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Effect of Different Smoking Methods and Levels of Fat on the Polycyclic Aromatic Hydrocarbon (PAHs) in Buffalo Meat Sausages

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ABSTRACT

Background: The study aimed to determine how different smoking methods and levels of fat content affect the generation of polycyclic aromatic hydrocarbons in smoked buffalo meat sausages.

Methods: The treatments included conventional smoking at 60°C for 40 minutes (T_1A), 3% diluted liquid smoke (T_1B) and 7% diluted liquid smoke (T_1C). The fat percentage in the sausages was substituted with inulin as a fat replacer in the successive treatments (T_2A, T_2B, T_2C, T_3A, T_3B, T_3C). Nine PAH compounds were analysed: Fluoranthene, chrysene, benzo(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, benzo(a)pyrene, benzo(ghi)perylene, and dibenzo(a,h)anthracene.

Result: Out of the nine PAHs, fluoranthene and chrysene were identified in the examined treated samples of PAHs. No substantial variance was noted regarding fat levels or smoking methods. The chrysene concentrations varied between 32.27 and 38.37 µg/kg, while fluoranthene levels fluctuated from 15.10 to 53.47 µg/kg. All treated samples were free of benzo(a)pyrene, which is considered the most potent carcinogen.

Key words: Buffalo meat, Chrysene, Liquid chromatography-mass spectrometry, Polycyclic aromatic hydrocarbons, Sausages, Smoked sausages.

INTRODUCTION

In many parts of the world, buffalo meat (carabeef) is becoming a significant red meat source. Likewise, the northeastern states of India are also progressively consuming more carabeef because of its red colour, low fat and cholesterol with poor marbling, low connective tissue, high protein content, ability to hold water, good myofibrillar fragmentation index, emulsifying ability, and desirable texture, buffalo meat is generally preferred over other meats (Kandeepan *et al*., 2013).

One of the earliest documented methods of preserving meat is smoking. There are numerous types of smoked products prepared from every type of meat in different methods (Kadirvel *et al*., 2025). All phenols, acetic acid, and carbonyls produced by smoking have antibacterial properties and work better synergistically (Kim *et al*., 2014). Presently, liquid smoke is used in foods to add flavour, and its antibacterial effect is provided by the carbonyls and acids in it (Milly *et al*., 2005). It is possible to utilize wood smoke as a hurdle system for preserving food. Even though acids and carbonyls are slightly present in the smoke, they give more flavour than phenols. However, an undesirable amount of smoke can produce a strong burnt and creosolic taste, while an ideal amount will deliver a pleasant smoky flavour.

Wood thermally decomposes at temperatures between 500 and 900°C, primarily in the absence of oxygen, which leads to the production of chemicals known as Polycyclic Aromatic Hydrocarbons (PAHs), which are among the most important carcinogens linked to human health (Bartle *et al*., 1991). With two or more fused aromatic rings, (Danyi *et al*., 2009; El-Badry N, 2010) these compounds are persistent, lipophilic, non-polar, and semi-volatile (Plaza *et al*., 2010; Palm *et al*., 2011). The Environmental Protection Agency (EPA) has identified 16 PAHs as high-priority pollutants, including benzo[a]pyrene (B[a]P), naphthalene (NAP), phenanthrene (PHEN), acenaphthylene (ACY), anthracene (ANTH), acenaphthene (ACE), fluorene

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(FLU), fluoranthene (FLTH), benzo[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), pyrene (PYR), benzo[g,h,i]perylene (B[ghi]P), chrysene (CHRY), dibenz[a,h]anthracene (D[ah]A) and indeno [1,2,3-c,d] pyrene (IND). Within the preceding list, the International Agency for Research on Cancer (IARC, 2010) has classified Benzo[a] pyrene (BaP) as "carcinogenic to humans" (group 1). The compounds that persist are either classified as "probably carcinogenic" (group 2A) or "possible carcinogen" (group 2B). In 2011, the European Union (EU) established the maximum levels of benzo[a]pyrene (BaP), benzo[a] anthracene (BaA), chrysene (CHR) and benzo[b] fluoranthene (BbF) in smoked meat and fish products at 5 µg/kg and 30 µg/kg, respectively (PAH4). PAHs account for approximately 1 to 20% of the total carcinogenic effect of smoked products (EU, 2011).

