

# **The Effect of Cooking Method and Food Additives on Formation of Polycyclic Aromatic Hydrocarbons in Chicken Meat**

By

**Hend Hassan Ali Ganbi**

Nutrition and Food Science Dept., Faculty of Education for Home Economics and Art Education , King Abd – Elaziz University, Jeddah , Saudi Arabia

**Abstract:** This research was performed in order to assess the rate of formation of the harmful and/or carcinogenic polycyclic aromatic hydrocarbons (PAHs) in chicken meat in Saudi Arabia. The affect of cooking method (pan-fat frying , butane gas oven grilling and charcoal grilling) and incorporating of some food additives prior to cooking process on the formation of the (PAHs) in chicken meat was investigated. The obtained results illustrated that the tested cooking methods , especially charcoal grilling , caused an exceptional rise in the total PAHs content. These methods encouraged the formation of PAHs derivatives at different levels. In addition, the tested food additives treatments with spices mixture or/and fresh garlic paste prior to cooking had a high inhibitory effect on the PAHs formation in cooked chicken. They caused significant reduction in the total PAHs content in cooked chicken by about 35.0 % to 64.8 % depending upon cooking method and food additives treatment. Therefore, the present results suggested that chicken meat should be treated prior to cooking and thermal processing with food additives that cause inhibition or prevention of the formation of harmful and/or carcinogenic PAHs. It should be avoided to cook chicken and other meat products by charcoal grilling , unless they are treated with food additives , which encouraged the PAHs formation at a high levels' causing health hazard and possible cancer.

**Key words:** polycyclic aromatic hydrocarbons ; cooked chicken ; food additives treatment ; pan-fat frying ; grilling ; charcoal grilling.

## **Introduction**

Polycyclic aromatic hydrocarbons (PAHs) represent a group of organic compounds consisting of two or more condensed aromatic rings that are widely geographically distributed and remain in the environment for a long time (Howsam *et al.*, 2000 ; FAO/WHO , 2005 and Djinovic *et al.*, 2008) . A number of them, such as benzo[a]pyrene, are carcinogenic

and mutagenic (Du-Four *et al.*, 2005), and they are widely believed to make a substantial contribution to the overall burden of cancer in humans (Phillips, 1999 ; FAO/WHO , 2005 and Djinovic *et al.*, 2008b). Due to these properties they are considered to be top of the list of the most hazardous substances. Harmful effects of PAHs on living organisms have been observed for years. (EC., 2005)

PAHs can be found throughout the environment in water (Fernandes *et al.*,1999 and Barth *et al.*, 2007), atmosphere (Chung-Yih, *et al.*, 2006) and sediment (Lauenstein & Kimbrough, 2007). Foods may be contaminated through different routes, which include the following: direct deposition of PAHs from the atmosphere as environmental contaminants on various fruits and vegetables (Fismes *et al.*, 2004; Tao *et al.*, 2006 and Rey-Salgueiro *et al.*, 2008) ; contamination from packaging materials and production of PAHs during the thermal processing of foods, e.g., roasting, frying , toasting , charcoal grilling, and smoking (Simko, 2002 ; Elhassaneen , 2004 ; Stolyhwo & Sikorski , 2005 and Rey-Salgueiro *et al.*, 2008). When food, particularly meat, and other animal food products is smoked, roasted, fried and grilled, the PAHs are formed as a result of incomplete combustion or thermal decomposition (pyrolysis) of the organic material. It had been reported that grilling, frying or roasting would generate PAHs in cooked meat and the charcoal grilling gave rise to the highest amount of PAHs when compared with frying, gas grilling or electric oven roasting. (Lijinsk , 1996 ; Elhassaneen , 2004 ; Janoszka *et al.*, 2004 and El-Badry *et al.*, 2006).

If the meat is in direct contact with the flame , pyrolysis of the fats in the meat generates PAHs that can become deposited on the meat. Even if not in direct contact , fat dripping on the flame or hot coals generates the compounds which are then carried back on to the meat .PAH production by cooking over charcoal is a function both of the fat content of the meat and the proximity of the food to the heat source, and can be reduced by cooking for longer period at lower temperature. Also the food is low in fat or cooked beneath the source of heat, contains many fewer PAHs , so the type of food cooked and the method of cooking are important (Howord & Fazio , 1980; Felton & Knize, 1991; Sinha & Rothman, 1999 ; Janoszka *et al.*, 2004 and El-Badry *et al.*, 2006).

