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Original Article

Screening the Release of Eight Polycyclic Aromatic Hydrocarbons Generated in Meat During Preparation by Different Methods in Jeddah, Saudi Arabia

Rema Abdulhameed Turkustani[1,*], Mohamed Hussein Madkour[2], Amani Alawi Alrasheedi[1], Said Salama Moselhy[3,*]

[1]Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[2]Department of Environment, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[3]Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, EGYPT.

ABSTRACT

Background and Aim: Polycyclic Aromatic Hydrocarbons (PAHs) are chemical compounds formed by pyrolysis during the cooking of meat using different methods. High consumption of PAHs may increase the risk of many diseases that impact human health. The rational of current study that screening the presence of twelve PAHs in 44 meat samples prepared using traditional cooking method as (mandi, madhbi, haneeth) and modern cooking as (smoked lamb, brisket, beef cheeks). **Materials and Methods:** The PAHs were extracted by saponification method, purified by SPE-C18 and subjected for GC/MS analysis. The Retention time of sample compared with supplied library standard. **Results:** Data obtained showed that, non-carcinogenic PAHs such as fluoranthene and phenanthrene were present in higher proportions in traditional meat samples than in modern meat samples. Data obtained showed that the detected level of PAHs or degree of meat contamination, expressed as the sum of eight priority PAHs (∑8PAHs), ranged between 3.9-34.5 µg/kg, 7.1-19.7 µg/kg, 6.0-23.4 µg/kg, 7.69-12.06 µg/kg, 9.4-31.9 µg/kg and 4.5-17.83 µg/kg for mandi, madhbi, haneeth, smoked lamb, brisket and beef cheek samples, respectively ($p<0.05$). The maximum levels were 10 µg/kg per meat sample. Brisket and mandi are considered to have the highest content of (∑8PAHs) compared with other types, with averages of 18.613 µg/kg and 12.054 µg/kg, respectively. Benzo (a)pyrene, the most toxic PAH, was highly present in smoked lamb and brisket with mean concentrations of 4.275 µg/kg and 4.163 µg/kg, respectively. All of the samples analyzed were found to be below the EU permitted limit (5 µg/kg) in terms of BaP. **Conclusion:** It is recommended to add natural products rich in antioxidants during marination to prevent or reduce the formation of these PAHs.

Keywords: Benzo(a) pyrene, Chrysene, Benzo[b]-fluoranthen, Benz[a]anthracene, Smoked meat, GC/MS.

Correspondence:
Prof. Said S. Moselhy
Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, EGYPT.
Email: moselhy6@hotmail.com

Dr. Rema Abdulhameed Turkustani,
Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, SAUDI ARABIA.
Email: rturkistani@kau.edu.sa.

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INTRODUCTION

Date seed powder was used as food additive due to its high biological value and ant oxidative potency. The main constituents of date seed include sugar, protein, fat, pectin, crude fiber and polyphenols. Especially the polyphenols have been recognized as strategically important as anti-diabetic, anti-inflammatory, anxiolytic, anti-spasmodic, hepato-, gastro- and nephroprotective and antiatherogenic nutrient that is specifically richly concentrated in the peel and seeds, reaching highest polyphenol concentrations in the stage compared to the fully mature *tamer* stage, regardless of cultivar.

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic, chemically related solid compounds, which are mostly white, pale-yellow, or even colorless.[1] They encompass more than 200 distinct compounds that are made up of two or more fused aromatic rings (consisting of 2 to 3 rings) or heavy compounds (consisting of 4 to 6 rings).[2] In the environment, PAHs can be produced from many natural sources, such as forest fires and volcanic emissions alongside a diverse range of sources linked to human activity. In humans, exposure to PAHs can occur through either intake of contaminated food or non-dietary routes such as inhalation or the skin. Out of these routes, the dietary intake of PAHs is regarded as the most common source of exposure.[3] More than 70% of PAH exposure among non-smokers was linked to dietary intake. The contamination of food by PAHs can occur from environmental pollution and throughout the steps of food preparation and processing such as smoking, drying, or grilling. Smoking is one of the food processing methods utilized

