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The existence of polycyclic aromatic hydrocarbons in commercially grilled beef kofta and chicken meat in El-Gharbia governorate markets.

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ABSTRACT

Polycyclic aromatic hydrocarbons are considered as harmful organic compounds that can occur during the heat treatment of meat, particularly grilling or barbecuing. The purpose of this research is to evaluate the degree of contamination of 12 PAH constituents (benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, Dibenzo [ae] pyrene, Dibenzo [al] pyrene, Dibenzo [ah] pyrene, Dibenzo [ai] pyrene, indeno [1,2, 3-cd] pyrene, benzo [ghi] perylene, Cyclopenta (c,d) pyrene and Dibenzo [a,h] anthracene) on 30 heat-treated meat samples using high-performance liquid chromatography (HPLC) in Charcoal grilled beef kofta, charcoal-grilled chicken meat and gas-grilled chicken meat (10 of each), PAH4 and PAH8 mean values were 90±93.17, 19.7±28. 1;69.6±74.4, 10.9±16.25;41. 3±44.9,3.4±5.1µg/g, respectively. Although the high levels of PAHs can be consumed, caution remains advised while eating grilled meat meals.

1. INTRODUCTION

Consumption of grilled meat is prevalent throughout the world, particularly in Egypt, due to its sensory property's excellence with nutritious worth for customers. Furthermore, present a risk to society because of the high amounts of cooking carcinogens (Farhadian et al., 2013).

Processing techniques include barbecue (Mottier et al., 2017), roasting (Jinap et al., 2019), grilling (Moazzen et al., 2015), and smoking are the main causes of PAHs in processed beef products (Djinovic et al., 2013). Several factors, including the type of fuel utilized (coal, gas, lumber, or electrical supply), the meat preparation techniques utilized (frying, barbecuing, and roasting), the cooking duration and temperature, the amount of fat in meat, the danger of dripping fat on a heat source, the proximity of the food to the heat source, and direct contact, have been identified as potentially influencing PAH formation during meal preparation and serving (Jinap et al., 2019).

Whenever food, especially meat, is grilled over direct heat, PAHs are formed. The breakdown of meat fat results in some kind of coating material that is extremely rich in PAHs. Even when there is no direct interaction, dissolving fats flow through into fire and heated coals, releasing PAHs that then return to the meat via smoke. Grilling leads to the production of PAHs because of the meat's fat content and its proximity to the heat source (Jägerstad and Skog, 2015).

The main source of exposure to PAHs for non-smokers and non-occupationally exposed adults is food. Diet contributes more than 90% of total PAHs exposures of the general population in various countries (Llobet et al., 2016). The average consumption across the European countries and the dietary exposure for the sum of eight carcinogenic and genotoxic PAHs (PAH8) (Chrysene, Benzo (a)pyrene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Dibenzo (a,h) anthracene, Benzo (g,h,i) perylene, Indeno (1,2,3- c,d) pyrene and Fluorene) was estimated as 1.73 mg/day (EFSA (2008).

2. MATERIAL AND METHODS

The present study was approved by Institutional Animals Care and Use Committee of Faculty of Veterinary Medicine, Benha University Ethical Approval No. BUFVTM 04-12-22.

2.1. Sample collection:

A collection of 30 fully prepared beef and Fresh chicken meat cuts samples (10 of each) representing charcoal-grilled beef kofta, charcoal-grilled chicken meat cuts, and gas-grilled chicken meat were obtained at random from several markets in El-Gharbia district, Egypt. Every sample was subsequently wrapped in a plastic bag, placed in a dry clean ice bucket, and transported to the lab without undue delay. All the meat samples that were gathered were examined for polycyclic aromatic hydrocarbons, their concentrations were compared to established standard limits, and their safety for human consumption was determined.

2.2. Polycyclic Aromatic Hydrocarbons Assessment (PAHS):

2.2.1. Samples preparation:

The meat samples product under test had been fully homogenized. To accommodate the detection method of gas chromatography-mass spectrometry (GC-MS), the

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sample preparation approach was developed in accordance with (Simko, 2002) and (Stumpe *et al*. 2008) with some adjustments.

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 µl of internal standard benzo[a]pyrene-d12 solution with concentration 10 ng/ µl and 125 µl of PAH mix with concentration 1 ng/µl 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 shaken 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 and transferred into a GC vial.

2.2.2. Gas chromatography with mass selective detector:

Thermo Scientific Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC/MS) Systems offer modularity was employed for analysis. Operating conditions were as follows: Varian Factor Four capillary column 30 m ×0.25 mm with film thickness of 0.25 lm, helium carrier gas 1 cm^3/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. The ionizing voltage was 1950 Volts.

2.2.3. Recovery:

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. In general, the average of triplicate analysis was calculated for each polycyclic aromatic hydrocarbon.

