# Detection and control of polycyclic aromatic hydrocarbons in meat products

Alaa K. Amin, Fatin S. Hassanin, Mohamed A. Hassan, Fahim A. Shaltout\*

Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, 13736, Egypt.

### **ARTICLE INFO**

Recieved: 03 March 2024

Accepted: 12 April 2024

#### \*Correspondence:

Corresponding author: Fahim A. Shaltout E-mail address: fahim.shaltout@fvtm.bu.edu.eg

Keywords:

Polycyclic aromatic hydrocarbons Grilled meat Lauryl oil Garlic oil Lemon oil

# Introduction

# ABSTRACT

Chemicals known as polycyclic aromatic hydrocarbons (PAHs) can be found in foods that have been grilled or barbecued, especially meats. One hundred and twenty samples of meat products represented by roasted kofta, roasted meat, charcoal grilled kofta and charcoal grilled meat (20 of each) were collected randomly from different markets at Kalyobia governorate, Egypt. The collected samples were examined for determination of Polycyclic Aromatic Hydrocarbons (PAHs). Natural oils as lauryl oil, garlic oil and lemon oil with concentration (0.5% -1% 1.5%) were used to reduce fat pyrolysis by minimizing the amount of meat drips. The recorded results revealed that mean values of PAH4 was 10.35 $\pm$ 0.89, PAH8 was 14.53 $\pm$ 1.17 and PAHs was 16.49 $\pm$ 1.33, respectively in untreated samples of charcoal grilled meat. Meanwhile PAH4 residual concentrations in samples treated with lauryl oil of 0.5, 1.0 and 1.5% were 13.7 $\pm$ 0.6, 10.3 $\pm$ 0.4 and 7.2 $\pm$ 0.3, and with reduction percentages of 26.4, 44.5 and 61.2%, respectively. Samples treated with garlic oil of 0.5, 1.0 and 1.5% showed PAH4 residual concentrations ere 12.1 $\pm$ 0.5, 8.9 $\pm$ 0.4, 5.5 $\pm$ 0.2, with reduction percentages of 34.9, 52.2 and 70.0%, respectively. However, samples treated with lemon oil in concentrations of 0.5, 1.0 and 1.5% revealed that PAH4 levels were10.3 $\pm$ 0.5,6.8 $\pm$ 0.4 and 3.9 $\pm$ 0.2, with reduction percentages of 45.7, 65.6 and 79.1%, respectively. It could be concluded that charcoal-grilled meat treated with natural oils decrease the levels of PAH4 in the examined samples.

Rapid urbanization and industrialization have spewed contaminants into the atmosphere, including polycyclic aromatic hydrocarbons, or PAHs (Mojiri *et al.*, 2019). PAHs are persistent pollutants with a broad range of biological toxicity due to their inherent properties, and their removal from the atmosphere has long been a source of worry. Furthermore, toasting, grilling, frying, baking, and roasting are processes used in both home and commercial food preparation that produce PAHs (Rose *et al.*, 2015). Three main factors contribute to the build-up of PAHs in charcoal beef steaks: the smoke from incomplete combustion of the heat source attached to the steak's surface, the pyrolysis of organic compounds (protein and fat) in the steak during cooking, and the heat source itself (Zelinkova and Wenzl, 2015).

Furthermore, the abrasive combustion of food molecules produces free radicals, which are the basic mechanism generating PAHs, and these byproducts eventually accumulate in food items (Singh *et al.*, 2016). because PAHs can mutate, cause cancer, and be cytotoxic. Consequently, the European Commission has established thresholds for the quantity of PAHs present food matrices. Meat that has been grilled by grilling or barbecuing is allowed to contain up to 5 ng g1 of benzo [a] pyrene (BaP) and 30 ng g1 of PAH4 (EC, 2023).

