

## Levels of Class 2A and 2B Polynuclear Hydrocarbons in Different Barbecued Meat by Microwave Assisted Extraction Coupled with Gas Chromatography/Mass Spectrometry

M.H. EL-Saeid

Faculty of Agriculture, AL-Azhar Univ., Nasr City, Cairo, Egypt and  
College of Food and Agricultural Sciences, King Saud University, Kingdom of Saudi Arabia

**Abstract:** The purpose of this research was to study the effect of grilling methods on the 2A and 2B Polycyclic Aromatic Hydrocarbons (PAHs) formation in different meat samples; and to determine the effectiveness of the use of microwave assisted extraction (MAE) and gas chromatography/mass spectrometry (GCMS) techniques in the extraction and analysis of class 2A Benzo[a]pyrene, Benz[a]anthracene and Dibenz[a,h]anthracene and 2B Indeno[1,2,3-cd]pyrene, Benzo[k]fluoranthene and Benzo[b]fluoranthene. Both 2 class are considered as probable humans carcinogens. Also, the analysis of class 2A and 2B polynuclear hydrocarbons (PAHs) levels in 4 types of barbecued meat; beef ribs, lamb ribs, goat ribs and half chickens was carried out. The effect of grilling methods ( charcoal-broiled and electrical roaster) on the PAHs levels were studied. A total of 160 meat samples were collected from 4 local restaurants in Cairo, Egypt. Laboratory analysis for PAHs was conducted by MAE as extraction technique coupled with GCMS. Results showed that meat grilling or roasting by charcoal-broiled gave rise to the highest amount of PAHs when compared with electric oven roasting in the 4 types of meats. Recoveries of the 6 PAHs in the investigated meat samples were ranged from  $91.7 \pm 3.6$  to  $101.3 \pm 1.4\%$ . GCMS run time of 6 PAHs was 43 min, with was highly sensitivity, accurate and rapid analytical techniques capable of detecting the minimum concentrations of MDL ( 0.01 to 0.04  $\mu\text{g/kg}$ ). The average of 6 PAHs formation in beef, lamb goat and half chicken grilled by charcoal ranged from 0.99-1.11 to 8.01-11.31; 1.05-1.31 to 9.77-13.26; 1.21-1.77 to 6.22-8.44 and 0.12-0.19 to 5.61-6.43( $\mu\text{g/kg}$ ) wet wt, respectively. Meanwhile, The average of the presence of 6 PAHs formation were 0.66-0.98 to 6.85-9.22; 0.84-1.11 to 7.88-12.55; 0.89-1.27 to 4.66-6.22 and 0.09-0.11 to 3.99-4.28 ( $\mu\text{g/kg}$ ) wet wt, respectively. All 4 types of fresh meat were free of the investigated polynuclear hydrocarbons.

**Key words:** Meat • PAHs • BaP • MAE and GCMS • Human health • Medical care • Genotoxicity and carcinogenicity

### INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) refer to a large group of organic chemicals that may cause genotoxicity and carcinogenicity and may be present in the environment as pollutants and they may be generated during the preparation of barbecued meats. The International Agency for research on Cancer (IARC) of the World Health Organization has evaluated the carcinogenicity of some PAHs based on evidence in human and experimental animals. Most of the PAHs evaluated by IARC's classification are classified as Group 2B namely Indeno[1,2,3-cd]pyrene, Benzo[k]fluoranthene and Benzo[b]fluoranthene are considered (possibly carcinogenic to humans). Three PAHs, namely, benzo [a]

pyrene, benz [a] anthracene and dibenz[a,h]anthracene, cause greater health concerns since they are classified as Group 2A (probably carcinogenic to humans). None of the PAHs are classified as group 1 carcinogenic to humans [1,2].