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to pass on organoleptic properties and preserve food items.[3] Throughout this process, PAHs carried out by the generated smoke can readily penetrate and contaminate smoked food items. The harmful effects of PAHs on human health are mostly associated with carcinogenesis and mutagenicity in addition to immunosuppressive effects. Even though not all PAHs exhibit carcinogenic effects, they can still significantly impact human health via the generation of free radicals and bioaccumulation. Globally, as a result of changes in the industrial, domestic and natural processes, there is a remarkable rise in the population's exposure frequency to carcinogenic agents, including PAHs. Out of the identified PAHs, the most intensively investigated compound is BaP.[4] BaP was found to interfere with the normal structure of the DNA and thus has been identified to exhibit genotoxic and mutagenic effects.[5] In practice, the accurate quantification of PAHs in food samples can be challenging due to the considerable variability of PAH levels within the sample. Thus, attempts to quantify BaP levels; the most potent carcinogenic compound, may not be feasible. Consequently, according to the recommendations of EFSA, analysis of BaP levels alone is insufficient, rather the amount of BaP should be analyzed as a part of the PAH4 or PAH8 systems (EFSA, 2008). In an effort to limit individuals' exposure to BaP and PAH4, multiple countries have identified the maximum levels of these compounds in food items. Based on the recommendations of the European Union Directiv,[6] BaP and PAH4 levels in contaminated foods should not exceed 5 μg/kg and 30 μg/kg, respectively.[7] Mandi, Madhabi and Haneeth are regarded as popular traditional dishes in the Kingdom of Saudi Arabia, particularly during social events. Moreover, there has been a recent surge in the popularity of smoked meat culture, with numerous restaurants specializing in serving such fare, including smoked lamb, brisket and beef cheeks. Identified traces of PAHs in smoked and grilled meats prepared over open flames were found to damage DNA and potentially increase risk of carcinoma.[8] The current evidence is insufficient to support whether meat consumption directly initiates this mechanism. However, red meat cooking methods, such as barbecue (directly contacting flames) along with grilling were found to produce carcinogenic agents such as PAHs.[9]

Nigella sativa is one of most popular spices used worldwide due its high biological effect as it contains thymoqunone that protect against some tumors.

The rational of current study was to reduce health problems produced during meat preparations by different methods. To achieve this purpose. We screened presence of twelve to PAHs in traditional and modern smoked and grilled meats in Jeddah, Saudi Arabia using Gas Chromatography/Mass Spectrum (GC/MS) in 44 samples from six different types of meat (Mandi, madhbi and Haneeth, smoked lamb, brisket and beef cheeks) and calculated the daily intake of these meats from a sample of Jeddah population to estimate their intake of PAH to calculate its risks on health.

MATERIALS AND METHODS

Samples collection

This study examined three traditional meats (mandi, madhbi, haneeth) and three modern smoked meats (smoked lamb, brisket, beef cheeks). Forty-four samples were obtained from 44 randomly selected restaurants in Jeddah, Saudi Arabia. Each type of meat had 8 samples, except for smoked lamb, which had only 4 samples. In addition, the sample collection occurred throughout the period of November to December 2022. The following items were obtained from these outlets: Mandi meat, Madhbi meat, Haneeth meat, smoked lamb, brisket and beef cheeks. The samples were subsequently stored at a temperature of 4°C while being transported to the laboratory. Each sample in the laboratory underwent analysis in three replicates and samples that were not immediately tested were held at a temperature of -20°C.

Cooking Method	Food item	Description
Charcoal grilled (indirect heat)	Mandi meat	Composed of meat and rice infused with a special mix of spices, cooked in a clay-based oven called Tandoor. The meat is suspended inside the Tandoor without touching the charcoal then the Tandoor is closed without letting any of the smoke go outside.
Stone grilled (direct heat)	Madhi meat	It is also a very common traditional dish of composed of lamb meat cooked over hot stones.
Charcoal grilled (indirect heat)	Haneeth meat	A traditional cuisine that's similar to Mandi, however, Haneeth is cooked in a tannour oven and infused with a different spice rub, known locally as Almarkh.
smoked oven (indirect heat)	Smoked lamb	which is cooked in the special smoking oven and usually takes more than 12 hr to prepare.
smoked oven (indirect heat)	Brisket meat	It is a type of meat isolated from the breast of the cow and it is considered one of the least tender cuts of beef.
smoked oven (indirect heat)	Beef cheeks	They are soft meat cuts isolated from the facial area of the cow, around the muscles of mastication.