Hundreds of individual PAHs may be formed and released during the incomplete combustion or thermal decomposition (pyrolysis) of the organic material. According to the Scientific Committee on Food (SCF) , benzo[*a*]pyrene can be used as a marker for the occurrence and impact of carcinogenic PAHs in food (Anderson *et al.*, 2002 and EC., 2005) . Benzo[*a*]pyrene ( B[*a*]Pyr ) a member of the PAH class, is one of the most

potent PAH carcinogens in animal experiments, and its relatively easy to separate and analyze. B[a]Pyr is present in a wide variety of food items, it has been also chosen as the general indicator of total PAHs presence in processed foods (Kazerouni *et al.*, 2001 and Simko, 2002). The emission factors of total PAHs , B[a]Pyr for broiling meat and other animal food stuffs were noticeably higher than those for broiling vegetables and non – fish seafood (Elhassaneen , 2004 ; El-Badry *et al.*, 2006 and Kuo *et al.*, 2006 ). It had been reported that the PAHs are mutagenic and carcinogenic agents in animals and human. Polycyclic aromatic hydrocarbons which formed in processed and cooked foods are very well known ecotoxicants which are harmful to health. In mammalian cells PAH undergo metabolic activation to diol epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (WCRF., 1997 and Janoszka *et al.*, 2004). It had been reported that there was a relationship between the intake of several foods that might have high level of PAH (e.g. smoked or grilled / barbecured meats) and risk for cancer at several sites including stomach and esophagus , colorectal, pancreatic , breast and bladder cancer (Steineck *et al.*, 1990 ; WCRF., 1997 ; Anderson *et al.*, 2002 ; Elhassaneen , 2004 and Jagerstad & Stog , 2005) . Because the PAHs are contained in wide variety of food, it is necessary to directly estimate the dietary intake of B[a]Pyr from all dietary sources to evaluate the relationship between dietary intake of B[a]Pyr and risk of cancer. (Janoszka *et al.*, 2004).

Therefore, this research was performed to assess the extent of formation of the harmful and/or carcinogenic polycyclic aromatic hydrocarbons (PAHs) in chicken tissues during cooking by different cooking methods (pan-fat frying , butane gas oven grilling and charcoal grilling) and to try to reduce or prevent the formation of these compounds by incorporating some food additives.

## Material and Methods

### Materials:

(1). **Chicken** : The fatted chicken used in this study were obtained during the season of 1429 / 1430 AH (2008/2009 A.D) from the local market in Jeddah City , Saudi Arabia. They weighed about 1400 – 1600 gm and aged about 8 weeks.

(2). **Refined Sunflower oil (RSO)** : The RSO produced on 11 February 2008 A.D by Abo - Dhabi Company for Plant Oils , Abo – Dhabi , UAR ,

was obtained from the local market in Jeddah City , Saudi Arabia , during the season of 2028 / 2029 A.H (2008 / 2009 A.D).

**(3). Refined Table Salt :** Sodium chloride ( Fine refined table salt ) produced on 20 May 2008 AD by Saudi Salt Refinery , Jeddah , Saudi Arabia , was obtained from the local market in Jeddah City , Saudi Arabia , during the season of 2028 / 2029 A.H (2008 / 2009 A.D).

**(4). Fresh Garlic Lobes :** They were obtained during the season of 2028 / 2029 A.H (2008 / 2009 A.D) from the local market in Jeddah City , Saudi Arabia. The garlic paste was prepared by grinding the decoated garlic lobes carefully.

**(5). Spices Mixture :** Spices ( Cumin , Coriander and black pepper ) were obtained during the previous mentioned season from the local market in Jeddah City , Saudi Arabia. The former Spices were cleaned individually and ground to a fine powder. Whereas , the homogenous spices mixture was made by mixing equal weight from each the former spices carefully with repeating the mixing process of individual spices in order to homogenize.

**(6). Vegetar Spice Coating Mix Powder :** It was produced on 10 May 2008 AD by Uniliver Meshrik Co. for Production of Food Products , Alex., Egypt and purchased during the season of 1429 / 1430 A.H from the local market in Jeddah city , Saudi Arabia.

**(7). Standard of the PAHs compounds :** The standard naphthalene ( Nap ) , acenaphthene (Ace) , anthracene (Ant) , fluoranthene (Fla) , pyrene (Pyr) , benzo[a]pyrene (B[a]Pyr) and dibenzo[a,h]anthracene (DB[a,h]Ant) were purchased from the Sigma Chemical Company (St. Louis, MO., USA) . While , the standard acenaphthalene (Acl) , fluorene (Flu) , phenanthrene (Phe) , benzo[a] anthracene (B[a]Ant) , chrysene (Chr) , benzo[b]fluoranthene (B[b]Fla) , benzo[k]fluoranthene (B[k]Fla) , benzo [g,h,i]pyrene (B[g,h,i]Pyr) and indeno[1,2,3-ed]pyrene (I-Pyr) were purchased from Fluka Ag Chem. Co., Buchs, Switzerland.

## **Methods:**

### **(1). Preparation of chicken samples for grilling processes :**

Chicken were slaughtered , plucked mechanically , eviscerated and washed carefully with tap water. Whereas , whole chicken carcass were immersed in 10 % NaCl solution for 10 minutes. After that , they washed carefully with tap water , let for about 2 minutes to drain and then divided into two equal main batches ; the first was taken for cooking by butane gas oven grilling and the second was used for cooking by charcoal grilling. Each main batch was divided also into approximately equal four sub-batches ; the first was cooked without any food additives treatment prior to

cooking process and taken as a control. The second sub-batch was treated by mashing it with spices mixture ; composed of equal weight from cumin , coriander and black pepper dried powders (T1).The third sub-batch was treated with fresh garlic paste (T2) , while the fourth one was mashed with both spices mixture and fresh garlic paste(T3) . All treated sub-batches were coated with wheat bran and then cooked by grilling process.