A number of authors have reported immunosuppressive effects for PAHs in a dose-range similar to that at which carcinogenicity has been observed. Furthermore, there appears to be a rough correlation between the potency of PAH as immunosuppressors and as carcinogens. Immunosuppression may therefore be an important toxic endpoint of PAH. Some authors also suggested that immunosuppression may be involved in the mechanisms by which PAH induce cancer. At present, however, the

data on immunosuppression are not sufficient for quantitative dose response assessment. It is extremely unlikely that short-term exposure to PAH in the environment would lead to death. On the other hand, eye irritation, photophobia and skin toxicity such as dermatitis and keratosis, have been demonstrated to be caused by occupational exposures to PAH. [1, 3-7]. PAHs is a class of cancer causing agents that are produced at high cooking temperatures and formed when meat fat drips onto hot barbecue coals and creates a smoke that deposits PAHs onto the meat [8].

PAHs never occur as individual compounds in the environment, they occur as a mixture of many other polynuclear aromatic hydrocarbons. Several findings have revealed that weakly or non carcinogenic PAHs present in a mixture can modify the carcinogenic activities of a given polynuclear aromatic hydrocarbons such as Benzo(a)pyrene [9-14].

Polycyclic Aromatic Hydrocarbons (PAHs) which cause health problems such as red blood cell damage, leading to anemia, DNA damage, genotoxicity, lung cancer, developmental and reproductive effects and the best known of the carcinogenic PAHs. Maximum Contaminant Level (MCL) has been set by USEPA at 0.2 ppb in drinking water and 1 ppm in fish [15-18].

Methods of extraction are subsequently used to isolate the PAH-containing fraction, these methods include liquid-liquid extraction, Soxhlet or sonication extraction, solid-phase extraction (SPE) and , more recently, supercritical-fluid extraction (SFE) microwave-assisted extraction (MAE), [19-21].

Microwave Solvent Extraction (MSE) achieves rapid extractions, potential advantages than conventional extraction, superior recovery, reduced solvent expenditure by using solvents at high temperature and pressure than other solvent extraction techniques, no cleanup, safe and high ability as food extraction technique [18, 22-25].

The isolation, identification and quantitative determination of PAH in a complex food matrix suffers from three main problems (i) most PAH so far identified occur in food at micro trace levels, i.e. ppb or ppt levels, which makes their selective separation very difficult; (ii) many other organic components are co-extracted from the matrix with the PAH and make identification of the PAH by chromatographic and spectral methods difficult; and (iii) PAH are characterized by structural similarity and many occur as isomers, which makes identification of individual compounds extremely difficult [21].

Processed foods, particularly meat and meat products, roasted, barbecued and grilled, PAH are formed as a result of incomplete combustion or thermal

decomposition (pyrolysis) of the organic material, if the meat is in direct contact with the flame, pyrolysis of the fats in the meat generates PAH that become deposited on the meat. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat and the proximity of the food to the heat source, it should be stressed that the standard for permissible PAH levels in food has not yet been established [21, 26-28].

The purpose of this research was to study the effect of grilling method on the 2A and 2B PAHs formation in different meat samples; and to determine the effectiveness of the use of MAE and GCMS techniques in the extraction and analysis of the levels of 6 PAHs formatted in fresh, barbecued 4 species of local meat collected from Cairo local restaurants.

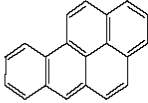

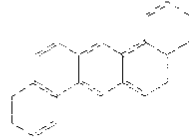
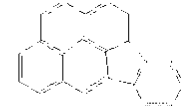
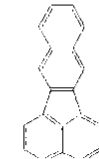
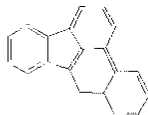
## MATERIALS AND METHODS

Sample collection and preparation: Hundred sixty meat samples ( $125 \pm 3.5$ g, 1.8 cm thick) were investigated in this study, including 4 meat types; barbecued beef ribs, lam ribs, goat ribs and half chickens ( $455 \pm 10.5$ g, 3.2 cm thick) collected from biggest local restaurants at Cairo, Egypt. Eighty meat samples grilled by charcoal of each barbecued meat types were collected from each restaurant as ready to eat samples. Also, 48 raw samples (ready for grilling) of each meat species were taken from same restaurants to be prepared by electrical grilling in the same restaurants for comparison and to compare and contribute the promotion of consumer safety by excluding the investigated 6 PAHs contamination in local markets. The effect of grilling methods (charcoal and electrical broiled) were taken into consideration. Control samples (32) of the same raw meats were also obtained.