Methods

Extraction of PAHs from meat samples

The PAHs were extracted by Saponification method according to.[10] Briefly, A 10 g beef sample was thoroughly mixed using

a Teflon homogenizer and then combined with 25 mL of a 1 molar ethanolic potassium hydroxide solution. The mixture was heated in a water bath at a temperature of 80°C for duration of 2 hr. The homogenate was transferred to a separator funnel and subjected to three extractions using 15 mL of cyclohexane each time. The cyclohexane was subsequently evaporated. The flask, which had oily residue after evaporating cyclohexane, was subjected to three washes with a mixture of acetone and acetonitrile in a ratio of 40:60 (1 mL). Subsequently, this mixture was transferred to a centrifuge tube. Following centrifugation at a speed of 5000 revolutions per minute for duration of 5 min, the liquid remaining above the sediment was filtered through a C_{18} column that had been activated and the resulting liquid that went through the column was collected. The purification of the material was conducted using Solid-Phase Extraction (SPE) with a C_{18} column that was activated with 10 mL of methanol and 10 mL of acetonitrile. The flask underwent a second round of washing, initially with 2 mL of cyclohexane (2 mL) followed by 1 mL of acetone/acetonitrile, which was repeated three times. The solution obtained was subjected to centrifugation and the test liquid was subsequently filtered through an activated C_{18}column and collected. Subsequently, the column was rinsed with a 5 mL solution of acetone/acetonitrile mixture. The resulting eluate was collected and the solvents were evaporated until completely dry. The remaining substance was diluted in 1mL of acetonitrile and then analysed using Gas Chromatography/Mass Spectrometry (GC/MS).

Gas chromatography- mass spectrometry

Agilent GCMS 5975 (Agilent, CA, USA) system including Agilent 7890 A gas chromatography equipped with Agilent 5975C-VL MSD mass spectrometer with Agilent 7693 A automatic liquid sampler was used for analysis. The Limit of Quantification (LOQ) 0.03 µg/kg.

Statistical analysis

Statistical analyses of the data were conducted using Statistical Packages for Social Sciences (SPSS, version 26, IBM Corp., Armonk, NY, USA). Nevertheless, descriptive statistics, including the minimum, maximum, mean, Standard Deviation (SD), median and Interquartile Range (IQR), were computed for every investigated item, namely Mandi, Madhbi, Haneeth, smoked lamb, brisket and beef cheeks. For anthropometric measurements, the percentages were computed alongside the mean±SDs of food item consumption, which were identified within the food group.

RESULTS

PAHs are created as the result of pyrolytic processes through the combination of three essential elements, namely high temperature, reduction in oxygen levels, in addition to the incomplete combustion of organic matter.

Table 1 shows the rotative retention time of standard PAHs. The results 0f PAH4 in mandi, madhbi, haneeth, smoked lamb, brisket and beef cheeks meats are presented in Table 2. Benzo (a) pyrene B [a] P was found in 2 out of 8 sample of each of the investigated mandi, madhbi and haneeth meats, the concentration of B[a]P in mandi, madhbi and haneeth was ranged from 3.2 to 6.1, 4.1 to 7.1 and 3.9 to 5.5 µg/kg, respectively. However, the level of B [a] P in brisket meat samples ranged between 0.9 and 11.1 µg/kg and was detected in 5 out of 8 samples. In the smoked lamb meat, the concentration of B [a] P ranged from 3.4 to 8.2 µg/kg and was found in 3 out of 4. Benzo[a] pyrene was not detected in Beef Cheeks expect in (BC3) sample (Table 2), where the measured concentration was found to be 3.5µg/kg. On average,

Table 1: Retention time of different PAHs separated by GC/MS.