2.2.4. Quality control:

The procedure and reagent blanks were analyzed for each batch of samples and subtracted from the sample analysis. The detection limits were 2.1 –5.9 ng/g for Polycyclic Aromatic Hydrocarbons in certain meat products, respectively. Method recoveries of the spiked standards were 69±9, 85±19, and 82±16 % for gaseous phase PAHs in air, particulate phase PAHs in air, and PAHs in meat, respectively. Method recoveries ± SD of the spiked standards were 77±13 and 87±15 % for gaseous and particulate phase derivative PAHs in air and 81±16 % in meat, Petric and Waston (2019).

3. RESULTS

The findings shown in table (1) demonstrated that the average concentration levels of potentially carcinogenic PAHs in charcoal grilled beef; benzo [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [a] pyrene, Dibenzo (a,h) anthracene, benzo (g,h,i) perylene and Indeno (1,2,3c-d) pyrene were 16.8± 10.4, 1.4± 2, 0.5± 1.1, 17.4± 2.8, 0.6± 0.98 and 7.5± 5, 5± 6.14 µg/kg-1 respectively. Regarding the mean concentrations values of the internationally recognized marker for their carcinogenicity PAH4 and PAH8 were 90 ± 93.14 and 19.7± 28.09 µg/kg^{-1} respectively. The maximum amount of PAH4 in meat samples is 12 µg/kg, while the permissible level of BaP is 2.0 µg/kg (EC, 2011).

Table 1 Statistical analytical results of PAHs µg/kg in examined samples of Charcoal Grilled beef Kofta (*n=10).

PAHs	Range		Mean	SD
	Minimum	Maximum		
BaP	5.9	28	17.4	2.8
Daep	0.21	0.38	0.08	0.14
DaIP	0.16	0.52	0.13	0.18
DahP	UDL	UDL	UDL	UDL
DaiP	0.22	0.65	0.16	0.24
IcdP	1.7	15.6	5	6.14
BghiP	2.6	15.3	7.5	5.0
CcdP	0.45	13.2	2.4	4.8
BaA	2.6	36.4	16.8	10.4
DahA	0.11	2.8	0.6	0.98
BbF	3.0	2.1	0.5	1.1
Chr	1.9	5.6	1.4	2
PAH4 sum	5.1	173.6	90	93.17
PAH8 sum	0.85	74.6	19.7	28.09

PAH4: BaA, Chr, BbF, and BaPPAHs, polycyclic aromatic hydrocarbons; BaP, benzo[a]pyrene; DaeP, Dibenzo [ae] pyrene; DaIP, Dibenzo [al] pyrene; DahP, Dibenzo [ah] pyrene; DaiP, Dibenzo [ai] pyrene; IcdP, indeno [1, 2, 3-cd] pyrene; BghiP, benzo [ghi] perylene; CcdP, Cyclopenta (c, d) pyrene; BaA, benzo[a]anthracene; DahA, Dibenzo [a, h] anthracene; BbF, benzo[b]fluoranthene; Chr, chrysene. UDL= under detected limits. SD = standard deviation= Number of samples.

According to the data in table (2), the average concentration levels of such potentially carcinogenic PAHs group in charcoal-grilled chicken meat samples; benzo [a] anthracene, chrysene, benzo [a] pyrene, benzo(g,h,i) perylene and Indeno (1,2,3c-d) pyrene were 12.67± 7.52, 1.1± 1.64, 14.1± 7.61, 4.34± 5.05 and 2.7± 3.86 µg/kg^{-1}, respectively. The recorded mean concentration levels of PAH4 and PAH8 were 69.6± 74.4 and 10.9± 16.25 µg/kg^{-1}, respectively.

Table 2 Statistical analytical results of PAHs µg/kg in examined samples of Charcoal Grilled Chicken meat (*n=10).

PAHs	Range		Mean	SD
	Minimum	Maximum		
BaP	2.8	26.6	14.1	7.61
Daep	UDL	UDL	UDL	UDL
DaIP	0.69	0.78	1.5	0.31
DahP	UDL	UDL	UDL	UDL
DaiP	0.35	0.57	0.1	0.2
IcdP	1.3	10.2	2.7	3.86
BghiP	1.6	12.7	4.34	5.05
CcdP	0.3	7.3	1.33	2.54
BaA	1.5	25.2	12.67	7.52
DahA	0.39	0.62	0.1	0.22
BbF	UDL	UDL	UDL	UDL
Chr	1.0	4.1	1.1	1.64
PAH4 sum	10.8	140.8	69.6	74.4
PAH8 sum	0.92	43.4	10.9	16.25

The results achieved in table (3), estimated the carcinogenic PAH individual concentrations in gas-grilled chicken meat were 7.78± 5.3, 0.49± 0.82, 8.24±5.52, 1.31± 2.21, and 0.83±1.56 $\mu g/kg^{-1}$ for benzo [a] anthracene, chrysene, benzo [a] pyrene, benzo (g,h,i) perylene and Indeno (1,2,3c-d) pyrene, respectively. While their PAH4 and PAH8 occur with a mean concentration level of 41.3± 44.9 and 3.4± 5.1 $\mu g/kg^{-1}$ respectively.