## **(2). Preparation of chicken patties for pan-fat frying process :**

Chicken patties were prepared from chicken breast meat muscles by the removal of skin and bones and then were washed well with tap water , sliced into approximately 1.5 cm thick-slices , according to the procedure reported by Conchillo *et al.* (2005). Then , prepared chicken patties were randomly divided into four approximately equal batches , each weighted about 1 kg , treated with different treatments prior to pan-fat frying process as follows : the first was pan-fat fried without any food additives treatment and taken as control. The second batch was mashed carefully with spices mixture (T1) , the third was mashed well with fresh garlic paste (T2) and the fourth one was mashed carefully with both spices mixture and fresh garlic paste (T3). After that , all batches of chicken patties were thinly covered with vegetar spice coating mix powder and then cooked by pan-fat frying.

## **(3). Cooking processes:**

**(a). Pan-fat frying method :** 200gm of refined sunflower seed oil (frying oil medium) placed in an electric stainless shallow pan (500 ml-capacity , 20 cm-diameter and 4 cm-high) and firstly heated to  $160\pm 5^{\circ}\text{C}$ . Then , chicken patties were fried individually at the former temperature for 8 min (4min for each side) , or to an internal temperature of about  $80^{\circ}\text{C}$ . Fried chicken patties samples were ground by grinder (Oster Heavy Duty Food Grinder , USA) , packaged in polyethylene bags and stored under freezing conditions (at  $-18\pm 2^{\circ}\text{C}$  ) until analyzed , as well the fresh unfried sample.

**(b). Butane gas oven grilling method :** Prepared whole chicken carcass were grilled in commercially thermostatic butane gas oven (Model Stuteskv , Russia) at about  $160\pm 5^{\circ}\text{C}$  for 15-20 min or until the desired bright brown color formed on the outer surface of chicken carcass ; as the cooking completion sign.

**(c). Charcoal grilling method :** In this procedures , prepared whole chicken carcass were divided into desired consistent parts and then cooked on manual grill-type fuelled with burnt charcoal (for 15-20 min) until cooking completion as previously illustrated , according to the procedure of Janoszk *et al.* (2004).

#### **(4). Determination of polycyclic aromatic hydrocarbons ( PAHs ) in tested food samples :**

The extraction and determination of the PAHs in fresh and cooked chicken samples were carried out according to the procedure of Howard & Fazio (1980) ; Lal & Khanna (1996) and Semko (2002).

**Extraction :** In brief , each sample was digested in alcohol and potassium hydroxide and then distilled water was added and the hydrocarbons partitioned into isoctane. The interfering materials were removed by column chromatography on florisil (60-100 mesh) followed by selective extraction of polycyclic aromatic hydrocarbons into dimethyl sulfoxide (DMSO). Further interfering materials were removed by column chromatography on sephadex LH-20. A solvent mixture of toluene and ethanol at ratio of 1:1 was used to obtain the pure extract of the PAHs which was used for determination of the PAHs components.

**Determination :** The 16 individual PAHs listed by the reference standard reported by the United States (US-EPA) , in the purified extracts of tested samples were determined qualitatively and quantitatively by using high performance liquid chromatography (HPLC). The PAHs identification system with millennium 3.2 software PAHs Standard were obtained from quantification performed using HPLC. The apparatus model used in determination was Waters HPLC. 600E , equipped with dual UV absorbance detector Waters 2487 and auto samples 717 plus attached to computerized supelco. The conditions of separation are as follows:

*Column :* Supelcosil LC-PAH.  $\mu\text{m}$  particles 15 cm length and 4.6 mm ID.

*Mobile phase :* Gradient acetonitrile ; water 60 to 100 % acetonitrile (v/v) over 45 min.

*Flow rate :* 0-2 min , 2.0 m/min , 2-45 min .1.0 ml/min.

*Detector :* it was set at 254 nm.

#### **(5). Statistical analysis:**

Statistical analysis was carried out using on IBM-PC computer and SAS program for the multiple comparison of polycyclic aromatic hydrocarbons in the tested chicken samples as the procedure of ANOVA and Duncan's Multiple Range. Tests were used according to Helwing (1983). The L.S.D at 5 % level of significant was also used to compare between the means of polycyclic aromatic hydrocarbons level in the fresh and cooked chicken samples according to Snedecor and Cochran (1986).

## Results and Discussion

### Formation extent of harmful and/or carcinogenic PAHs in chicken meat throughout cooking process as affected by cooking method and some food additives :

Sixteen polycyclic aromatic hydrocarbon (PAH) compounds were detected in fresh uncooked and cooked chicken meat samples including ; naphthalene (Nap) , acenaphthalene (Acl) , acenaphthene (Ace) , fluorene (Flu) , phenanthrene (Phe) , anthracene (Ant) , fluoranthene (Fla) , pyrene (Pyr) , benzo[*a*]pyrene (B[*a*]Pyr) , chrysene (Chr) , benzo[*b*]Fluoranthene (B[*b*]Fla) , benzo[*k*]fluoranthene (B[*k*] Fla) , benzo[*a*]pyrene (B[*a*]Pyr) , dibenzo[*a,h*]anthracene (DB[*a,h*]Ant ) , benzo[*g,h,i*]pyrene (B[*g,h,i*]Pyr) and indeno[*1,2,3-ed*]pyrene (I-Pyr).

