



Polycyclic aromatic hydrocarbons (PAHs) in Visočka pečenica, a traditional dry-cured meat product from Bosnia and Herzegovina

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ABSTRACT

Visočka pečenica is a traditional dry-cured beef meat product from Bosnia and Herzegovina, and is protected by a geographical indication label at the national level. In this research, the content of 16 EU priority polycyclic aromatic hydrocarbons (PAHs) in Visočka pečenica from traditional and industrial production was examined. The content of PAH compounds has not been analysed in Visočka pečenica to date. Determination and quantification of PAHs in Visočka pečenica were performed by a GC/MS method. The content of all individual PAH compounds was higher in *Musculus longissimus dorsi* than in beef round in both production methods, and their contents increased with the longer smoking process. In addition, higher contents of PAHs were found in Visočka pečenica from traditional than in industrial production, with some exceptions. In Visočka pečenica from traditional production, higher contents of individual PAH compounds and their sums were determined when they were smoked at a higher shelf level in the smokehouse, both halfway through and at the end of the smoking process. Contrarily, in Visočka pečenica from industrial production, PAH contents were higher at the lower rather than the higher shelf level, with some exceptions. The most common PAH compound in the Visočka pečenica from both production methods was chrysene (CHR) (3.00–14.08 µg/kg).

1. Introduction

Visočka pečenica is a traditional dry-cured beef meat product from Bosnia and Herzegovina that has been produced in the municipality of Visoko for many years. It is produced from the highest quality parts of beef carcasses, dry salted only with kitchen salt and cold smoked and dried. The product is characterized by the special production technology characteristic of the aforementioned location, is top quality and is especially valued for its aroma, taste and smoke aroma. Smoking is one of the oldest technol-

ogies for preserving meat and meat products and is defined as the process of penetration of meat products by volatiles resulting from thermal destruction of wood (Andrée *et al.*, 2010). Smoking gives special colour, taste and aroma to food, and enhances preservation due to its dehydrating, bactericidal and antioxidant properties (Škaljac *et al.*, 2019; Roseiro *et al.*, 2011; Puljić *et al.*, 2019). However, in addition to the positive effect of the smoking process and a large number of useful compounds that are created by burning wood, harmful components are also creat-

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ed, which include polycyclic aromatic hydrocarbons (PAHs). PAHs comprise the largest class of chemical compounds, containing two or more fused aromatic rings made up of carbon and hydrogen atoms, known to be genotoxic agents (Ciecierska and Obiedzinski, 2007). About 660 different compounds belong to the PAH group (Jira et al., 2013). Human exposure to PAHs occurs in three ways, i.e., inhalation, dermal contact and consumption of contaminated foods, which accounts for 88–98% of such contamination; in other words, diet is the major source of human exposure to these contaminants (Tareq et al., 2020). Due to the lipophilic and hydrophobic characteristics of PAHs, they tend to accumulate in the food chain (Bansal and Kim, 2015). The PAH content in smoked food is determined by various technological factors, such as the species of wood and its humidity, the fat content of the food, the temperature of combustion and oxidation, the method of smoke flow through the chamber and the duration and smoking technique, as well as the humidity and size of the product surface (García-Falcón and Simal-Gándara, 2005; Zachara et al., 2017; Sojimu et al., 2019; Zdolec et al., 2019; Kafouris et al., 2020). The European Food Safety Authority (EFSA) identified several PAHs (the content of benzo[a]pyrene (BaP) and the sum of contents of four PAHs, BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)) that would be considered as a reference for the determination of PAHs in food (EFSA, 2008). According to European Commission (EU) regulation (European Commission, 2006; 2011; 2014), the maximum permissible content of BaP in meat products is 2 µg/kg, and the sum of the PAH4 content should not exceed 12 µg/kg. The International Agency for Research on Cancer (IARC) classified one PAH of 16 examined PAHs in this study (BaP) as carcinogenic to humans (Group 1), three (cyclopenta[cd]pyrene (CPP), dibenzo[a,l]pyrene (DIP), and dibenz[a,h]anthracene (DhA)) (Group 2A) as probably carcinogenic and nine (BaA, CHR, BbF, 5-methylchrysene (5MC), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), indeno[1,2,3-cd]pyrene (IcP), dibenzo[a,i]pyrene (DiP) and dibenzo[a,i]pyrene (DhP)) as possibly carcinogenic to humans (Group 2B), while three (benzo[ghi]perylene (BgP), dibenzo[a,e]pyrene (DeP) and benzo[c]fluorene (BcF)) were not classifiable according to their carcinogenicity to humans (IARC, 2010). The aims of the research were to determine the influence of production technology on the content of PAH compounds in traditional Visočka pečenica. In addition, the research exam-