Most PAHs are mutagenic, carcinogenic, teratogenic, and immunotoxic to microorganisms, animals and humans (Burchiel and Gao, 2014; Rengarajan *et al*., 2015; Bolden *et al*., 2017). Both humans and animals have skin sensitivities to naphthalene, anthracene and benzo(a) pyrene. Inhalation or ingestion of higher naphthalene concentrations can also destroy red blood cells. Exposure to PAHs during pregnancy causes low birth weight, early delivery, and heart malformations (Rengarajan *et al*., 2015).

The age and health status of the individual can have an impact on the toxicity of PAHs. Eye irritation, vomiting, diarrhoea, disorientation, skin irritation, and inflammation are among the acute health effects of PAHs. Conversely, the long-term consequences consist of lung malfunctions, reduced immune function, breathing issues, asthma-like symptoms, cataracts and liver and kidney damage (Abdel-Shafy and Mansour, 2016). The highest risk for cancer arises through ingestion of PAH, *i.e*., 98.1-99.3%, followed by contact through the skin (0.66-1.83%) and inhalation (0.03–0.04%) (Zhang *et al*., 2019). Long-term exposure to PAHs can also cause tumours in the lungs, skin, oesophagus, colon, pancreas, bladder and breasts (Rajpara *et al*., 2017). This study looked at how different smoking methods and fat levels affect the production of fluoranthene, chrysene, benzo(b)fluoranthene, benzo(a) anthracene, benzo (k) fluoranthene, benzo(a)pyrene, benzo (ghi) perylene, indeno (1,2,3-cd) pyrene and dibenzo (a,h) anthracene in smoked buffalo meat sausages.

This study aims to present important discoveries about the safety of smoked meat products, specifically, the formation of PAHs-as well as the safety measures to be taken while smoking meat and meat products.

MATERIALS AND METHODS

The study was conducted at the Department of Livestock Product Technology and the All India Coordinated Research Project on Post Harvest Engineering and Technology (AICRP on PHET) at the Khanapara Center, College of Veterinary Science, Assam Agricultural University, located in Khanapara, Guwahati, India. The extraction and quantification of polycyclic aromatic hydrocarbons (PAH) were performed at the National Meat Research Institute (NMRI) in Hyderabad, India.

Source of raw material

The buffalo meat along with its fat, was procured from the Kokrajhar district of Assam, India. Before further processing, the beef was sliced into 2-3 cm chunks and stored at -18°C. Cellulose casing of 21 mm diameter was used for stuffing the buffalo meat emulsion. Liquid smoke named SMOKEZ ENVIRO 24PB (Make: Red Arrow International, Manitowoc, USA) obtained from Indi-Pure Resources LLP, Ahmedabad, was utilized in this study. Best-quality spices (namely Jeera, Black Pepper, Dhania and Kashmiri red chilies, Make: Everest spices), condiments (Onion, Ginger and Garlic powder, Make: Keya Foods), binder and Inulin (Make: Urban Platter), were obtained from the local market. The PAH compounds were extracted using Quechers Disque AOAC salt and solid phase extraction (SPE) HLC6cc Cartridges (Waters India). Sigma Aldrich (USA) provided the standards for the nine PAHs utilized in the investigation.

Preparation of the sausage

The meat pieces and fat, held at -18°C overnight, were thawed at 4±1°C. The thawed meat chunks were chopped using a mechanical meat mincer (Model: TC12, Sun Labz) with a 4 mm diameter sieve. The curing agent was added to the minced meat in the quantity specified in Table 1 and allowed to settle for a full day to ensure adequate curing. Subsequently, the cured meat was ground in a bowl chopper to produce an emulsion that contained fat, inulin, seasonings, sauces and non-meat items. The liquid smoke was incorporated into the emulsion at a ratio of 1:4, as illustrated in Table 1. Ten treatment groups were prepared for this investigation (Table 2).

The emulsion treatments meant for conventional smoking, T_1A, T_2A, T_3A and the control were directly stuffed into the cellulose casing using a mechanical sausage stuffer (Make: Sun Labz, India). However, the treatments with liquid smoke were stuffed only after proper mixing of liquid smoke directly with the emulsion mix. Subsequently, the sausages were cooked in a cooking vat at 85°C for forty-five minutes. The sausages were promptly immersed in cooled water (4±1°C) after heating to neutralize the latent heat, prevent overcooking, subject any microorganisms that may be present to a thermal shock and prevent further cooking. After taking out the sausages from the chilled water, the control and treatments with liquid smoke were peeled off, packed in vacuum packages and kept in refrigeration storage (4±1°C). At the same time, the treatments with conventional smoking were further intended for smoking at 60°C for 40 minutes and later cooled, peeled off, and packed in vacuum packages.

