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AUTHORS: Škaljac, S., Jokanović, M., Tomović, V., Kartalović, B., Ikonić, P., Ćućević, N., Vranešević, J., Ivić, M., Šojić, B., Peulić, T.

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Influence of traditional smoking on the content of polycyclic aromatic hydrocarbons in dry fermented beef sausage from Serbia

Snežana Škaljac [a], Marija Jokanović [a, *], Vladimir Tomović [a], Brankica Kartalović [c], Predrag Ikonić [b], Nedim Ćućević [a], Jelena Vranešević [c], Maja Ivić [a], Branislav Šojić [a], Tatjana Peulić [b]

[a] *University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 2100, Novi Sad, Serbia*
[b] *University of Novi Sad, Institute of Food Technology in Novi Sad, Bulevar cara Lazara 1, 21000, Novi Sad, Serbia*
[c] *Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000, Novi Sad, Serbia*

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ABSTRACT

This study examined the safety of dry fermented beef sausage (*Sjenički sudžuk*), produced and smoked in traditional conditions, from PAH point of view and the possibility of PAH reduction in this type of traditional product. The contents of 16 polycyclic aromatic hydrocarbons, from Environmental Protection Agency list (16 US-EPA PAH), and instrumental colour characteristics in nine groups of sausages, smoked at different distance from source of smoke (2 m - A, D, and G groups; 3 m - C, F, and I groups; 4–5 m - B, E, and H groups) were determined.

Results of this study showed that sausages smoked in traditional condition at distance of 2 m from the fire had significantly ($p < 0.05$) the highest contents of 16 US-EPA PAHs (D group −2065.74 μg/kg; G group - 2556.44 μg/kg) and PAH4 - sum of benz[a]anthracene, chrysene, benzo[a]pyrene and benzo[b]fluoranthene (D group - 7.31 μg/kg; G group - 7.42 μg/kg), while the sausages smoked at distance of 4–5 m had significantly ($p < 0.05$) the lowest contents of 16 US-EPA PAHs (B group - 342.92 μg/kg; E group - 739.41 μg/kg) and PAH4 (lower than limit of detection).

These results indicated safety of this traditional meat product, considering EU Regulation 835/2011 criteria for PAH content and pointed out that distance of 4–5 m between smoke sours and dry fermented beef sausages influenced on significant reduction of 16 US-EPA PAH compounds content.

The second part of this study showed that sausages with the highest content of PAH didn't have the highest total color change (ΔE) or total lightness change (ΔL^*). Therefore, it could not be concluded that fermented beef sausage with lighter surface colour has lower PAH content, compared with darker sausages.

1. Introduction

Sjenički sudžuk is traditional dry fermented beef sausage, from the south of Serbia, produced without additive or starter culture addition, according to the traditional recipe and experience of the local population. This product is very appreciated in Serbia because of its recognizable and specific sensory characteristics (Ikonić et al., 2022).

When making decision for buying dry fermented sausages consumers usually decide based on colour characteristics. That is the reason why colour formation is an important quality indicator of meat products (Bozkurt & Bayram, 2006; Gøtterup et al., 2008; Ledesma, Laca, Rendueles & Díaz, 2016a, 2016kaljac et al., 2018a). The desirable dark red color of the *Sjenički sudžuk* surface is highly dependent on the traditional smoking process. Traditional smoking provides unique colour and aroma characteristics of dry fermented sausages, but also it affects on the production of certain types of potentially carcinogenic chemicals, such as polycyclic aromatic hydrocarbons (PAH) (Ledesma, Laca, Rendueles, & Diaz, 2016; Sikorski & Kołakow, 2010, 2010kaljac et al., 2018b). PAH are organic compounds comprised of two or more fused aromatic rings, and are created during smoking as a result of incomplete wood combustion. Their contents in meat products very significantly depending on the way (traditional or industrial) and conditions of the smoking (type of wood, temperature of smoke, smoke intensity, type of casing ets.) (Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Ledesma, Rendueles, & Diaz, 2016; Malarut & Vangnai, 2018; Mastanjević et al., 2019; Zhu et al., 2022; Škaljac et al., 2014). In traditional smoking process dry fermented sausages are in direct contact with all smoke components and there can be a greater amount of their penetration into the sausages (European Commission - European Commission – EC, 2002; Codex Alimentarius Commission - CAC, 2009).

* Corresponding author. Marija Jokanović, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000, Novi Sad, Serbia.
E-mail address: marijaj@tf.uns.ac.rs (M. Jokanović).

