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Original scientific paper

Polycyclic aromatic hydrocarbons in dry fermented sausages from the Serbian market

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ABSTRACT

In this study, levels of benzo(a)pyrene and sum of PAH4 compounds (benzo(a)pyrene benzo(a)anthracene, chrysene and benzo(b)fluoranthene) in four types of dry fermented sausages (n=126) collected from market in Belgrade, Serbia, were analysed. The levels of benzo(a)pyrene and PAH4 compounds ranged < 0.2–0.6 μg/kg and < 0.2–2.7 μg/kg, respectively. The levels of benzo[a]pyrene and sum of PAH4 in all samples were < 2 μg/kg and < 12 μg/kg, respectively, which is the MRL in smoked meat and smoked meat products regulated both by Serbian and EU legislation. PC analysis showed that kulen and sremska sausage have a higher capacity for absorption of the analysed compounds during the process of meat smoking than do the other investigated sausages.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are numerous groups of compounds consisting of two or more condensed aromatic rings. According to the number of aromatic rings, they can be classified as light (2–3 rings) or heavy (4–6 rings) compounds (*Purcaro et al.,* 2013). PAHs are formed the during incomplete combustion of organic matter which is result of a pyrolytic process and involves high temperature and reduced oxygen levels. They are slightly water-soluble compounds, have a lipophilic character, and consequently, they tend to accumulate in the food chain. Food contamination by PAHs can originate from environmental pollution as well as during food preparation and processing. High-protein food like meat and fish which are smoked, dried or cooked are the main sources of PAHs (*Rose et al.,* 2015). Some of them are mutagenic and carcinogenic compounds. The main chemical and physical properties of 16 EU PAHs as well as their International Agency for Research on Cancer (IARC) carcinogenicity classification are summarized in Table 1 (*IARC,* 2010). In addition to IARC, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO), Scientific Committee on Food (SCF), and European Food Safety Authority (EFSA) also classify and evaluate the carcinogenicity of PAHs according to their occurrence (*Zelinkova & Wenzl,* 2015). These organizations classify BaA, CHR, BbF and BaP as mutagenic, genotoxic and carcinogenic compounds.

Smoked meat and meat products are widely distributed in the diet among the population in Serbia (*SORS,* 2022). Across the country, there are traditional smokehouses as parts of individual house-

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Table 1. List of the 16 EU priority PAHs frequently analysed in food samples with their abbreviation, chemical formula, molecular weight and IARC toxicity classification (*IARC,* 2010).

Compound Name	Abbreviation	Chemical formula	Molecular weight (g mol^{-1})	Melting Point (°C)	Boiling Point (°C)	Toxicity (IARC)*
Benzo[a]pyrene	BaP	$C_{20}H_{12}$	252.3	179	495	1
Cyclopenta[cd]pyrene	CPP	$C_{18}H_{10}$	226.3	170	439	2A
Dibenz[a,h]anthracene	DhA	$C_{22}H_{14}$	278.3	262	535	2A
Dibenzo[a,l]pyrene	DlP	$C_{24}H_{14}$	302.4	162	595	2A
Benzo[b]fluoranthene	BbF	$C_{20}H_{12}$	252.3	168	481	2B
Benzo[k]fluoranthene	BkF	$C_{20}H_{12}$	252.3	216	480	2B
Benzo[j]fluoranthene	BjF	$C_{20}H_{12}$	252.3	165–166	480	2B
Benz[a]anthracene	BaA	$C_{18}H_{12}$	228.3	158	438	2B
Chrysene	CHR	$C_{18}H_{12}$	228.3	254	448	2B
5-Methylchrysene	5MC	$C_{19}H_{14}$	242.3	118	458	2B
Dibenzo[a,i]pyrene	DiP	$C_{24}H_{14}$	302.4	281–284	594	2B
Dibenzo[a,h]pyrene	DhP	$C_{24}H_{14}$	302.4	308	596	2B
Indeno[1,2,3-cd]pyrene	IcP	$C_{22}H_{12}$	276.3	164	530	2B
Benzo[ghi]perylene	BgP	$C_{22}H_{12}$	276.3	273	550	3
Dibenzo[a,e]pyrene	DeP	$C_{24}H_{14}$	302.4	233–244	592	3
Benzo[c]fluorene	BcF	$C_{17}H_{12}$	216.3	125–127	398	3