#### 1. Formation extent of the PAHs in chicken patties throughout pan-fat frying.

As given in table (1) , fresh uncooked chicken patties contained only Ace , Flu , Phe , Ant , Chr , B[*k*] Fla and I-Pyr at concentration of 16.93 , 28.27 , 0.49 , 0.24 , 0.17 , 10.65 and 2.18  $\mu\text{g} \times 10^{-2} / \text{kg}$  , on wet weight basis ; respectively. The other determined PAHs were not detected in fresh uncooked chicken patties.

The obtained results (Table1) illustrate that thermal processing applied in pan-fat frying encouraged the formation of the PAHs in chicken tissues. The total PAHs were increased from  $58.93 \times 10^{-2} \mu\text{g}/\text{kg}$  in fresh uncooked chicken patties to  $505.70 \times 10^{-2} \mu\text{g}/\text{kg}$  in pan-fat fried chicken patties which were not treated with any food additives prior to frying process. The encouragement effect of the thermal pan-fat frying process on the formation of PAHs in cooked chicken patties was a result of incomplete combustion or thermal decomposition (Pyrolysis) of the organic material (Lijinski , 1996 ; Janoszka *et al.*, 2004 and El-Badry *et al.*, 2006). Moreover ; B[*b*]Fla , B[*k*]Fla , DB[*g,h*]Ant and B[*g,h,i*]Pyr were not detected in all fried chicken patties. On the other hand , the treatments of chicken patties with spices mixture or/and fresh garlic paste caused a high considerable reduction in the formation extent of the PAHs in chicken patties at rates of 35.0 , 39.6 and 64.8 % , when treated with spices mixture(T1),fresh garlic paste(T2) and with the combination of the two former treatments (T3); respectively. The inhibitory effect of spices and garlic paste treatments on the PAHs formation during pan-fat frying could be attributed to the naturally occurrence of antioxidants which prevented or inhibited the oxidation and polymerization of hydrocarbons resulted from the incomplete combustion and pyrolysis of organic matter

**Table (1): Formation of the PAHs in Pan - fat fried chicken Patties ( $\mu\text{g} \times 10^{-2} / \text{kg}$  ; on wet weight basis ) as affected by cooking method and some food additives.**

PAH Compound	PAH Compound Level ( $\mu\text{g} \times 10^{-2} / \text{kg}$ )					L.S.D at 5 %
	Fresh Uncooked Patties	Pan - Fat Fried Chicken Patties				
		Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Nap	ND	9.08	7.39	5.06	2.86	0.59
Acl	ND	316.9	228.1	173.6	105.13	16.06
Ace	16.93	52.17	20.42	36.04	29.59	3.93
Flu	28.27	67.58	32.15	49.10	21.04	5.17
Phe	0.49	19.21	10.98	15.48	7.76	2.24
Ant	0.24	4.66	4.61	3.35	ND	0.37
Fla	ND	12.93	8.47	10.61	5.48	1.02
Pyr	ND	1.30	0.83	1.30	0.22	0.09
B[a]Ant	ND	0.62	0.49	0.62	0.19	0.13
Chr	0.17	13.85	9.92	7.37	3.83	0.42
B[b]Fla	ND	ND	ND	ND	ND	---
B[k]Fla	10.65	ND	ND	ND	ND	---
B[a]Pyr	ND	3.45	2.56	1.83	1.07	0.29
DB[g,h]Ant	ND	ND	ND	ND	ND	---
B[g,h,i]Pyr	ND	ND	ND	ND	ND	---
I - Pyr	2.18	3.93	2.80	0.99	0.58	0.16
<b>Total PAHs</b>	<b>58.93</b>	<b>505.7</b>	<b>328.7</b>	<b>305.3</b>	<b>177.8</b>	<b>40.47</b>

*Control* :The cooked sample without any food additives treatment. *T1* : Cooked sample treated with spices mixture prior to cooking process. *T2* : Cooked sample treated with fresh garlic paste prior to cooking process. *T3* : Cooked sample treated with both spices mixture and fresh garlic paste prior to cooking process. *L.S.D at 5 %* : Least significant difference between fresh and cooked chicken samples at significant level of 5%. *ND* : Not detected.

including proteins and fats. These results are in accordance with those reported by Phillips (1999) and El-Badry *et al.* (2006).

## **2. Formation extent of the PAHs in butane gas oven grilled whole chicken carcass :**

As shown in table (2) , the commercially butane gas oven grilling caused a high rise in the formation of deleterious carcinogenic PAHs in whole chicken carcass at a higher extent than those in pan-fat fried chicken samples. This is the result of direct contact of chicken tissues with the flame. Pyrolysis of the fats in chicken meat generates PAHs that become deposited on the chicken meat (Felton & Knize , 1999 ; Sinha & Rothman , 1999 ; Janoszka *et al.*, 2004 and El-Badry *et al.*, 2006).