Smoking

The cooked sausages were introduced to the H-30 Smoke Oven Machine (Make: Henan Xuanhua) at a temperature of

60°C for 40 minutes by burning teakwood sawdust. The relative humidity was maintained at 80-90% using a tray of water inside the smoke machine. The sausages were vacuum-packed in polyethylene bags after being allowed to cool to room temperature and then peeled off. The subgroups A, B, and C were based on the smoking method: A (conventional smoking at 60°C for 40 minutes), B (using 3% diluted (1:4) liquid smoke), and C (using 7% diluted (1:4) liquid smoke).

Extraction method of PAHs for LC-MS

The AOAC procedure (AOAC 2012) was employed to extract and clean the samples. The homogenization of 15 grammes of the treated buffalo meat sausage was followed by adding 15 milliliters of chilled acetonitrile (ACN). The mixture was vortexed for ten minutes and then shaken for one minute. Subsequently, 1.5 g of sodium acetate and 6 g of magnesium sulphate (Waters India) were introduced to the Quechers Disque AOAC salt. The mixture was subsequently vigorously agitated by hand for two minutes. Following a 10-minute centrifugation of the mixture at 4°C at 5000 rpm, 3 ml of the supernatant was subjected to solid phase extraction (SPE) using an Oasis Prime HLC 6cc Cartridge (Waters India). Glass tubes were used to capture two milliliters of the eluent, which was subsequently transferred to glass vials for injection into the LC-MS after the initial one milliliter was discarded.

LC-MS Quantification of PAHs

The extract was analyzed using a mass spectrometer in conjunction with Acquity UPLC (Wu *et al*., 2017). A 150* 2.1 mm, 5 µm column of "PAH C-18" was employed to connect the LC system. Helium served as the carrier gas. The flow rate was maintained at 0.6 ml/min while 10 µl of the aliquot was injected into the column. The voltage in the capillary was 3.13 KV, while the voltage in the cone was 25 V. The desolvation flux was set at 650L/hr, the desolvation temperature was set at 300°C and the collision energy was set at 14V. The inflow temperature was maintained at 275°C. The column temperature protocol for PAH analysis was increased to 80% ACN at 3 minutes and 98% ACN at 8 minutes after commencing at 70% ACN at 0.01 minutes. Subsequently, it was maintained at 9 minutes and subsequently decreased to 70% ACN at 9.5 minutes. A null sample was run for 12 minutes and matrix-matched standards were employed. The concentrations of individual PAH standards varied from 5 to 100 µg/kg. Twelve minutes were allotted as the run time. It was feasible to identify numerous PAHs, such as fluoranthene, chrysene, benzo(a) anthracene, benzo(k) fluoranthene, benzo(b) fluoranthene,

Table 1: Recipe for carabeef sausages.

Ingredients (%)	Control	Treatments		
		T_1	T_2	T_3
Lean buffalo meat	80	80	80	80
Buffalo fat	20	20	10	7.5
Inulin	-	-	10	12.5
Total	100	100	100	100
Spices	1.5	1.5	1.5	1.5
Condiments	3	3	3	3
Binder (Corn flour)	3	3	3	3
Ice cubes	10	10	10	10
Sugar	0.5	0.5	0.5	0.5
Salt	2.3	2.3	2.3	2.3
Sodium nitrite	0.15	0.15	0.15	0.15
Sodium triphosphate (STPP)	0.5	0.5	0.5	0.5
Ascorbic acid	0.2	0.2	0.2	0.2

Table 2: Formulationsof control and different treatment.