Table 3 Statistical analytical results of PAHs µg/kg in examined samples of Gas grilled chicken meat (*n=10).

PAHs	Range		Mean	SD
	Minimum	Maximum		
BaP	1.3	17.4	8.24	5.52
Daep	UDL	UDL	UDL	UDL
DaIP	UDL	UDL	UDL	UDL
DahP	UDL	UDL	UDL	UDL
DaiP	UDL	UDL	UDL	UDL
IcdP	1.1	4.5	0.83	1.56
BghiP	1.1	5.6	1.31	2.21
CcdP	0.16	3.2	0.58	1.11
BaA	1.0	16.6	7.78	5.3
DahA	UDL	UDL	UDL	UDL
BbF	UDL	UDL	UDL	UDL
Chr	0.3	2.1	0.49	0.82
PAH4 sum	4.9	82.4	41.3	44.9
PAH8 sum	5.83	13.1	3.4	5.1

4. DISCUSSION

The results obtained in table (1) were not consistent with those found by Eldaly et al., (2016) as benzo[ghi]perylene for any samples of charcoal grilled kofta without marination, except at maximum quantities of 33.2±4 and 26±16 $\mu g/kg^{-1}$ for benzo(a)anthracene and benzo (a) pyrene, respectively. The results were higher than those presented in samples of charcoal-grilled beef collected by Darwish et al., (2019) for PAH4 and PAH8 levels. The quantities of PAH8, total PAH4 and B[a] P found during this study exceeded the maximum permissible limits (2$\mu g/kg^{-1}$) of PAHs in grilled meat and meat products specified by (EC, 2011).
These variations in PAH concentration levels were attributed to the time of grilling, the amount of fat content, and high thermal contact, which resulted in the fat being dropped from the kofta into the fire, resulting in a smoke generation, or to combustion of organic compounds, which develops free radicals liable for PAH initiation.
According to results in table (2), lower findings were reached via (Farhadian *et al.,* 2010) found the total PAH concentrations of charcoal grilled chicken meat was between 4.63-12.4 $\mu g/kg^{-1}$; (Hamzawy *et al.,* 2016) found that the benzo (a) Pyrene concentration in charcoal grilled chicken ranged from 0.49-7.20$\mu g/kg^{-1}$ and with a mean value of 2.01$\mu g/kg^{-1}$. Hassan, (2016) found the mean PAHs concentrations of charcoal grilled chicken meat was 9.941 $\mu g/kg^{-1}$, (Anjum *et al.,* 2019) found the mean values of benzo [a] anthracene and chrysene in charcoal grilled chicken samples were 0.510 and 0.742 $\mu g/kg^{-1}$, respectively. The observed PAH8, total PAH4, and B [a] P levels in this investigation exceeded the maximum allowed limits of PAHs in grilled meat and meat products (2 $\mu g/kg^{-1}$) specified by (EC, 2011).
The variations in the PAH amounts varied substantially according to the kind of charcoal used, the duration of the grilling procedure, the quantity of fat in the poultry meat, and the grade of doneness.

The results are given in table (3) lower findings were achieved via (Farhadian *et al.,* 2010) found the total PAH concentrations in gas-grilled chicken meat was between 14.3-37.6 $\mu g/kg^{-1}$. (Mishref, 2014) detected B[a]P with a mean value of 9.2 $\mu g/kg^{-1}$ (Ahmad *et al.,* 2014) estimated the mean PAH concentrations in gas-grilled chicken meat was 1.68 $\mu g/kg^{-1}$. (Anjum *et al.,* 2019) found the mean values of benzo [a] anthracene and chrysene in gas-grilled chicken samples were 0.317 and 0.285 $\mu g/kg^{-1}$, respectively. The measured PAH8, PAH4 and B[a] P concentrations in this investigation exceeded the maximum allowed limits (2 $\mu g/kg^{-1}$) of PAHs in grilled meat and meat products specified by (EC, (2011).
The variations in the PAH amounts were significantly attributed to the distance between fire gas and the chicken pieces, the time of the grilling process, the amount of fat content and direct or indirect gas grilling that encouraged PAHs formation and caused hazardous health problems.

5. CONCLUSION

The three kinds of grilled meat had significantly different PAH concentrations (p 0.05). Dishes flame-gas grilled with chicken meat seemed to have the lowest number of PAHs when the gas-flame supplier oriented vertically, followed by dishes charcoal-grilled with beef kofta and chicken meat. Moreover, the consequences of different heat providers and meat amounts of fat play a potential role in reducing PAH contamination inside charcoal grilled meat meals.

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