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This knowledge is the reason for number of studies determining the contents of PAH in traditionally smoked meat products in different European countries in recent years. Meat producers are very interested for the scientific results of PAH contents in traditional dry fermented sausages and for possible solutions for the safety improvement of the these products which are very popular in European population nutrition (Alves et al., 2017; Djinovic, Popovic, & Jira, 2008; Lorenzo et al., 2011; Lorenzo, Purrinos, García-Falcón, & Franco, 2010; Rozentāle et al., 2015; Rozentāle, Zacs, Bartkiene, & Bartkevics, 2018; Santos, Gomes, & Roseiro, 2011; Zachara, Gałkowska & Juszczak, 2017, 2017krbić et al., 2014). The European Commission has set limits for the PAH contents in different food varieties (EC, 2011a). The limits for smoked meat and meat products are: 2 μg/kg for BaP (benzo[a]pyrene) and 12 μg/kg for PAH4 (benzo[a]pyrene, benz[a]anthracene, benzo[b] fluoranthene and chrysene) (EC, 2011a). Some European countries (Croatia, Cyprus, Finland, Ireland, Latvia, Portugal, Poland, Spain, Slovak Republic and Sweden) limited higher levels of PAH, than previously mentioned, for traditional meat product at levels of 5.0 μg/kg for BaP and 30 μg/kg for PAH4. The reason for this decision states that it is impossible to produce traditional meat products of typical sensory characteristics with a lower content of PAH compounds (EC, 2020). Nevertheless, reduction of PAH levels in meat products, without changes of typical organoleptic characteristics, is the important task of many traditional meat producers.

Therefore, the aim of this research was to determine the content of PAH compounds, from European Protection Agency list, in *Sjenički sudžuk* smoked in traditional conditional. Also, the connection between color characteristics changes and content of PAH compound was examined.

2. Materials and methods

2.1. Sausage preparation

Sjenički sudžuk sample sausages were made following original procedure in three different small/micro processing plants located in the town of Sjenica, during three production seasons (2018–2020) in traditional practice. Thus, 9 batches were manufactured in total and marked with capital letters from A to I (A, B, C – first season; D, E, F – second season; G, H, I – third season). Fresh boneless beef (approx. 75% lean) was salted using 35 g/kg of common salt (NaCl) and maintained at 4 °C for 7 days (pre-ripening period). Afterward, salted meat was ground (4 mm diameter mincing plate) and mixed with the other ingredients (raw garlic paste - 4 g/kg, black pepper - 3 g/kg, red sweet paprika powder - 2 g/kg), until a homogenous mixture was obtained. The prepared mixture was stuffed into natural casings, i.e. small intestines of beef with a diameter of approx. 40 mm and a length of approx. 50 cm. The ends of the sausage were tied off and bound together, forming a horseshoe shape. Raw sausages were entirely processed in a traditional smoking/drying room for 23 days. After one day of air-drying, sausages were subjected to smoking process. It lasted for 9 consecutive days in case of batches A, C, D, F, G, H and I, while batches B, E and H were smoked 11 days with two pauses, after 3 and 6 days of production. Throughout smoking, sausages came in direct contact with smoke (direct smoking), at a distance of 2 m (batches A, D and G) and 3 m (batches C, F and I) between smoke source (fire) and sausages. Only in case of batches B, E and H the distance between fire and products was larger (4–5 m), since the smoke was produced in a room on the ground floor, passing through the mesh barrier to reach the sausages on the first floor. The mesh barrier was made of thin wire and the pore size was 2 mm × 2 mm. The smoke was produced using beech wood for each batch of sausages.

The ambient (thermo-hygrometric) conditions in traditional smoking/drying room were registered continuously during the entire period of processing. Air temperature and relative humidity during smoking processes are presented in Table 1. After the smoking process, sausages underwent drying/ripening process in the same room, with an average overall processing temperature ranging from 7.58 °C to 21.7 °C and average relative humidity varying from 75.0% to 87.5%, up to 23rd day. At the end of drying period (23 day of production) sausages have content of moisture from 32,33% to 34,98%, content of total fat from 24,43% to 26,68%, a content of protein from 29,52% to 32,50% and content of total ash from 6.83% to 7,82% (determined according to methods of International Organization for Standardization).

Instrumentally obtained colour characteristics and contents of 16 US-EPA PAH were determined at the beginning of production (0 day) and at the end of drying period (23 day of production).

2.2. PAH determination

The contents of 16 PAH compounds designated by the United States Environmental Protection Agency: naphthalene (Nap); acenaphthylene (Acy); acenaphthene (Ace); phenanthrene (Phe); anthracene (Ant); fluorene (Fln); fluoranthene (Flt); pyrene (Pyr); benz[a]anthracene (BaA); chrysene (CHR); benzo[a]pyrene (BaP); benzo[k]flouranthene (BkF); benzo[b]fluoranthene (BbF); indeno[1,2,3-cd]pyrene (IcP); dibenz [a,h]anthracene (DhA) and benzo[ghi]perylene (BgP) were determined. The analysis of 16 EPA PAH was carried out using 7890B/5977A gas chromatograph–mass selective detector (GC–MSD) (Agilent, Palo Alto, CA, USA). PAH extraction method and chromatographic conditions were performed according to the method described in detail by Mastanjević et al. (2019).