*IARC, International Agency for Research on Cancer carcinogenicity classification [1 – The agent (mixture) is carcinogenic to humans; 2A – The agent (mixture) is probably carcinogenic to humans; 2B – The agent (mixture) is possibly carcinogenic to humans; 3 – The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans]

holds. Also, industrial meat companies produce different types of smoked meat and meat products both in traditional and industrial smokehouses (*Đinović et al.,* 2008*; Jira & Djinovic,* 2008). Presently, though, there is very little authentic and scientific information available on the PAH content of dry fermented sausages from the Serbian market (*Skrbic et al.,* 2014*; Skaljac et al.,* 2023). As production practices change with time, periodic surveillance and studies on PAH contents in smoked meat and meat products are needed to assess the safety of such products with respect to humans. Hence, the main objectives of this study were to determine the levels of BaP as well as sum of PAH4 (BaP, BaA, CHR, BbF) in different dry fermented sausages obtained from the Serbian market during 2022 and to assess the safety of the products in the line both with the Serbian and EU legislation on PAHs. In addition, the distribution of the analysed PAH compounds in the analysed samples was analysed by applying PCA analysis.

2. Materials and methods

A total of 126 dry fermented sausages (budimska, n= 14; cajna, n= 40; kulen, n = 36; sremska, n = 8 and homemade salami, n = 28) were collected in Serbian markets during 2022. After collection, samples were labelled and stored in polyethylene bags and frozen at −18°C prior to analysis. Sam-

ples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method. 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 sample was shaken and centrifuged. Supernatant was cleaned up by solid phase extraction (transferring into a 15 mL centrifuge tube containing 150 mg primary/secondary amine, 150 mg C18 and 900 mg $MgSO_4$,) with the aim of eliminating the possible interfering compounds from the sausage extract. After centrifugation, extracts were evaporated under a stream of N_2 at 40°C to dryness and then dissolved in 1 mL of hexane. The extracts were then analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS). Determination of PAH compounds was performed using gas chromatography with a triple quadrupole mass detector.

Principal components analysis (PCA) was used to group the observed samples and to discover the distribution of polycyclic aromatic hydrocarbons in dry fermented sausages. PCA analysis and result visualization were performed using software Past 3.15 (*Hammer et al.,* 2001).

3. Results and discussion

Levels of BaP and sum of PAH4 compounds in 126 samples of dry fermented sausages are shown in Table 1. BaP and PAH4 levels were in the range from < 0.2 to 0.6 µg/kg and < 0.2 to 2.7 µg/kg, respectively. The obtained PAHs levels did not exceed maximum residue limits currently in force. The legal maximum contents of BaP (2 µg/kg) and of the sum of all four compounds (PAH4; 12 µg/kg) were established by European Commission Regulation No. 835/2011 (*EC,* 2011). The maximum residue limits (MRL) both for BaP and the sum of PAH4 compounds in smoked meat and meat products were defined by the legislation of Serbia (*SGRS,* 2014), as well, and are in accordance with the EU regulation.

Measured BaP and PAH4 levels of all analysed dry fermented sausages from this study were in the range with data reported by *Skrbic et al*. (2014) for dry fermented sausages with protected designation of origin, *Petrovska klobasa* from Serbia (BaP: < 0.17–0.51 µg/kg; PAH4: < 0.17–1.68 µg/kg). However, *Skaljac et al.* (2023) determined much higher PAH4 content (1.48–7.42 µg/kg) in dry fermented beef sausage from Serbia (*Sjenicki sudzuk*) in comparation to results from our study.

PCA analysis

Principal component analysis (PCA) was applied to establish the possible correlations among the measured BaP and PAH4 levels and the different types of dry fermented sausages. In PCA plots, points which are geometrically close to each other indicate the similarity of patterns that represent these points. The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles corresponding to high correlations). To visu-

Table 1. Levels (max – min, µg/kg) of BaP and sum of PAH4 compounds in dry fermented sausages purchased in Serbian markets

Dry fermented sausages	Levels, [µg/kg]	
	BaP	PAH4
Budimska sausage, n=14	< 0.2–0.3	< 0.2–1.8
Cajna sausage, n=40	< 0.2–0.3	< 0.2–1.3
Kulen, n=36	< 0.2–0.6	< 0.2–2.6
Sremska sausage, n=8	< 0.2–0.4	< 0.2–2.7
Homemade salami, n=28	< 0.2–0.3	< 0.2–1.6

