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Evaluation of Different Cooking Methods to Control the Formation of Polycyclic Aromatic Hydrocarbons (PAHs) During Heat Treatment

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Abstract

POLYCYCLIC aromatic hydrocarbons (PAH) are considered harmful, carcinogenic organic compounds that can occur during the heat treatment of meat, particularly grilling or barbecuing. This study aims to assess the level of contamination of sixteen PAH constituents (Chrysene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Naphthalene, Benz[a]anthracene, Benzo[g,h,l]perylene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene. Indo[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene, Acenaphthylene, Pyrene) on 20 heat-treated meat product samples using Gas chromatography (GCMS) in charcoal-grilled beef burgers and beef burgers cooked in hot air oven (10 of each) in Cairo governorate supermarkets. Polycyclic aromatic hydrocarbons 4 (PAH4) and Polycyclic aromatic hydrocarbons 8 (PAH8) mean values in charcoal grill and hot air oven samples were 73.56±13.64, 82.64±14.42. 15.83±5.13, 16.42±5.68 µg/kg, respectively. The maximum amount of PAH4 in meat samples according to the European Commission is 12 µg/kg, so the use of a hot air oven reduces PAH compared to a charcoal grill. Although high levels of PAHs can be produced in cooked meat, it is nevertheless advisable to reduce their formation by choosing appropriate cooking methods.

Keywords: PAHs, Ready-to-Eat, Charcoal, GC-MS, Burger, Hot air oven.

Introduction

Organic molecules known as polynuclear aromatic hydrocarbons (PAHs) are mostly solid, colourless, white, or light yellow. It is a wider class of organic compounds with two or more fused aromatic rings made of hydrogen and carbon [1]. The presence of PAHs in food is caused by manufacturing procedures or cooking techniques was reported by [2]. However, due to the higher concentration of carcinogenic compounds in grilled meat compared to meat prepared using alternative cooking methods, it presents an elevated health risk to the general population [3].

Polycyclic aromatic hydrocarbons (PAHs) may form in meat in several ways, such as the thermal breakdown of organic matter (such as proteins, fats, and carbohydrates) at temperatures of 150 °C or higher or the dripping of fat over a heat source, which deposits PAHs on the meat [4, 5]. Or by inadequate charcoal combustion, which might result in PAHs adhering to food's surface [6, 7].

Because processed and red meat include N-nitroso compounds, heterocyclic aromatic amines, and polycyclic aromatic hydrocarbons (PAHs), the International Agency for Research on Cancer (IARC) has categorized these substances as category 1 carcinogens to humans. Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), Group 2B (maybe carcinogenic to humans), and Group 3 (unclassified as carcinogenic to humans) are the few groups into which the International Agency for Research on Cancer has divided the toxicant PAHs [8]. PAHs have been categorized by several regulatory organizations according to their potential for cancer. The four main PAHs that show evidence of carcinogenic activity have been identified by the European Commission (EC) as being Benz [a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), and benzo[a]pyrene (BaP) [9].

The European Food Safety Authority (EFSA) has also divided these toxicant PAHs into PAH2 (BaP and Chr), PAH4 (BaA, BaP, BbF, and Chr), and

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PAH8 (BaA, BaP, Chr, BkF, BbF, IcdP, DahA, and BghiP) [10].

Human exposure to PAHs has been associated with long-term adverse effects on neuro differentiation occurring during late gestation or early infancy, as well as oxidative DNA damage [11], and a 30- to 50% increase in the risk of breast cancer [12, 13]. Furthermore, they can result in oxidative stress and inflammatory reactions by generating reactive oxygen species, which can lead to cardiopulmonary and cardiovascular disorders [14].

Therefore, this study aimed to influence of cooking procedures, like charcoal grilling and in hot air oven at 200˚C, on the generation of PAHs levels.

Material and Methods

In this study, 20 beef and Kofta samples (10 of each) were collected from different supermarkets in Cairo governorate, Egypt. These samples were examined for the level of contamination of polycyclic aromatic hydrocarbons and for validation of their effects on consumers' health.

Prepared beef burger samples

Ground beef meat and bread crumbs were mixed until well blended. Then four 3/4-inch-thick patties were formed. Twenty beef burger samples total (ten samples each, cooked at 200°C in a hot air oven or on a charcoal grill) were frozen at 4°C until the analysis was completed. Analysis of the polycyclic aromatic hydrocarbons (PAHs) residues was performed by extraction methods, cleanup, and estimation of PAHs levels was carried out by gas chromatography in the Pesticide Residue Department Central Pesticide Lab., Agriculture Research Center, Giza, Egypt. The contents of the samples were compared to recommended standard limits and their acceptability for human consumption.