ined the influence of fat content, smoking height and smoking duration on the content of PAH compounds.

2. Materials and methods

2.1. Production and sampling of Visočka pečenica

Experimental production of Visočka pečenica was performed in traditional and industrial conditions. Forty samples were sampled twice for research needs, halfway through and at the end of the smoking process. Accordingly, the total number of analysed samples was 80. Samples were taken from different cuts on the carcass (round — *Musculus gluteobiceps*, *Musculus gluteus medius*, *Musculus semitendinosus* and back musculature — *Musculus longissimus dorsi*; MLD). During the smoking phase, the meat pieces were spread over shelves at two levels. The first level was in relation to the fireplace, at a height of two meters. The height of the second level was four meters. Looking at the phases, the experimental set-up meant that during smoking, five samples of both anatomical regions were placed on both levels of the smokehouse. The set-up of the experiment, from the choice of raw material to the technological processing to the method of sampling, was identical for both productions. In the comparison of traditional and industrial production, the differences were related to the amount of added salt, the length of smoking, and the parameters of the smoking process (temperature and relative humidity inside the smokehouse, outside and the temperature of the stokehole).

2.2. Methods

The content of PAH compounds in the samples of Visočka pečenica was determined by an internal method in the laboratory of the Institute of Meat Hygiene and Technology in Belgrade. Samples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. Determination of 16 EU PAH compounds (BaA, CHR, BbF, BaP, BcL, CPP, 5MC, BjF, BkF, IcP, DhA, BgP, DIP, DeP, DiP, DhP) was performed using gas chromatography with a triple quadrupole mass detector. Briefly, 2.5 g of homogenized sample was weighed into a 50 mL centrifuge tube; 10 mL of acetonitrile were added and the mixture was shaken vigorously for 1 min; after that 1 g NaCl and 4 g MgSO₄ were added, with the tube being shaken immediately after addition of the salt. Then each tube was shaken and centrifuged. Superna-

Table 2. Contents ($\mu\text{g}/\text{kg}$) of the examined PAHs in Visočka pečenica