Reagents and analytical standards: All chemicals and reagents were of analytical grade and of highest purity possible. LC grade acetone and petroleum ether used for extractions was obtained from Fischer Scientific. A PAHs standard mixture dissolved in isooctane (NIST, Baltimore, MD) containing benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, Indeno[1,2,3-cd]pyrene, Benzo[k]fluoranthene and Benzo[b]fluoranthene standards (Table 1) with purity of 99.9 % was obtained from ChemService Inc. (ChemService, Westchester, PA).

**Minimum Detection Limit (MDL):** To determine the MDL of the 6 investigates PAHs, the dilution levels ranged from 0.001-1.0 ppm was used. The MDL by MAE and GCMS methods of the individual PAHs in the investigated meat samples were from 0.01-0.04 ppm as

Table 1: Chemical structures of 2A and 2B polynuclear aromatic hydrocarbons

PAHs Name	Structure	MW	TEF	CAS No.
Benzo[a]pyrene B[a]P*	C <sub>20</sub> H <sub>12</sub> 	252.3	1.0	50-32-8
Benzo[a]anthracene B[a]A*	C <sub>18</sub> H <sub>12</sub> 	228.29	0.1	56-55-3
Dibenz[a,h]anthracene DB[a,h]A*	C <sub>22</sub> H <sub>14</sub> 	278.35	1.0	53-70-3
Indeno[1,2,3-cd]pyrene I[1,2,3-cd]P**	C <sub>22</sub> H <sub>12</sub> 	276.3	0.1	193-39-5
Benzo[k]fluoranthene B[k]F**	C <sub>20</sub> H <sub>12</sub> 	252.3	0.1	207-08-9
Benzo[b]fluoranthene B[b]F**	C <sub>20</sub> H <sub>12</sub> 	252.3	0.1	205-99-2b

\* Class 2A \*\* Class 2B TEF: Toxic Equivalence Factor. MW: Molecular Weight

Table 2: PAHs Recovery and relative standard deviation (RSD) percentages and MDL of meat samples extracted by MSE and determined by GC/MS

PAHs	Recovery % $\pm$ RSD	MDL (ppm)
B[a]P	101.3 $\pm$ 1.4	0.001
B[a]A	98.2 $\pm$ 3.2	0.002
DB[a,h]A	92.5 $\pm$ 3.3	0.003
I[1,2,3-cd]P	91.7 $\pm$ 3.6	0.004
B[k]F	96.7 $\pm$ 3.4	0.003
B[b]F	95.1 $\pm$ 3.8	0.003

showing in Table 2. MDL of BaP in meat spiked and investigated meat samples was higher than 100% by GC-MS, probably because of the higher detection limit and several orders of magnitude was found by GC-MS fragments [21].

**Recovery Assays:** To evaluate the recovery of PAH separated and analyzed by use of this procedure and to prevent matrix effects affecting peak positions in GC-MS chromatograms, untreated 4 species meat samples were spiked with 6 PAHs under

our investigation at levels of 0.001-1.0 mg/kg. Identification of six PAH determined in 4 species of meat samples were achieved on the standards and of PAH determined in spiked and un spiked meat samples. The spiked samples were homogenized and allowed to equilibrate for 2 h before analysis. Three replicates were analyzed to calculate the recovery and relative standard deviation (RSD) % by MAE and GCMS methods. Recoveries of the individual PAHs in the investigated meat samples were 91.7 $\pm$ 3.6 to 101.3 $\pm$ 1.4% as showing in Table 2.

**Extraction:** CEM application note No. E003 [29] was modified and used during conducting of the present study [18]. A Microwave Solvent Extraction (MSE) system model MES-1000 (CEM Corporation, Matthews, NC, USA) with Lined Extraction Vessels (LEV) was used. This system consists of a 950 watt microwave instrument which has been specifically designed for use with organic solvents. Extraction vessels are double-walled vessels specifically adapted for use with organic solvents. Extraction conditions was conducted with 50g raw or grilled meat sample, extraction solvent was 60 ml of acetone: petroleum ether (1:1), pressure was 130 psi, microwave power was 95%, temperature was 128°C and time of extraction 22 min.