Briefly, 3 g of sample was mixed with 6 ml acetonitrile and 3 ml water on vortex for 1 min. Then anhydrous $MgSO_4$ and anhydrous CH_3COONa were added and the sample was centrifuged at 252 rcf for 5 min. The supernatant (1 ml) was transferred to a 5 ml tube containing anhydrous $MgSO_4$ (150 mg), primary and secondary amine-PSA (100 mg) and C18 (50 mg), and again it was centrifuged at 252 rcf for 5 min. Obtained supernatant (0.5 ml) was transferred to a glass vial, evaporated at room temperature under nitrogen gas and reconstituted in hexane and analyzed on Agilent DB-5MS column. The sample was injected on 280 °C using spit-less mode and the following temperature program was applied: hold at 50 °C for 0.4 min; at 25 °C/min from 50 °C to 195 °C and hold for 1.5 min; at 8 °C/min from 195 °C to 265 °C; and maintain at 315 °C for 1.25 min after increasing at 20 °C/min. The MSD temperature was 280 °C. A carrier gas helium (He) flow of 1.2 mL/min was used. The identification of PAH was verified by comparing target ions and retention times.

Performance for validation method (precision, reproducibility, accuracy, linearity, LOQ-limit of quantification, LOD-limit of detection) of analysis for polycyclic aromatic hydrocarbons were presented in the paper by Mastanjević et al. (2019). The method precision was calculated as differences in PAH content in spiked and un-spiked samples of smoked meat. The limit of detection-LOD were from 0.29 to 0.53 μg/kg and the limit of quantification-LOQ were from 1.05 to 2.00 μg/kg for

Table 1
Temperature and relative humidity in traditional chamber during smoking process.

Sausage sample	Temperature (°C)			Relative humidity (%)		
	min.	max.	average	min.	max.	average
A	5.5	22.5	12.1	40.2	85.6	67.5
B	−2.20	21.7	12.6	48.8	89.9	74.3
C	4.5	21.0	12.0	45.2	87.6	70.5
D	22.1	37.3	28.5	65.5	87.1	78.3
E	14.3	29.0	21.0	53.4	92.5	73.1
F	22.5	35.3	27.8	73.4	89.3	81.2
G	10.1	29.5	21.4	66.4	93.1	85.8
H	3.30	23.1	13.6	55.4	92.9	78.7
I	8.1	25.5	20.4	68.4	95.1	86.8

the PAH components. The recoveries were from 85.9 to 100% for the PAH components indicating a good result in PAH measurement (EC, 2011b).

2.3. Instrumental measurement of colour characteristics

Colour characteristics were determined by the method described by Škaljac, Jokanovic, et al. (2018) using the MINOLTA CR-400 Chroma Meter (Konica Minolta Inc., Japan), with standard observer angle of 2° (illuminant - D65) and aperture of 8 mm in the measuring head. Measurements of colour characteristics (CIE $L^*a^*b^*$ system: L^* - lightness; a^*redness and yellowness-b^*) were performed on 12 different surface areas (with casings) of sausages samples. Presented data were obtained from lean meat, avoiding fat parts.

Total color change (ΔE) and total lightness change (ΔL^*) were calculated using CIE $L^*a^*b^*$ values (Jokanović et al., 2020; Ledesma, Laca, et al., 2016):

$$\Delta E = \sqrt[2]{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \tag{1}$$

$$\Delta L^* = \sqrt[2]{\left(L_0^* - L^*\right)^2} \tag{2}$$

where:
L_0^*; a_0^*; b_0^* - average value of colour characteristic measured at the 0 day of production
L^*; a^*; b^*- average value of colour characteristic measured at the end of drying period (23rd day).

2.4. Statistical analysis

Statistical analysis was performed using software package (STATISTICA 12.0). All results were presented as mean value ± standard deviation (SD). The variance analysis (ANOVA) and Duncan's multiple range test were used to test the differences, where a criteria of $p < 0.05$ was considered to indicate statistical significance of 95%.

3. Results and discussion

3.1. PAH analysis

The results of the content of 16 US EPA priority PAH of dry fermented beef sausages (*Sjenički sudžuk*), smoked in traditional conditions, obtained for raw sausage mixture and at the end of drying period are presented in Table 2 and Table 3. The PAH contents determined in raw sausage mixture, before smoking (0 day of production) were: Acy (1.53–16.22 μg/kg), Phe (0.69–12.41 μg/kg), Ant (11.39–26.83 μg/kg), Fln (4.87–11.67 μg/kg), Flt (1.32–9.23 μg/kg), Pyr (ND-1.53 μg/kg), BkF (ND-1.38 μg/kg), while for other investigated PAH contents were below the limit of detection in raw sausages samples. These findings were consistent with the results of Mastanjević et al. (2019), who in Croatian raw dry fermented sausage determined only light PAH (Anl, Ane, Flu, Ant, Phe), and similar content of Σ16 US-EPA PAHs (71.93 μg/kg). Martorell et al. (2010) reported lower value of Σ16 US-EPA PAHs (1.25 μg/kg) in Spanish raw sausages mixture, while Roseiro, Gomes, and Santos (2011) reported higher value of Σ16 US-EPA PAHs (250,63 μg/kg dry matter) in Portugal raw sausages mixture comparing to values obtained in the present study (25.75–49.06 μg/kg). Contents of PAH in raw sausages mixture depend on used spices and meat, feed used in animal breeding, environmental contamination such as wildfire, exhaust fumes and another combustion process (Fasano, Yebra-Pimentel, Martinez-Carballo, & Simal-Gandara, 2016; Lee, Jeong, Park, & Lee, 2018; Mastanjević et al., 2019; Roseiro et al., 2011).

