | <LOQ                              |
| BaA | 22.10 ± 0.13                      | 13.43 ± 0.14                      | <LOQ                             | <LOQ                              |
| PY  | 177.67 ± 2.33                     | 5.57 ± 0.17                       | <LOQ                             | <LOQ                              |
| CHR | 21.38 ± 0.73                      | <LOQ                              | <LOQ                             | <LOQ                              |
| BbFA| 10.17 ± 0.46                      | <LOQ                              | <LOQ                             | <LOQ                              |
| BkFA| 12.38 ± 0.15                      | <LOQ                              | <LOQ                             | <LOQ                              |
| BaP | 5.06 ± 0.11                       | 7.79 ± 0.17                       | <LOQ                             | <LOQ                              |
| DBahA| 7.54 ± 0.67                       | <LOQ                              | <LOQ                             | <LOQ                              |
| BhgIP| 5.46 ± 0.19                       | 0.76 ± 0.20                       | <LOQ                             | <LOQ                              |
| IP  | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                              |

ΣPAH4  | 44.86 ± 2.16                      | 21.46 ± 0.93                      | <LOQ                             | <LOQ                              |

ΣPAH16 | 2028.20 ± 12.55                   | 255.14 ± 5.34                     | 167.47 ± 5.48                    | 94.31 ± 3.26                      |

a–c Means ± standard deviation within rows with different superscripts are significantly different (p < 0.05); LOQ—limit of quantification.

Similar to BaP, the PAH4 content was only quantified in traditionally smoked samples. The recorded concentrations for BaP in traditionally smoked samples is worryingly high, amounting to almost four times above the legally prescribed concentration. In traditionally smoked collagen samples, the BaP concentration was significantly lower than for the traditional, but still almost 2.5 times higher than the legally prescribed concentration. The sum of PAH4 was 21.46 µg/kg for natural casings and significantly lower for collagen casings, at 13.88 µg/kg. The legally prescribed values for PAH4 are 12 µg/kg; traditionally smoked samples showed almost two-fold higher concentrations. The samples in the collagen casing showed significantly lower values for PAH4, but it was still above the legally prescribed level. Thus, worryingly high concentrations of PAH4 were exceeded those in most Spanish, Croatian, Serbian, and Portuguese traditionally smoked dry sausages [3,4,11,17,19,21,22]. The higher PAH4 values were reported by Roserio et al. (2012) for Portuguese smoked blood sausage Moura of “Trás-os-Montes” (271.83 µg/kg) [24] and Wretling et al. (2010) for sauna smoked Swedish ham (209 µg/kg) [20].

Skaljac et al. (2018) [26] reported similar lower values for PAH4 and PAH16 in samples of Serbian sausage smoked in industrial conditions and in samples stuffed in artificial casing. A similar reduction of PAH content with the use of artificial casing were reported for frankfurter types, Spanish, Portuguese, and Croatian smoked dry sausages [7,18,27,28]. The sum of PAH16 was higher in natural casings...
for both smoking procedures (traditional at 245.19 µg/kg and industrial at 145.48 µg/kg). However, in industrially smoked samples, the concentrations of BaP and PAH4 were not quantified and thus made them less harmful for human consumption. This may be related to the high porosity of natural casings. High concentrations for BaP, PAH4, and PAH16 may be the result of prolonged smoking in traditional procedures (30 days). Similar results, with the increase of PAH concentration in smoked meat products with an increase of smoking time, were reported Hitzel et al. (2013) [29], Pohlmann et al. (2012) [30], and Fraqueza et al. (2020) [31]. On the other hand, the PAH formation in smoked meat products may be related to the type of wood used for smoke generation [32–35]. The worryingly high concentrations of BaP, PAH4, and PAH16 in traditionally smoked sausages in this investigation may also be related to the use of oak wood to generate smoke; similarly high concentrations of BaP and PAH4 were reported by Reserio et al. (2012) [24] for Portuguese sausages smoked with oak wood.

### 4. Conclusions

The results of this study indicate that traditional smoking and the use of natural casings can result in elevated and potentially harmful BaP and PAH4 concentrations in Hercegovačka kobasica. Producers who utilize the traditional smoking method should pay attention to smoking duration and wood combustion temperature. The potential use of smoke filters and smoking time reduction are also options that may help reduce the formation of PAHs in traditionally smoked sausages. Samples in collagen casings showed significantly lower values for PAHs, meaning that the smoking manner (industrial or traditional) plays a crucial role in PAH formation. The industrially smoked samples, both collagen and natural casings, had <LOQ concentrations of PAH4 and BaP.

### Supplementary Materials

The following are available online at http://www.mdpi.com/2227-9717/8/8/918/s1, Table S1: The average values for precision, reproducibility, accuracy, linearity, LOQ and LOD for PAH method validation.

### Author Contributions

Conceptualization, K.M.; methodology, B.K.; software, K.M.; validation, B.K.; investigation, L.P. and D.K.; data curation, K.H. and D.K.; writing—original draft preparation, K.M.; writing—review and editing, K.H.; supervision, K.M.; All authors have read and agreed to the published version of the manuscript.

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**Table 2. PAH contents (µg/kg) in Hercegovačka kobasica.**

| PAH   | Traditional Smoking—Natural Casing | Traditional Smoking—Collagen Casing | Industrial Smoking—Natural Casing | Industrial Smoking—Collagen Casing |
|-------|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| NA    | 26.11 ± 0.51                      | <LOQ                              | 113.05 ± 1.84                    | 44.40 ± 0.53                     |
| ACL   | 65.77 ± 0.25                      | 23.55 ± 0.17                      | 32.83 ± 0.59                     | 22.05 ± 0.56                     |
| AC    | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                             |
| FL    | 23.33 ± 0.43                      | <LOQ                              | <LOQ                             | <LOQ                             |
| AN    | 15.95 ± 0.23                      | 5.91 ± 0.37                       | <LOQ                             | <LOQ                             |
| PHE   | 79.24 ± 1.24                      | 31.59 ± 0.31                      | <LOQ                             | <LOQ                             |
| FA    | 8.95 ± 0.14                       | 6.10 ± 0.22                       | <LOQ                             | <LOQ                             |
| BaA   | 13.43 ± 0.40                      | 8.40 ± 0.19                       | <LOQ                             | <LOQ                             |
| PY    | 5.56 ± 0.28                       | 3.50 ± 0.19                       | <LOQ                             | <LOQ                             |
| CHR   | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                             |
| BbFA  | 0.23 ± 0.08                       | 0.51 ± 0.10                       | <LOQ                             | <LOQ                             |
| BkFA  | 1.06 ± 0.10                       | <LOQ                              | <LOQ                             | <LOQ                             |
| BaP   | 7.79 ± 0.45                       | 4.98 ± 0.55                       | <LOQ                             | <LOQ                             |
| DBahA | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                             |
| BghiP | 0.76 ± 0.16                       | <LOQ                              | <LOQ                             | <LOQ                             |
| IP    | <LOQ                              | <LOQ                              | <LOQ                             | <LOQ                             |
| ΣPAH4 | 21.46 ± 0.75                      | 13.88 ± 0.61                      | <LOQ                             | <LOQ                             |
| ΣPAH16| 245.19 ± 2.93                     | 84.54 ± 0.82                      | 145.48 ± 1.17                    | 64.46 ± 1.25                     |

*a,c* Means ± standard deviation within rows with different superscripts are significantly different (*p* < 0.05); LOQ—limit of quantification.
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