Countries are inconsistent with different food categories and limit values when it comes to PAH limit standards in food (Racovita *et al.*, 2021). The European Commission's Scientific Committee on Food (SCF) declared 15 PAHs to be genotoxic and carcinogenic, with Benzo[a]pyrene (BaP) identified as a food detection index. (Li *et al.*, 2021; Zhao *et al.*, 2014). As a result, the SCF added a PAH to the European Union (EU) standard called "15 + 1 EU priority PAHs" in 2005. However, the European Food Safety Authority (EFSA) questioned the logic of using BaP as the only PAH de-

tection indicator in food. Therefore, EFSA, in 2008, proposed to use eight PAHs (PAH-8, including Benz[a]anthracene (BaA), Chrysene (CHR), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), BaP, Dibenz[a, h]anthracene (DhA), Benzo[g, h, i]perylene (BgP), and Indeno[1,2,3-c, d]pyrene (IcP)) or four (PAH-4, including BaA, CHR, BkF, and BaP) as the detection index of PAHs in food (EFSA, 2008). PAH-8 (BaP, CHR, BaA, BbF, BbF, DhA, BgP, and IcP) and PAH-4 (BaP, CHR, BaA, BbF) have since become widely used as PAH detection standards in food. Detection standards for PAHs in foods are still limited to only one BaP type in China (National Food Safety Standard, PR China, GB 2762-2017). So, in this study, natural methods were used to control PAHs levels in charcoal grilled meat and control the carcinogenic effects that cause.

# Materials and methods

This study was carried out in the Center of Experimental Animal Research, Faculty of Veterinary Medicine, Benha University, Egypt, in accordance with ethical rules (NO BUFVTM 40-06-23).

## Collection of samples

One hundred and twenty samples of meat products represented by roasted kofta, roasted meat, charcoal grilled kofta and charcoal grilled meat (30 of each) were collected randomly from different markets at Kalyobia governorate, Egypt. Each sample was separately wrapped in a plastic bag and transferred directly to the laboratory. The collected samples were examined for determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Heterocyclic Aromatic Hydrocarbons (HAAs). Accordingly, the comparison of such dangerous residues in the examined meat products with the maximum residual limits stipulated by international regulations

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. ISSN: 2090-6277/2090-6269/ © 2011-2024 Journal of Advanced Veterinary Research. All rights reserved.

was applied to evaluate of their acceptability for human consumption. Further, certain trials using natural oils and antioxidants to control these residues to gain excess to meat products were performed.

Thus, this study provided an overview of important variables to reduce the amount of PAHs, particularly those that cause cancer, in grilled meat.

### Determination of Polycyclic Aromatic Hydrocarbons (PAHS)

For the sample preparation cyclohexane (ECD tested), N,N-dimethylformamide, methanol (HPLC grade), sodium chloride (ACS), ethanol sodium-sulphate (ACS) potassium hydroxide were purchased El-Gomhurya, Al-Amirya, Egypt. Silica solid phase extraction (SPE) tubes (500 mg) and ultra-pure water was obtained with a MilliQ filter system. Mixture of 15 PAH standards were used (purchased from AccuStandard, CT06513, USA). The standard mix of PAHs consisted of a solution in acetonitrile with concentration 50 mg/l and the concentration of deuterated benzo[a]-pyrene-d12 dissolved in cyclohexane was 1000 ng/µl.

The tested meat product was thoroughly homogenized. The sample preparation procedure was elaborated according to Simko (2002) and Stumpe *et al.* (2008) with some modifications in order to adapt to the gas chromatography–mass spectrometry (GC–MS) detection method.

An Agilent 1200 HPLC system (Germany) consisting of a quaternary pump G1311A, a vacuum degasser G1322A, an automatic injector G1329A with sample tray G1330A, a column thermostat G1316A, a fluorescence detector G1321A, a multiple wave detector G1365B and integration software (Chem Station G2170AA and G2180AA) was used.

Twenty five grams of the sample were placed into round bottomed flask, 12 g of potassium hydroxide and 100 ml of ethanol were added. Accordingly, 25  $\mu$ l of internal standard benzo[a]pyrene-d12 solution with concentration 10 ng/  $\mu$ l and 125  $\mu$ l of PAH mix with concentration 1 ng/  $\mu$ l were added. Therefore, the mixture was subjected to an alkaline treatment with potassium hydroxide and ethanol by heating for 2 hours at 40°C under reflux and filtered. After cooling to room temperature, the solution was transferred to a 500 ml separating funnel, 100 ml of water and 100 ml of cyclohexane were added. The funnel was shaken, and the layers were allowed to separate.