During smoking and drying period an increasing of PAH compound contents in dry fermented sausages occurs, consequently, in this study the PAH content in sausage samples at the end of drying period, 23rd day of production (moisture content <35%), was determined. The PAH compounds determined in dry fermented beef sausages at the end of drying period were: Nap, Acy, Phe, Ant, Fln, Flt, Pyr, CHR and BkF, while contents of other investigate PAH were below limit of detection. The highest content of Nap was determined in C group of sausages (3 m from the fire - 37.07 μg/kg), while in D, E, F, G, H and I groups of samples it was below limit of detection. The contents of Phe (248.21 μg/kg), Ant (1034.74 μg/kg) and Flt (153.01 μg/kg) were the highest in sausages of D group (2 m from the fire), while content of Acy (486.885 μg/kg) and Fln (527.98 μg/kg) were the highest in G group of

Table 2
Content of polycyclic aromatic hydrocarbons (PAH) in raw sausage mixtures before smoking process.

0 day of production																		
Polycyclic aromatic hydrocarbons (μg/kg)																		
Sausage sample	Nap	Acy	Ace	Phe	Ant	Fln	Flt	Pyr	BaA	CHR	BaP	BkF	BbF	IcP	DhA	BgP	Σ 16 US EPA	PAH4
A	ND	4.43^{c} ±1.20	ND	9.86^{f} ±0.47	19.00^{d} ±0.30	5.57^{b} ±0.10	2.58^{d} ±0.07	1.45^{c} ±0.06	ND	ND	ND	ND	ND	ND	ND	ND	42.90^{c} ±0.55	ND
B	ND	2.90^{b} ±0.02	ND	0.98^{ab} ±0.10	14.85^{b} ±0.37	4.87^{a} ±0.11	1.32^{a} ±0.00	0.83^{b} ±0.02	ND	ND	ND	ND	ND	ND	ND	ND	25.75^{a} ±0.32	ND
C	ND	4.14^{c} ±0.14	ND	12.41^{g} ±0.60	11.39^{a} ±0.58	7.31^{d} ±0.48	2.28^{c} ±0.15	1.53^{c} ±0.01	ND	ND	ND	ND	ND	ND	ND	ND	39.05^{b} ±0.71	ND
D	ND	12.41^{e} ±0.53	ND	1.68^{cd} ±0.21	18.39^{d} ±0.68	11.32^{g} ±0.60	3.95^{e} ±0.12	ND	ND	ND	ND	1.31^{a} ±0.26	ND	ND	ND	ND	49.06^{d} ± 0.79	ND
E	ND	16.22^{f} ±0.06	ND	1.55^{cd} ±0.04	18.20^{d} ±0.81	11.67^{g} ±0.08	9.23^{h} ±0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	56.87^{e} ±0.83	ND
F	ND	11.62^{d} ±0.24	ND	1.24^{bc} ±0.04	11.85^{a} ±0.21	10.20^{f} ±0.63	6.77^{g} ±0.28	ND	ND	ND	ND	1.38^{a} ±0.06	ND	ND	ND	ND	43.05^{c} ±0.92	ND
G	ND	1.53^{a} ±0.11	ND	0.69^{a} ±0.02	16.29^{c} ±0.27	5.61^{b} ±0.05	1.72^{b} ±0.01	0.53^{a} ±0.02	ND	ND	ND	ND	ND	ND	ND	ND	26.38^{a} ±0.10	ND
H	ND	4.32^{c} ±0.02	ND	3.90^{e} ±0.10	26.83^{f} ±0.07	7.93^{e} ±0.01	4.27^{f} ±0.18	1.46^{c} ±0.44	ND	ND	ND	ND	ND	ND	ND	ND	48.71^{d} ±0.77	ND
I	ND	2.31^{b} ±0.02	ND	1.76^{d} ±0.10	24.55^{e} ±0.05	6.60^{c} ±0.29	2.24^{c} ±0.05	0.93^{b} ±0.19	ND	ND	ND	ND	ND	ND	ND	ND	38.39^{b} ±0.41	ND

In the same column different letters mean that values are significantly different ($p < 0.05$); ND - content of PAH < LOD detected.
Results are expressed as means ± standard deviations (n = 3); PAH4 - the sum of BaA. CHR. BbF. BaP.

CORRECTED PROOF

Table 3
Content of polycyclic aromatic hydrocarbons (PAH) in dry fermented beef sausage (*Sjenički suđuk*) at the end of drying process.