Chemicals and Reagents

The solvents utilized were n-hexane (purity > 99.0%), toluene (Merck), dichloromethane chromate-graphy grade, acetone (Riedel-de Häen, purity 99.8%), and acetonitrile (Sigma-Aldrich, purity > 99.9%). Salts and buffers from Agilent QuEChERs were bundled in anhydrous containers for EN 15662, including 0.5 g of disodium citrate sesquihydrate, 1 g of sodium chloride (NaCl), 4 g of magnesium sulphate (MgSO4), and 1 g of sodium citrate. Prior to usage, silica gel (60–120 mesh, Fluka) was activated for 12 hours at 150◦C.

Apparatus

For sample extraction, Polytetrafluoroethylene or polyethylene, 50 mL screw-capped tubes and 15 mL tubes containing 1 g of magnesium sulphate were acquired. 10 mL solvent dispenser (Hirschmann Laborgerate) for acetonitrile, centrifuge (Heraeus Labofuge 400) with a maximum speed of 4000 rpm, Vortex, automatic pipettes (Hirschmann Laborgerate) appropriate for volumes ranging from 10 μL to 100 μL and 100 μL to 1000 μL. Before being used, the glassware was washed with detergent and water then rinsed with acetone and dried at 90◦ C.

Sample Extraction Steps [15]

It is necessary to take into account the cost-benefit criteria, the validation process, and the context of fitness for purpose. Accurately 50 mL Teflon centrifuge tube containing about 10 g of weighted meat sample was used. Fifty μL of 10 μg/mL pyrened10 was then added, serving as a surrogate standard of 50 μg/ Kg. Each set of six replicates was then spiked with 20, 100, and 500 μL of a 1 μg/mL spiking mixture to get 2, 10, and 50μg/ kg, respectively. 10 mL of acetonitrile was used for extraction, and after shaking for 2 minutes, mixed with Agilent QuEChERs. Then the mixture was shaken for one minute and centrifuged for five minutes at 4000 rpm. After transferring aliquots of the resultant supernatant to a Teflon tube containing MgSO4, the tube was vortexes for 30 seconds and centrifuged for 2 minutes at 4000 rpm. Meanwhile, 4 mL of the acetonitrile layer was transferred to a 50 mL flask and evaporated almost completely.

PAHs Samples were cleaned up by Packed Solid Phase Extraction (SPE) Steps [16].

All meat extracts were exposed to a packed solid-phase cleanup cartridge, prepared in-house using the following recipe. Plug a glass wool on a 10 mL length syringe, one gram of 20% deactivated silica gel and 0.2 gram of $MgSO_4$ were weighted and conditioned with 5 mL of n-hexane/dichloromethane (3:2), the sample extract loaded to the cartridge using 10 mL of elute (n-hexane/dichloromethane). The fractions were Gathered into a 50 mL flask, evaporated on a rotary evaporator at 40 °C until they were almost dry, dissolved in 2 mL of toluene, and then submitted to GCMS for examination.

GC-MSD Conditions [16]

For both qualitative and quantitative analysis of PAHs, an Agilent 6890N series gas chromatography instrument with a 5975 series mass selective detector and an Agilent GC column of model J&W HP-5ms Ultra Inert with the specifications (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) were utilized. The carrier gas, helium gas, was maintained in the column at a constant flow rate of 1.3 mL/min. At 260˚ C, the back injector line was kept constant. One μL of injection was used in the split less mode. After the column temperature was initially held at 90˚C for 2 min, the temperature was ramped up to

180˚C at a rate of 15˚C /min. It was then held at 180˚C for 15 min, ramped up to 250˚C at a rate of 10˚C/min, maintained for two minutes, ramped up to 290˚C at a rate of 10˚C /min, and held for 10 min. The mass spectrometer was operated in the ionization mode and spectra were acquired using a mass range of 45–450 m/z. The base peak of each targeted PAH was compared to complete the SIM acquisition process.

Results

The results shown in Tables (1) declared that the average concentration levels of PAHs in charcoal grilled beef burger (Chrysene, Acenaphthene, Fluorene, Phenant-hrene, Anthracene, Fluoranthene, Benz[a]anthracene, Benzo[g,h,l]perylene, Benzo[b] fluoranthene, Benzo [k]fluoranthene, Benzo[a]pyrene, Indo[1,2,3-cd] pyr-ene (IP), Dibenzo[a,h] anthracene, Acenaphthylene and Pyrene) were 18.15± 7.79, 18.1± 8.16, 86.25 ± 60.95, 228.4± 80.30, 49.34± 23.73, 109.26±23.12, 13.92± 4.26, 2.36± 3.01, 32.27± 9.45, 9.22± 5.91, 6.71± 5.93, 18.52± 6.10 and 39.37± 17.83 respectively. But Naphthalene, Benzo[k]fluoranthene and Dibenzo[a,h]anthracene were under the detection limit. The mean concentration values of carcino-genicity hydrocarbons (PAH4 and PAH8) were 73.56± 13.64 and 82.64± 14.42µg/kg^{-1}.