The ethanol/water phase was transferred into a 250 ml separating funnel and shaked with another 50 ml of cyclohexane. The ethanol/water phase was discarded, and the cyclohexane phases were combined. Thus, the cyclohexane solution was washed successively with 50 ml water, 50 ml of methanol/water (4:1) and 50 ml of water. The cyclohexane extract was shaken with 50 ml of N, N-dimethylformamide/ water (9:1) solution. The layer of N, N-dimethylformamide/water solution was transferred into a 250 ml separating funnel, 50 ml of 1% NaCl solution were added, and PAH were extracted with 75 ml of cyclohexane. The cyclohexane phase was dried over anhydrous sodium sulphate and concentrated by rotary evaporator under reduced pressure (40°C, 235 mbar). The extract was applied to a silica SPE column previously conditioned with cyclohexane (5 ml). The flask was rinsed with cyclohexane (3 ml), and the PAH were eluted with 6 ml cyclohexane. The collected fraction was evaporated under a light stream of nitrogen at 40°C temperature, dissolved in 50 µl of cyclohexane.

An Agilent 1200 HPLC system (Germany) consisting of a quaternary pump G1311A, a vacuum degasser G1322A, an automatic injector G1329A with sample tray G1330A, a column thermostat G1316A, a fluorescence detector G1321A, a multiple wave detector G1365B and integration software (Chem Station G2170AA and G2180AA) was used. Varian Factor Four capillary column 30 m×0.25 mm with film thickness of 0.25 lm, helium carrier gas 1 cm3/min, injector and detector temperature 280°C, temperature program: 120°C (1 min), 120-250°C (15°C/ min), 250°C (13 min), 250-280°C (20°C/ min), 280°C (1 min), 280-300°C (35°C/ min), 300°C (20 min). Total run time was 48 minutes. matograph. The data were acquired operating the MS in selected ion monitoring mode. Peak spectra were compared to the mass spectra of PAH standards and library supplied with the instrument. The results of recovery of PAHs from the different meat products under investigation were evaluated according to the technique adopted by Chantara and Sangchan (2009). Accurately, the recovery percentages were ranged from 91% to 103% for the various studied meat products. Thus, the average of triplicate analysis was calculated for each polycyclic aromatic hydrocarbon.

### Experimental part

Accurately, 30 samples of meat fillets represented by untreated 3 samples (control), 9 samples treated with lauryl oil at concentrations of 0.5, 1 and 1.5% (3 of each), 9 samples treated with garlic oil at concentrations of 0.5, 1 and 1.5% and 9 samples treated with lemon oil at concentrations of 0.5 and 1.0%. All treated samples were sprayed for 15 min in the prepared natural oils (0.5, 1.0 and 1.5%) and cooked by ordinary charcoal grilling. Therefore, all charcoal grilled meat fillets were examined for determination of their contents of BaP, BaA, BpF and CHR Polycyclic Aromatic Hydrocarbons (PAH4). The reduction% of PAHs was calculated to study the effect of such treatments on these serious residues. (The source of essential oils is Food Analysis Center, Faculty of Veterinary Medicine, Benha University)

## Statistical Analysis

The collected data were analyzed using one-way analysis of variance (ANOVA) with Duncan by SPSS<sup>®</sup> version 16.0 according to the methods recommended by Feldman *et al.* (2003).

#### Results

Results achieved in Table 1, showed the mean concentration of potentially carcinogenic PAHs, PAH4 was  $21.92\pm1.48$ , PAH8 was  $31.16\pm1.85$  and PAHs was  $36.97\pm2.32$  µg/kg.

The recorded results in Table 1, showed that the mean concentration of the PAHs group in the examined samples of roasted meat (n=30), PAH4 was  $17.05\pm1.32$ , PAH8 was  $24.35\pm1.67$  and PAHs was  $28.86\pm1.95 \mu g/kg$ .

The results in Table 1, revealed that PAH individual concentrations in the examined samples of charcoal grilled kofta (n=30), PAH4 was  $13.91\pm1.14$ , PAH8 was  $13.91\pm1.14$  and PAHs was  $22.75\pm1.62 \mu g/kg$ .

The results achieved in Table 1, showed the mean concentration of PAHs in the examined samples of charcoal grilled meat (n=30), PAH4 was  $10.35\pm0.89$ , PAH8 was  $14.53\pm1.17$  and PAHs was  $16.49\pm1.33$  µg/kg.

Additionally, the findings of the present investigation presented in Table 2, demonstrated the influence of lauryl oil in concentrations of 0.5, 1 and 1.5% on PAHs levels in the examined samples of charcoal grilled meat fillets (n=5). PAH4 was  $13.7\pm0.6$  with reduction percentage 26.4% for lauryl oil concentration 0.5%.