End of drying process																		
Polycyclic aromatic hydrocarbons (μg/kg)																		
Sausage sample	Nap	Acy	Ace	Phe	Ant	Fln	Flt	Pyr	BaA	CHR	BaP	BkF	BbF	IcP	DhA	BgP	Σ 16 US EPA	PAH4
A	1.14^{a} ±0.17	236.57^{e} ±3.01	ND	89.01^{e} ±0.27	384.31^{e} ±0.59	243.37^{h} ±5.15	23.75^{b} ±0.08	45.90^{c} ±2.69	ND	5.10^{b} ±0.13	ND	1.60^{c} ±0.09	ND	ND	ND	ND	1030.75^{d} ±9.17	5.10^{b} ±0.13
B	13.19^{b} ±0.33	66.74^{a} ±2.27	ND	23.00^{a} ±1.41	119.31^{a} ±3.93	40.21^{a} ±1.16	8.83^{a} ±0.39	70.91^{d} ±0.19	ND	ND	ND	0.73^{a} ±0.02	ND	ND	ND	ND	342.92^{a} ±8.15	ND
C	37.07^{c} ±1.96	205.09^{d} ±1.65	ND	50.43^{b} ±0.42	294.15^{b} ±1.85	82.72^{b} ±2.23	32.47^{c} ±0.31	39.85^{b} ±0.56	ND	ND	ND	ND	ND	ND	ND	ND	741.79^{b} ±3.94	ND
D	ND	248.25^{f} ±0.28	ND	248.21^{h} ±0.55	1034.74^{i} ±8.71	232.87^{g} ±9.15	153.01^{i} ±2.86	137.13^{f} ±0.14	ND	7.31^{e} ±0.06	ND	4.22^{e} ±0.24	ND	ND	ND	ND	2065.74^{f} ±19.93	7.31^{e} ±0.06
E	ND	69.12^{a} ±1.29	ND	81.96^{d} ±0.61	374.14^{d} ±1.99	106.78^{c} ±0.45	61.87^{f} ±0.10	45.54^{c} ±1.13	ND	ND	ND	ND	ND	ND	ND	ND	739.42^{b} ±2.79	ND
F	ND	210.79^{d} ±7.86	ND	144.47^{g} ±3.05	637.38^{g} ±3.97	187.31^{f} ±2.03	95.53^{g} ±1.86	86.10^{e} ±0.25	ND	1.48^{a} ±0.03	ND	3.43^{d} ±0.21	ND	ND	ND	ND	1366.49^{e} ±14.70	1.48^{a} ±0.03
G	ND	486.85^{g} ±8.54	ND	246.40^{h} ±7.83	1001.01^{h} ±10.28	527.98^{i} ±7.19	144.77^{h} ±2.04	137.21^{f} ±1.63	ND	7.42^{e} ±0.11	ND	4.82^{f} ±0.38	ND	ND	ND	ND	2556.44^{g} ±17.66	7.42^{e} ±0.11
H	ND	110.73^{b} ±0.54	ND	75.27^{c} ±0.20	337.50^{c} ±1.11	122.39^{d} ±0.81	40.98^{d} ±0.12	33.80^{a} ±0.15	ND	6.86^{d} ±0.16	ND	1.20^{b} ±0.07	ND	ND	ND	ND	728.72^{b} ±2.73	6.86^{d} ±0.16
I	ND	133.46^{c} ±2.44	ND	105.74^{f} ±1.69	503.02^{f} ±7.66	154.45^{e} ±1.45	49.00^{e} ±0.72	38.44^{b} ±0.50	ND	5.75^{c} ±0.36	ND	0.97^{ab} ±0.02	ND	ND	ND	ND	990.84^{c} ±10.71	5.75^{c} ±0.36

In the same column different letters mean that values are significantly different ($p < 0.05$); ND - content of PAH < LOD detected.
Results are expressed as means ± standard deviations ($n = 3$); PAH4 - the sum of BaA. CHR. BbF. BaP.