In general, spices mixture or/and fresh garlic paste treatments prior to gas-oven grilling caused a high inhibition to the formation of determined PAHs in grilled chicken at a variable rates depending upon food additive treatment prior to grilling and the PAHs compound itself. This is a result of the antioxidant effect for the active naturally occurrence ingredients in spices and fresh garlic paste. The total PAHs decreased from  $750.2 \mu\text{g} \times 10^{-2} / \text{kg}$  for grilled chicken sample without any food additives to 383.5 , 480.9 and 314.7  $\mu\text{g} \times 10^{-2} / \text{kg}$  on wet weight basis , when chicken carcass was treated with spices mixture (T1) , fresh garlic paste (T2) and with the combination of both spices mixture and fresh garlic paste (T3) ; respectively. In addition , the tested former food additives treatments prevented the formation of Nap , Ace , Fla , B[a] Ant , B[b] Fla , Db[a,h] Ant and B[g,h,i] Pyr in grilled chicken samples. The predominant PAHs in all butane gas oven-grilled chicken samples were I-Pyr , Chr , Flu , Acl , Pyr and Phe ; at different concentrations affected by food additive treatment prior to grilling and the PAH compound itself. The present findings are in a good agreement with those reported by Lijinsky (1996) ; Janoszka *et al.* (2004) and El-Badry *et al.* (2006).

## **3. Formation extent of the PAHs in charcoal grilled chicken carcass:**

As illustrated in table (3) , the charcoal grilling process encouraged the formation of the most determined PAH derivatives in grilled chicken carcass at different concentrations affected by the food additive treatment prior to grilling and the nature and chemical structure of the PAHs derivative itself. It caused also a highly exceptional rise in total PAHs content from  $56.94 \mu\text{g} \times 10^{-2} / \text{kg}$  for fresh uncooked chicken sample to 1228.7  $\mu\text{g} \times 10^{-2} / \text{kg}$  in the charcoal grilled chicken sample (not treated with any food additives prior to grilling) and to 792.3 , 855.8 and 545.2  $\mu\text{g} \times 10^{-2} / \text{kg}$  in

**Table (2): Formation of the PAHs in butane gas oven grilled chicken ( $\mu\text{g} \times 10^{-2} / \text{kg}$  ; on wet weight basis ) as affected by cooking method and some food additives.**

PAH Compound	PAH Compound Level ( $\mu\text{g} \times 10^{-2} / \text{kg}$ )					L.S.D at 5 %
	Fresh Uncooked Patties	Gas Oven Grilled Chicken				
		Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Nap	ND	ND	ND	ND	ND	---
Acl	ND	93.58	26.10	42.76	17.33	3.95
Ace	13.09	5.32	ND	1.31	ND	0.81
Flu	28.27	106.80	39.42	64.09	33.85	5.07
Phe	0.60	70.49	54.80	30.36	21.09	7.33
Ant	0.32	1.70	0.97	1.04	0.72	0.19
Fla	ND	ND	ND	ND	ND	---
Pyr	ND	82.96	23.28	27.92	19.46	5.38
B[a]Ant	ND	ND	ND	ND	ND	---
Chr	0.17	140.63	67.01	91.80	66.38	4.92
B[b]Fla	ND	ND	ND	ND	ND	---
B[k]Fla	11.49	8.10	7.33	6.98	4.72	1.16
B[a]Pyr	ND	32.98	19.60	12.75	9.90	3.04
DB[g,h]Ant	ND	0.45	ND	0.29	ND	0.23
B[g,h,i]Pyr	ND	ND	ND	ND	ND	---
I - Pyr	3.02	207.17	144.99	201.64	141.20	9.18
<b>Total PAHs</b>	<b>56.96</b>	<b>750.2</b>	<b>383.5</b>	<b>480.9</b>	<b>314.7</b>	<b>41.26</b>

*Control* :The cooked sample without any food additives treatment. *T<sub>1</sub>* : Cooked sample treated with spices mixture prior to cooking process. *T<sub>2</sub>* : Cooked sample treated with fresh garlic paste prior to cooking process. *T<sub>3</sub>* : Cooked sample treated with both spices mixture and fresh garlic paste prior to cooking process . *L.S.D at 5 %* : Least significant difference between fresh and cooked chicken samples at significant level of 5%. *ND* : Not detected.

**Table (3): Formation of the PAHs in charcoal grilled chicken ( $\mu\text{g} \times 10^{-2} / \text{kg}$  ; on wet weight basis ) as affected by cooking method and some food additives .**