No.	Compounds	RT	Height	Area	Area %	Molecular mass
1	Naphthalene	10.66	570,313	46,76	0.841	128
2	Phenanthrene	11.54	443,038	21,95	0.395	178
3	Anthracene	13.39	851,376	36,72	0.660	178
4	Fluoranthene	14.31	564,178	32,22	0.579	202
5	Pyrene	15.08	440,551	20,84	0.375	202
6	Benz[a]anthracene	15.47	520,950	24,23	0.436	228
7	Chrysene	16.39	677,252	20,49	0.369	228
8	Benzo[b] fluoranthene	17.00	1,436,755	56,02	1.007	252
9	Benzo[k] fluoranthene	17.66	1,064,106	41,43	0.745	252
10	Benzo[a]pyrene	17.71	21,302,946	686,24	12.338	252
11	Benzo[ghi]perylene	18.91	848,409	32,99	0.593	276
12	Dibenz [a,h]anthracene	21.43	650,253	23,11	0.416	278

Inst. ACQUISITION PARAMETERS. Inst (Perkin Elmer model: clarus 580/560S), Injection=°C, volume=1 µL, Split=:1, Carrier Gas, Solvent Delay=6.00 min, Transfer Temp=150°C, Source Temp=180°C, Scan: 50 to 620Da, Column (Elite-5MS, 30 m 0.25 mm ID 0.25 um df).

Table 2: The levels of PAHs detected in different meat samples cooked by different methods.

Meat Samples			
Smoked Lamb	SL1	2.8 µg	7.69 µg
	SL2	3.4 µg	8.9 µg
	SL3	8.2 µg	12.06 µg
	SL4	5.5 µg	9.6 µg
	range	2.8-8.2 µg	7.69-12.06 µg
	mean	4.975	9.5625
Brisket	B1	3.7 µg	17 µg
	B2	12.4 µg	12.4 µg
	B3	8 µg	14.9 µg
	B4	12.6 µg	29.1 µg
	B5	2.5 µg	9.4 µg
	B6	9.1 µg	16.4 µg
	B7	1.8 µg	17.8 µg
	B8	14.1 µg	31.9 µg
	range	1.8-14.1 µg	9.4-31.9 µg
	mean	8.025	18.6125
Beef Cheeks	BC1	0 µg	9.9 µg
	BC2	0.99 µg	11.79 µg
	BC3	8.17 µg	8.17 µg
	BC4	0 µg	4.5 µg
	BC5	8.73 µg	17.83 µg
	BC6	2.11µg	5.81 µg
	BC7	3.8 µg	12.7 µg
	BC8	0 µg	7.1 µg
	range	0.99-8.73 µg	4.5-17.83 µg
	mean	2.9875	9.7375
Meat Samples		**PAH4**	**PAH8**
Mandi	Mn1	7.3 µg	7.3 µg
	Mn2	3.9 µg	3.9 µg
	Mn3	0 µg	0 µg
	Mn4	0 µg	0 µg
	Mn5	6.1 µg	18.8 µg
	Mn6	0.65 µg	13.45 µg
	Mn7	10.6 µg	34.5 µg
	Mn8	8.6 µg	18.48 µg
	range	0.65-10.6 µg	3.9-34.5 µg
	mean	4.64375	12.05375
Madhabi	Mad1	0 µg	7.56 µg
	Mad2	0 µg	0 µg
	Mad3	15.7 µg	19.7 µg
	Mad4	4.1 µg	13.7 µg
	Mad5	0 µg	0 µg
	Mad6	7.1µg	7.1 µg
	Mad7	0 µg	8.1 µg
	Mad8	11.2 µg	16.7 µg
	range	4.1-15.7 µg	7.1-19.7 µg
	mean	4.7625	9.1075
Haneeth	H1	7.46 µg	7.46 µg
	H2	4.9 µg	9.3 µg
	H3	0.74 µg	6.24 µg
	H4	6.8 µg	9.93 µg
	H5	13.3 µg	23.4 µg
	H6	3.4 µg	7.3 µg
	H7	0 µg	6.0 µg
	H8	0 µg	7.6 µg
	range	0.74-13.3 µg	6.0 -23.4 µg
	mean	4.47375	9.65375

the concentration of B[a]P was found to be the lowest in the beef cheeks samples. In comparison, higher concentrations of B[a]P were observed in the smoke lamb meat samples (Table 3).

The level of Benzo (a) anthracene B[a]A in brisket meat samples ranged between 1.4 and 3.1 µg/kg and was detected in 5 out of 8 samples. The concentration of B[a]A ranged from 0.67 to 2.11 µg/kg in beef cheeks and it was found in 4 out of 8 samples. In haneeth, the levels of B[a]A ranged from 2.8 to 3.4 µg/kg and it was detected in 3 out of 8 haneeth samples. B[a]A was not detected in mandi, madhbi and smoked lamb, except in (Mn 6) mandi meat, where the measured concentration was found to be 0.65 µg/kg. On average, the highest concentration of B[a]A was in brisket samples, followed by haneeth and then beef cheeks meat (Table 3).

