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Effect of Household Cooking Methods and Some Food Additives on Polycyclic Aromatic Hydrocarbons (PAHs) Formation in Chicken Meat

N. EL Badry

Department of Food Science and Technology,
Faculty of Agriculture, AL-Azhar University, Cairo, Egypt

Abstract: This research was carried out to threw the light on formation of polycyclic aromatic hydrocarbons (PAHs) in the cooked chicken meat as affected by the household common cooking methods (microwave oven, pan-frying after boiling process and household gas grilling as direct and indirect with gas flame). In addition, the effect of some food additives (spices mixture, garlic paste, garlic paste and spices mixture and marinades) on the formation of the total and carcinogenic PAHs in cooked chicken meat was also investigated. The obtained results showed that thermal processing used in cooking caused a highly rise in the total and carcinogenic PAHs content and encouraged the formation of the most determined PAHs derivatives at a considerable concentrations in chicken meat, especially, after direct gas grilling with gas flame used in cooking of the chicken meat. Treatments with either spices mixture or garlic paste or garlic paste and spices mixture and marinades prior to cooking caused a highly noticeable reduction in the total and carcinogenic PAHs level. It was concluded that chicken meat should be treated prior to cooking and thermal processing with food additives treatment that causing inhibition or prevention the formation of the PAHs during cooking and also, cooking of chicken meats by direct gas grilling must be avoided due to it encouraged PAHs formation and causing health hazards.

Key words: Polycyclic aromatic hydrocarbons (PAHs) · Grilled chicken · Carcinogen · Microwave · Food additives · Household cooking methods

INTRODUTION

Chicken is one of the world favorite foods, according to USDA, chicken is the species number one consumed by many people worldwide Chicken can be cooked in many different ways, either by itself or combined with other foods such as grains, vegetables or fruits. It can be used in appetizers, soups, salads, sandwiches and main dishes. Deep-fat frying, grilling, broiling, roasting, baking, stir-frying and braising are the more common cooking methods. Among these, deep fat frying and grilling are probably the most popular, dry-heat cooking methods [1].

Cooking and food processing at high temperatures have been shown to generate various kinds of genotoxic substances or cooking toxicants, including PAHs. Grilling (broiling) meat, fish or other foods with intense heat over a direct flame results in fat dripping on the hot fire and yielding flames containing a number of PAHs [2]. These chemicals adhere to the surface of the food. The more intense the heat, the more PAHs are present [3]. Although PAHs migrate through the food chain into hydrophobic compartments [4-7] and thus, accumulate in lipid components due to their lipophilic nature, but significant amounts of PAHs do not usually accumulate in the meat, milk, or egg of food animals because PAHs are rapidly metabolized in these species [8]. PAHs are present in grilled meat or fish in varied amounts (0-130 ng/g). The concentration of the benzo(a)pyrene [B(a)P] in these foods ranges from 0.2 to 50 ng/g [9]. Grilled meat in general is estimated to contain 10.5 ng/g B (a) P [10]. Some of the highest concentrations of PAHs have been found in food cooked over open flames. For example, in barbecued meat total PAHs were found to be present at concentration up to 164 ppb and B(a)P being present at levels as high as 30 ppb [11].

Corresponding Author: Nasser EL Badry, Department of Food Science and Technology, Faculty of Agriculture, AL-Azhar University, Cairo, Egypt. E-mail address: Nasserelbadry70@yahoo.com

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. They are primarily formed by incomplete combustion or pyrolysis of organic matter and during various industrial processes. PAHs generally occur in complex mixtures which may consist of hundreds of compounds [12]. 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 (genotoxic substances or cooking toxicants [2] which are harmful 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 [13].

Food can be contaminated by environmental PAHs that are present in air, soil or water, by industrial food processing methods (e.g. heating, drying and smoking processes) and by home food preparation and from certain home cooking practices. (e.g. grilling and roasting processes) [14, 15]. High PAH concentrations have been reported in charcoal grilled/barbecued foods (such as fatty meat and meat products grilled under prolonged and severe conditions [16, 17].

Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources of PAH [18, 19]. The risk of exposure to these compounds depends on the kind of diet, eating habits and cooking practices, which often result from regional traditions. Persons, for whom fish, meat, chicken and its products are the main source of organic compounds, may be exposed to polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HAs,) which are formed under household and restaurant cooking methods conditions from compounds naturally occurring in muscle tissues [20,21]. A pathway including condensation of some organic compound with free radical intermediates generated by the Maillard reaction [22].

Phytochemicals derived from plant origin such as fruits, vegetables and herbs are known to contain many different components that can act as antioxidants. Among alliums, garlic (*Allium sativum*), onion (*Allium cepa*) [23-25]. Because they act as free radical scavengers, singlet oxygen quenchers, or metal chelators consumption of plant products possessing antioxidant potential protects from the oxidative damage of reactive oxygen species (ROS) [26]. Organosulfur compounds present in alliums have been considered to be the main factors responsible for its health-promoting and related biological effects, including antimutagenic [27] and antioxidant [28-30] effects. In some recent reports, it has been demonstrated that phenolic compounds undergo oxidative polymerization during food processing or storage that increase chain-breaking activity as well as antioxidant activity [31-33].