Concerning 1% lauryl oil concentration; PAH4 was  $10.3\pm0.4$  with reduction percentage 44.5%. The best concentration of lauryl oil to reduce the carcinogenic PAHs in fillets of charcoal-grilled meat fillets was 1.5%; PAH4 was 7.2±0.3 with reduction percentage 61.2 %.

Furthermore, results in Table 3, showed the Influence of garlic oil in concentrations of 0.5, 1, and 1.5% on PAHS levels in the examined samples of charcoal grilled meat fillets (n=5). PAH4 level was  $12.1\pm0.5$  with a reduction percentage of 34.9% for garlic oil concentration.

In contrast, for those with 1% garlic oil concentration; PAH4 level was  $8.9\pm0.4$  with a reduction percentage of 52.2%.

The optimal concentration of garlic oil to reduce the carcinogenic PAHs in fillets of charcoal-grilled meat (n=5) was 1.5%, with PAH4level of  $5.5\pm0.2$  and a reduction percentage of 70.4%.

One microliter of the sample solution was injected into gas chro-

The recorded results in Table 4, showed the influence of lemon oil in

A.K. Amin et al. /Journal of Advanced Veterinary Research (2024) Volume 14, Issue 6, 950-953

Table 1. Analysis of PAHs levels (µg/kg) in the examined samples of roasted kofta, roasted meat, charcoal grilled kofta and charcoal grilled meat (n=30).

PAHs	Roasted kofta	Roasted meat	Charcoal grilled kofta	Charcoal grilled meat
Benzo[a]Pyrene (BaP)	10.69±0.73	8.19±0.41	7.02±0.36	4.93±0.28
Benz[a]anthracene (BaA)	8.07±0.61	6.37±0.29	4.89±0.22	3.76±0.19
Benzo(b)fluoranthene (Bbf)	$1.35 \pm 0.04$	$0.96{\pm}0.08$	$0.74{\pm}0.05$	$0.61 \pm 0.04$
Chrysene (CHR)	$1.81{\pm}0.06$	1.53±0.11	$1.26{\pm}0.08$	$1.05 \pm 0.06$
Sum PAH4	21.92±1.48	17.05±1.32	13.91±1.14	10.35±0.89
Benzo[k]fluoranthene (BkF)	2.13±0.15	$1.88 \pm 0.10$	1.51±0.12	$1.18{\pm}0.07$
Dibenz[a,h]anthracene (DahA)	0.52±0.03	$0.45 \pm 0.02$	0.36±0.01	$0.25 \pm 0.02$
Benzo[g,h,i] perylene (BghiP)	3.76±0.31	2.91±0.18	2.40±0.16	1.84±0.13
Indenol [1,2,3 cd] pyrene (IcdP)	2.83±0.26	$2.06 \pm 0.14$	1.25±0.11	$0.91 {\pm} 0.06$
Sum PAH8	31.16±1.85	24.35±1.67	$19.43 \pm 1.45$	14.53±1.17
Dibenzo[ae]pyrene (DaeP)	$0.58{\pm}0.04$	$0.46{\pm}0.02$	0.32±0.01	UDL
Dibenzo[al]pyrene (DaIP)	$0.93{\pm}0.07$	$0.78{\pm}0.05$	$0.59{\pm}0.04$	$0.41 \pm 0.03$
Dibenzo[ah]pyrene (DahP)	$0.41 \pm 0.02$	$0.33 \pm 0.02$	0.26±0.01	0.16±0.01
Dibenzo[ai]pyrene (DaiP)	0.24±0.01	$0.19{\pm}0.01$	$0.14{\pm}0.01$	UDL
Cyclopenta(c,d)pyrene (CcdP)	2.89±0.15	2.27±0.13	$1.63{\pm}0.09$	$1.18{\pm}0.07$
Fluoranthene (Flt)	$0.76{\pm}0.08$	$0.48{\pm}0.03$	$0.38 \pm 0.04$	0.23±0.01
Sum PAHs	36.97±2.32	28.86±1.95	22.75±1.62	16.49±1.33

Data are expressed as Mean±SE. PAH4: BaP, BaA, Bbf & CHR; PAH4: BaP, BaA, Bbf, CHR, BkF, DahA, BghiP & Icd; UDL: Undetected limit

Table 2. Influence of lauryl oil on PAHS levels in the examined samples of charcoal grilled meat fillets (n=5).