Benzo (b) flurathene B[b]F was detected in half of the haneeth meat samples (Table 2). The concentration of B[b]F ranged between 0.74 and 1.3 µg/kg in haneeth meat samples considered in this study. B[b]F was found in 2 out of eight mandi, madhbi, brisket and beef cheeks meat samples and in 1 out of 4 smoked lamb meat samples, where the measured concentration was found to be 2.8 µg/kg. The arithmetic mean of Mandi had the highest value, while haneeth meats had the lowest value (Table 2).

The data in Tables 2 and 3 showed that Chrysene (CHR) was detected in 3 out of 8 mandi meat samples with concentrations that ranged from 3.9 to 8.6 µg/kg. CHR was found in 2 out of 8 samples of madhbi and brisket meat samples. The concentrations

Table 3: PAHs Incidence and concentration µg/ kg in different meat samples cooked by different methods.

PAH	Food group	Food item	Min µg/ kg	Max µg/ kg	Mean µg/ kg
Benzo (a) anthracene	Traditional meats	Mandi	0.00	0.65	0.081
Benzo (a) pyrene			3.2	6.1	1.163
Benzo (b) flurathene			4.1	5.4	1.188
Chyrsene			3.9	8.6	2.213
Fluorathene			3.3	7.14	3.793
Phenanthrene			1.6	11.1	4.275
Naphthalene			0.10	0.43	0.15
Anthracene			2.45	4.3	1.581
Pyrene			4.9	12.1	2.888
Benz[k]flourance			4.6	5.00	1.788
Dibenz[a,h]anthracene			7.7	10.3	4.498
Benzo[ghi]perylene			0.0	9.0	1.125
Benzo (a) anthracene		Madhbi	-	-	-
Benzo (a) pyrene			4.1	7.1	1.4
Benzo (b) flurathene			2.2	2.4	0.575
Chyrsene			8.8	13.5	2.788
Fluorathene			2.8	3.9	1.573
Phenanthrene			4.3	7.8	3.588
Naphthalene			0.00	8.1	1.013
Anthracene			-	-	-
Pyrene			8.1	9.1	3.163
Benz[k]flourance			8.1	9.6	2.213
Dibenz[a,h]anthracene			4.00	7.56	2.133
Benzo[ghi]perylene			-	-	-
Benzo (a) anthracene		Haneeth	2.8	3.4	1.163
Benzo (a) pyrene			3.9	5.5	1.175
Benzo (b) flurathene			0.74	1.3	0.474
Chyrsene			0.00	13.3	1.663
Fluorathene			4.9	6.3	2.713
Phenanthrene			0.34	10.43	3.51
Naphthalene			0.00	7.2	0.9
Anthracene			-	-	-
Pyrene			7.86	9.77	3.326
Benz[k]flourance			2.2	5.3	2.3
Dibenz[a,h]anthracene			0.93	4.4	1.316
Benzo[ghi]perylene			5.5	6.2	1.463
Food item	Min µg/ kg	Max µg/ kg		Food group	Mean µg/ kg

PAH	Food group	Food item	Min µg/ kg	Max µg/ kg		Mean µg/ kg
Smoke lamb	-	-		Modern meats	-	
	3.4	8.2			4.275	
	0.00	2.8			0.7	
	-	-			-	
	2.9	3.3			1.55	
	0.45	0.91			0.34	
	-	-			-	
	-	-			-	
	6.2	12.5			6.875	
	1.1	2.3			1.325	
	2.76	4.1			3.263	
	-	-			-	
Brisket	1.4	3.1			1.425	
	0.9	11.1			4.163	
	1.6	2.5			0.513	
	2.8	12.6			1.925	
	-	-			-	
	1.5	2.1			0.688	
	1.1	8.56			2.401	
	7.3	9.1			2.05	
	9.3	10.9			3.813	
	7.3	9.2			2.063	
	5.3	8.7			4.538	
	6.9	8.6			3.988	
Beef cheeks	0.67	2.11			0.638	
	0.00	3.5			0.438	
	3.8	4.1			0.988	
	0.00	7.4			0.925	
	-	-			-	
	3.22	6.6			2.365	
	0.77	0.92			0.323	
	2.4	4.1			1.2	
	6.4	9.1			2.838	
	3.7	5.5			2.275	
	4.4	6.2			1.325	
	4.5	8.9			3.15	