Sample	BaA	CHR	BbF	BaP	BcL	CPP	5MC	BjF	BkF	IcP	DhA	BgP	D/P	DeP	DiP	DhP
TRHH	2,47 ^a Axx±0	3,00 ^a Axx±0	0,80 ^a Axx±0	1,36 ^a Axx±0	2,25 ^a Axx±0	2,10 ^a Axx±0	0,13 ^a Axx±0	0,35 ^a Axx±0	0,65 ^a Axx±0	0,94 ^a Axx±0	0,16 ^a Axx±0	0,82 ^a Axx±0	0,20 ^a Axx±0	0,13 ^{aAx} X±0,01	<	<
	,49	,31	,10	,39	,57	,77	,03	,04	,08	,13	,04	,12	,06		0.1	0.1
TMHH	5,42 ^b Ayx±0	6,14 ^b Ayx±0	1,56 ^b Ayx±0	3,04 ^b Ayx±0	4,80 ^b Ayx±0	5,30 ^b Ayx±1	0,28 ^b Ayx±0	0,66 ^b Ayx±0	1,16 ^b Ayx±0	1,78 ^b Ayx±0	0,33 ^b Ayx±0	1,54 ^b Ayx±0	0,41 ^b Ayx±0	0,23 ^{bAy} X±0,06	<	<
	,85	,81	,21	,41	,89	,05	,05	,10	,12	,21	,05	,21	,06		0.1	0.1
TRLH	2,95 ^a Axx±0	3,77 ^a Axx±0	0,94 ^a Axx±0	1,65 ^a Axx±0	2,33 ^a Axx±0	2,54 ^a Axx±1	0,16 ^a Axx±0	0,40 ^a Axx±0	0,76 ^a Axx±0	1,13 ^a Axx±0	0,18 ^a Axx±0	1,02 ^a Axx±0	0,23 ^a Axx±0	0,13 ^{aAx} X±0,03	<	<
	,73	,45	,13	,57	,71	,07	,03	,06	,10	,17	,05	,17	,05		0.1	0.1
TMLH	4,43 ^b Ayx±1	4,89 ^b Ayx±1	1,27 ^b Ayx±0	2,48 ^b Ayx±0	3,99 ^b Ayx±1	4,51 ^b Ayx±1	0,25 ^b Ayx±0	0,55 ^b Ayx±0	0,96 ^b Ayx±0	1,42 ^b Ayx±0	0,25 ^b Ayx±0	1,24 ^b Ayx±0	0,32 ^b Ayx±0	0,16 ^{bAy} X±0,04	<	<
	,19	,28	,36	,61	,10	,28	,08	,15	,26	,34	,07	,25	,08		0.1	0.1
TRHE	7,82 ^a Axx±1	9,20 ^a Axx±1	2,20 ^a Axx±0	4,25 ^a Axx±0	6,96 ^a Axx±1	6,93 ^a Axx±1	0,42 ^a Axx±0	0,94 ^a Axx±0	1,71 ^a Axx±0	2,62 ^a Axx±0	0,45 ^a Axx±0	2,25 ^a Axx±0	0,57 ^a Axx±0	0,32 ^{aAx} Y±0,08	<	<
	,45	,56	,39	,69	,25	,24	,09	,17	,27	,38	,06	,31	,10		0.1	0.1
TMHE	12,39 bAyY±	14,08 bAyY±	3,36 ^b bAyY±	6,62 ^b bAyY±	11,80 bAyY±	11,79 bAyY±	0,64 ^b bAyY±	1,44 ^b bAyY±	2,56 ^b bAyY±	3,90 ^b bAyY±	0,66 ^b bAyY±	3,41 ^b bAyY±	0,84 ^b bAyY±	0,45 ^{bAy} Y±0,13	<	<
	2,12	2,19	,57	,17	1,66	1,92	,09	,24	,47	,65	,08	,70	,16		0.1	0.1
TRLE	6,20 ^a Axx±0	7,46 ^a Axx±0	1,64 ^a Bxx±0	3,16 ^a Bxx±0	5,75 ^a Axx±0	4,97 ^a Axx±0	0,31 ^a Axx±0	0,69 ^a Bxx±0	1,38 ^a Bxx±0	1,95 ^a Bxx±0	0,31 ^a Bxx±0	1,74 ^a Bxx±0	0,34 ^a Bxx±0	0,15 ^{aBx} Y±0,04	<	<
	,69	,68	,19	,39	,75	,98	,03	,07	,13	,20	,03	,23	,05		0.1	0.1
TMLE	10,97 bAyY±	12,06 bAyY±	2,64 ^b ByY±0	5,03 ^b ByY±1	12,27 bAyY±	10,78 bAyY±	0,55 ^b AyY±0	1,13 ^b ByY±0	1,94 ^b ByY±0	2,74 ^b ByY±0	0,49 ^b ByY±0	2,20 ^b ByY±0	0,58 ^b ByY±0	0,32 ^{bBy} Y±0,12	<	<
	3,03	3,43	,69	,38	3,37	3,14	,19	,31	,51	,79	,11	,68	,19		0.1	0.1