**Analysis:** The GC/MS system used for the analyses and quantification of the 6 PAHs in meat samples extract was performed on a HP 5890 series II plus GC coupled to an HP 5972 Mass Selective Detector. The GC columns were a DB-5 fused silica capillary column (30m x 0.32mm i.d., 1µm film thickness; J and W Scientific, Folsom, CA) One micro liter of the meat sample extract was injected split less injector temperature of 250 °C, on the GC/MS for analysis. The temperature program for the GC was as follows: isothermal for 4 min at 70 °C, increased at a rate of 10 °C/min to 280 and isothermal for 10 min. Helium was used as the carrier gas and the column head pressure was maintained at 10 psi to give an approximate flow rate of 1 mL/min. The injector and transfer line were maintained at 290 and 250°C, respectively. The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of m/z 50-400 and automatic gain control (AGC) [14, 30].

## RESULTS

This work concerns the use of analytical procedures to determine the effect grilling methods on the formation of PAHs in heat-treated meat prepared by 2 types of grilling conditions. Application of MAE coupled with GC-MS enabled selective isolation of PAH fractions from the meat sample matrix. Identification and quantitative analysis of the individual compounds were achieved GC-MS. Six PAHs were identified and quantified in 160 meat samples (beef, lamb, goat and chicken) prepared according to recipes used for cooking in Egypt. The PAHs concentrations among the four species of grilled meat were found to be significantly different, furthermore, there were high significant ( $p > 0.05$ ) difference in PAH concentrations between beef and chicken grilled by

charcoal and electrical roaster. Different levels of fat contents caused interspecies differences in the PAHs content in the 4 types of grilled meats under the same grilling methods. The largest levels of PAHs were found in the highest level of fat content meat. All the 4 types of fresh meat were free of any polynuclear heterocyclic compounds. The detection and run time of PAHs was 43 min. with only 0.001 to 0.004 ppm as a MDL. The highest concentration of PAHs was detected in charcoal grilled followed by electrical roaster. In charcoal grilled samples, beef had the highest concentration of investigated PAHs followed by lamb, goat and chicken samples. PAHs concentrations of electrical grilled samples were found to be low concentration in all tested meat species than charcoal grilled samples.

### Effect of Grilling Methods on the Formation Levels of PAHs in Beef ribs:

Table 4 presents the effect of grilling methods, such as charcoal and electrical grilling on the PAHs formation levels in the beef ribs samples collected from local restaurants in Cairo, Egypt. The results showed the concentration of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in beef samples were 2.35-5.28, 8.01-11.31, 1.99-2.98, 1.67-2.78, 1.99-2.91 and 1.98-3.37 µg/kg in charcoal grilled beef, respectively. Meanwhile, the concentration and formation level of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in electrical grilled beef samples were 1.85-4.03, 6.85-9.22, 1.32-2.28, 1.30-1.97, 1.74-2.47 and 1.66-2.98 µg/kg, respectively. On the other hand, the 4 types of fresh meat were free of the investigated PAHs. The highest concentration of tested PAHs were detected in charcoal grilled beef ribs followed by electrical grilling. All beef samples grilled by charcoal stay had the highest level of B[a]A, followed by B[a]P, B[b]F, DB[a,h]A, B[k]F and B[k]F, as shown in Table 4.

### Effect of Grilling Methods on the Formation Levels of PAHs in Lamb Ribs:

The effect of charcoal and electrical grilling methods, on the PAHs formation levels in the lamb ribs samples results were showed in table 5. The concentration of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in lamb samples were 2.44-4.97, 9.77-10.26, 2.20-2.66, 2.22-3.57, 1.05-1.31 and 1.31-1.96 µg/kg in charcoal grilled beef, respectively. Meanwhile, the concentration and formation level of the same investigated PAHs in the electrical grilled lamb samples were 1.06-3.55, 7.88-9.55, 1.86-2.33, 1.83-2.84, 0.84-1.11 and 0.89-1.21 µg/kg, respectively. On the other hand, the 4 types of fresh meat were free of the investigated PAHs.