Treatmen	ent Control	Control 0.50% Lauryl oil		1% Lauryl oil		1.50% Lauryl oil	
РАПЗ	Average level	Mean	R %	Mean	R%	Mean	R%*
Benzo[a]Pyrene (BaP)	9.2±0.5 <sup>A</sup>	6.7±0.3 <sup>B</sup>	27.2	5.1±0.3 <sup>c</sup>	44.6	3.6±0.2 <sup>D</sup>	60.9
Benz[a]anthracene (BaA)	5.8±0.3 <sup>A</sup>	$4.1 \pm 0.2^{B}$	29.3	$3.0{\pm}0.2^{\circ}$	48.2	$1.9{\pm}0.2^{\rm D}$	67.2
Benzo(b)fluoranthene (Bbf	) 2.1±0.1 <sup>A</sup>	1.6±0.1 <sup>B</sup>	23.8	$1.2 \pm 0.1^{\circ}$	42.9	$0.8 \pm 0.1^{D}$	61.9
Chrysene (CHR)	1.5±0.1 <sup>A</sup>	1.3±0.1 <sup>B</sup>	13.3	$1.0\pm0.1^{\circ}$	33.3	0.9±0.1 <sup>D</sup>	40
Sum PAH4	18.6±0.9 <sup>A</sup>	13.7±0.6 <sup>B</sup>	26.4	$10.3 \pm 0.4^{\circ}$	44.5	7.2±0.3 <sup>D</sup>	61.2

\*Means with different superscripts in the same column are significantly different (P<0.05). R %\*= Reduction %

## Table 3. Influence of garlic oil on PAHS levels in the examined samples of charcoal grilled meat fillets (n=5).

Treatmen	Treatment	Control	Control 0.50% Garlic oil		1% Garlic oil		1.50% Garlic oil	
	_	Average level	Mean	R %	Mean	R%	Mean	R%*
Benzo[a]Pyrene (B	BaP)	9.2±0.5 <sup>A</sup>	6.1±0.2 <sup>B</sup>	33.7	4.5±0.2°	51.1	2.9±0.2 <sup>D</sup>	68.5
Benz[a]anthracene	e (BaA)	5.8±0.3 <sup>A</sup>	3.3±0.2 <sup>B</sup>	43.1	2.6±0.2°	55.2	$1.5 \pm 0.2^{D}$	74.1
Benzo(b)fluoranthe	ene (Bbf)	2.1±0.1 <sup>A</sup>	1.5±0.1 <sup>B</sup>	28.6	1.0±0.1 <sup>°</sup>	52.4	0.6±0.1 <sup>D</sup>	71.4
Chrysene (CHR)		1.5±0.1 <sup>A</sup>	1.2±0.1 <sup>B</sup>	20	0.8±0.1 <sup>c</sup>	46.7	$0.5 \pm 0.1^{D}$	66.7
Sum PAH4		18.6±0.9 <sup>A</sup>	$12.1 \pm 0.5^{B}$	34.9	$8.9 \pm 0.4^{\circ}$	52.2	5.5±0.2 <sup>D</sup>	70.4

\*Means with different superscripts in the same column are significantly different (P<0.05). R %\*= Reduction %

Table 4. Influence of lemon oil on PAHS levels in the examined samples of charcoal grilled meat fillets (n=5).

Treatment	reatment	Control 0.50% Lemon oil			1% Lemon oil		1.50% Lemon oil	
	_	Average level	Mean	R %	Mean	R%	Mean	R%*
Benzo[a]Pyrene (BaP	<b>)</b>	9.2±0.5 <sup>A</sup>	5.6±0.2 <sup>B</sup>	39.1	3.9±0.2°	57.6	2.2±0.1 <sup>D</sup>	76.1
Benz[a]anthracene (B	BaA)	5.8±0.3 <sup>A</sup>	2.7±0.1 <sup>B</sup>	53.4	$1.8 \pm 0.2^{\circ}$	68.9	1.0±0.2 <sup>D</sup>	82.7
Benzo(b)fluoranthene	e (Bbf)	2.1±0.1 <sup>A</sup>	$1.1 \pm 0.1^{B}$	47.6	$0.7{\pm}0.1^{\circ}$	66.7	$0.4{\pm}0.1^{\text{D}}$	80.9
Chrysene (CHR)		1.5±0.1 <sup>A</sup>	0.9±0.1 <sup>B</sup>	40	$0.4{\pm}0.1^{\circ}$	73.3	0.3±0.1 <sup>D</sup>	80
Sum PAH4		18.6±0.9 <sup>A</sup>	$10.3\pm0.5^{B}$	45.7	$6.8 \pm 0.4^{\circ}$	65.6	3.9±0.2 <sup>D</sup>	79.1