PAH Compound	PAH Compound Level ( $\mu\text{g} \times 10^{-2} / \text{kg}$ )					L.S.D at 5 %
	Fresh Uncooked Patties	Charcoal Grilled Chicken				
		Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Nap	ND	ND	ND	ND	ND	---
Acl	ND	271.9	190.3	164.8	84.57	23.03
Ace	13.09	ND	ND	ND	ND	---
Flu	28.27	154.3	91.90	129.6	66.43	11.07
Phe	0.60	112.7	50.12	74.35	40.18	6.52
Ant	0.32	39.46	32.36	19.18	12.74	4.19
Fla	ND	5.51	3.80	2.72	ND	1.33
Pyr	ND	67.18	57.94	40.06	28.59	9.06
B [a] Ant	ND	ND	ND	ND	ND	---
Chr	0.17	54.60	48.27	46.61	36.06	7.21
B [b] Fla	ND	18.95	12.14	9.40	5.81	2.64
B [k] Fla	11.49	43.09	36.72	30.39	18.25	5.18
B [a] Pyr	ND	119.3	80.49	58.76	43.98	13.03
DB [g,h] Ant	ND	51.07	12.26	26.08	ND	2.95
B [g,h,j] Pyr	ND	ND	ND	ND	ND	---
I - Pyr	3.02	310.6	176.3	253.9	208.6	14.13
<b>Total PAHs</b>	<b>56.94</b>	<b>1228.7</b>	<b>792.3</b>	<b>855.8</b>	<b>545.2</b>	<b>77.34</b>

*Control* : The cooked sample without any food additives treatment. *T1* : Cooked sample treated with spices mixture prior to cooking process. *T2* : Cooked sample treated with fresh garlic paste prior to cooking process. *T3* : Cooked sample treated with both spices mixture and fresh garlic paste prior to cooking process. *L.S.D at 5 %* : Least significant difference between fresh and cooked chicken samples at significant level of 5%. *ND* : Not detected.

charcoal grilled chicken samples treated with spices mixture (T1) , fresh garlic paste (T2) and with both spices mixture and fresh garlic paste together (T3) ; respectively. The present result also indicate that tested food additives treatment were highly effective in inhibiting the formation

of the PAHs in chicken meat during charcoal grilling. On the other hand , charcoal grilling method encouraged the formation of the harmful or/and carcinogenic PAHs in cooked chicken samples much higher than pan-fat frying and butane gas oven grilling. This is because of the direct contact of chicken tissues with the heat source or hot coals' as explained before. Besides fat dripping on the hot coals and the pyrolysis of fats in chicken meat generates the PAH compounds which are then carried back on to chicken samples. Therefore , the PAHs formation by cooking over charcoal is a function of both the fat content of food and the proximity of food to the heat source (Lijinsky , 1991 ; Phillips , 1999 ; Anderson *et al.*, 2002 ; Elhassaneen , 2004 ; Janoszka *et al.*, 2004 ; Jagerstad & Skog , 2005 and El-Badry *et al.*, 2006).

**Food legislations and health safe limitations for the PAHs in tested cooked chicken products and the daily intake of some carcinogenic PAHs from cooked chicken products :**

Until now , the maximum permissible level (MPL) and the health hazard dietary intake of the PAHs in cooked and processed food are unidentified accurately and varied from country to another. In this concern , Janoszka *et al.* (2004) mentioned that the health hazard level of PAHs daily ingested in diet was found to be 3.7 µg in Great Britain , 5.17 µg in Germany , 1.2 µg in New Zealand and 3 µg in Italy. Also , FAO/WHO (2005) reported that total PAHs level of 14 µg/kg in cooked and processed foods considered to be carcinogenic and mutagenic. In addition , it has been reported that the maximum permissible levels (MPLs) of total PAHs and B[a]Pyr are 10 and 1 µg/kg for cooked or processed foodstuffs ; respectively (FAO/WHO , 2005). While , the MPL of either Acl or Flu is reported to be 2 µg/kg for the former products (Stolyhwo & Sikroski , 2005).

The healthy safe quality of the tested chicken products cooked by either pan-fat frying , butane gas oven grilling or charcoal grilling conventional or microwave grilling was evaluated in relation to food legislation and most considerable identified limitations for total PAHs and their hazardous derivatives in the tested cooked chicken products including ; the carcinogenic level (µg/kg) , health hazard level (µg/day) based on the daily consumption of 200 gm from cooked chicken products and the MPL (µg/kg) for them as illustrate in table (4).

**Table(4) : Food legislations and health safe limitations for the PAHs and the daily intake (D.I) of some deleterious carcinogenic PAHs , based on daily consumption of 200 gm from cooked chicken sample .**