Control (C)	Sausage with 20% added fat without any smoke treatment
T_1A	Sausage with 20% added fat and conventional smoking
T_1B	Sausage with 20% added fat and 3% liquid smoke
T_1C	Sausage with 20% added fat and 7% liquid smoke
T_2A	Sausage with 10% added fat, 10% inulin and conventional smoking
T_2B	Sausage with 10% added fat, 10% inulin and 3% liquid smoke
T_2C	Sausage with 10% added fat, 10% inulin and 7% liquid smoke
T_3A	Sausage with 7.5% added fat, 12.5% inulin and conventional smoking
T_3B	Sausage with 7.5% added fat, 12.5% inulin and 3% liquid smoke
T_3C	Sausage with 7.5% added fat, 12.5% inulin and 7% liquid smoke.

benzo(a) pyrene, indeno (1,2,3-cd) pyrene, benzo (ghi) perylene and dibenzo (a,h) anthracene, by contrasting the retention times and mass spectra of unknown peaks with those of the reference standards. After LC-MS analysis, standard curves for each PAH were obtained and correlation coefficients were computed with an Excel software application.

Limits of detection (LODs) and quantification (LOQs) were determined using signal-to-noise ratios of 3 and 10, respectively. The LODs and LOQs were be 5 µg/kg and 10 µg/ kg, respectively. Table 3 gives the retention time of the 9 PAH compounds. Fig 1 shows the chromatographs for the standards of all the 9 PAHs studied.

Statistical analysis

The data obtained for the studied PAH compounds in smoked carabeef sausages were analyzed statistically in the IBM SPSS (16) software. The data was statistically analyzed ($P<0.05$) by the One-way ANOVA tool of the software, where the PAHs data was studied between the treatment groups.

RESULTS AND DISCUSSION

The mean values for PAH compounds, Fluoranthene, and Chrysene detected in the treated samples of the buffalo meat sausage with different smoking methods and fat levels are presented in Table 4 and the chromatographs for the same are given in Fig 2.

The control sample did not show the presence of any of the studied PAH compounds, namely Fluoranthene, Benz(a) anthracene, Chrysene, Benzo (a) pyrene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Indeno (123cd) pyrene, Benz (ghi) perylene and Dibenz (a,h) anthracene. Also, none of the treatments was detected for Benzo (a) pyrene, Benzo (k) fluoranthene, Indeno (123 cd) pyrene, Benz (ghi) perylene and Dibenz (a,h) anthracene. Benz (a) anthracene was below the limit of quantification (BLQ), *i.e.*, below 10 µg/kg for treatments T_1A, T_1B, T_1C and T_2A, while for the other treatments, it was not detected. All the treatments except T_2B had Benzo (k) fluoranthene below the level of quantification (BLQ).

The estimated mean concentration (µg/kg) for fluoranthene in the treated samples was above 15 µg/kg for all treatments except T_3B, where fluoranthene was below the limit of quantification. The concentration of fluoranthene was found to be highest in treatment T_2C and lowest in treatment T_1B. A significant ($P<0.05$) difference was observed in treatments T_1A, T_1B and T_2C compared to the remaining treatments. The estimated mean concentration (µg/kg) for chrysene in the treated samples was above 32 µg/kg for all treatments. It was observed that the concentration of chrysene was highest in treatment T_3A and lowest in treatment T_2C. However, no significant difference ($P<0.05$) was observed between treatments.

Table 3: Retention time of the studied polycyclic aromatic hydrocarbons (pah) compounds.

Compound name	Retention time (min)
Flouranthene	1.707
Benzo (A) anthracene	2.52
Chrysene	2.73
Benzo (A) pyrene	3.47
Benzo (B) fluoranthene	3.904
Benzo (K) fluoranthene	4.39
Indeno (1,2,3- CD) pyrene	5.86
Benzo (G,H,I) perylene	5.53
Dibenz (A, H) anthracene	5.179

In the present study, out of the 9 PAH compounds, Fluoranthene and Chrysene were detected and quantified in the treated samples. However, no considerable variation was noted regarding fat levels or smoking methods. The chrysene concentrations varied between 32.27 and 38.37 µg/kg, while fluoranthene levels fluctuated from 15.10 to 53.47 µg/kg. All treated samples were free of benzo(a) pyrene, which is considered the most potent carcinogen. Tareq *et al*. (2020) employed gas chromatography-mass spectrometry (GC-MS) to analyze PAHs (naphthalene, fluorene, phenanthrene, anthracene, pyrene, benzo(a) anthracene, chrysene and benzo (a) pyrene). They observed that the minimum and maximum mean concentrations of the PAHs mentioned above were 19.23 and 294.50 mg/kg in smoked meat, which were much higher than the findings of the present study.