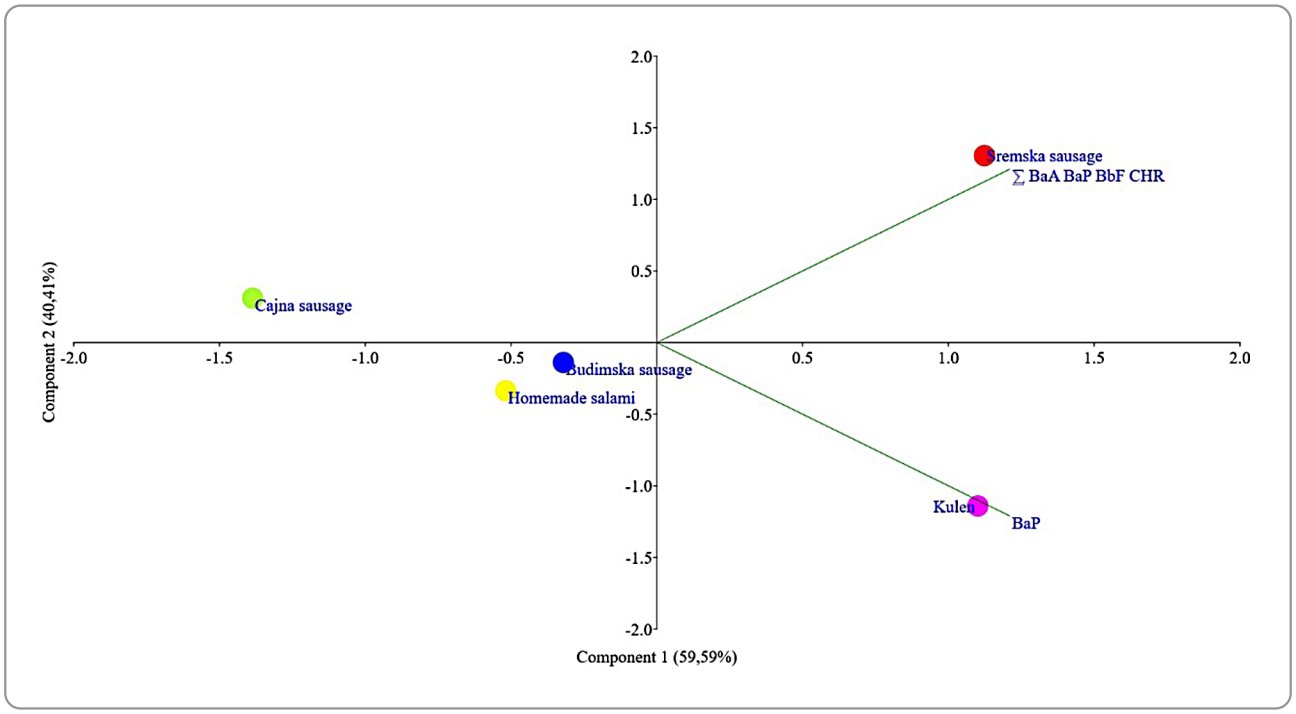

Figure 1. Biplot graph for BaP and PAH4 levels in dry fermented sausages

alise the data trends and the discriminating efficiency of the used descriptors, a scatter plot of the results from the samples using the first two principal components (PCs) from PCA of the data matrix for dry fermented sausages was obtained (Figure 1).

The influence of different parameters that describes the dry fermented sausages studied can be evaluated from the scatter plot (Figure 1), in which the sausages with higher BaP and PAH4 levels are located on the right side of the figure (sremska sausage and kulen). The lowest BaP and PAH4 levels were observed in cajna sausage, while higher levels were found in homemade salami and budimska sausage.

4. Conclusion

This study aimed to provide information on polycyclic aromatic hydrocarbons (BaP, PAH4) in five different types of dry fermented sausages from the Serbian market. PC analysis showed that high correlation exists in the levels of BaP in kulen sausage and sum of PAH4 compounds in sremska sausage. The levels of benzo[a]pyrene and sum of PAH4 in all samples were < 2 µg/kg and < 12 µg/kg, respectively, which are the maximum limits in smoked meat and smoked meat products regulated both by Serbian and European Union legislation.

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