\*Means with different superscripts in the same column are significantly different (P<0.05). R %\*= Reduction %

concentrations of 0.5, 1 and 1.5% on PAHS levels in the examined samples of charcoal grilled meat fillets.

PAH4 level was 6.8±0.4 with a reduction percentage of 65.6%.

The most efficient concentration of lemon oil for lowering the car-

PAH4 level was10.3±0.5 with a reduction percentage of 45.7% for cinogenic PAHs in charcoal grilled meat fillets was 1.5%, with PAH4 level 0.5% lemon oil concentration While for lemon oil concentration of 1.0%, of 3.9±0.2 and a reduction percentage of 79.1%.

#### Discussion

Charcoal grilling is a popular method in Egypt and other Arabian countries for improving the organoleptic quality, palatability, and digestibility of beef steaks. However, it can also lead to the creation of carcinogenic compounds, such as PAHs. As a result, the identification of these compounds offers details on possible health hazards associated with eating processed meat, knowledge that may be helpful for consumers' health management (IARC, 2018).

This study's primary focus was on BaP and PAH4 (the combined content of BaP, BaA, CHR, and BbF). Additionally, an analysis was done on the contents of DaeP, DalP, DahP, IcdP, DaiP, BghiP, CcdP, BaA, and DahA, which caused by fat-induced flame generation, incomplete combustion of the heat source, or organic compound pyrolysis.

In terms of their carcinogenicity, PAH4 and PAH8, which are internationally known indicators, had mean concentration values of 90.0±93.14 and 19.7±28.09 µg/kg-1, respectively. The results obtained are not likely to be the same as those reported by Eldaly et al. (2016), who found only benzo(a) pyrene and benzo(g,h,i) anthracene at maximum concentrations of 26.0±16.0 µg/kg and 33.2±4.0 µg/kg in the examined non-marinated charcoal grilled kofta samples.

The results in Table 1, showed greater levels of PAH4 and PAH8 than those seen in samples of beef cooked over charcoal (Darwish et al., 2019). The maximum allowable limits of PAHs in grilled meat and meat products established by the EC (2023) were surpassed by the discovered B[a] P, total PAH4, and PAH8 in this investigation.

The results obtained in Table 1 were greater than those reported by Alomira et al. (2011), who stated that the mean concentration of B[a]P was 1.33 µg/kg<sup>-1</sup>, and lower than those recorded by Shawish et al. (2022), who found B[a]A, B[a]P, and CHR with mean values of 10.7, 9.7, and 2.3 µg/kg<sup>-1</sup>, respectively. Furthermore, the study's detection of B[a]P, total PAH4, and PAH8 exceeded the maximum allowable levels for PAHs in grilled meat and meat products (EC, 2023). The quantity of fat content, the duration and distance of the grilling process, and the type of contact heat (direct or indirect) were found to be strongly associated with these changes in PAH concentration levels. These factors are what cause PAH development in meat.

On the other hand, Janoszka et al. (2004) found that cooked beef products in Poland had a lower concentration of BaP (0.11 to 3.93 µg/kg), and the overall PAH content ranged from 2.43 to 16.10  $\mu$ g/kg. Additionally, Eldaly et al. (2016) found that only benzo(a)anthracene and benzo(a) pyrene, at maximum concentrations of 33.2±4.0 and 26.0±16.0 µg/kg, respectively, were detected in the analyzed non-marinated charcoal-grilled meat samples. They did not identify benzo[ghi]perylene. The amount of fat in the steak, the length of time it was grilled, and the high heat contact caused the fat to drip off onto the flames and produce smoke were all strongly linked to these differences in the concentration levels of PAHs or because of organic matter pyrolyzing, which produces free radicals that cause PAH production.

