



Polycyclic Aromatic Hydrocarbons in Grilled Meats from Restaurants

Abdel-Azeem M. Abdel-Latif^{1*}, Fatma H.M.A.², Jehan M.M. Ouf³, Mohamed H. H. Roby⁴
and Nasser S. Abdel-Atty²

¹Animal Health Research Institute, Fayoum, Agriculture Research Center, Egypt

²Food Hygiene Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

³Food Hygiene Dep., Animal Health Research Institute, Agriculture Research Center, Dokki, Egypt

⁴Food Sciences and Technology Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt

*Corresponding Author: Abdel-Azeem M. Abdel-Latif, E-Mail: abdomeat82@yahoo.com

ABSTRACT

Polycyclic aromatic hydrocarbons are a group of lipophilic compounds that can be generated during the preparation of food items at elevated temperatures. They are regarded as potentially genotoxic and carcinogenic to human beings, related to increased incidence of breast and colorectal cancers, oxidative DNA injury, and bad effects on children neuro-differentiation. Thus, they are considered a public health concerns. A total of thirty samples of grilled beef steak, beef kofta and chicken (ten each) were collected from different restaurants. The samples were extracted by magnesium sulfate and sodium acetate in acetonitrile then purified in magnesium sulfate, primary, secondary amine and silica gel, and finally measured by gas chromatography-mass spectrometry (GC-MS). Benzo[a]pyrene was recorded with the highest average level (3.63µg/kg) in grilled kofta samples, but it was not detected in chicken samples. On the other hand, PAH4, PAH8 and ΣPAHs content were more abundant in grilled beef steak (5.32, 9.97 and 56.91µg/kg). Meanwhile, they recorded the least concentrations of grilled chicken from different restaurants. Furthermore, benzo[a]pyrene exceeded the permissible limits of the European Commission and Egyptian National Food Safety Authority in grilled kofta samples; further studies are needed to investigate the limits of exposure to these harmful compounds from meats and other food items.

Keywords: Beef steak, Benzo[a]pyrene, GC-MS, Grilled chicken, kofta, PAHs.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are very stable pollutants of foodstuffs and included various sources such as contaminated soils, polluted air and water, cooking procedures, food processing, pseudo-curing with smoking flavor agents or wood generation (Reinik *et al.*, 2007). Foodstuff contamination with PAHs may occur during extreme heat processing, drying, toasting, roasting or frying by direct pyrolysis of food nutrients and due to direct deposition of PAHs from smoke produced via incomplete combustion of different thermal agents (Rey-Salgueiro *et al.*, 2008). Also, these harmful compounds in grilled meats result from partial charcoal pyrolysis that leads to the formation of PAHs in grilled food; the heat on the surface of muscle foods forms these contaminants by direct pyrolysis of food nutrients. Additionally, meat drips that are formed

during the grilling and fall on hot charcoal lead to the formation of PAHs, which are carried by the smoke, and adsorbed on the surface of the meat (Costa *et al.*, 2009).

Human exposure to PAHs is related to a 30-50% increase in the incidence of breast cancer (White *et al.*, 2016), oxidative DNA injury (Yu *et al.*, 2021), and bad effects on neuro-differentiation which occur over a long period in late gestation or early childhood (Slotkin *et al.*, 2017). Additionally, they can cause cardiopulmonary and cardiovascular diseases by producing reactive oxygen species, resulting in oxidative stress and inflammatory responses (Ramesh *et al.*, 2004).

This study aimed to investigate the content and profile of polycyclic aromatic hydrocarbons (PAHs) in different grilled meat types, including beef steak, beef kofta and broiler chicken from different restaurants in

Fayoum Governorate, Egypt. Beef steak is made by slicing beef into thin chips. Salt and black pepper are added, while kofta is made by adding chopped vegetables and ground spices to minced beef, then molded into bars and grilled or fried.

MATERIALS AND METHODS

1. Chemicals

Mixed 18 PAHs standards containing 10mg/L of each PAHs congener (Dissolved in cyclohexane) manufactured by Supelco[®] (Bellefonte, PA, USA) was used, deionized water was obtained by pobel[®] water distiller (Desa 0040 model, Spain), the salts kits were got from Agilent[®] Technologies (Palo Alto, CA, USA) and finally, acetonitrile obtained from Merck[®] (Darmstadt, Germany).