of CHR ranged from 8.8 to 13.5µg/kg for madhbi meat samples and from 2.8 to 12.6 µg/kg for brisket meat samples. CHR was not detected in smoked lamb, beef cheeks and haneeth, except in (BC5) in beef cheeks sample and (H5) in haneeth, where the measured concentration was found to be 7.4 and 13.3 µg/kg, respectively (Table 2). The results indicate that the means of traditional meat samples was higher than the mean of modern meat samples (Table 3). The PAH4 levels ranged from 0.65 to 10.6 µg in mandi meat, 4.1 to 15.7 µg in madhbi meat, 0.74 to 13.3 µg in haneeth meat, 2.8 to 8.2 µg in smoked meat, 1.8 to 14.1 µg in brisket and 0.99 to 8.73 µg in beef cheeks. The mean concentration of ∑8 PAHs in brisket and beef cheeks varied between 9.4 and 31.9 µg, 4.5 and 17.83 µg, respectively. Concentrations of ∑8 PAHs in madhbi, haneeth and smoked lamb samples varied from

7.1 to 19.7 µg, 6.0 to 23.4 µg and 7.69 to 12.06 µg, respectively. The results indicated that the lowest mean ∑8 PAHs was obtained for Mn2 (3.9), however, the highest ∑8 PAHs was (34.5 µg) in Mn8 in mandi meat samples (Table 3).

DISCUSSION

As a consequence of this process, a diverse mixture of PAHs is generated and can subsequently accumulate in the environment, contaminating water, soil and air and ultimately the food chain.[11,12] These differences can be attributable to multiple factors, most notably, the methodological inconstancies across the utilized surveys.[11] Furthermore, the level of intake of PAHs contaminated food were suggested to vary based on the cooking method and the characteristics of distinct food items.[13]

According to the European Food Safety Authority (EFSA, 2008) classified them based on their potential health effects into 3 different types. These 3 groups are the carcinogenic PAH2 (BaP and Chr), the mutagenic PAH4 (BaA, BaP, BbF and Chr) and the toxic PAH8 (BaA, BaP, Chr, BbF, Benzo[k]fluoranthene (BkF), Indeno[1,2,3-cd] pyrene (IcdP), Dibenzo[a,h]anthracene (DahA) and Benzo[ghi]perylene (BghiP).[13] Another classification system used by International Agency for Research on Cancer (IARC) classifies different PAHs based on their carcinogenicity to humans. For instance, BaP belongs to Group 1 and it is labeled as carcinogenic, whereas members of Group 2B, namely BaA, BbF and Chr, are labeled potentially carcinogenic. Similarly, other health related organizations, such as the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO), Scientific Committee on Food (SCF) and EFSA classify different PAHs based on their carcinogenicity. Accordingly, BaA, Chr, BbF and BaP were considered to exhibit mutagenic, genotoxic and carcinogenic effects.[14] In practice, the accurate quantification of PAHs in food samples can be challenging due to the considerable variability of PAH levels within the sample. Thus, attempts to quantify BaP levels; the most potent carcinogenic compound, may not be feasible.

Smoking is a food processing method utilized to pass on organoleptic properties and preserve food items. Throughout this process, PAHs carried out by the generated smoke can readily penetrate and contaminate smoked food items.[15] In an attempt to reduce the levels of food contamination by PAHs, the Codex Alimentarius Commission (CAC) recommended to control a number of variables during smoking, most importantly smoking method.[16] In general, conventional food smoking can be classified into direct and indirect smoking methods. In the direct method, food items and the smoke source are enclosed within the same compartment. This approach can be divided based on the absence or presence of the source of fire throughout the process into either cold (temperatures reach 30°C) or hot (temperatures reach 130°C) techniques, respectively. Contrastingly, in the indirect method, food items and the electrostatic generated smoke source are placed into separate compartments.[17] Another contemporary indirect method utilized by the food industry is adding liquid smokey flavors to pass on organoleptic properties to food items. These flavored liquids are created from condensed smoke and exhibit lower levels of PAHs when compared to direct smoking methods.[18] Consequently, unlike the direct smoking method, indirect methods are considered safer and more popular in the food industry.[19]

