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ORIGINAL ARTICLE

Effect of Different Grilling Methods on Polycyclic Aromatic Hydrocarbons Content in Grilled Broiler Chicken

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Abstract
Grilled chicken became widespread desired dish for wide section of consumers, however, they may act as a probable source of some chemical carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) and other harmful chemicals. The content of these materials may differ according to cooking methods, temperature and grilling duration. The main aim of the present study was to evaluate the influence exerted by different grilling methods that usually practiced by consumers on the content and profile of polycyclic aromatic hydrocarbons in experimentally marinated then grilled broiler chicken. Thirty samples were marinated and grilled by charcoal, gas and electric grill (Ten samples each), the samples were extracted using magnesium sulfate and sodium acetate in an organic solvent, then purified by primary secondary amine, magnesium sulfate and endcapped octadecylsilane silica gel particles and finally measured by gas chromatography-mass spectrometry(GC-MS). Benzo[a]pyrene, Sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene (PAH4), the genotoxic PAHs (PAH8) and total PAHs (ΣPAHs) recorded the highest average levels (1.38, 3.09, 3.09 and 36µg/kg) respectively, in charcoal grilled chicken samples, while lowest mean values were detected in electric machine grilled chicken samples (0, 0.44, 0.55 and 26.36µg/kg), respectively. Also, benzo[a]pyrene exceeded the permissible limits in 40% of charcoal grilled chicken samples. From the obtained results of the current study, electric grilling added less content of benzo[a]pyrene, PAH4, PAH8, non-carcinogenic PAHs and total PAHs, while samples grilled using charcoal grill showed the highest contamination with these compounds in terms of the previous parameters.

Keywords
Broiler chicken, Charcoal grilling, Electric grilling, Gas grilling, GC-MS, PAHs

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are complex blend with countless number of congeners that have multiple united rings of benzene, with extensive presence, long-lasting persistence, long range transportation potential, and carcinogenic potential **(Dennis, 2007)**. They are formed by the inadequate ignition of organic substance, they arise when coal is burned, and are also existing unintentionally in food **(Phillips, 1999)**. Also food preparation at extreme temperatures produce several types of harmful materials that are responsible for human genotoxicity and carcinogenicity, comprising polycyclic aromatic hydrocarbons **(Jägerstad and Skog, 2005)**. Possible mechanisms of their formation in grilled foods may include heat decomposition of organic substances like fats, carbohydrate and protein at heating levels exceeding 200^0C, in addition, dripping of lipids on very hot surfaces, have the ability to form volatile compounds of these materials, which then stick to the meat surface, and finally imperfect incineration of charcoal can have great role in their creation, then they spread onto the surface of cooked materials **(Ferrarese et al., 2008)**.

Exposure to PAHs had a significant relation to increased risk of obesity and cardiometabolic risk factors (including increase in blood pressure both systolic and diastolic, low

density lipoproteins level, whole cholesterol and triglycerides) in children **(Poursafa et al., 2018)**. Also, frequent long term exposure to PAHs is greatly connected with diabetes **(Stallings-Smith et al., 2018)** and with DNA damage, defects in arterial oxygen saturation, blood pressure, cardiac frequency and bronchial asthma **(Oliveira et al., 2020)**. PAHs can be generated during cooking and the amounts produced and occurring in the cooked food is governed by fuel used and cooking method **(Rose et al., 2015)**. Therefore, this study was performed to discover the influence of multiple grilling ways (Charcoal, gas and electric grilling) on concentrations of 18 PAHs congeners, PAH4, PAH8 and ΣPAHs in grilled boiler chicken.