The present investigation was carried out to monitor the effect of some commonly home cooking practices of chicken and the treatments with some food additives prior to cooking on the formation of polycyclic aromatic hydrocarbons (PAHs) to try to reach the most preferable treatments and cooking methods which prevent or reduce the total and carcinogenic PAHs formation in the tested chicken.

MATERIALS AND METHODS

Materials:

Chicken Meat Samples: Fattened broilers chicken used in this study were obtained from local market at Nasr city, Cairo, Egypt. Their weight ranged between 1000 - 1200 g and their average age was 8 weeks.

Garlic and Onion: They were obtained from the local market at Nasr city, Cairo, Egypt, whereas, the garlic paste were prepared by grinding the decoated garlic cloves.

Sodium Chloride: It was obtained from the Egyptian Salts and Minerals, Co., Egypt.

Spices: spices (Cumin, Coriander, Black pepper and Rosemary) were obtained from the local market at Nasr city, Cairo, Egypt. The spices were cleaned individually and ground to a fine powder whereas, the spices mixture was prepared by mixing equal weight from the former spices and then mixing again carefully.

Prepared Marinades: Marinades used in seasoning of chicken meat at different commonly household preparation cooking methods conditions in this study were formulated from tomato juice, garlic paste, onion, sodium chloride, some spices (Cumin, Coriander and Black Pepper).

Methods:

Preparation of Chicken Samples: Chicken were slaughtered, mechanically plucked, eviscerated and washed. Whole chicken samples were immersing in 10 % NaCl solution for 15 min and then washed with tap water and divided into five equal batches. Each batch was consisted of four chickens. The first batch was untreated with any food additives treatments and used as a control.

The second batch was treated by seasonings mixture. The third batch was mashed with garlic paste. The forth batch was treated with garlic paste and spices mixture and the fifth batch was treated by commonly household preparation marinades Every one of five equal batches was divided into four portions 'one of them cooked by microwave oven while the second cooked by pan-frying after boiling process. The third batch was cooked by direct flame household gas grilling and forth cooked by indirect flame household gas grilling as applied in most commonly household cooking methods conditions in Egypt. This process repeated three times to give replicate samples.

Cooking Methods: Cooking processes of chicken were carried out using the most commonly used methods in Egypt as follows:

Microwave Cooking: The microwave cooking methods were used for cooking of chicken samples treatments. Microwave oven used for frying chicken samples was UPO Samsung, Model No. A2213 A1A (Republic of Korea); the frequency of radiation emitted in this oven was 50Hz. In this method, prepared whole chicken samples treated by food additives (spices mixture or garlic paste or garlic paste and spices mixture or marinades) for 60 min. then halfed and placed in shallow glass microwave cooking dish (500 ml capacity, 20 cm diameter and 3cm high) Whereas the prepared chicken treatments were fried to reach well done cooking for 20 min (10 min for each side) in microwave oven at full power. Then samples after cooking samples were ground and homogenized by grinder (Oster Heavy Duty Food Grinder, USA) and packed in polyethylene bags and stored under freezing condition at -18±2°C until analyzed [34].

Pan-Frying after Boiling Process: Chicken samples were treated by food additives (Spices mixture or garlic paste or garlic paste and spices mixture or marinades) for 60 min. and boiled in water for 15 min from the beginning of boiling and then former prepared treatments were placed in stainless shallow-pan (1000 ml capacity, 25 cm diameter and 6 cm high) with small portion of frying oil medium and heated, then the prepared chicken samples were fried individually for 10 min (5 min for each side), to the golden color. After pan-frying the samples were exposed to the same behavior of grilled samples previously mentioned.

Gas Grilling Method: The former prepared whole chicken samples were grilled in a household horizontal gas-flame cooker. Gas-fueled grills typically use natural gas (NG) as their fuel source, with gas-flame cooking food directly for 40 min. and indirectly for 50 min. (until well done cooking.) in this case chicken is skewered on rotate rod (called a spit), which is used to hold food when it is being cooked over fire in an oven or in a fireplace The gas-flame cooker was kept closed during cooking and opened only when it was time to turn or remove samples from the grill. After grilling the grilled samples were prepared as previously mentioned until analyzed.

Analytical Methods:

Polycyclic Aromatic Hydrocarbons (PAHs):

Extraction: The PAHs were extracted according to the method described by Howard, *et al.* [35, 36] with slight modification carried out by Egyptian Petroleum Research Institute, Ministry of Scientific Research, Egypt. In brief, each sample was digested in alcohol and potassium hydroxide and then distilled water was added and the hydrocarbons partitioned into iso octane. Interfering materials were removed by column chromatography on florisil (60-100 mesh) followed by selective extraction of the PAH into dimethyl sulfooxide (DMSO). Further interfering materials were removed by column chromatography on Sephadex LH-20, utilizing a solvent mixture of toluene and ethanol at ratio of 1:1 to obtained the purified extracts of the PAH which used for determination of the PAH components.