2. Sampling and sample preparation

Grilled meats samples (Beef steak, Beef kofta and chicken) were purchased from various restaurants in Fayoum Governorate, Egypt during the period from January to July 2021. Ten samples were taken from each product (Sample weight was 1kg of grilled beef kofta and beef steak and one whole grilled broiler chicken, grilled chicken were deboned, then all samples were homogenized and kept in a deep freezer in foil-wrapped bottles till examination at Faculty of Agriculture Laboratories, Fayoum University.

3. Extraction and clean-up procedures

The technique formerly designed by **Chen et al. (2013)** was used, five grams of homogenized sample were mixed with 10 mL of deionized water in a polypropylene tube for 1 minute, then 10 mL acetonitrile solvent was poured and the mixture was exposed to strong shaking for another minute. The content was replaced in a QuEChERS column having 6 grams of MgSO₄ and 1.5 grams of sodium acetate, subsequently vortexed for 1 minute and centrifuged at 1431 g for 5 minutes. Then 6 mL of the uppermost layer was transferred into the clean-up column with 0.4g of PSA, 1.2g of magnesium sulfate and 0.4g of silica gel particles for purification. The content was then put in a centrifuge at 1431 g for 5 minutes and 1µl aliquot from the uppermost layer was injected into a gas chromatograph coupled with a mass spectrometer for PAHs analysis.

4. Gas chromatographic analysis

The PAHs standard curve was obtained by integrating the compound concentration alongside the peak area. The content of each congener in the sample was estimated according to its particular calibration curve, 1µl of the sample extract was introduced into an Agilent HP-5MS gas chromatograph, and eighteen PAHs congeners were identified before elapsing for forty minutes period. Helium gas was used as the carrier gas with a flowing speed of one milliliter per minute; the temperature of the injector was adjusted at

290°C. The splitless mode was selected, and the temperature programming was adjusted on the following: 70°C at first, then elevated to 195°C with the level of fifteen centigrade per minute then continued for another two and half minutes. After then increased to 240°C at the level of fifteen centigrade per minute, then lasted for 17 minutes, upraised to 270°C at the level of five centigrade per minute, and elevated to 310°C at the level of fifteen centigrade per minute then was fixed for ten minutes, PAHs concentrations in tested samples were quantified by matching times of retention and mass spectrum of masses for unidentified peaks to that of previously injected reference materials.

5. Statistical analysis

The analysis of variance (ANOVA) was made and the means were calculated via SPSS 13.0 statistical package (SPSS, 2008); for descriptive statistics, PAHs concentrations that were lower than the apparatus limit of detection were given a zero value.

RESULTS

Regarding beef steak samples, and from data shown in table 1, naphthalene is the highest level recorded 20.23±2.67µg/kg. Among the eight carcinogenic and genotoxic compounds (PAH8), benzo[b]fluoranthene recorded the highest mean value of 4.87±0.39µg/kg, while the most potent PAHs carcinogen, benzo[a]pyrene recorded 0.02±0.01µg/kg. From the data presented in table 2, the carcinogenic PAH4 recorded a mean value of 5.32µg/kg, while PAH8 measured 9.97µg/kg. Unsurprisingly, the non-carcinogenic PAHs constitute the highest portion of the analyzed members with an average level of 46.94µg/kg constituting 82.48% of the total PAHs concentrations was 56.91µg/kg wet weight of the beef steak samples. Benzo[a]pyrene and PAH4 levels were within the permissible limits established by **European Commission (2011 and 2014)** and the **Egyptian National Food Safety Authority (2022)**.

Regarding grilled kofta samples and from data found in table (1), naphthalene recorded the highest value of 18.13±0.91µg/kg wet weight; while benzo[a]pyrene was 3.63±2.18µg/kg wet weight. From data in table (2), PAH4 recorded an average level of 4.93µg/kg; meanwhile, PAH8 was 5.60µg/kg, and the non-carcinogenic PAHs represented the highest content with a mean value of 49.94µg/kg and a percent of 89.91% of the total PAHs concentration that was 55.54µg/kg wet weight of the analyzed kofta samples. From data in this study, 40% of kofta samples exceeded benzo[a]pyrene permissible limits set by **European Commission (2011 and 2014)** and **Egyptian National Food Safety Authority (2022)**, while 20% of samples exceeded PAH4 permissible limits established by **European Commission (2014)** and **Egyptian National Food Safety Authority (2022)**.