Alongside the cooking methods, which takes into consideration the smoking compartment design and equipment, the levels of PAHs can be influenced by numerous other variables. These include the duration, temperature and humidity of the smoking process, the cleanliness and maintenance of the smoking apparatus along with the utilized fuel type, namely charcoal or biomass. Furthermore, the amount of fat impeded in the food products and their proximity to heat are other important indictors of PAHs levels.[20] A study conducted aimed to investigate the effects of meat grilling on the levels of PAHs, specifically PAH production. The results revealed that the use of a special grilling apparatus to remove meat drippings, in which fat was the main constituent, alongside the generated smoke, lead to a significant reduction in the sum of the PAH. Compared to conventional grilling methods, the amount of PAH generated following the removal of meat drippings and smoke was decreased by 48-89% and 41-74%, respectively. Therefore, it was concluded that the main culprit for PAHs production was the smoke generation secondary to the incomplete combustion of grease dripped onto flames.[21] A study conducted by Roseiro *et al.*, indicated that the levels of PAHs were higher when food items were placed closer to the source of smoke.[22] The source of combustible materials used in the smoking process can also influence the amount PAHs. For instance, the combustion of apples and alder shells produce lower levels of PAHs compared to spruce wood.[23] These recommendations should be utilized by the food industry based on Hazard Analysis Critical Control Point (HACCP) principles in order to minimize PAHs contamination of food items during smoking. It was reported that, chicken drumsticks treated with either green or white tea were prepared prior to grilling. The results indicated that the levels of BaP detected in the samples were significantly lower with the electric grilling compared to charcoal grilling. Similarly, owning to its free radicals scavenging properties, samples pretreated with white tea exhibited lower BaP levels.[23] Therefore, these findings support the use of electric grilling and the marination of food items with white tea marinades prior to grilling, in order to reduce BaP levels and in turn increase the grilling safety profile.

Prior to cooking, meatballs were infused with a 0.5% blend of spices, namely ginger, garlic, black pepper, red-chili, paprika and onion powders. The use of spices influenced the production level of both BaA and BaP along with the overall PAHs level across the samples. Furthermore, the type of meat was another crucial

factor in determining the levels of BaA, which was primarily inhibited by the addition of ginger powder. It was concluded that the use of spice powders during meat processing can potentially result in the reduction of PAHs production. This effect was attributable to antioxidant properties of each spice and their potential inhibitory effects on the formation of PAHs.[24-27] In comparison to the control (marinades without vegetable oil), the use of palm oil and sunflower oil resulted in a substantial increase of PAH levels from 190.1 µg/kg to 457.6 µg/kg and 376.6 µg/kg, respectively. The observed difference in PAH levels between both oil types was attributed to the presence of antioxidants in the sunflower oil. It was also observed that the addition of an alkaline marination mixture (pH over 7.5) led to a significant increase in detected heavy PAH and BaP levels by more than 70% and 80%, respectively. This study concluded that the addition of grease and alkaline marination mixtures can result in a significant increase of PAHs contamination in grilled foods.[25] The amount of PAHs in canned and smoked food items, namely chicken, fish, pork and sausages. It was concluded that traditionally smoked food items, especially sausages, demonstrated the highest average of PAH4 levels of around 24.27 µg/kg.[28-31]

CONCLUSION

In conclusion, the saponification and SPE method was found to be the most effective in Extraction. Brisket and Mandi are considered the highest content of ∑8PAHs compared with other types. Benzo(a) pyrene and chrysene, the most toxic PAHs are highly present in (smoked lamb. brisket) and (madhbi and mandi) respectively. Among compounds, the highest for Dibenz[a,h]anthracene folowed by Benz(a) Pyrene. It was recommended that, the amount consumed of brisket and mandi should be reduced in minimum rate to decreased bioaccumulation of these PAHs and decreased risk of chronic diseases. It is preferred to add a natural component rich with antioxidants that reduced or prevented formation of PAHs during cooking

ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of King Abdulaziz University, Jeddah, Saudi Arabia. The protocol was done according to the ethical guidelines of the 1975 Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

MM and AA design the experiments. RAT running the practical section and SSM, data analysis and interpretation. ALL authors share in write and review manuscript.

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