Determination: The 16 individual PAHs listed by the reference 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) according to the procedure described by Lal and Khanna, [37]. The PAHs identification and system with millennium 3.2 software PAHs standard were obtained from quantification performed using HPLC. The apparatus model used in determination was waters HPLC 600 E, equipped with dual UV absorbance detector water 2487 and auto samplers 717 plus attached to computerized supelco. The condition of separation is as follow:

Column: Supelcosil LC-PAH,5µm particles 15 cm length and 4.6 mm ID.

Mobile Phase: Graient acetonitrile;water 60 to 100% acetonitrile (v/v)over 45 min.

Flow Rate: 0-2 min, 0.2 m/min, 2-45 min.1.0 ml/min.

Detector: It was set at 254 nm.

Statistical Analysis: Data were subjected to the statistical analysis according to Analysis of Variance (ANOVA) of Completely Randomized Design [38].Treatment means were compared using the Least Significant Differences (LSD) at 0.05 level of probability and Standard Error. Computations and statistical analysis of data were done using facilities of computer and statistical analysis system package [39].

RESULTS

Effect of Microwave Cooking and Some Food Additives Treatments on Polycyclic Aromatic Hydrocarbons (PAHs) Formation in Cooked Chicken Meat: Data presented in Table 1 show the influence of microwave cooking and some food additives treatments on the formation of polycyclic aromatic hydrocarbons (PAHs) in cooked chicken meat under household cooking method conditions. It could be observed that total and carcinogenic PAHs (μg/kg) increased by microwave cooking as compared by contents in raw sample, however, total and carcinogenic PAHs content of cooked chicken meat decreased by treatments with food additives under investigation as compared by control samples of cooked chicken meat. Total PAHs content of chicken meat was 0.2090, 43.5610, 11.544, 13.3860, 4.3 030 and 3.8830 μg/kg in raw chicken meat, chicken meat treated by spices mixture, garlic paste, (garlic paste and spices mixture) and marinades, respectively while carcinogenic PAHs was 0.1441, 17.626, 7.769, 4.204, 2.034 and 1.938(μg/kg), respectively.

Concerning the Effect of food additives treatments on total and carcinogenic PAHs formation in chicken meat prior to cooking in microwave oven, it could be noticed that chicken meat treated with garlic paste showed considerably reduced total and carcinogenic PAHs formation and the reduction become more obvious in samples treated by spices mixture followed by mixture of garlic and spices together and marinades.

Effect of Pan-Frying Cooking and Some Food Additives Treatments on Polycyclic Aromatic Hydrocarbons (PAHs) Formation in Cooked Chicken Meat: The effect of pan-frying cooking and some food additives treatments on the formation of total and carcinogenic PAHs in chicken meat is shown in Table 2; it could be observed that chicken samples treated by food additives (Spices mixture or garlic paste or garlic paste and spices mixture or marinades) had considerably reduced total and carcinogenic PAHs values which ranged from 59 - 92 and 31 - 90 % respectively, as compared to the control sample (untreated with any additives). The increment effect of treatment with spices mixture or garlic paste or garlic paste and spices mixture or marinades resulted in lowing level of total and carcinogenic PAHs in chicken meat. Total and carcinogenic PAHs content of chicken meat cooked by pan-frying cooking was 0.2090 and 0.144; 62.4063 and 21.4163; 14.6367 and 8.965 ; 14.6367 and 8.965; 25.3410 and 5.2090 and 4.7653 and 1.9933 (μg/kg) in raw chicken meat, chicken meat treated by spices mixture, garlic paste, garlic paste and spices mixture and commonly marinades respectively.

Effect of Direct and Indirect Gas-Flame Cooking Methods and Some Food Additives Treatments on Polycyclic Aromatic Hydrocarbons (PAHs) Formation in Cooked Chicken Meat: The effect of direct and indirect gas-flame cooking process and some food additives treatments before grilled chicken meat on the formation of total and carcinogenic PAHs in chicken meat as shown in Tables 3 and 4; directly and indirectly gas-flame cooking method (until well done cooking.) arise considerably amounts of total and carcinogenic PAHs in control sample (untreated with any additives) 71.7950, 25.4070 and 63.9380, 28.2610 μg/kg for directly and indirectly gas-flame cooking method, respectively, as compared to 0.2090, 0.1440 μg/kg of raw sample as affected by cooking methods. Meanwhile, grilled chicken samples treated with food additives treatments before direct and indirect gas-flame cooking methods caused, in general, a highly inhibition of the total and carcinogenic PAHs forming in grilled chicken samples at variable rates depending upon direct or indirect gas-flame cooking methods. These treatments caused a highly considerable reduction in the total and carcinogenic PAHs formation at level of 15.4600, 8.211 μg/kg for samples treated by spices mixture, 29.1260, 15.4690 μg/kg for samples treated by garlic paste, 8.7860, 4.6230 μg/kg for samples treated by spices mixture and garlic paste and 6.9790, 3.966 μg/kg for samples treated by marinades and grilled by direct gas-flame cooking methods and 10.4600, 5.7650 μg/kg for samples treated by spices mixture; 23.6900, 15.0590 μg/kg for samples treated by garlic paste; 5.3340, 3.9120 μg/kg for samples treated by spices mixture and garlic paste and 3.1930, 2.2830 μg/kg for samples treated by marinades and grilled by indirect gas-flame cooking methods.