Regarding 1% lauryl oil concentration, Benzo[a]Pyrene (BaP), Benz[a] anthracene (BaA), Benzo(b)fluoranthene (Bbf), Chrysene (CHR) were  $5.1\pm0.3$ ,  $3.0\pm0.2$ ,  $1.2\pm0.1$  and  $1.0\pm0.1$  with reduction percentages of 44.6%, 48.2%, 42.9% and 33.3% respectively While PAH4 was 10.3±0.4 with a reduction percentage of 44.5%. The best concentration of lauryl oil to reduce the carcinogenic PAHs in fillets of charcoal-grilled meat fillets was 1.5%; Benzo[a]Pyrene (BaP), Benz[a]anthracene (BaA), Benzo(b) fluoranthene (Bbf), Chrysene (CHR) levels were 3.6±0.2, 1.9±0.2, 0.8±0.1 and 0.9±0.1 with reduction percentages of 60.9%, 67.2%, 61.9% and 40% respectively, While PAH4 level was 7.2±0.3 with a reduction percentage of 61.2%.

The type of wood used, the smoking technique, the temperature at which it burns, the smoke's composition, and the length of time that food is exposed to smoke all affect how much PAHs are carried by the smoke particles (Duedahl-Olesen et al. 2006). The amount of PAHs is further influenced by factors such as the fuel utilized, the length and kind of processing, the processing level, and the distance from a heat source. However, certain food products contain more PAHs due to operations like crushing, storing, recycling, and concentrating (Singh et al. 2016). The European Food Safety Authority (EFSA, 2008) states that benzo[a]pyrene (BaP) is considered a carcinogen to humans.

The carcinogenic PAHs in the charcoal beef steak were more successfully reduced by the altered marinade treatment. The addition of lemon juice tenderized the beef steak, facilitating faster protein digestion and retaining moisture so the meat did not dry out or become tough. This contributed to the reduced PAH levels in the beef steak treated with a modified marinade. These findings so concur with those published by Farhadian et al. (2012) in that they decrease the amount of time required to finish cooking

It also keeps fat droplets from coming into touch with charcoal, which inhibits the production of smoke and the buildup of PAHs on the surface of beef steaks. Further, the addition of lemon juice to the essential marinade treatment had a positive effect on the concentration of PAHs. These oddities could be caused by differences in the acidity of the samples. Higher acidity may also have an impact on the kinetics and mechanism of nonenzymic browning (Maillard) processes. The reaction rate frequently increases when pH rises (Li et al., 2021).

PAHs are involved in the aroma products of Maillard reactions, according to studies on the kinetics and mechanism of PAH formation (Britt et al., 2004). But as Nursten (2005) points out, lemon juice contains organic compounds like sulfur dioxide, which are commonly used to stop the Maillard processes and may help reduce the amount of PAHs in the samples of beef steak.

# Conclusion

Charcoal-grilled meat treated with natural oils decrease the levels of PAH4 in the examined samples. It is recommended that meat should be marinated prior grilling to minimize the hazards of PAHs on human health.

#### Acknowledgments

The authors are grateful to the Faculty of Veterinary Medicine, Benha University, for supporting this work.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### References