Variables	Total PAHs		B [a] Pyr		Acl		Flu	
	content µg / kg	D.I ( µg / day )	content µg / kg	D.I ( µg / day )	content µg / kg	D.I ( µg / day )	content µg / kg	D.I ( µg / day )
<b>Pan - Fat Fried Chicken Patties :</b>								
Control	5.057	1.0114	0.0345	0.0069	3.1692	0.6338	0.6758	0.1352
Treated (1)	3.287	0.6574	0.0256	0.0051	2.2806	0.4561	0.3215	0.0643
Treated (2)	3.053	0.6106	0.0183	0.0037	1.7359	0.3472	0.4910	0.0982
Treated (3)	1.778	0.3556	0.0107	0.0021	1.0513	0.2103	0.2104	0.0421
<b>Gas Oven - Grilled Chicken :</b>								
Control	7.502	1.5004	0.3298	0.0660	0.9358	0.1872	1.0680	0.2136
Treated (1)	3.835	0.7670	0.1960	0.0392	0.2610	0.0522	0.3942	0.0788
Treated (2)	4.809	0.9618	0.1275	0.0255	0.4276	0.0855	0.6409	0.1282
Treated (3)	3.147	0.6294	0.0990	0.0198	0.1733	0.0347	0.3385	0.0677
<b>Charcoal - Grilled Chicken :</b>								
Control	12.287	2.4574	1.1932	0.2386	2.7790	0.5438	1.5432	0.3086
Treated (1)	7.923	1.5846	0.8049	0.1610	1.9025	0.3805	0.9190	0.1838
Treated (2)	8.558	1.7116	0.5876	0.1175	1.6481	0.3296	1.2957	0.2591
Treated (3)	5.452	1.0904	0.4398	0.0880	0.8457	0.1691	0.6643	0.1329
<b>Carcinogenic level (µg/kg)</b>	<b>14.0</b>		<b>≤ 2</b>		<b>---</b>		<b>---</b>	
<b>Health hazard level (µg/kg)</b>	<b>5.7</b>		<b>&lt; 1</b>		<b>&lt; 2</b>		<b>&lt; 2</b>	
<b>MPL (µg/kg)</b>	<b>10</b>		<b>1</b>		<b>2</b>		<b>2</b>	

**Control:** The cooked sample without any food additives treatment. **T1:** Cooked sample treated with spices mixture prior to cooking process. **T2:** Cooked sample treated with fresh garlic paste prior to cooking process. **T3:** Cooked sample treated with both spices mixture and fresh garlic paste prior to cooking process. **MPL:** Maximum permissible level (µg/kg).

As shown from the obtained data (Table 4) , the cooked chicken products by charcoal grilling method were having , somewhat , a poorly health safe quality and the ingestion of them are possible causing hazards and deleterious effects on consumer's health as they as contain a considerable high

concentration (12.29  $\mu\text{g}/\text{kg}$ ) of total polycyclic aromatic hydrocarbons (PAHs) which was nearly, somewhat, to the reported carcinogenic level (14  $\mu\text{g}/\text{kg}$ ) and as they contain B[a]Pyr, Acl and Flu at an exceptional levels of 1.19, 2.72 and 1.54  $\mu\text{g}/\text{kg}$ , which were exceeded or nearly to the reported health hazard level (<1  $\mu\text{g}/\text{kg}$  for B[a]Pyr and < 2  $\mu\text{g}/\text{kg}$  for Acl & Flu). While, chicken products cooked by either pan-fat frying or butane gas oven grilling had a good health safe quality, especially when treated with tested spices mixture and fresh garlic paste prior to cooking process, as they are effective in inhibiting the PAHs formation in foodstuffs throughout cooking.

### **Conclusion and Recommendation**

The present results recommended that further investigations should be carried out to find the optimum treatments prior to cooking and the best cooking conditions that decrease and inhibit the formation of the PAHs in food products throughout cooking; especially charcoal grilling, as the accumulation nature of these compounds in human and mammalian tissues. Furthermore, it should be avoided to cook chicken and the other meat products by charcoal grilling method as it encourages the formation of PAHs and their deleterious carcinogenic derivatives at a higher level which cause health hazard and cancer diseases, unless they are treated by food additives. In addition, it should be tried to find the best pre-treatments that prevent or inhibit the PAHs formation throughout processing and cooking; such as spices mixture and fresh garlic paste treatments.

### **References**

- Anderson, K.E.; Sinha, R.; Kulldorff, M.; Gross, M.; Lang, N.P.; Barber, C.; Harnack, L.; Di-Magno, E.; Bliss, R. and Kadlubar, F.F. (2002). Meat intake and cooking techniques: Associations with pancreatic cancer. *Mutation Research*, 506/507: 225-231.
- Barth, J. A. C.; Steidle, D.; Kuntz, D.; Gocht, T.; Mouvet, C. and Von-Tuempling, W. (2007). Deposition, persistence and turnover of pollutants: First results from the EU project Aquaterra for selected river basins and aquifers. *Science of the Total Environment*, 376(1–3): 40–50.
- Chonchillo, A.; Ansorena, D. and Astiasaran, I. (2005). Use of microwave in chicken breast and application of different storage conditions: consequences on oxidation. *Eur. Food Res. Technol.*, 221: 592 – 596.
- Chung-Yih, K.; Hong-Shen, L., and Jeang-Hung, L. (2006). Emission of polycyclic aromatic hydrocarbons and lead during Chinese midautumn festival. *Science of the Total Environment*, 366: 233–241.