Table 1: The polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat grilled by microwave oven under commonly household cooking methods conditions as affected by some food additives

	PAHs components level (µg/kg)					
	Grilled chicken by microwave oven					
Pretreatment						
PAHs Components	Raw chicken	Control (without additives)	Spices	Garlic	Spices + Garlic	Marinades
Naphthalene	ND	2.3290±0.0104		4.5660±0.0254	ND	0.6350±0.00577
Acenaphthylene	ND	1.5140±0.0087	0.1780±0.0158	ND	ND	ND
Acenaphthene	ND	0.0150±0.0023	0.2770±0.0648	ND	1.0730±0.0127	ND
Fluorene	0.0394±0.00139	0.0310±0.0017	ND	4.6160±0.0208	ND	ND
Phenanthrene	0.0254±0.0014	0.0070±0.0006	ND	ND	ND	0.0830±0.0052
Anthracene	ND	0.3090±0.0006	2.7710±0.0052	ND	0.7900±0.0855	0.8630±0.00808
Fluoranthene	ND	0.0720±0.0040	ND	ND	ND	ND
Pyrene	ND	0.1020±0.0017	0.5490±0.0169	ND	0.4060±0.0046	0.4640±0.00751
Benzo (a) anthracene *	ND	0.0630±0.0046	0.0630±0.0027	0.1610±0.0040	1.4110±0.0058	0.0540±0.00289
Chrysene**	0.0289±0.0017	11.1650±0.0058	0.5340±0.0052	ND	ND	1.1000±0.00012
Benzo (b) fluoranthene**	0.0570±0.00061	3.3500±0.0346	ND	ND	ND	ND
Benzo (k) fluoranthene**	ND	3.0050±0.0006	0.0080±0.00068	1.0720±0.01212	0.6230±0.0069	0.7840±0.01328
Benzo (a) pyrene*	ND	0.0260±0.0029	ND	ND	ND	ND
Dibenzo (a,h) nthracene*	0.0582±0.0013	0.0170±0.0017	0.0250±0.00058	ND	ND	ND
Benzo (g,h,i) pyrene	ND	22.5560±0.0058		ND	ND	ND
Indeno (1,2,3- cd) yrene**	ND	ND	7.1390±0.00808	2.9710±0.02483	ND	ND
Total PAHs	0.2090^{F}	43.5610^{A}	11.544^{C}	13.3860^{B}	4.3030^{D}	3.8830^{E}
Total carcinogenic PAHs	0.1441^{F}	17.626^{A}	7.769^{B}	4.204^{C}	2.034^{D}	1.938^{E}

(SE): Standard error. (ND): Not detectable. (*) : IARC Group 2a: Probably carcinogenic to humans according to the [40] (**): IARC Group 2b: Possibly carcinogenic to humans according to the [40]. (*and **): Classified as carcinogenic to humans by US EPA [41] and WHO/IPCS [42] and Larsen and Larsen [43]

Table 2: The polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat grilled by pan -frying under commonly household cooking methods conditions as affected by some food additives

	PAHs components level (µg/kg)					
	Grilled chicken by household pan -frying					
Pretreatment						
PAHs Components	Raw chicken	Control (without additives)	Spices	Garlic	Spices + Garlic	Marinades
Naphthalene	ND	20.8957± 0.67279	ND	0.425±0.0025	1.9530±0.00346	1.5820±0.005
Acenaphthylene	ND	ND	ND	ND	ND	1.3733±0.00567
Acenaphthene	ND	10.0140±±0.00351	0.0100±0.00057	ND	ND	ND
Fluorene	0.0393±0.00145	12.0897±0.00717	0.0210±0.00577	ND	0.6650±0.00231	ND
Phenanthrene	0.0253±0.00145	8.0180±0.00208	0.0050±0.00057	0.031±0.0025	ND	0.0923±0.00567
Anthracene	ND	ND	0.0120±0.00115	9.899±0.8965	1.7390±0.00577	0.8290±0.004
Fluoranthene	ND	2.2663±0.00801	0.0077±0.00033	ND	ND	0.1053±0.00267
Pyrene	ND	ND	0.1070±0.0052	ND	0.6710±0.00577	0.1633±0.00167
Benzo (a) anthracene *	ND	ND	0.726± 0.0023	2.651±0.5630	0.1810±0.00173	0.1180± 0.004
Chrysene**	0.029±0.0017	5.2857±0.04884	1.0630± 0.0052	3.5264±0.256	ND	ND
Benzo (b) fluoranthene**	0.0570±0.0057	ND		2.2301±0.7456	ND	ND
Benzo (k) fluoranthene**	ND	ND	5.0020 ±0.0057	1.2307±0.278	ND	0.5020± 0.002
Benzo (a) pyrene*	ND	3.8373±0.01217	0.1780±0.00981	1.156±0.0369	ND	ND
Dibenzo (a,h) nthracene*	0.0580±0.0011	12.123±0.9120	1.9960±0.01674	2.2954± 0.3691	1.9530±0.00346	ND
Benzo (g,h,i) pyrene	ND	ND	5.5090± 0.0103	ND	ND	ND
Indeno (1,2,3- cd) yrene**	ND	5.456±0.7580	ND	1.8954±0.08965	ND	1.3733±0.00567
Total PAHs	0.2090^{E}	62.4063^{A}	14.6367^{C}	25.3410^{B}	5.2090^{D}	4.7653^{D}
Total carcinogenic PAHs	0.144^{F}	21.4163^{A}	8.965^{C}	14.6822^{B}	2.134^{D}	1.9933^{E}