sausages (2 m from the fire). The lowest content of Acy (66.74 μg/kg), Phe (23.00 μg/kg), Ant (119.31 μg/kg) Fln (40.21 μg/kg) and Flt (8.83 μg/kg) were determined in B group of sausages (4–5 m from the fire). Predominantly, over 90%, of PAH compound obtained in analyzed sausages samples were light PAH (with two or three rings), and from highest molecular PAH compounds only Pyr, CHR and BkF were detected in *Sjenički sudžik*. This was in accordance with the results for traditional fermented sausages previously reported by other authors (Mastanjević et al., 2019; Roseiro et al., 2011; Santos et al., 2011, 2011kaljac et al., 2014). All examined samples of *Sjenički sudžuk* at the end of drying period (23rd day) had BaP content lower than limit of detection, while contents of PAH4 were from ND (B, C and E groups) to 7.42 μg/kg (G group), with significant difference ($p < 0.05$) between all investigated groups except between groups B, C and E, and also D and G. These results confirmed safety of traditional fermented beef sausages (*Sjenički sudžuk*), from PAH point of view, which were subjected to direct smoke per 9 days because all examined samples meet the requirements of the EU Regulation 835/2011 and 2020/1255. Also, this study confirmed that it is possible to produce traditional fermented beef sausages, of typical sensory characteristics, with a lower content of PAH compounds (BaP < 2 μg/kg; PAH4 < 12 μg/kg) and that it is not necessary to apply the regulatory criteria EU Regulation 2020/1255 (BaP < 5 μg/kg; PAH4 < 30 μg/kg). The total content of 16 US-EPA PAH in dry fermented beef sausages was in the range from 342.92 μg/kg (B group; 4–5 m from the fire) to 2556.44 μg/kg (G group; 2 m from the fire), with significant difference ($p < 0.05$) between all investigated groups, except between groups C, H and E. Škaljac et al. (2014) determined that dry fermented pork sausages in natural casing had lower total content of 13 US-EPA from 125 μg/kg to 220 μg/kg. This difference could be explained with the fact that they used a different type of wood (cherry) and that the smoking process was of lower intensity (Fasano et al., 2016; Ledesma, Rendueles, & Diaz, 2016). Mastanjević et al. (2019) reported similar values for the 16 EPA PAH (679 μg/kg) and PAH4 (9.90 μg/kg) for Croatian dry fermented sausages and also Santos et al. (2011) for Portuguese traditional sausages (total 16 US-EPA PAH from 877.37 μg/kg to 2609.81 μg/kg; PAH4 from 3.47 μg/kg to 4.80 μg/kg). On contrary, traditional sausages from Bosnia and Hercegovina, Spain and Italy had lower total contents of 16 US EPA PAHs (< 300 μg/kg) compared with results determined in present study (Lorenzo et al., 2010, 2011; Mastanjević, Kartalović, Puljić, Kovačević, & Habschied, 2020; Purcaro, Moret, & Conte, 2009). The differences in the PAH compounds contents could be related with traditional smoking procedure which is specific for each geographical area and the type of meat product. Many parameters during traditional smoking procedure such as temperature, humidity, type of wood, smoke intensity, numbers of smoking days, fat content in product, diameter of product ets, affected on the type and the content of PAH compounds in traditional meat products (Fasano et al., 2016; Ledesma, Rendueles, & Diaz, 2016; Malarut & Vangnai, 2018). Results of this study showed that sausages smoked in traditional condition at the distance of 2 m (D and G groups) from the fire had significantly ($p < 0.05$) the highest contents of 16 US-EPA PAHs (2065.74 μg/kg; 2556.44 μg/kg, respectively) and PAH4 (7.31 μg/kg; 7.42 μg/kg, respectively). On the other hand, the sausages smoked at the distance of 4–5 m (B and E groups) from the fire had significantly ($p < 0.05$) the lowest contents of 16 US-EPA PAH (342.921 μg/kg; 739.41 μg/kg, respectively) and PAH4 (lower than limit of detection). Obtained results indicated that the increased distance between the sausages and the fire caused the deposition of smoke particles and with them PAH compounds (especially light PAH compounds), resulting with partially purified smoke which come in contact with the sausages. So, it would be justified to separate the source of the smoke from the smoking chamber, because if only this procedure is applied it would surely affect the reduction of the PAH compounds content in smoked sausages. It was also observed that the sausages from the first season (A, B and C groups) generally had significantly ($p < 0.05$) lower PAH compounds content compared to sausages smoked at the same distance but in the second (D, E and F groups) and third season (G, H and I groups). In the first season, the average temperatures during smoking (A = 12.0 °C; B = 12.6 °C; C = 12.0 °C) were noticeable lower compared to the second season (D = 28.5.0 °C; E = 21.0 C; F = 27.8 °C) and the third season (G = 21.4 °C; H = 13.6 °C; I = 20.4 °C). Lower temperatures during smoking indicated that the smoking process itself was less intensive in the first season compared to the other two seasons. This influenced the PAH compounds content to be lower in sausages from the first season compared to sausages from the second and third seasons even though all sausages were processed at the same distance from fire. Also, air-drying period, prior to smoking process, which in traditional

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Table 4
Instrumental colour characteristics (CIE $L^*a^*b^*$ system) on the surface of dry fermented beef sausage (*Sjenički suđuk*).