IRHH	2,81 ^a Axx±1	3,21 ^a Axx±1	0,78 ^a Axx±0	1,59 ^a Axx±0	1,97 ^a Axx±0	2,72 ^a Axx±0	0,15 ^a Axx±0	0,33 ^a Axx±0	0,74 ^a Axx±0	0,87 ^a Axx±0	0,15 ^a Axx±0	0,85 ^a Axx±0	0,12 ^a Axx±0	0,14 ^{aAx} X±0,04	<	<
	,00	,09	,28	,57	,82	,99	,06	,12	,25	,37	,06	,26	,07		0.1	0.1
IMHH	4,18 ^b Ayx±1	4,61 ^b Ayx±1	1,16 ^b Ayx±0	2,23 ^b Ayx±0	3,17 ^b Ayx±1	4,01 ^b Ayx±1	0,22 ^b Ayx±0	0,50 ^b Ayx±0	1,01 ^b Ayx±0	1,32 ^b Ayx±0	0,24 ^b Ayx±0	1,29 ^b Ayx±0	0,19 ^b Ayx±0	0,20 ^{bAy} X±0,05	<	<
	,47	,50	,38	,70	,24	,43	,08	,17	,30	,44	,09	,32	,07		0.1	0.1
IRLH	3,51 ^a Axx±0	3,98 ^a Axx±0	0,98 ^a Axx±0	1,96 ^a Axx±0	2,60 ^a Axx±0	3,39 ^a Axx±0	0,20 ^a Axx±0	0,42 ^a Axx±0	0,89 ^a Axx±0	1,14 ^a Axx±0	0,20 ^a Axx±0	1,18 ^a Axx±0	0,17 ^a Axx±0	0,15 ^{aAx} X±0,03	<	<
	,64	,66	,17	,30	,62	,68	,04	,07	,12	,18	,04	,15	,04		0.1	0.1
IMLH	4,23 ^b Ayx±0	4,76 ^b Ayx±0	1,19 ^b Ayx±0	2,40 ^b Ayx±0	3,00 ^b Ayx±0	4,06 ^b Ayx±0	0,24 ^b Ayx±0	0,51 ^b Ayx±0	1,07 ^b Ayx±0	1,46 ^b Ayx±0	0,25 ^b Ayx±0	1,49 ^b Ayx±0	0,22 ^b Ayx±0	0,17 ^{bAx} X±0,02	<	<
	,84	,92	,25	,54	,65	,79	,06	,11	,23	,39	,07	,46	,07		0.1	0.1
IRHE	3,99 ^a Axx±0	4,40 ^a Axx±0	1,11 ^a Axx±0	2,26 ^a Axx±0	2,94 ^a Axx±0	4,04 ^a Axx±0	0,22 ^a Axx±0	0,48 ^a Axx±0	1,02 ^a Axx±0	1,37 ^a Axx±0	0,23 ^a Axx±0	1,39 ^a Axx±0	0,20 ^a Axx±0	0,16 ^{aAx} Y±0,03	<	<
	,76	,75	,19	,36	,90	,90	,04	,08	,14	,20	,03	,19	,04		0.1	0.1
IMHE	6,79 ^b Ayx±1	7,15 ^b Ayx±1	1,84 ^b Ayx±0	3,58 ^b Ayx±0	5,71 ^b Ayx±1	6,90 ^b Ayx±1	0,34 ^b Ayx±0	0,79 ^b Ayx±0	1,62 ^b Ayx±0	2,18 ^b Ayx±0	0,38 ^b Ayx±0	2,09 ^b Ayx±0	0,36 ^b Ayx±0	0,31 ^{bAy} Y±0,08	<	<
	,61	,64	,41	,74	,58	,69	,10	,18	,30	,46	,08	,49	,10		0.1	0.1
IRLE	4,36 ^a Axx±0	4,64 ^a Axx±0	1,17 ^a Axx±0	2,45 ^a Axx±0	3,65 ^a Axx±0	4,68 ^a Axx±1	0,22 ^a Axx±0	0,50 ^a Axx±0	1,08 ^a Axx±0	1,53 ^a Axx±0	0,25 ^a Axx±0	1,49 ^a Axx±0	0,24 ^a Axx±0	0,19 ^{aAx} Y±0,09	<	<
	,92	,91	,26	,52	,76	,00	,05	,11	,23	,38	,06	,30	,08		0.1	0.1
IMLE	6,94 ^b Ayx±2	7,33 ^b Ayx±2	1,85 ^b Ayx±0	3,70 ^b Ayx±1	6,03 ^b Ayx±2	7,28 ^b Ayx±2	0,38 ^b Ayx±0	0,78 ^b Ayx±0	1,58 ^b Ayx±0	2,20 ^b Ayx±0	0,38 ^b Ayx±0	2,22 ^b Ayx±0	0,39 ^b Ayx±0	0,32 ^{bAx} Y±0,13	<	<
	,43	,43	,64	,29	,35	,68	,12	,26	,50	,78	,11	,88	,16		0.1	0.1