(SE): Standard error. (ND): Not detectable. (*) : IARC Group 2a: Probably carcinogenic to humans according to IARC [40] (**): IARC Group 2b: Possibly carcinogenic to humans according IARC [40]. (*and **): Classified as carcinogenic to humans by US EPA [41] and WHO/IPCS [42] and Larsen and Larsen [43]

Table 3: The polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat grilled by direct flame butane gas-grilling under commonly household cooking methods conditions as affected by some food additives

	PAHs components level (µg/kg)					
	Grilled chicken by direct flame household butane gas-grilling					
Pretreatment						
PAHs Components	Raw chicken	Control (without additives)	Spices	Garlic	Spices + Garlic	Marinades
Naphthalene	ND	ND	0.0970±0.01097	0.3110±0.0052	0.0500±0.00289	ND
Acenaphthylene	ND	ND	6.0750±0.00635	ND	1.2740±0.01155	1.5600±0.0052
Acenaphthene	ND	0.0070±0.00058	0.0060±0.00058	ND	2.8390±0.01386	1.0020±0.00058
Fluorene	0.0393±0.00145	ND	ND	5.0490±0.00635	ND	ND
Phenanthrene	0.0253±0.00145	1.0110±0.00577	0.0670±0.00693	0.0190±0.00404	ND	0.1110±0.0052
Anthracene	ND	ND	0.5620±0.00577	7.5440±0.00462	ND	0.1460±0.00462
Fluoranthene	ND	43.1310±0.01097	0.4420±0.00173	0.7340±0.00346	ND	0.0370±0.00404
Pyrene	ND	0.9320±0.02598	ND	ND	ND	0.1570±0.00404
Benzo (a) anthracene *	ND	ND	3.8400±0.0052	10.1260±0.0052	3.610±0.0513	ND
Chrysene**	0.0290±0.00173	1.2950±0.0127	ND	ND	ND	ND
Benzo (b) fluoranthene**	0.0570±0.00058	0.0050±0.00058	ND	ND	ND	3.0100±0.00058
Benzo (k) fluoranthene**	ND	20.1000±0.00635	ND	ND	ND	ND
Benzo (a) pyrene*	ND	5.3020±0.01386	2.2500±0.03464	3.2300±0.025	1.0130±0.0015	0.9560±0.0052
Dibenzo (a,h) nthracene*	0.0580±0.00115	ND	ND	2.1130± 0.0089	ND	ND
Benzo (g,h,i) pyrene	ND	0.0120±0.00115	ND	ND	ND	ND
Indeno (1,2,3- cd) yrene**	ND	ND	2.1210±0.01155	ND	ND	ND
Total PAHs	0.2090^{F}	71.7950^{A}	15.4600^{C}	29.1260^{B}	8.7860^{D}	6.9790^{E}
Total carcinogenic PAHs	0.1440^{F}	25.4070^{A}	8.211^{C}	15.4690^{B}	4.6230^{D}	3.9660^{E}

(SE): Standard error. (ND): Not detectable. (*): IARC Group 2a: Probably carcinogenic to humans according to IARC [40] (**): IARC Group 2b: Possibly carcinogenic to humans according to IARC [40]. (*and **): Classified as carcinogenic to humans by US EPA [41] and WHO/IPCS [42] and Larsen and Larsen [43]

Table 4: The polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat grilled by indirect flame butane gas-grilling under commonly household cooking methods conditions as affected by some food additives