Sausage sample	0 day of production			End of drying process				
	L^*	a^*	b^*	L^*	a^*	b^*	ΔE	ΔL^*
A	$46.16^{ay} \pm 1.91$	$9.85^{ay} \pm 1.95$	$9.75^{aby} \pm 2.24$	$28.80^{cdx} \pm 1.59$	$7.64^{bx} \pm 1.32$	$5.73^{bx} \pm 1.33$	$18.05^{a} \pm 1.49$	$17.36^{a} \pm 1.59$
B	$51.35^{cy} \pm 3.21$	$13.60^{dy} \pm 1.67$	$12.50^{dy} \pm 1.71$	$27.69^{bcx} \pm 1.35$	$8.60^{bcx} \pm 1.01$	$5.10^{abx} \pm 0.47$	$20.55^{b} \pm 1.21$	$23.66^{d} \pm 1.35$
C	$47.95^{aby} \pm 2.53$	$11.15^{acx} \pm 1.24$	$12.71^{dy} \pm 2.54$	$29.31^{dx} \pm 1.19$	$10.94^{ex} \pm 1.14$	$6.93^{cx} \pm 0.84$	$19.56^{b} \pm 1.28$	$18.64^{b} \pm 1.19$
D	$47.40^{aby} \pm 1.86$	$11.57^{cy} \pm 1.60$	$8.82^{ay} \pm 1.82$	$25.50^{ax} \pm 1.87$	$8.35^{bcx} \pm 1.98$	$5.48^{bx} \pm 1.11$	$22.47^{c} \pm 2.17$	$21.91^{c} \pm 1.87$
E	$46.85^{aby} \pm 1.01$	$11.95^{cy} \pm 0.78$	$11.65^{cdy} \pm 1.18$	$25.81^{ax} \pm 1.45$	$5.22^{ax} \pm 0.51$	$4.56^{ax} \pm 0.61$	$23.20^{c} \pm 1.50$	$21.04^{c} \pm 1.45$
F	$48.09^{aby} \pm 1.98$	$11.76^{cy} \pm 0.98$	$10.65^{bcy} \pm 1.77$	$27.20^{bx} \pm 1.01$	$4.84^{ax} \pm 0.79$	$5.06^{abx} \pm 0.60$	$22.72^{c} \pm 0.98$	$20.89^{c} \pm 1.01$
G	$47.24^{aby} \pm 2.04$	$10.80^{abcx} \pm 1.07$	$12.95^{dy} \pm 1.89$	$28.76^{cdx} \pm 1.01$	$10.16^{dex} \pm 1.67$	$5.85^{bx} \pm 0.86$	$19.88^{b} \pm 1.13$	$18.48^{b} \pm 1.01$
H	$48.34^{by} \pm 1.41$	$10.13^{aby} \pm 0.83$	$11.54^{cdy} \pm 1.23$	$29.68^{dx} \pm 1.39$	$8.79^{bcx} \pm 1.22$	$5.57^{bx} \pm 0.90$	$19.69^{b} \pm 1.48$	$18.67^{b} \pm 1.39$
I	$50.73^{cy} \pm 2.17$	$11.87^{cy} \pm 0.88$	$9.79^{aby} \pm 0.97$	$29.23^{dx} \pm 0.73$	$9.30^{cdx} \pm 1.46$	$5.51^{bx} \pm 0.91$	$22.13^{c} \pm 0.95$	$21.50^{c} \pm 0.73$

In the same column, different letters (a-e) means that values are significantly different ($p < 0.05$).
In the same row, different letters (x-y) means that values are significantly different ($p < 0.05$) at the 0 day of production compared with end of drying period.
Results are expressed as mean ± standard deviation (n = 12).

conditions lasts for approximately 24h (17h–24h), affects the surface of the sausage. Longer air-drying period results with drier sausage surface, therefore the smoke particles and with them PAH compounds more weakly stick to sausage surface, what was most likely to be the case with sausages in the first season.

The results of this research have applied interest for the traditional meat producers, because they defined that distance of 4–5 m between smoke sours and dry fermented beef sausages influenced on significant reduction of 16 US-EPA PAH compounds content.