Table 1: Average concentrations of PAHs ($\mu\text{g}/\text{kg}$, wet weight) as means \pm standard error in grilled meat types from different restaurants:

Compound name	Grilled steak	Grilled kofta	Grilled chicken
Naphthalene	20.23 ^a \pm 2.67	18.13 ^a \pm 0.91	16.54 ^a \pm 1.37
2-methyl Naphthalene	5.61 ^a \pm 0.47	5.76 ^a \pm 0.42	2.95 ^b \pm 0.56
1-methyl Naphthalene	1.66 ^a \pm 0.05	3.42 ^b \pm 0.66	1.06 ^a \pm 0.05
Acenaphthylene	0.25 ^a \pm 0.01	0.39 ^a \pm 0.03	0.68 ^a \pm 0.27
Acenaphthene	1.94 ^a \pm 0.09	1.51 ^a \pm 0.02	2.22 ^a \pm 0.38
Fluorene	1.86 ^a \pm 0.08	3.95 ^b \pm 0.25	1.56 ^c \pm 0.06
Phenanthrene	7.04 ^a \pm 0.90	8.01 ^a \pm 0.39	1.41 ^b \pm 0.10
Anthracene	6.14 ^a \pm 0.66	7.11 ^a \pm 0.37	2.85 ^b \pm 0.26
Fluoranthene	1.43 ^a \pm 0.12	1.10 ^a \pm 0.03	0.48 ^b \pm 0.23
Pyrene	0.80 ^a \pm 0.09	0.65 ^a \pm 0.04	0.10 ^b \pm 0.03
Benzo[a]anthracene	0.03 ^a \pm 0.01	0.11 ^a \pm 0.09	0.02 ^a \pm 0.01
Chrysene	0.40 ^a \pm 0.03	0.32 ^a \pm 0.02	0.04 ^b \pm 0.01
Benzo[b]fluoranthene	4.87 ^a \pm 0.39	0.87 ^b \pm 0.03	0.02 ^b \pm 0.01
Benzo[k]fluoranthene	3.83 ^a \pm 0.42	0.67 ^b \pm 0.05	0.0 ^b \pm 0.0
Benzo[a]pyrene	0.02 ^a \pm 0.01	3.63 ^a \pm 2.18	0.0 ^a \pm 0.0
Indeno[1,2,3-cd]pyrene	0.17 ^a \pm 0.09	0.0 ^a \pm 0.0	0.0 ^a \pm 0.0
Dibenz[a,h]anthracene	0.65 ^a \pm 0.11	0.0 ^b \pm 0.0	0.0 ^b \pm 0.0
Benzo[ghi]perylene	0.0 ^a \pm 0.0	0.0 ^a \pm 0.0	0.0 ^a \pm 0.0

Means with dissimilar superscripts are significantly variant at $p \leq 0.05$

Table 2: Average concentrations of benzo[a]pyrene, PAH8, PAH4 and total PAHs in grilled meats from different restaurants:

	Beef steak	Beef kofta	chicken
Benzo[a]pyrene	0.02	3.63	0.0
PAH4	5.32	4.93	0.10
PAH8	9.97	5.60	0.10
Non carcinogenic PAHs	46.94	49.94	29.85
Total PAHs	56.91	55.54	29.95
Non carcinogenic PAHs%	82.48%	89.91%	99.66%

A-PAH4= Sum of average content of Benzo[a]pyrene+ Benzo[a]anthracene+ Benzo[b]fluoranthene +Chrysene.

B-PAH8= Sum of average content of PAH4+ Benzo[ghi]perylene+ Benzo[k]fluoranthene+ Dibenz[a,h]anthracene+ Indeno[1,2,3-cd]pyrene.

C-Total PAHs= Sum of 18 investigated PAHs compounds.

D-Non carcinogenic PAHs= Sum of first 10 compounds as arranged table (1).

Concerning grilled chicken, and from data in table (1), PAHs were predominated by naphthalene, 16.54 \pm 1.37 $\mu\text{g}/\text{kg}$ wet weight. From values in table (2), non-carcinogenic PAHs constitute more than 99% of the total PAHs in grilled chicken samples collected from different restaurants with a mean level of

29.85 $\mu\text{g}/\text{kg}$. None of the tested samples detected Benzo[a]pyrene. In addition, benzo[a]pyrene and PAH4 levels are all within the permissible limits established by **European Commission (2011 and 2014)** and **Egyptian National Food Safety Authority (2022)**.