- Djinovic , J.; Popovic , A. and Jira , W. (2008) . Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia . In press in Meat Science . Available online at : [www.sciencedirect.com](http://www.sciencedirect.com)
- Du-Four , V. A. ; Janssen , C. R. ; Brits , E. and Van Larebeke , N. (2005). Genotoxic and mutagenic activity of environmental air samples from different rural, urban and industrial sites in Flanders, Belgium. Mutation Research, 588(2), 106–117.
- EC . (2005a)European Commission . Commission Recommendation 2005/108/ EC of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Official Journal of the European Union, L34, 43–45.
- EC . (2005b). European Commission . Commission Regulation No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons. Official Journal of the European Union, L34, 3–5.
- El-Badry , N.; Abul-Fadl, M.; Sharaf, A.M. and El-Saied, M.H. (2006). Polycyclic aromatic hydrocarbons formation in cooked tilapia fish and beef fingers as affected by cooking methods and some food additives. J. Agric. Sci. Mansoura Univ., 31(8): 5601-5614.
- Elhassaneen , Y.A. (2004) . The effects of charcoal-broiled meat consumption on antioxidant defense system of erythrocytes and antioxidant vitamins in plasma . Nutrition Research , 24: 435 – 446.
- FAO/WHO (2005). Report of the Joint FAO/WHO Food Standard Programmed, Codex Committee on Food Additives and Contaminants, 37<sup>th</sup> Session The Hague, the Netherlands, 25-28 April.
- Felton , J.S. and Knize , M.G. (1991). Occurrence, identification and bacterial mutagenicity of heterocyclic amines in cooked food. Mutat.Res., 259: 205-217.
- Fernandes , M. B. ; Sicre , M. A. ; Broyelle , I. ; Lorre , A. and Pont, D. (1999). Contamination by polycyclic aromatic hydrocarbons (PAHs) in French and European rivers. Hydrobiologia, 410: 343–348.
- Fismes , J.; Schwartz , C.; Perrin-Ganier , C.; Morel , J. L.; Charissou , A. M. and Jourdain , M. J. (2004). Risk of contamination for edible vegetables growing on soils polluted by polycyclic aromatic hydrocarbons. Polycyclic Aromatic Compounds, 24(4–5): 827–836.
- Helwing , J.T. (1983) . SAS introductory guide. Revised Edition SAS institute INC. Cary, North Coloina, USA 27511 , p. 55 , 61.
- Howard , J.W. and Fazio,T. (1980). Analytical methodology and reported finding of polycyclic aromatic hydrocarbons in foods, J. Assoc. of Anal. Chem., 63:1077-1104.

- Howsam , M. ; Jones , K. C. and Ineson , P. (2000). PAHs associated with the leaves of three deciduous tree species. I – Concentrations and profiles. *Environmental Pollution*, 108(3): 413–424.
- Janoszka , B.;Warzecha , L.;Blaszyk,U. and Bodzek , D. (2004). Organic compounds formed in the thermally treated high-protein food Part 1: Polycyclic aromatic hydrocarbons. *ACTA Chromatographica*. No. 14, pp. 115-128.
- Kazerouni , N.; Shiha , R.; Hsu , C.H.;Greenberg , A. and Rothman , N.(2001). Analysis of 200 items for benzo(a)pyrene and estimation of its intake in an epidemiologic study. *Food and Chemical Toxicology*, 39: 423-436.
- Kuo , C.Y.;Lee,H.S. and Lai,J.H. (2006). Emission of polycyclic aromatic hydrocarbons and lead during Chinese mid autumn festival. *Science of The Total Environment*, 366(1): 233-241.
- Lal , B. and Khanna , S. (1996). Degradation of crude oil by acinetobacter calcoaceticus and alcaligenes odorans. *J. Appl. Bacterol.*, 81: 355-362.
- Lauenstein , G. G. and Kimbrough, K. L. (2007). Chemical contamination of the Hudson–Raritan Estuary as a result of the attack on the World Trade Center: Analysis of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in mussels and sediment. *Marine Pollution Bulletin*, 54(3): 284–294.
- Lijinsky , W. (1996). The formation and occurrence of polycyclic aromatic hydrocarbons associated with food. *Mutation Research*, 259: 251-261.
- Phillips , D. H. (1999). Polycyclic aromatic hydrocarbons in the diet. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 44(1–2):139–147.
- Rey-Salgueiro , L.; Martinez-Carballo , E.; Garcia-Falcon , M.S. and Simal-Gambara , J. (2008). Effects of a chemical company fire on the occurrence of polycyclic aromatic hydrocarbons in plant foods . *Food Chemistry* , 108: 347 – 353.
- Simko , P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B*, 770: 3–18.
- Simko , P. (2005). Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. *Molecular Nutrition and Food Research*, 49: 637–647.
- Sinha , R. and Rothman , N. (1999). Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer, *Cancer Lett.*, 143: 189-194.
- Snedecor , G.W. and Cochran , W.G. (1986). *Statistical methods* 6<sup>th</sup> Ed. Iowa State Univ. press , Ames. Iowa , USA., p. 23 – 29.

- Steineck , G.; Hagman , U.; Gemardsson , M. and Norell , S.E. (1990) . Vitamin A supplements , fried foods , fat and urothelial . Cancer of casereferent study in Stockholm in 1985 – 1987. International Journal of Cancer , 45: 1006 – 1011.
- Stolyhwo , A. and Sikorski , Z. E. (2005). Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Article. Food Chemistry, 91(2): 303-311.
- Tao , S. ; Jiao , X. C.; Chen , S. H.; Liu , W. X.; Coveney , R. M., Jr. and Zhu , L. Z. (2006). Accumulation and distribution of polycyclic aromatic hydrocarbons in rice (*Oryza sativa*). Environmental Pollution, 140: 406–415.