2. Material and Methods

2.1. Chicken Preparation and Grilling

Slaughtered ready to cook broiler chickens (about 1.7-1.8kg live weight) were bought from local chicken slaughterhouses in Yousef El sedeek District, Fayoum Governorate, Egypt during the period from January to June 2021. The chicken were washed thoroughly under running tape water. The samples were marinated for 1 hour prior to grilling in a marinade mixture consisting of 250g mashed tomatoes, 60g minced onion, 20g minced garlic, squeezed one lemon juice, 20g salt, 3g black pepper, 3g ground cumin, and 3gm ground coriander. The whole chicken was spread on the grilling network and grilled by charcoal, electric or gas grill. The grilling continued until chicken was grilled well done as commonly accepted by consumers without regard to time or temperature (ten whole chickens were used in every grilling method).

For charcoal grilling, a handmade rectangular iron garden grill (local industry) with dimensions of about 60x40x30 cm and charcoal was ignited till glowing then topped directly with the stainless steel grilling network. Electric grilling was performed using I home® electric grill (Egypt) with 5 levels of temperature and water tray under grilling network to receive the dripping, on the other hand, gas grilling was done using grilling network fixed on cooker tray and located under the upper burner of home cooker Universal® (Egypt).

Grilled chicken meat samples (a whole chicken was considered as one sample) were bone-removed, then sliced, minced then kept at -20°C for consequent GC-MS examination. All processed samples were homogenized separately by Toshiba® food processor, and then wrapped in aluminum foil to be protected from light to prevent photo degradation of PAHs. Extraction, cleanup of samples, GC-MS determination of PAHs were carried out at Faculty of agriculture Laboratories, El Fayoum University.

2.2. Chemicals

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

2.3. Extraction and Clean-up Procedures

The technique formerly designed by **Chen et al., (2013)** was used. Five grams of homogenized sample were mixed with 10 ml of deionized water in a polypropylene tube for 1 minute, then 10 ml acetonitrile solvent were poured and the mixture was exposed to for strong shaking for another minute. The content was replaced in a QuEChERS column having 6 grams of MgSO4 and 1.5 grams acetates of sodium, subsequently vortexed for 1 minute and centrifuged at 1431 g for 5 minutes. Then 6 mL of the uppermost layer were transferred into clean-up column with 0.4g of PSA, 1.2g of magnesium sulfate and 0.4g of silica gel particles for purification. The content was then put in centrifuge at 4000 rpm for a period of 5 min and 1µl aliquot from the uppermost layer was injected into gas chromatograph coupled with mass spectrometer for PAHs analysis.

2.4. Gas Chromatographic Analysis

The PAHs standard curve was obtained by integrating the compound concentration alongside the area of peak. The content of each congener in the sample was estimated according to its particular calibration curve, 1µl of the sample extract was introduced into an Agilent HP-5MS gas chromatograph, eighteen PAHs congeners were identified before elapsing of forty minutes period. Helium gas was used as the carrier gas with flowing speed of one milliliter per minute; temperature of the injector was adjusted at 290°C. The spilt less mode was selected, the temperature programming was adjusted on the following : 70°C at first, then elevated to 195°C with the level of fifteen centigrade per minute then continued for another two and half minutes. After then increased to 240°C at with the level of fifteen centigrade per minute, then lasted for 17min, upraised to 270°C at the level of five centigrade per minute, and elevated to 310°C at the level of fifteen centigrade per minute then was fixed for a period of ten minutes **(Kao et al., 2012)**. PAHs concentrations in tested samples were quantified by matching times of retention and mass spectrum of masses for unidentified peaks to that of previously injected reference materials.

2.5. Statistical Analysis

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

3. Results

Regarding charcoal-grilled sections, and from data shown in **Table (1)**, it could be concluded that all non-carcinogenic PAHs compounds were found in investigated samples, additionally; the peak level was recorded for naphthalene, 17.08±2.31µg/kg, wet weight. Furthermore, benzo[a]pyrene was detected with average content of 1.38±0.66µg/kg wet

weight. From data shown in **Table (2),** it could be noticed that carcinogenic PAH4 level was 3.09 µg/kg, carcinogenic and genotoxic PAH8 content was 3.09µg/kg, while the non-carcinogenic PAHs had an average concentration of 32.91µg/kg and constituted about 91% of ΣPAHs concentration that recorded 36µg/kg, wet weight of the examined charcoal grilled sections. Forty percent of charcoal-grilled items were higher than the benzo[a]pyrene permissible limits set by **European Commission (2014)**.