Table 3: PAHs Quantification Ions (QI) and Confirmation Ions (CI) by SIM-GC/MS

PAHs	Rt	QI (m/z)	CI (m/z)
B[a]P	37.63	252	126,250
B[a]A	30.42	228	114,226
DB[a,h]A	41.91	278	139,279
I[1,2,3-cd]P	41.75	276	138,277
B[k]F	35.93	252	126,250
B[b]F	36.33	252	126,250

SIM: Single Ion Monitoring. Rt: Retention time

Table 4: Effect of grilling methods on the formation levels of PAH in beef ribs samples

Grilling methods	Total samples	Number of restaurants	Range of PAH levels (µg/kg)					
			B[a]P	B[a]A	DB[a,h]A	I[1,2,3-cd]P	B[k]F	B[b]F
Charcoal	20	4	2.35-5.28	8.01-11.31	1.99-2.98	1.67-2.78	1.99-2.91	1.98-3.37
Electrical	12	4	1.85-4.03	6.85-9.22	1.32-2.28	1.30-1.97	1.74-2.47	1.66-2.98
Control	8	4	ND	ND	ND	ND	ND	ND

ND: Not Detected

Table 5: Effect of grilling methods on the formation levels of PAH in lamb ribs samples

Grilling methods	Total samples	Number of restaurants	Range of PAH levels (µg/kg)					
			B[a]P	B[a]A	DB[a,h]A	I[1,2,3-cd]P	B[k]F	B[b]F
Charcoal	20	4	2.44-4.97	9.77-10.26	2.20-2.66	2.22-3.57	1.05-1.31	1.31-1.96
Electrical	12	4	1.06-3.55	7.88-9.55	1.86-2.33	1.83-2.84	0.84-1.11	0.89-1.21
Control	8	4	ND	ND	ND	ND	ND	ND

ND: Not Detected.

Table 6: Effect of grilling methods on the formation levels of PAH in goat ribs samples

Grilling methods	Total samples	Number of restaurants	Range of PAH levels (µg/kg)					
			B[a]P	B[a]A	DB[a,h]A	I[1,2,3-cd]P	B[k]F	B[b]F
Charcoal	20	4	1.98-2.66	6.22-8.44	2.11-3.34	2.88-4.77	1.21-1.77	1.39-1.94
Electrical	12	4	1.55-2.21	4.66-6.22	1.23-2.03	1.76-3.33	1.03-1.19	0.89-1.27
Control	8	4	ND	ND	ND	ND	ND	ND

ND: Not Detected

Table 7: Effect of grilling methods on the formation levels of PAH in half chicken samples

Grilling methods	Total samples	Number of restaurants	Range of PAH levels (µg/kg)					
			B[a]P	B[a]A	DB[a,h]A	I[1,2,3-cd]P	B[k]F	B[b]F
Charcoal	20	4	0.65-1.18	5.61-6.43	1.39-2.55	0.68-0.96	0.12-0.19	0.23-0.42
Electrical	12	4	0.45-0.89	3.99-4.28	1.22-2.03	0.60-0.88	0.09-0.11	0.15-0.30
Control	8	4	ND	ND	ND	ND	ND	ND

ND: Not Detected

Table 8: Comparison of formation levels (µg/kg) of PAHs in beef, lamb goat ribs and half chicken samples grilled by charcoal and electrical

Meat samples	PAHs level (µg/kg)	
	Charcoal	Electrical
Beef	0.99-1.11 to 8.01-11.31	0.66-0.98 to 6.85-9.22
Lamb	1.05-1.31 to 9.77-13.26	0.84-1.11 to 7.88-12.55
Goat	1.21-1.77 to 6.22-8.44	0.89-1.27 to 4.66-6.22
Chicken	0.12-0.19 to 5.61-6.43	0.09-0.11 to 3.99-4.28

The highest concentration of tested PAHs were detected in charcoal grilled beef ribs followed by electrical grilling. All lamb samples were grilled by charcoal had the highest level of B[a]A, followed by B[a]P, B[b]F, DB[a,h]A, B[k]F and B[k]F.