- Alomirah H., Al-Zenki S., AlHooti S., Zaghloul S., Sawaya W., Ahmed N., Kannan K., 2011. Concenrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. Food Control 22, 2028-2035.
- Britt, F., Buchanan, A. C., Clyde, V., Owens, J., Jr., Skeen, T., 2004. Does glucose enhance the formation of nitrogen containing polycyclic aromatic compounds and polycyclic aromatic hydro-carbons in the pyrolysis of proline. Fuel 83,1417-1432.
- Chantara, S., Sangchan, W., 2009. Sensitive analytical method for particle- bond polycyclic aromat-ic hydrocarbon. A case study in Chiang Mai, Thailand. J. Sci Asia. 35, 32-48. Darwish, W.S., Chiba, H., El-Ghareeb, A., Elhelaly, E., Hui, S.P., 2019. Determination of polycyclic ar-
- omatic hydrocarbon content in heat-treated meat retailed in Egypt, Health risk assessment, benzo[a]pyrene induced mutagenicity and oxidative stress in human colon (CaCo-2) cells and protection using rosmarinic and ascorbic acids, Food Chemistry 290,114–124. Duedahl-Olesen, L., White, S., Binderup, M., 2006. Polycyclic aromatic hydrocarbons (PAH) in Dan-
- ish smoked fish and meat products. Polycyclic Aromatic Compounds 26, 163-184. EC, 2023. European Commission: Commission regulation (EU) No 2023/915, of 25 April 2023,
- amending regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Off. J. Eur. Union 215, 4–8.
- EFSA, 2008. European Food Safety Authority: Polycyclic Aromatic Hydrocarbons in Food, Scientific Opinion of the Panel on Contaminants in the Food Chain'. EFSA Journal. 724, 1-114. Eldaly, E.A., Hussein, M.A., El-Gaml, A., El-Hefny, D.E., Mishref, M., 2016. Polycyclic Aromatic Hydro-
- Eddiy, E.A., Hussein, M.A. Er-Gann, A., Er-Henry, D.E., Misiner, M., 2010. Polycyclic Aromatic Hydro-carbons (PAHs) in Charcoal Grilled Meat (Kebab) and Kofta and the Effect of Marinating on their Existence". Zagazig Veterinary Journal 44, 40-44.
  Farhadian, A., Jinap S., Faridah, A., Zaidul, I.S., 2012. Effects of marinating on theformation of poly-cyclic aromatic hydrocarbons (benzo[a]pyrene, benzo [b]fluoranthene and fluoranthene) in
- grilled beef meat'. Journal of Food Control 28, 420-425.
- Feldman, D.; Ganon, J.; Haffman, R., Simpson, J., 2003. The solution for data analysis and presenta-tion graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
- IARC (International Agency for the Research on Cancer), 2018. Monographs on the Evaluation of Carcinogenic Risks to Humans, Lyon, France'.
- Janoszka, B., Warzecha, L., Blaszczyk, U., Bodzek, D., 2004. Organic Compounds Formed in Thermally Treated High-protein Food'. Part I, Polycyclic Aromatic Hydrocarbons. Acta. Chromato graphic a 14,115-128
- Li, L., Belloch, C., Flores, M., 2021. The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions'. Molecules 26, 223.
- Mojiri, Á., Zhou, J. L., Ohashi, Á., Ozaki, N., Kindaichi, T., 2019. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Science Total Environ. 696, 133971
- National Food Safety Standard, 2017. Limits of contaminants in food. GB 2762-2017http://db.
- foodmate.net/2762/.
  Nursten, H., 2005. The Maillard reaction, chemistry, biochemistry and implications. The University of Reading. Reading, UK, Loyal Society of Chemistry. pp. 151-152
- Racovita, R. C., Secuianu, C., Israel-Roming, F., 2021. Quantification and risk assessment of car-cinogenic polycyclic aromatic hydrocarbons in retail smoked fish and smoked cheeses. Food
- Control 121, 107586. https://doi.org/10.1016/j. foodcont.2020.107586.
  Rose, M., Holland, J., Dowding, A., Petch, S.R., White, S., Fernandes, A. Mortimer, D., 2015. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting'. Food Chem. Toxicol. 78, 1-9.
  Shawish, R.R., Hamed, R.E. Elbayoumi, Z.H., 2022. Recognition of aromatic hydrocarbon compounde in score orilled bafe required to the formation product on the formation product on the formation of parts.
- pounds in some grilled beef products on the Egyptian market by gas chromatography-mass spectrophotometry technique'. Adv. Anim. Vet. Sci. 10, 1769-1773.
- Simko, P., 2002. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavoring food additives. J. Chromatography 770, 3-18.
- Singk, L., Varshney, J.G. Agarwal, T., 2016. Polycyclic aromatic hydrocarbons' formation and occurrence in processed food'. Food Chemistry 199, 768-781.
  Stumpe, V., Bartkevics, V., Kukare, A., Morozovs, A., 2008. Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. Food Chemistry 110, 794-797.
  Zelinkova, Z., Wenzl, T., 2015. The occurrence of 16 EPA PAHs in food a review. Polycycl'. Aromat. Comm. 25: 249. 264.
- Comp. 35, 248–284. Zhao, Z., Zhang, L., Cai, Y., Chen, Y., 2014. Distribution of polycyclic aromatic hydrocarbon (PAH) residues in several tissues of edible fishes from the largest freshwater lake in China, Poyang Lake, and associated human health risk assessment. Ecotoxicology and Environmental Safety. J. Ecoenv. 104, 323-331. https://doi.org/10.1016/j. ecoenv.2014.01.03.