In contrast to results obtained by Pohlmann *et al*. (2013b), where the PAHs content in Frankfurter-type sausages with varying back fat content increased as the fat content increased, no significant difference ($P>0.05$) was observed for the various fat content used in the present study of smoked buffalo meat sausages. Similarly, Babaoglu *et al*. (2017) reported that the content of fat in the Kokorec and the smoking regime alone did not influence the concentrations of PAH.

Ikeogu *et al*. (2023) recorded that the average concentration of PAH in traditionally smoked fish (14.568 µg/kg) was significantly higher ($P<0.05$) than the average PAH concentration of the samples in the modern smoked fish (4.404 µg/kg). However, the concentration for most PAHs did not exceed the maximum acceptable concentration.

Pohlmann *et al*. (2012) reported that the sum of the five phenolics was affected by the casing type and not by the fat contents of the sausages. They noted that cellulose-peelable casing could help reduce the PAH contents of hot-smoked sausages because a higher percentage of the PAHs (BaP: 77%; PAH4: 61%) do not pass the casing into the meat product. In a similar study, Ledesma et al. (2015) reported that no BaP was found inside the smoked meat products stuffed in synthetic casings. On the other hand, Santos *et al*. (2011) estimated the incidence of polycyclic aromatic hydrocarbons in Portuguese traditional smoked meat products, specifically the 'Paio traditional' meat type sausage with a fat content of 40% and smoked for 30 days. The total PAH content was 2609.81 µg/kg and

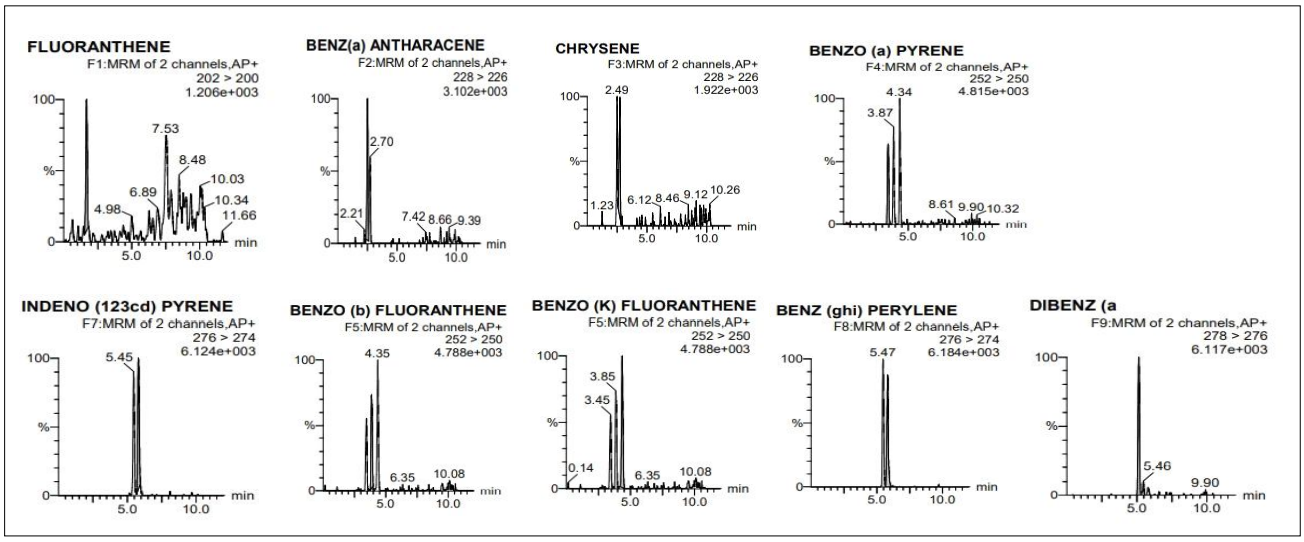


Fig 1: Chromatographs of differentstandards of the pahs (10 µg/kg).

Table 4: Concentration of polycyclic aromatic hydrocarbons (pahs) (µg/kg) of buffalo meat sausage with different method of smoking and levels of fat (MEAN±SE).