**Effect of Grilling Methods on the Formation Levels of PAHs in Goat Ribs:** The effect of charcoal and electrical grilling methods, on the PAHs formation levels in the goat ribs samples results were showed in Table 6. The concentration of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in goat samples were 1.98-2.66, 6.22-8.44, 2.11-3.34, 2.88-4.77, 1.21-1.77 and 1.39-1.94 µg/kg in charcoal grilled beef, respectively. Meanwhile, the concentration and formation level of the same investigated PAHs in the electrical grilled beef samples were 1.55-2.21, 4.66-6.22, 1.23-2.03, 1.76-3.33, 1.03-1.19 and 0.89-1.27 µg/kg, respectively. The highest concentration of tested PAHs were detected in charcoal grilled goat ribs followed by electrical grilling. All beef samples were grilled by charcoal had the highest level of B[a]A, followed by B[a]P, B[b]F, DB[a,h]A, B[k]F and B[k]F.

**Effect of Grilling Methods on the Formation Levels of PAHs in Half Chicken ribs:** Table 7 presents the effect of charcoal and electrical grilling methods on the PAHs formation levels in the half chicken samples. The results showed the concentration of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in chicken samples were 0.65-1.18, 5.61-6.43, 1.39-2.55, 0.68-0.96, 0.12-0.19 and 0.23-0.42 µg/kg in charcoal grilled chicken, respectively. Meanwhile, the concentration and formation level of B[a]P, B[a]A, DB[a,h]A, I[1,2,3-cd]P, B[k]F and B[b]F in electrical grilled chicken samples were 0.45-0.89, 3.99-4.28, 1.22-2.03, 0.60-0.88, 0.09-0.11 and 0.15-0.30 µg/kg, respectively. The tested 4 types of fresh meat were free of the investigated PAHs. The highest concentration of tested PAHs were detected in charcoal grilled chicken samples followed by electrical grilling. All chicken samples were grilled by charcoal stay had the highest level of B[a]A, followed by B[a]P, B[b]F, DB[a,h]A, B[k]F and B[k]F.

Table 8 Summarizes the comparison of the effect of charcoal and electrical grilling on the concentration of the investigated PAHs in beef, lamb, goat and chicken. The results showed the highest level of PAHs were found in beef samples followed by lamb, goat and chicken. Chicken samples grilled by both grilling methods register the lowest levels of all analyzed PAHs.

## DISCUSSION

Grilling of meat at a lower temperature or further away from the heat source would result in lower levels of PAHs. Raw meats prepared by boiling was found to contain lower of PAHs levels than those prepared by charcoal and electrical grilling. PAHs were mainly found in the outer part of barbecued meat. Results indicated that the thermal process and method of grilling has a significant effect on the concentration of class 2A and 2B PAHs generated in the meat samples. some of the highest levels of PAHs reported in meat have been detected in meat cooked over open flames such as barbecued meat. These findings are in agreement with those has been previously reported [26, 31-33]. Grilled beef, goat, lamb and chicken samples grilled by electrical oven (oven grilled) had the lowest concentration of PAHs when compared with charcoal grilled samples. This can be probably due to the oven grilled foods were indirect contact with the flame [34].

In a another study [35], bread subjected to direct toasting (flame-toasting, coal-grilling or gas oven toasting) had high levels of PAHs (350 ng/g of total PAHs) than indirect toasting (electric oven-toasting).

In the study by Janoszka *et al.* [21] Flt and B(a)P were found at concentrations of 0.57 ng/g and 0.15 ng/g, respectively, in grilled chicken breast on charcoal. In addition, Kazerouni *et al.* [28] found that B(a)P was present in 200 different meat dishes, of which very well-grilled or barbecued meat had the highest concentrations of PAH, ranging from 0.40 to 1.15 ng/g. When comparing our results with the other studies, the tested chicken grilled on charcoal had higher concentration of PAHs than electrical grilling. Some ingredients, especially those used in grilled meats, may be responsible for generating high concentration of PAHs. It is known that recoating with sauces can often result in burned meat surface [34]. Fat content of the sample is also an important factor for PAHs formation in grilled meat [36,37]. The author suggest these compounds arise from the pyrolysis of the fat drippings.