DISCUSSION

Many authors studied the effect of grilling on PAHs level in beef. In this respect, **Terzi et al., (2008)** recorded higher levels of benzo[a]pyrene in samples of grilled meat in Northern Turkey, that was 24.2 ± 0.48 $\mu\text{g}/\text{kg}$, wet weight, in addition, higher levels were detected by **Alomirah et al., (2011)** in grilled meat samples from Kuwait City restaurants and the mean levels of benzo[a]pyrene, PAH8, and total PAHs were 1.37, 13.7 and $241 \mu\text{g}/\text{kg}$. Meanwhile, **Aaslyng et al. (2013)** recorded higher PAHs content in beef grilled at home by Danish consumers. They recorded that the average values of benzo[a]pyrene, PAH4 and ΣPAHs were 6.3, 17.3 and $229 \mu\text{g}/\text{kg}$, wet weight, respectively. On the other hand, lower values were obtained by **Gosetti et al., (2011)** in grilled beef samples, where the mean values of pyrene, chrysene, dibenzo[a,c]anthracene, dibenzo[a,h]anthracene, fluoranthene, fluorene, acenaphthene, anthracene, benzo[a]pyrene, benzo[e]pyrene, benzo[g,h,i]perylene, benzo[k]fluoranthene, phenanthrene, and ΣPAHs in grilled beef steak were 1.303, 0.152, 0.261, 0.434, 0.608, 0.348, 0.261, 0.087, 0.035, 0.193, 0.261, 0.304, 0.174, 0.261, and $4.412 \mu\text{g}/\text{kg}$ wet weight, respectively.

Moazzen et al., (2013) found much lower figures for benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, acenaphthylene, acenaphthene, fluorene, phenanthrene anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, PAH4 and ΣPAHs in grilled beef with averages of 0.131 ± 0.128 , 0.925 ± 0.754 , 0.535 ± 0.547 , 0.284 ± 0.236 , 0.854 ± 0.931 , 1.511 ± 1.386 , 1.584 ± 0.913 , 1.886 ± 1.572 , 1.578 ± 0.999 , 1.819 ± 1.374 , 2.24 ± 1.933 , 0.91 ± 0.585 , 1.55 ± 1.339 , 0.615 ± 0.778 , 0.377 ± 0.316 , and $16.79 \mu\text{g}/\text{kg}$, wet weight, respectively, On contrary, **Oz and Yuzer (2016)** could not detect any of PAH8 members in grilled beef steak samples from Turkish cities.

Concerning grilled chicken, **Loutfy et al., (2007)** obtained higher levels in grilled chicken samples from Ismailia City of Egypt, where acenaphthylene and ΣPAHs recorded 46.66 and $56.7 \mu\text{g}/\text{kg}$ wet weight, additionally chrysene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[a]anthracene, benzo[b+k+j]fluoranthene, and benzo[g,h,i]perylene were detected at levels of 0.38, 0.16, 0.34, 0.24, 0.17, 0.33 and $0.857 \mu\text{g}/\text{kg}$, wet weight, respectively. **Perelló et al., (2009)**, investigated slightly higher figures in grilled chicken samples from Catalonia in Spain. The mean levels of indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[g,h,i]perylene were 0.05, 0.05, 0.04, 0.05, and $0.16 \mu\text{g}/\text{kg}$ wet weight, however, **Aaslyng et al., (2013)** recorded lower values in chicken grilled at

home by Danish consumers. PAHs concentration was $19.7 \mu\text{g}/\text{kg}$ wet weight. Also, low levels were recorded by **Janoszka et al., (2004)** in grilled chicken from Poland, ΣPAHs content was $8.26 \mu\text{g}/\text{kg}$ wet weight. It was also estimated by **Reinik et al., (2007)** in Estonian grilled chicken, where ΣPAHs level was $13 \mu\text{g}/\text{kg}$, but they obtained a higher level of PAH4 in the same study, that recorded $9.3 \mu\text{g}/\text{kg}$ wet weight.