In chicken grilled using gas-grill, and from data available in **Table (1)**, it could be summarized that all non-carcinogenic PAHs were detected in analyzed samples with exception of fluoranthene. The highest level was recorded for naphthalene, 13.97±1.52µg/kg, wet weight; but benzo[a] pyrene was not identified in the tested items, also all PAH8 members were below the limit of detection, except, chrysene and indeno[1,2,3-cd]pyrene which recorded an average content of 1.68±0.14 and 0.10±0.02µg/kg, wet weight, in the same order.

Table (1). Mean concentrations of PAHs (µg/kg, wet weight) ± standard error in chicken samples experimentally-grilled by different grilling methods

Compound name	Charcoal grilled	Gas grilled	Electric grilled
Naphthalene	17.08±2.31^{a}	13.97±1.52^{a}	14.76±0.92^{a}
2-methyl Naphthalene	4.68±0.65^{a}	4.94±0.44^{a}	4.26±0.70^{a}
1-methyl Naphthalene	1.29±0.09^{a}	0.79±0.09^{b}	1.08±0.08^{ab}
Acenaphthyelene	0.23±0.02^{a}	0.15±0.01^{a}	0.96±0.32^{a}
Acenaphthene	1.67±0.24^{ab}	2.12±0.08^{a}	1.37±0.16^{b}
Fluorene	1.31±0.09^{a}	2.36±0.18^{b}	0.83±0.07^{c}
Phenanthrene	2.88±0.26^{a}	0.79±0.04^{b}	0.86±0.28^{b}
Anthracene	2.87^{a}±0.15	0.63^{b}±0.03	0.66^{b}±0.09
Fluoranthene	0.56±0.02	-	-
Pyrene	0.34±0.04^{a}	0.99±0.04^{b}	1.03±0.09^{b}
Benzo[a]anthracene	-	-	0.18±0.09
Chrysene	1.71±0.09^{a}	1.68±0.14^{a}	0.26±0.03^{b}
Benzo[b]fluoranthene	-	-	-
Benzo[k]fluoranthene	-	-	0.06±0.05
Benzo[a]pyrene	1.38±0.66	-	-
Indeno[1,2,3-cd]pyrene	-	0.10±0.02^{a}	0.05±0.04^{b}
Dibenz[a,h]anthracene	-	-	-
Benzo[ghi]perylene	-	-	-

Means with dissimilar superscripts are significantly different at *p≤0.05,* Means with dissimilar superscripts are significantly different at p≤0.05

From data shown in **Table (2)**, it was evident that carcinogenic PAH4 level was 1.68µg/kg, in addition, carcinogenic and genotoxic PAH8 content was 1.78µg/kg, however, the non-carcinogenic PAHs had an average concentration of 26.74µg/kg and constituted about 94% of ΣPAHs concentration that recorded 28.52µg/kg, wet weight of the examined gas-grilled chicken samples. Benzo[a] pyrene and PAH4 levels in gas-grilled chicken items were all within permissible limits established by **European Commission (2011 and 2014)**.

Concerning chicken samples grilled using electric-grilling machine, and from **Table (1)**, all non-carcinogenic PAHs were detected in analyzed samples with exception of fluoranthene, the highest level was recorded for naphthalene, 14.76±0.92µg/kg, wet weight. The PAH4 group with carcinogenic potential, were absent in all analyzed samples except for chrysene and benzo[a]anthracene with average levels of 0.26±0.03 and 0.18±0.09µg/kg, wet weight.

Finally, the PAH8 group, that include the aforementioned PAH4 group and another four congeners, it was evident that benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene, were found with average levels of and 0.06±0.05 and 0.05±0.04 µg/kg, wet weight, in the same order, while dibenz[a,h] anthracene benzo[ghi]perylene were absent in all tested chicken sections grilled with electric machine.