Treatments	Concentration of PAHs µg/kg								
	Flu	BaA	Chr	BaP	BbF	BkF	IcdP	BghiP	DahA
C	ND	ND	ND	ND	ND	ND	ND	ND	ND
T_1A	29.2±6.31^{a}	BLQ	33.03±2.87	ND	BLQ	ND	ND	ND	ND
T_1B	15.10±0.00^{b}	BLQ	34.50±3.68	ND	BLQ	ND	ND	ND	ND
T_1C	24.93±6.54^{abc}	BLQ	34.43±1.82	ND	BLQ	ND	ND	ND	ND
T_2A	22.23±3.21^{abcd}	BLQ	37.17±4.01	ND	BLQ	ND	ND	ND	ND
T_2B	25.90±0.00^{abcde}	ND	35.97±2.71	ND	ND	ND	ND	ND	ND
T_2C	53.47±9.04^{f}	ND	32.27±0.97	ND	BLQ	ND	ND	ND	ND
T_3A	22.97±5.29^{abcdef}	ND	38.37±1.77	ND	BLQ	ND	ND	ND	ND
T_3B	BLQ	ND	37.03±3.20	ND	BLQ	ND	ND	ND	ND
T_3C	48.97±7.69^{afh}	ND	36.30±3.81	ND	BLQ	ND	ND	ND	ND

ND- Not detected, BLQ- Below the level of quantification. Flu-Fluoranthene, BaA- Benz (a)antharacene, Chr- Chrysene, BaP- Benzo (a) pyrene,BbF- Benzo (b) fluoranthene, BkF- Benzo (k) fluoranthene, IcdP- Indeno (123 cd) pyrene, BghiP- Benz (ghi) perylene, DahA- Dibenz (a, h) anthracene n=5, Means with superscript bearing different alphabet (small) column wise differ significantly (P<0.05).

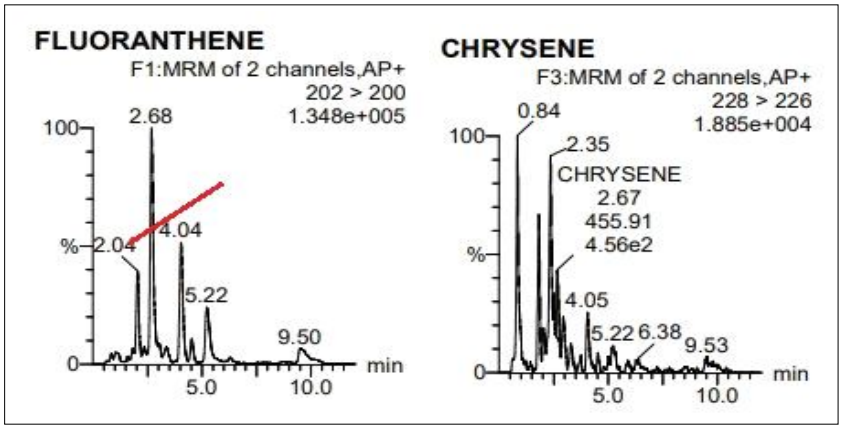


Fig 2: Chromatographs of fluoranthene and chrysene of carabeef sausages with different methods of smoking and levels of fat.

the BaP was 0.36 µg/kg. They opined that greater fatness and higher temperatures during the drying/smoking stage could be the source of such greater PAH. Similarly, Nithin *et al*. (2018) found that commercial liquid smoking (SMOKEZ ENVIRO 24PB) had lower levels of PAHs as compared to traditional smoked masmin.

In the Indian scenario, little work has been done on studying the polycyclic aromatic hydrocarbons (PAHs) in meat and meat products, particularly buffalo meat. However, the present food consumption trend requires more studies to prevent carcinogenic compounds from entering the food chain.

CONCLUSION

Among the nine polycyclic aromatic hydrocarbons (PAHs) estimated in the current study, the control had no PAHs. The PAH compounds BaP, BkF, IcdP, BghiP and DahA were absent in all treatments. Fluoranthene and chrysene were found in the treatments; however, no significant difference was observed with respect to fat content or smoking method. Therefore, from the present study, it can be concluded that the amount of added fat in carabeef sausages may not directly increase the concentration of PAHs in the product, and not all PAHs may be produced at a similar time and temperature combination during the smoking process. Nevertheless, conventional smoking with appropriate time and temperature combinations and liquid smoke can reduce the production of carcinogenic PAHs. Further, limiting the production of PAHs is possible using hurdle technologies. It is, therefore, necessary to conduct additional research on the PAH content of different liquid smokes available in the market and various hurdle technologies that can be applied while smoking meat and meat products.

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Ethical approval

This article contains no studies with human participants or animals.

Conflict of interest

The authors reported no potential conflict of interest.

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