	PAHs components level (µg/kg)					
	Grilled chicken by indirect flame household butane gas-grilling					
Pretreatment						
PAHs Components	Raw chicken	Control (without additives)	Spices	Garlic	Spices + Garlic	Marinades
Naphthalene	ND	7.9320±0.00693	ND	ND	0.0131±0.00167	ND
Acenaphthylene	ND	2.0120±0.00635	0.5620±0.00346	0.5440±0.01905	ND	ND
Acenaphthene	ND	16.1310±0.00346	ND	ND	ND	0.2450±0.00981
Fluorene	0.0393±0.00145	ND	ND	5.7340±0.0052	0.9840±0.00404	0.1460±0.00693
Phenanthrene	0.0253±0.00145	4.3020±0.00981	1.1210±0.0052	ND	ND	ND
Anthracene	ND	0.0050±0.00058	ND	ND	ND	ND
Fluoranthene	ND	5.2950±0.00635	2.8400±0.00462	1.1190±0.01097	0.4250±0.00404	0.3380±0.01097
Pyrene	ND	ND	0.0970±0.00462	ND	ND	ND
Benzo (a) anthracene *	ND	ND	0.4420±0.00289	0.3110±0.01328	ND	1.0100±0.01328
Chrysene**	0.0290±0.00173	6.0070±0.00577	2.0670±0.00462	15.7480±0.03464	1.4410±0.00981	0.5600±0.02598
Benzo (b) fluoranthene**	0.0570±0.00058	7.1000±0.0052	ND	ND	ND	ND
Benzo (k) fluoranthene**	ND	6.1250±0.01386	1.2500±0.0052	ND	ND	0.1570±0.01039
Benzo (a) pyrene*	ND	1.0110±0.00577	ND	0.3110±0.01328	0.0500±0.0002	ND
Dibenzo (a,h) nthracene*	0.0580±0.00115	7.0070±0.00346	ND	ND	ND	0.5560±0.00981
Benzo (g,h,i) pyrene	ND	ND	0.0750±0.00808	0.2340±0.00346	ND	1.1210±0.01155
Indeno (1,2,3- cd) yrene**	ND	1.0110±0.00924	2.0060±0.00058	ND	2.421±0.0052	ND
Total PAHs	0.2090^{F}	63.9380^{A}	10.4600^{C}	23.6900^{B}	5.3340^{D}	3.1930^{E}
Total carcinogenic PAHs	0.1440^{F}	28.2610^{A}	5.7650^{C}	15.0590^{B}	3.9120^{D}	2.2830^{E}

(SE): Standard error. (ND): Not detectable. (*): IARC Group 2a: Probably carcinogenic to humans according to IARC [40] ( ).*IARC Group 2b: Possibly carcinogenic to humans according to IARC [40]. (*and **): Classified as carcinogenic to humans by US EPA [41] and WHO/IPCS [42] and Larsen and Larsen [43]

Table 5: The limitation of total, carcinogenic PAHs and benzo[a]pyrene based on consumption of 250 gm from the grilled chicken meat as affected by commonly household preparation cooking methods and some food additives

	Total PAHs		Total carcinogenic PAHs		Benzo[a]pyrene	
Pretreatment	Content (µ/kg)	Daily intake* (µ/kg)	Content (µ/kg)	Daily intake* (µ/kg)	Content (µ/kg)	Dailyintake* (µ/kg)
Microwave oven						
Raw uncooked chicken	0.2090	0.0523	0.1441	0.0360		
Without any additives	43.5610	10.8903	17.6260	4.4065	0.0260	0.0065
With spices treatment	11.5440	2.8860	7.7690	1.9423	-	-
With garlic treatment	13.3860	3.3465	4.2040	1.0510	-	-
With Spices and Garlic	4.3030	1.0758	2.0340	0.5085	-	-
With marinade	3.1930	0.7983	1.9380	0.4845	-	-
Pan - frying						
Without any additives	62.4063	15.6016	21.4163	5.3541	3.8373	0.9593
With spices treatment	14.6367	3.6592	8.9650	2.2413	0.1780	0.0445
With garlic treatment	25.3410	6.3353	14.6822	3.6706	1.1560	0.2890
With Spices and Garlic	5.2090	1.3023	2.1340	0.5335	-	-
With marinade	4.7653	1.1913	1.9933	0.4983	-	-
Direct - flame butane gas						
Without any additives	71.7950	17.9488	25.4070	6.3518	5.3020	1.3255
With spices treatment	15.4600	3.8650	8.2110	2.0528	2.2500	0.5625
With garlic treatment	29.1260	7.2815	15.4690	3.8673	3.2300	0.8075
With Spices and Garlic	8.7860	2.1965	4.6230	1.1558	1.0130	0.2533
With marinade	6.9790	1.7448	3.9660	0.9915	0.9560	0.2390
Indirect - flame butane gas						
Without any additives	63.9380	15.9845	28.2610	7.0653	1.0110	0.2528
With spices treatment	10.4600	2.6150	5.7650	1.4413	-	-
With garlic treatment	23.6900	5.9225	15.0590	3.7648	0.3110	0.0778
With Spices and Garlic	5.3340	1.3335	3.9120	0.9780	0.0500	0.0125
With marinade	3.1930	0.7983	2.2830	0.5708	-	-
Carcinogenic level (µ/kg)	14.0000	-	= 2			
Health hazard (µ/day)	5.7000	-	< 1			
Maximum permissible level (µ/kg)	10.0000	-	1			

* Daily intake (µg) based on consumption of 250gm of product per day

The highest reduction was accrued in samples treated with marinade followed by spices mixture and garlic paste, spices mixture and garlic paste for direct gas-flame cooking methods. Also the same trained was found in samples cooked by indirect gas-flame cooking methods in addition samples cooked by direct gas-flame cooking methods was contained a higher level than that total and carcinogenic PAHs found in indirect gas-flame cooking methods (Tables 3 and 4).