Table (2). Average concentrations of benzo[a]pyrene, PAH8, PAH4 and total PAHs in experimentally grilled chicken samples by different grilling methods:

	Charcoal-grilled	Gas-grilled	Electric-grilled
Benzo[a]pyrene	1.38	0.0	0.0
PAH4	3.09	1.68	0.44
PAH8	3.09	1.78	0.55
Non carcinogenic PAHs	32.91	26.74	25.81
Total PAHs	36	28.52	26.36
Non carcinogenic PAHs%	91.41%	93.75%	97.91%

A-PAH4= Sum of average content of benzo[a]pyrene+ benzo[a]anthracene+ benzo[b]fluoranthene +chrysene
B-PAH8= Sum of average content of PAH4+ Benzo[ghi]perylene+ Benzo[k]fluoranthene+ Dibenz[a,h]anthracene+ Indeno[1,2,3-cd]pyrene,
C-Total PAHs= Sum of 18 investigated PAHs compounds, D-Non carcinogenic PAHs= Sum of first 10 compounds as arranged **Table (1)**

From data shown in **Table (2),** carcinogenic PAH4 level was 0.44µg/kg, in addition, carcinogenic and genotoxic PAH8 content was 0.55µg/kg, the non-carcinogenic PAHs had an average concentration of 25.81µg/kg and constituted about 98% of ΣPAHs concentration that recorded 26.36µg/kg, wet weight. Benzo[a]pyrene and PAH4 levels in electric-grilled chicken items were all below the permissible limits established by **European Commission (2011 and 2014).**

4. Discussion

Many authors recorded variable values of PAHs in grilled chicken, in this respect, higher PAHs content were estimated by **Kazerouni et al., (2001)** in charcoal grilled chicken from Maryland, where benzo[a]pyrene level was 4.57µg/kg wet weight, furthermore, higher levels were measured by **Jahurul et al., (2013)** in charcoal grilled chicken from Malaysia, the mean concentrations of fluoranthene, benzo[a]pyrene, benzo[b]fluoranthene, and ΣPAHs were 33.92, 3.70, 4.69 and 42.31µg/kg wet weight, respectively.

Alternatively, lower PAHs levels were obtained by means of **Perelló et al., (2009)** in Catalonia, Spain, the mean levels of acenaphthyelene, naphthalene, acenaphthene, phenanthrene, fluorene, anthracene, fluoranthene, pyrene, benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene, dibenz[a,h]anthracene, and ∑PAHs in grilled chicken were 0.1, 0.44, 0.1, 2.95, 0.4, 0.12, 0.87, 0.87, 0.53, 0.05, 0.16 0.05, 0.04, 0.05, 0.05, 0.16, 0.05, and 6.25µg/kg wet weight, respectively. Nearly similar results were found in grilled chicken analyzed by **Alomirah et al., (2011)** from Kuwait, the average concentration for benzo[a]pyrene in that study was 1.32µg/kg wet weight.

Higher PAHs content were detected by **El Badry (2010)** in gas grilled chicken, the mean levels of naphthalene, acenaphthyelene, fluorene, acenaphthene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene, benzo[a] pyrene, dibenz[a,h]anthracene, and ΣPAHs were 7.93±0.006, 2.01±0.006, nd, 16.13±0.0003, 0.005±0.0005, 4.30±0.009, 5.29±0.006, nd, nd, 6±0.005, 7.1±0.005, 6.12±0.013, nd, 1.011±0.009, 1.011±0.005, 7±0.003, and 63.93µg/kg wet weight, respectively, and by **Mohammadi and Valizadeh-kakhki (2018)** in gas grilled samples from Bushehr City in Iran, the average values of naphthalene, ∑PAHs were 156.12 and 159.1µg/kg wet weight, respectively, but lower figures were recorded by **Farhadian et al., (2010)** in Malaysian gas grilled chicken where the mean levels of fluoranthene, benzo[b]fluoranthene and benzo[a]pyrene, sum of 3 PAHs were7.64, 0.40, 0.40 and 8.51µg/kg wet weight, respectively. Also, much higher levels for ∑PAHs that was 222µg/kg wet weight were estimated by **Alomirah et al., (2011)**.