3.2. Colour analysis

In the second part of this research, the connection between the colour characteristics changes and the PAH compound content was examined. The colour development of *Sjenički sudžuk* was determined by colour characteristics lightness-L^*; redness-a^* and yellowness-b^* in CIE $L^*a^*b^*$ system (Table 4). Values of colour characteristics at the 0 day of production on the surface of *Sjenički sudžuk* were in range 46.16 (A group) to 51.35 (B group) for L^* values, from 9.85 (A group) to 13.60 (B group) for a^* values and from 8.82 (D group) to 12.95 (G group) for b^* values. During processes of smoking and drying values of L^*, a^* and b^* on the surface of *Sjenički sudžuk* were significantly ($p < 0.05$) decreased, what was in accordance with the results previously reported by Ledesma, Laca, Rendueles, and Diaz (2016). Colour formed on the surface of *Sjenički sudžuk* at the end of drying period was darker, with lower values of redness and yellowness compared with 0 day of production (dark brownish-red colour). Decrease of water content and pH value during the drying period influences on formation of darker colour in fermented sausages (Bozkurt & Bayram, 2006; Chen et al., 2016). The sausages of D ($L^* = 25.50$) and E ($L^* = 25.81$) groups had significantly lower ($p < 0.05$) values of L^*, while sausages of E ($a^* = 5.22$) and F ($a^* = 4.84$) groups had significantly lower ($p < 0.05$) values of redness compared to all other examined groups of sausages at the end of drying period. Also, significantly higher ($p < 0.05$) values of yellowness had sausages of C group ($b^* = 6.93$) compared to all examined groups of sausages. According to the literature data, the values of lightness-L^* determined on the surface of *Sjenički sudžuk* at the end drying period were lower than values reported for some other traditional dry fermented sausages from Argentina (Palavecino et al., 2016), China (Chen et al., 2016; Hu et al., 2022; Huang & Huan, 2016), Croatia (Lešić et al., 2020), Portugal (Fraqueza et al., 2020) and Spain (Casquete et al., 2011; Cava, Garci-Parra, & Ladero, 2020), but similar with L^* values determined for some Spanish (Ledesma, Laca, et al., 2016) and Turkish (Bozkurt & Bayram, 2006; Sucu & Turp, 2018) traditional dry fermented sausages. Furthermore, the values of redness-a^* and yellowness-b^* were also lower than for other traditional dry fermented sausages from Spain, China, Croatia, Portugal, Argentina and Turkey (Casquete et al., 2011; Cava et al., 2020; Chen et al., 2016; Fraqueza et al., 2020; Hu et al., 2022; Huang & Huan, 2016; Ledesma, Laca, et al., 2016; Lešić et al., 2020; Palavecino et al., 2016; Sucu & Turp, 2018). Colour on the surface dependents on many parameters: type of smoking, temperature during fermentation process, moisture content of sausages, type of used salt, pH values and redox potential of meat and type of meat, pigment concentration, type of dominant microbiological culture in sausage, type of spicy pepper etc. (Bozkurt & Bayram, 2006; Chen et al., 2016; Ledesma, Laca, et al., 2016; Lešić et al., 2020; Sánchez & Leroy, 2015, 2015kaljac et al., 2022). The total colour changes (ΔE) were significantly ($p < 0.05$) higher in sausages D (ΔE = 22.47), E (ΔE = 23.20), F (ΔE = 22.72), and I (ΔE = 22.13) compared with A (ΔE = 18.05), B (ΔE = 20.55), C (ΔE = 19.56), G (ΔE = 19.88), and H (ΔE = 19.69) groups. The total lightness change was the highest ($p < 0.05$) on the surface of the sausage of B group ($\Delta L^* = 23.66$) compared with all examined groups of sausages. Ledesma, Laca, et al. (2016) in their study reported that the total colour change (ΔE) depended on the intensity of the smoking period, also Pohlmann, Hitzel, Schwagele, Speer, and Jira (2012) concluded that smoking affected the formation of a darker color of sausages, which is in accordance with present research. To the best of our knowledge, there are not studies about connection of total colour change (ΔE) and content of the PAH compound. Results of this study showed that sausages with the highest content of PAH didn't have highest total color change (ΔE) and total lightness change (ΔL^*). Therefore, it could not be concluded that *Sjenički sudžuk* with lighter surface colour has lower PAH content, compared with darker sausages.

The consumers cannot draw a conclusion about PAH content only based on the surface colour of dry fermented sausages - *Sjenički sudžuk*. Conditions during production of *Sjenički sudžuk* were important factors for ensuring dark brownish-red colour on the surface and have dominant influence on total colour change (ΔE) and total lightness change (ΔL^*) compared with intensity of smoking.

4. Conclusion

The overall results indicated that the total content of 16 US-EPA PAHs was significantly ($p < 0.05$) lower in sausages smoked in camber at distance of 4–5 m from the fire compared with sausages smoked at distance of 2 m from the fire. Examined samples of traditional fermented beef sausage, at the end of drying period, had content of BaP lower than limit of detection, while contents of PAH4 were from ND to 7.42 μg/kg. This study confirmed that it is possible to produce traditional fermented beef sausage of typical sensory characteristics with a lower content of PAH compounds (BaP < 2 μg/kg; PAH4 < 12 μg/kg) and that for this traditional product it is not necessary to apply the regulatory criteria EU Regulation 2020/1255 (BaP < 5 μg/kg; PAH4 <

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30 μg/kg). Also, the study showed that sausages with the highest content of PAH didn't have the highest total color change (ΔE) or total lightness change (ΔL*). Therefore, it could not be concluded that fermented beef sausage with lighter surface colour has lower PAH content, compared with darker sausages.

CRediT authorship contribution statement

Snezana Škaljac : Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization. **Marija Jokanović :** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **Vladimir Tomović :** Conceptualization, Formal analysis, Funding acquisition. **Brankica Kartalović :** Formal analysis, Investigation, Visualization, Validation, Supervision. **Predrag Ikonić :** Conceptualization, Methodology, Investigation, Resources. **Nedim Ćućević :** Formal analysis, Investigation, Resources. **Jelena Vranešević :** Formal analysis, Investigation, Project administration. **Maja Ivić :** Formal analysis, Investigation. **Branislav Šojić :** Methodology, Formal analysis. **Tatjana Peulić :** Methodology, Validation.

Data availability

The authors do not have permission to share data.

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