For kofta, higher PAHs values were obtained by **Alomirah et al., (2011)** in grilled meat from Kuwait City, the mean concentrations of PAH8 and ΣPAHs were $13.7 \mu\text{g}/\text{kg}$ and $241 \mu\text{g}/\text{kg}$, in the same order, but they recorded lower figure for benzo[a]pyrene that was only $1.37 \mu\text{g}/\text{kg}$ wet weight. Also, higher PAHs content was obtained by **Farhadian et al., (2012)** in grilled meat, where ΣPAHs value was $70.3 \mu\text{g}/\text{kg}$, but also recorded lower benzo[a]pyrene level that was $1.53 \pm 0.12 \mu\text{g}/\text{kg}$ wet weight, also higher PAHs concentrations were estimated by **Manda et al., (2012)** in grilled meat samples gathered from Côte d'Ivoire, the mean concentrations of benzo[g,h,i]perylene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, benzo[k]fluoranthene, and chrysene in grilled meat were nd, 12.6, 7.15, 0.73, 32.31, 19.22, nd, and $4.04 \mu\text{g}/\text{kg}$ wet weight, respectively.

The highest content was reported by **Eldaly et al., (2016)** in charcoal-grilled Egyptian kofta, where benzo[a]anthracene, benzo[a]pyrene anthracene, fluoranthene, chrysene, and ΣPAHs recorded 16.8 ± 7.0 , 9.2 ± 5.67 , 18.2 ± 11.2 , 57 ± 28.59 , 18.6 ± 1.69 and $119.8 \pm 54.15 \mu\text{g}/\text{kg}$, respectively, but lower values were detected by **Viegas et al., (2012)** in grilled beef samples, where PAH4 and ΣPAHs recorded 2.33 and $47.18 \mu\text{g}/\text{kg}$ wet weight, respectively. But lower PAHs values were obtained by **Duedahl-Olesen et al., (2015)** grilled meat samples from Denmark, the mean level of benzo[a]pyrene, PAH4 and ΣPAHs were 0.20 ± 0.50 , 0.50 ± 1.50 and $10.20 \pm 2.05 \mu\text{g}/\text{kg}$ wet weight, respectively, while the least PAHs amount was obtained by **Oz and Yuzer (2016)** grilled beef meat and they could not detect any of the 16 analyzed PAHs congeners.

Significant variances ($P \leq 0.05$) were present in the mean values of PAHs congeners between grilled samples of beef steak, chicken and kofta from different restaurants, which can be attributed to the usage of different coal kinds, as the production of parent PAHs from the coal burning includes both the parent PAHs released from the coal itself and the parent PAHs formed during the combustion process, because the PAHs level and composition within coal itself fluctuates depending on coal rank, and fuel source (**Stout and Emsbo-Mattingly, 2008**). The presence of these harmful compounds in grilled meats results from partial charcoal pyrolysis that leads to the formation of

PAHs in grilled food; the heat on the surface of muscle foods forms these contaminants by direct pyrolysis of food nutrients. Additionally, meat drips that are formed during the grilling and fall on hot charcoal lead to the formation of PAHs, which are carried by the smoke, and adsorbed on the surface of the meat (Costa *et al.*, 2009).

Also, this variation in total PAHs levels in different grilled meat types from different restaurants, which ranged from 29.95 to 56.91 µg/kg, is probably due to variation in fat amount, as fatty meat when grilled, more fat exudes on the hot coal and, by the high temperature, PAHs are generated then carried by smoke to the surface of the grilled meats (Reinik *et al.*, 2007). Additionally, this difference may be attributed to the fact that some restaurants marinate meats with different edible oils before grilling, which creates several levels of PAHs depending on the oil type (Farhadian *et al.*, 2012).

The abundance of light non-carcinogenic PAHs in grilled meats that ranged from 83-99% in this study could be explained, by the fact that smoke composition itself affects their content, as these low molecular weight compounds are usually found in higher levels in the smoke (Gomes *et al.*, 2009). Moreover, the high levels of PAHs, especially benzo[a]pyrene in kofta may result from the high lipid amounts in kofta because beef fat is added to kofta components to facilitate binding and molding; this fat is pyrolyzed to produce more PAHs (EC-SCF, 2002).

CONCLUSION

It could be concluded that PAHs concentrations between the three groups of grilled meats dishes were found to be significantly different at $P \leq 0.05$, also, benzo[a]pyrene recorded the highest level in grilled kofta samples, but it was not detected in chicken samples. On the other hand, PAH4, PAH8 and ΣPAHs were more abundant in grilled steak and recorded; meanwhile, they recorded the lowest concentrations in grilled chicken from different restaurants. Furthermore, benzo[a]pyrene exceeded the permissible limits in grilled kofta but was below permissible limits in other investigated food items.

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Conflict of interest

The authors declare that they are not involved in any potential conflicts of interest.

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