Nearly similar PAHs concentrations were obtained by **Farhadian et al., (2010)** in electric oven grilled chicken; benzo[a]pyrene and benzo[b]fluoranthene were under the limit of the apparatus, while **Pouzou et al., (2018)**, recorded lower levels in micro-waved chicken, the mean concentration of ∑PAHs was 7.59µg/kg wet weight.

Higher PAHs content were estimated by **El Badry (2010)** in microwaved chicken samples, the average levels of benzo[b]fluoranthene, chrysene, ΣPAHs and benzo[g,h,i] perylene recorded 3.55±0.034, 11.16±0.005, 43.56 and 22.55±0.005 µg/kg wet weight, respectively. Furthermore, much higher PAHs levels were analyzed by **Wang et al., (2018)** in grilled wings of chicken, the mean concentration of dibenz[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2, 3-cd]pyrene, benzo[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a] pyrene, PAH4 and ∑PAH8 were 38.87±4.50, nd, 1.69±0.70, 16.03±0.41, 5.92±0.09, 34.45±1.84, 90.05±3.78, 3.68±0.58, 146.45 and 190.69µg/kg wet weight, respectively.

There were significant differences in average levels of PAHs congeners between grilled samples of chicken grilled by charcoal, gas and electric grill at $p \leq 0.05$, this variation can be attributed to the space between heat source and chicken, time lasting for finishing the grilling process, and the grill dirtiness condition **(Ledesma et al., 2015).** The predominance of benzo[a]pyrene, PAH4 and PAH8 in charcoal grilled chicken can be attributed to fat burning then dropping on hot charcoal, besides, the difference in grilling periods, leads to greater aromatization of these compounds that accumulate in charcoal, then release and located on the surface of the grilled chickens **(Wenzl et al., 2006)**. Also, difference of PAHs amount and profile in chicken grilled by different methods can be related to the temperature variation between coal, gas and electric grill that plays an important role, PAHs content increase with the temperature **(Ledesma et al., 2016).**

Higher PAHs levels in charcoal grilled chicken may result from the very high temperature of coal that reaches 500-900^{o}C which tend to produce heavier molecular weight PAHs from thermal decomposition of lignin and lignocelluloses during wood burning and also from fats charring **(European Commission-Scientific Committee on Foods, 2002),** also higher levels of PAH4, PAH8, benzo[a]pyrene and ΣPAHs in charcoal grilled chicken samples, can be explained partially by the fact that charcoal has a volatile materials of about 3-18% that lead to their formation in smoke ascending during grilling, resulting in higher content of PAHs in grilled meats **(Kim et al., 2021).**

The relatively low PAHs content in chicken meat obtained in this study, and being mostly within permissible limits can be attributed to the marination mixture that contain vegetables and crushed seeds containing multiple active substances, these bioactive substances can inhibit free radicals, which lead to the creation of primary pyrolysis yields due to Maïllard reaction and so, these antioxidant substances may decline their development in chicken during grilling **(Falowo et al., 2014)**.

5. Conclusion

From the obtained results of the current study, it could be concluded that electric grilling added less contents of benzo[a]pyrene, PAH4, PAH8, non-carcinogenic PAHs and total PAHs to grilled chicken, while charcoal grilling shown the highest contamination with these compounds in terms of previous parameters concentrations. Furthermore, benzo[a] pyrene exceeded the permissible limits in 40% of charcoal grilled chicken samples; further studies should be performed to bring the content of PAHs in grilled chicken to more safe limits.

6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

8. Acknowledgment

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