a-b Different lowercase letters in the columns indicate statistically significant differences between samples of "Visočka pečenica" produced from round musculature and MLD at different heights at the halfway through and end of the smoking process; A-B Different capital letters in the columns indicate statistically significant differences between the samples of "Visočka pečenica" at higher and lower smoking heights at the halfway through and end of the smoking process; x-y Different lowercase letters in the columns indicate statistically significant differences between samples of "Visočka pečenica" produced from round musculature and MLD at different lengths of smoking at higher and lower smoking heights; X-Y Different uppercase letters in the columns show statistically significant differences between the "Visočka pečenica" samples at the halfway through and end of the smoking process at higher and lower smoking heights; (T-traditional, I-industrial production; M-MLD, R-round samples; H-higher, L-lower smoking heights; H-halfway through, E-end of the smoking process)

tant was cleaned up by SPE (transferring into a 15 mL centrifuge tube containing 150 mg PSA, 150 mg C18 and 900 mg MgSO₄) with the aim of eliminating the possible interfering compounds from the sample extract. After centrifugation, extracts were evaporated under a stream of N₂ at 40°C to dryness and then dissolved in 1 mL of hexane. The extracts were then analysed by GC-MS/MS. A two-factor analysis of variance was used to test the differences between the means and the Tukey test was used as a post hoc test. Statistical analyses were performed using Past software 3.15 (Hammer et al., 2001).

3. Results and discussion

The contents of the analysed PAHs in the samples of Visočka pečenica are shown in Table 2. In the tested samples, chrysene (CHR) was the most abundant of all tested PAH compounds in Visočka pečenica made by both production processes halfway through (4.45 $\mu\text{g}/\text{kg}$ -traditional and 4.14 $\mu\text{g}/\text{kg}$ -industrial) and at the end of the smoking process (10.70 $\mu\text{g}/\text{kg}$ -traditional and 5.88 $\mu\text{g}/\text{kg}$ -industrial). The content of all individual PAHs in all samples of Visočka pečenica from both anatomical regions

smoked on both shelf levels in both production processes increased with longer smoking, so there were statistically significantly higher contents of all PAH compounds at the end compared to the halfway through the smoking process. The higher content of PAH compounds in the Visočka pečenica at the end of the smoking process is a consequence of longer exposure of the meat to smoke, so these compounds accumulated to a greater extent. The trend where the content of PAH compounds during smoking mainly increased in smoked meat products was established by *et al.* (2008), *et al.* (2008a), *Fraqueza et al.* (2020), *Hokkanen et al.* (2018) and *Ledesma et al.* (2014) in their research. In the research by *et al.* (2008), the content of BaP in beef ham was 0.02 µg/kg at the beginning of the smoking process, while at the end of the smoking process, the content was 1.09 µg/kg. The content of all individual 16 EU PAH compounds except DiP and DhP in beef ham smoked in a traditional and industrial smokehouse established by *et al.* (2008a) was lower compared to the content in the Visočka pečenica in this work.

In the comparison of both smoking heights, Visočka pečenica from traditional production had a higher content of PAH compounds at a higher height, while Visočka pečenica from industrial production at a lower height contained more PAHs, with some exceptions. *Purcaro et al.* (2013) state that greater distance from the heat source leads to a decrease in the content of PAH compounds in the final product. Of course, it was to be expected that the content of PAH compounds in Visočka pečenica would be higher at a lower height, which was not the case with traditional production. Namely, this difference in the content of PAH compounds in terms of smoking heights is probably a consequence of the different temperature regimes during smoking, the duration of smoking, the position of the products, the smoke source, the structure of the smokehouse as well as the part of the product actually tested (in our study, the entire sample was used for the test without separating the inner and outer parts). Research on the penetration of PAH compounds into the interior of smoked meat products shows that about 99% of all PAH compounds are found in the outer part, which makes up 22% of the total mass of the examined product (*Jira et al.*, 2006). In their research, *Toroman et al.* (2013) recorded the highest content of BaP in Bosnian prosciutto which was located in the closest point under the hearth, with amounts of 5.1 µg/kg (uncontrolled process) and 3.6 µg/kg (controlled process). *Hokkanen et al.* (2018) and *Mastanjević et*