In conclusion, analysis of 4 different meat grilling types (beef, lamb, goat and chicken from Egypt) indicated that beef samples contain the highest values of 6 PAH compounds followed by lamb, goat and chicken. The highest concentration of PAHs was detected in charcoal grilled followed by oven grilled in all tested meat samples. With the MAE and GCMS multiresidue method, the optimum conditions were met to extract and determined 6 PAHs in more than 160 meat samples in short time and sensitive detection limit (0.001-0.004 ppm)

## REFERENCES

1. IARC, (International Agency for Research on Cancer), 1987. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Supplement No. 7. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. International Agency for Research on Cancer, Lyon, France.
2. WHO, 2000 Regional Office for Europe. Chapter 5.9 PAHs. In WHO Regional Office for Europe. Air Quality Guidelines 2nd ed. Denmark: WHO Regional Office for Europe; 2000. IRAC.
3. MOE, (Ministry of the Environment), 1997. Scientific Criteria Document for Multimedia Standards Development. Polycyclic Aromatic Hydrocarbons (PAH). Part1: Hazard Identification and Dose-Response Assessment. Ministry of the Environment, Toronto, Ontario.
4. ATSDR, (Agency for Toxic Substances and Disease Registry), 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, August 1995.
5. CEPA, (Canadian Environmental Protection Act), 1994. Polycyclic Aromatic Hydrocarbons. Environment Canada and Health Canada, Ottawa, Ontario. EN40-215/42E.
6. WHO, (World Health Organization), 1996. Updating and Revision of the Air Quality Guidelines for Europe. Report on a WHO Working Group on Volatile Organic Compounds. Brussels, Belgium, 2-6 October, 1995.
7. NIPHEP, (National Institute of Public health and Environmental Protection), 1989. Integrated Criteria Document PAHs. NIPHEP, Bilthoven, Netherlands. # 758474011.
8. Agricultural Research Service, 2001. Cooked-to-Brown Burgers May Not Tell the Truth. Retrieved June 16, 2003/010202.htm
9. Van Duuren B.L. and M.M. Goldschmidt, 1976. Carcinogenic and tumour promoting agents in tobacco-carcinogenesis. *J. Natl. Cancer Inst.*, 56: 1237-1242.
10. DiGiovanni, J., J. Rymer, T.J. Slaga and R.K. Boutwell, 1982. Anticarcinogenic and carcinogenic effect of benzo(a)pyrene and dibenz(a,c)anthracene on skin tumor initiation by polycyclic hydrocarbons. *Carcinogenesis*, 3: 371-375.
11. Slaga, T.J. and J. DiGiovanni, 1984. Inhibition of chemical carcinogenesis. *ACS Monogr.*, 102: 1279-1321.
12. Nesnow, S., 1990. Mouse skin tumour and human lung cancer: Relationship with complex environmental emissions. In: Complex mixtures and cancer risk (eds. H. Vainio, M. Sorsa and A.J. McMichael), pp. 44-54, IARC Scientific Publications No. 104, IARC, Lyon.
13. Mottier, P., V. Parisod and R.J. Turesky, 2000. Quantitative Determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. *J. Agric. Food Chem.*, 48: 1160-1166.
14. Anyakora, C., A. Mohsen and C. Herbert, 2008. A screen for Benzo(a)pyrene in Fish Samples From Crude Oil Polluted Environments. *American J. Environ. Sci.*, 4(2): 145-150.
15. Yang, S.C., S.N. Jeng, Z.C. Kang and H. Lee, 2000. Identification of benzo[a]pyrene 7,8-Diol 9,10-Epoxyde N2-Deoxyguanosine in human lung adenocarcinoma cells exposed to cooking oil fumes from frying fish under domestic condition. *Chem. Res. Toxicol.*, 13: 1046-1050.
16. Telli-Karakoc, F., P.J. Ruddock, D.J. Brid, A. Hewer, A. Van Schanck, D.H. Phillpis and L.D. Peters, 2002. Correlative changes in metabolism and DNA damage in turbot exposed to benzo[a]pyrene. *Marine Environ. Res.*, 45(3-5): 511-515.
17. Anastasio, A., R. Mercogliano, L. Vollano, T. Pepe and M.L. Cortesi, 2004. Levels of Benzo(a)pyrene in Mozzarella di Bufala Campana Cheese Smoked According to Different Products. *J. Agric. Food Chem.*, 52: 4452-4455.
18. EL-Saeid, M.H., 2006. Microwave solvent extraction (MSE) and HPLC as a rapid determination methods of Benzo[a]Pyrene in Fish. *Bulletin of College of Food Sciences Research Center, King Saud University*, 143: 1-21.
19. Eskilsson, C.S. and E. Björklund, 2000. *J. Chromatogr. A.*, 902: 227.
20. Camel, V., 2000. *Trends Anal. Chem.*, 19: 229.
21. Janoszka, B.L. Warzech, U. Błaszczyk and D. Bodzek, 2004. Organic compounds formed in thermally treated high-protein foods part 1: Polycyclic Aromatic Hydrocarbons. *Acta Chromatographica*, No. 14.
22. USEPA, 1986. Test methods for evaluating solid waste. SW-846, 3<sup>rd</sup> ed. Method, 3540: 3450-3.