The Healthy Safe Limitations and the Daily Intake of total, carcinogenic polycyclic aromatic hydrocarbons and benzo[a]pyrene based on consumption of 250 g from the tested chicken meat samples as affected by household cooking methods and some food additives.

Data in Table 5 showed that, the healthy safe quality of the tested cooked chicken products under household cooking methods conditions was evaluated in relation to the most considerable limitation of total and carcinogenic PAHs and BaP based on possible daily intake of these items and some of their derivatives with consumption of 250 g of the tested cooked chicken products. The control (untreated with any additives.) cooked chicken products under commonly household cooking methods conditions contained a higher concentration of total polycyclic aromatic hydrocarbons (PAHs) than the Carcinogenic level (14.0 µg/kg), [44]. Maximum permissible level (10.0 µg/kg) as reported by FAO/WHO [44] and Stolyhwo and Sikorski [45] and Health hazard (5.7 µg/day as reported by Janoszka *et al.* [13] in all tested samples except chicken meat cooked by microwave oven was higher than Maximum permissible level (10.0 µg/kg) and health hazard (5.7 µg/day) on contrast control samples was contained a lower concentration of BaP than the Maximum permissible level (1 µg/kg) as reported by FAO/WHO [44] and Stolyhwo and Sikorski [45] in all tested samples except chicken meat cooked by direct flame butane gas-grilling (1.3255 µg/kg), in addition all tested samples pre-treatments was contained total PAHs and BaP at a safe limit (lower than health hazard) except samples pre-treated with garlic and cooked by pan- frying cooking method, direct and indirect flame butane gas-grilling which contained 6.3353, 7.2815 and 5.9225 µg/kg respectively.

DISCUSSION

The mean concentrations of 16 PAH compounds were determined in the raw and cooked chicken meat by microwave cooking, pan-frying after boiling process and gas grilling as direct or indirect with heat source) and treated with some food additives (spices mixture, garlic paste, garlic paste and spices mixture and marinades used in house hold preparation) prior to cooking including; naphthalene (NA), acenaphthylene (ACL), acenaphthene (AC), fluorine (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), bonzo(k) fluoranthene (BkFA), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DbahA), benzo(g,h,i)pyrene (BghiP) and indeno(1,2,3-cd)pyrene (IP). Also total carcinogenic PAHs were calculated according to the classification of IARC [40,46] which classified the Benzo(a)anthracene (BaA), Benzo(a)pyrene (BaP) and Dibenzo(a,h) anthracene (DBahA) as Group 2a Probably carcinogenic to humans and benzo(b)fluoranthene (BbFA), bonzo(k) fluoranthene(BkFA) and indeno(1,2,3-cd)pyrene (IP) as Group 2b Possibly carcinogenic to humans, while previously components additionally to chrysene (CHR) Classified as carcinogenic to humans [41-43].

The 16 individual PAHs, in raw and cooked chicken samples by microwave cooking, pan-frying, direct gas grilling and indirect gas grilling) and the treated with some food additives (spices mixture, garlic paste, garlic paste and spices mixture and marinades used in house hold preparation) prior to cooking are shown in Tables (1, 2, 3 and 4).

The current results revealed that chicken meat cooked by microwave oven appear that IP only no detected in control samples and was contained the highest amount of total and carcinogenic PAHs compared with samples treated by food additives under investigation and the same trained also found in samples cooked by pan-frying and direct or indirect gas grilling cooking methods.

The [47] reported that cooking processes, especially the high temperature ones (e.g. grilling, baking, etc.) are known to induce the production of potential carcinogens. There have been concerns that microwave cooking may also increase the production of carcinogens or mutagens in foods. Also EC [48] revealed that food processing or cooking steps such as roasting, grilling, barbecuing and smoking generate PAHs and increase the level of PAHs in the food being cooked. Tested samples cooked by microwave oven and treated by food additives under investigation reduced total PAHs and total carcinogenic PAHs formation at range 69 - 92 and 55 - 89 %, 59 - 92 and 31 - 90 % for samples cooked by pan-frying, 59 - 90 and 39 - 84 % for samples cooked by direct gas grilling and 62 - 95 and 46 - 91 % for samples cooked by indirect gas grilling. In addition the most frequently components of sixteen detected PAHs were BaA and BkFA in samples cooked by microwave oven, PHE and DBahA in samples cooked by pan-frying, PHE and BaP in samples cooked by direct gas grilling and FA and CHR in samples cooked by indirect gas grilling. The inhibition effect of pre-treatment on the formation of the PAHs and carcinogenic PAHs in cooked chicken may be attributed to the naturally occurrence antioxidant in garlic, spices and in content of marinades which are prevented or inhibited the oxidation and polymerization of hydrocarbons resulted from in the complete composition and pyrolysis.