al. (2019) also found a higher content of PAH compounds in smoked meat products that were closer to the smoke source in their research.

A statistically significantly higher content of all individual PAH compounds was found in MLD samples compared to round samples in both production methods smoked on both shelf levels and both at halfway through and at the end of the smoking process. The MLD samples were characterized by a higher fat content compared to the round samples, confirmed by their chemical analysis. PAHs are highly lipophilic (*Puljić et al.*, 2019; *Reinik et al.*, 2007; *Martorell et al.*, 2010; *Đinović-Stojanović et al.*, 2016; *Kartalović et al.*, 2015), and generally become more lipophilic, less soluble and less volatile with increasing molecular weight (*Đinović et al.*, 2008a). Despite PAHs accumulating mainly on product surfaces, due to their lipophilic nature, some diffusion of these compounds can take place to inner layers (*Gomes et al.*, 2013), where water activity and fat content have a determinant role (*Hokkanen et al.*, 2018). The higher content of all PAH compounds in MLD samples is a consequence of their higher fat content and their lipophilic nature. In the comparison of both production methods, the content of individual PAH compounds was generally higher in the samples of Visočka pečenica from traditional production compared to industrial production. The higher content of PAH compounds in the samples of Visočka pečenica from traditional production is probably a consequence of longer smoking (for two days), higher temperature of the stokehole during each smoking day, the construction of the smokehouse, the position of the stokehole and the position of the meats inside the smokehouse. *Šuvalija* (2002) found a higher content of BaP in samples of Bosnian prosciutto from traditional compared to industrial production. A higher content of PAH compounds in smoked meat products from traditional compared to industrial production was established by *Đinović et al.* (2008a), *Puljić et al.* (2019), *Zachara et al.* (2017), *Kubiak and Polak-Šliwińska* (2015), *Mastanjević et al.* (2020) and *Roseiro et al.* (2011) in their research.

The maximum permitted amount (MPA) for smoked meat and smoked meat products according to the *Rulebook on Maximum Permitted Amounts for Food Contaminants (Official Gazette of BiH No. 68/14)* for BaP is 2.0 µg/kg. Based on the results presented in the samples of Visočka pečenica, the BaP content ranged from 1.36 µg/kg to 6.62 µg/kg in both production methods. According to our results,

four groups of Visočka pečenica in this research met the requirements of the *Rulebook* regarding BaP content because they contained BaP at <2.0 µg/kg.

4. Conclusion

The conducted research presents the first results of the content of 16 EU priority PAH compounds in Visočka pečenica. The production technology for this product has retained all the characteristics of centuries-old tradition, regardless of the type of production. In the end, such an approach resulted in some high quality products, and the conducted tests showed that some Visočka pečenica was an acceptable product from the health and safety aspect as well. Still, the research found a higher content of PAH compounds with longer smok-

ing, as well as in Visočka pečenica that had a higher fat content. A lower content of PAH compounds was found in Visočka pečenica from industrial production where lower combustion chamber temperatures were recorded. In Visočka pečenica from traditional production, the higher content of PAH compounds occurred in products smoked a higher shelf level, but in industrial production, smoking at a lower shelf level produced Visočka pečenica with higher PAH contents. The content of BaP ranged from 1.36 to 6.62 µg/kg in Visočka pečenica from traditional production and from 1.59 to 3.70 µg/kg in Visočka pečenica from industrial production. In the future, it is recommended that research is continued regarding the qualitative and quantitative presence of PAH compounds in Visočka pečenica and other cured meat products in Bosnia and Herzegovina.

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