23. Colis, M.J. and E.D. Neas, 1988. Introduction to microwave sample preparation: theory and practice, chap 2. In Kingston H.M; Jassie, L.B. eds. Washington, DC: American Chemical Society.
24. Hasty, E. and R. Revesz, 1995. Total petroleum hydrocarbon determination by Microwave Solvent Extraction. American Laboratory, (2): 66-74.
25. EL-Saeid, M.H., 1999. New Techniques for residue analysis of pesticides in foods (Ph.D. Dissertation) Al-Azhar Univ., Cairo, Egypt.
26. Phillips, D.H., 1999. Polycyclic aromatic hydrocarbons in the diet. Mutation Research-Genetic Toxicol. Environ. Mutagenesis, 44(1-2): 139-147.
27. Puzanowska-Tarasiewicz, H. and M. Tarasiewicz, 1999. Polish J. Environ. Stud., 8: 57.
28. Kazerouni, N., R. Sinha, H. Che-Han, A. Greenberg and N. Rothman, 2001. Food Chem. Toxicol., 39: 423.
29. CEM, application note # E003. PAHs from Sludge HPLC method. CEM Corporation, Friendswood, TX 77549.
30. Anyakora, C., A. Ogbeche, P. Palmer, H. Coker, G. Ukpo and C. Ogah, 2005. GC/MS analysis of polynuclear aromatic hydrocarbons in sediment samples from the Niger Delta region. Chemosphere, 60: 990-997.
31. Farhadian, A., S. Jinap, F. Abas and Z. Sakar, 2010. Determination of polycyclic aromatic hydrocarbons in grilled meat. Food Control, 21: 606-610.
32. Chen, B.H. and Y.S. Lin, 1997. Formation of polycyclic aromatic hydrocarbons during processing of duck meat. J. Agric. Food Chem., 45: 1394-1403.
33. Wretling, S., A. Eriksson G.A. Eskhult and B. Larsson, 2010. Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish J. Food Composition and Analysis, 23: 264-272.
34. Agerstad, M.J. and K. Skog, 2005. Review genotoxicity of heat-processed foods. Mutation Research, 574: 156-172.
35. Rey-Salgueiro, L., M.S. Garc -Falco, E.M. Nez-Carballo and N.J. SimalGa, 2008. Effects of toasting procedures on the evels of polycyclic aromatic hydrocarbons in toasted bread. Food Chemistry, 108: 607-615.
36. Fretheim, K., 1983. Polycyclic aromatic hydrocarbons in grilled meat products: A review. Food Chemistry, 10: 129-139.
37. Djino ic, J., P. Aleksandar and J. Wolfgang, 2008. Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia Meat Science, 80: 449-456.