The antiradical potential of phenolic compounds is due to their ability to donate hydrogen, which closely depends on the number of hydroxyl groups, the substitution pattern of hydroxyl groups and the chemical structure of the compound. Natural alliums contain a number of polyphenolic compounds, in addition to various organosulfur compounds, most of which are known to possess antioxidant and antiradical properties. These compounds are partially destroyed during heat processing, which, however, induces the formation of new compounds such as caramels and melanoidins. Melanoidins consist of brown compounds that can be detected in biological material and, in particular, in heat-treated foods, whereas they are the main end products of the Maillard reaction [49-51].

Until now, the maximum permissible level and the health hazard dietary intake of the PAHs in cooked and processed food are indefinite accurately and varied from country to another. In this concern, it was reported [13] that the health hazard level of the PAHs daily ingested in the diet was found to be 3.7 μg in great Britain, 5.17 μg in Germany, 1.2 μg in New Zealand and 3μg in Italy. Also, it was recorded that total PAHs level of 14μg/kg in cooked and processed foods considered is carcinogenic and mutagenic.

In relation to the carcinogenic PAHs predominated in cooked chicken meat under commonly household cooking methods conditions, were BaP. In addition, the BaP compound had been reported as a good general indicator of the formation level of total PAHs in cooked and thermal processed fish and meat products and that compound represented 1-20% of total PAHs in the former

thermal-treated product [52-55]. It has been reported that the maximum permissible levels (MPLs) of total PAHs and BaP are 10 and 1 μg/kg wet cooked or processed meat and fishery products respectively [44, 45].

The healthy safe quality of the tested grilled chicken products under commonly household cooking methods conditions was evaluated in relation to the most considerable limitation of total and carcinogenic PAHs and BaP based on possible daily intake of these items and some their derivatives with consumption of 250 g of the tested grilled chicken products.

As illustrated in Table 5; when the carcinogenic, health hazard and maximum permissible levels of total and carcinogenic PAHs and BaP were taken in our consideration, the cooked chicken samples should be treated prior to cooking process with seasonings mixture, garlic paste, garlic paste and spices mixture and marinades or with any treatment inhibit the formation of these compounds, to reduce the formation of the PAHs in cooked chicken below carcinogenic level or the healthy hazard level. Also, it should be avoided the cooking of chicken meat by cooking method as it encourages the formation of PAHs and their derivatives at a higher levels which causing health hazard and cancer diseases and should be tried to find the best pre-treatments that prevent or inhibit the PAHs formation throughout thermal processing and cooking.

In general terms, in control samples of cooking methods under investigation the highest concentrations were found after cooking by direct flame gas grilling followed by indirect flame gas grilling and samples cooked with pan- frying method while the lowest was found in samples cooked by microwave oven as compared with samples treated prior cooking. These values appear the effecting of household cooking methods conditions on PAHs formation in cooked chicken meat.

Charred food of almost any composition contains PAHs [19] while only very low level of PAHs was detected when food was cooked by some cooking steps such as steaming and microwave cooking. A study found that significant amount of PAHs was formed when beef cooked in corn oil by conventional frying and reheating whereas negligible amount was formed when cooked by microwave cooking and reheating [56]. As well as shows that there was a significant difference ($p < 0.05$) between control samples and others treated with food additives (spices mixture or garlic paste or garlic paste and spices mixture or marinades) in all tested household cooking methods. These findings are in agreement with those has mentioned by Phillips [19] and Farhadian *et al.* [57]. Normal roasting or frying food does not produce copious quantities of PAHs some of the highest levels of PAHs reported in foods have been detected in food cooked over open flames. For example, in barbecued meat total PAHs were found to be present at levels up to 164 ppb with Benzo (a) pyrene being present at levels as high as 30 ppb and also Chen and Lin [58] in their study found that for total PAHs, smoking contained the highest amount (154 ng/g), followed by charcoal grilling (151 ng/g), roasting (88.5 ng/g) and steaming (4.4 ng/g). Charred food of almost any composition will contain PAHs; however, normal roasting or frying food does not produce copious quantities of PAHs [59]. Some of the highest levels of PAHs reported in foods have been detected in food cooked over open flames. For example, in barbecued meat total PAHs were found to be present at levels up to 164 ppb [11], with benzo *a* pyrene being present at levels as high as 30 ppb.

From data in Tables (1, 2, 3 and 4), it was noticed that treatments of chicken meat prior to cooking with food additives under investigation, considerably reduced total and carcinogenic PAHs formation when cooked by microwave cooking or pan-frying after boiling process or gas grilling as direct or indirect with heat source compared with control samples. In all cooking methods samples treated with marinades showed to be the lowest content in total and carcinogenic PAHs followed by samples treated by garlic paste and spices mixture, spices mixture and the highest content of total and carcinogenic was formed in sample treated by garlic paste as affected by addition of food additives prior cooking process. There was a significant difference among all pre-cooked tested samples in microwave oven, pan-frying and direct or indirect gas grilling cooking methods.

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