The results presented in Table (2) indicated that the average concentration levels of PAHs in beef burger cooked in hot air oven, Chrysene, Acenaphthene, Phenanthrene, Benz[a]anthracene, Benzo[a]pyrene, Indo[1,2,3-cd] pyrene, Acenaphthylene and Pyrene were 1.26± 1.60, 2.08± 2.45, 16.13± 3.98, 4.02± 3.19, 10.10± 4.65, 1.05± 0.99, 5.88± 1.93 and 8.11± 2.36 respectively. Although Benzo[g,h,l] perylene, Benzo[b] fluoranth-ene, Benzo[k]fluoranthene, Fluorene, Anthracene, Naphthalene, Benzo[k] fluoranthene and Dibenzo [a,h]anthracene were under the detection limit. While their PAH4 and PAH8 occur with a mean concentration level of 15.38± 5.13 and 16.42 ± 5.68 µg/kg.

Discussion

The results demonstrated that meat cooked on charcoal had far higher levels of PAHs than meat cooked on gas, especially carcinogenic PAHs. Variations were noted between the PAH4 and PAH8 concentrations of burgers cooked on a charcoal grill and those cooked in a hot air oven depending on the type of cooking and PAH type according to [17]. On the other hand, [18] mentioned that whereas benzo [ghi]perylene was not detected in the charcoal-grilled Kofta examined samples, maximum concentrations of benzo(a)anthracene and benzo(a)pyrene were determined to be 33.2± 4 and 26± 16 µg/kg, respectively. The results were higher than those reported on charcoal-grilled beef burgers in this study.

According to [19] The maximum limit of BaP and PAH4 in meat samples is 2 µg/kg and 12µg/kg respectively; however, elevated concentrations were observed in this investigation in both grilling methods.

Applying high temperatures caused an increase in PAHs creation due to the pyrolysis of organic components that included nitrogen, such as proteins and amino acids were examined by [20]. The incomplete combustion of charcoal compared to gas may be the cause of the increased quantity of PAHs in meat cooked over charcoal as opposed to hot air oven cooking.

The mean concentration of BaP, BaA, Chr, BbF and PAH4 in beef grilled at 200º C were 3.0 ± 0.8 1.7± 0.1 1.9 ± 0.4 0.6 ± 0.01 7.1 (ng/g wet weight) ± standard deviation according to those found by [21]. This exhibited lower quantities than those detectable by this investigation.

The average concentrations of BaP and PAH4 in beef steaks cooked on gas were 0.20, 0.73 ng/g, while those cooked using charcoal heat sources were 0.46, 6.0 ng/g [2]. This demonstrated a lower concentration than what was identified in this study.

According to the results published by [22], the average concentrations of the carcinogenic PAH4 and PAH8 in Charcoal Grilled beef, Kofta were 90± 93.14 and 19.7± 28.09 µg/ kg^{-1}, individually. In this investigation, the values of Charcoal Grilled beef burger were 73.56± 13.64 and 82.64± 14.42 µg/kg^{-1}, in that order. Both studies agreed that the results were above the maximum amount of PAH4 in meat samples, above the permissible level [19].

Lower concentrations than those reported in this study were observed by [23] for benzo[a]pyrene, indeno[1,2,3-cd]pyrene, di-benzo[a,h]anthracene, benzo[g,h,i]perylene, acenaph-thylene,acenaphthene, fluorene, phenanthrene anthr-axcesne, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranth-ene, PAH4 and PAHs in grilled beef with averages of 0.131± 0.128, 0.925± 0.754, 0.535± 0.547, 0.284± 0.236, 0.854± 0.931, 1.511± 1.386, 1.584± 0.913, 1.886± 1.572, 1.578± 0.999, 1.819± 1.374, 2.24± 1.933, 0.91± 0.585, 1.55± 1.339, 0.615± 0.778, 0.377± 0.316,4 and 16.79µg /kg, wet weight, respectively. In contrast, samples of grilled beef steak from Turkish cities did not contain any of the PAH8 members [24].

Conclusion

The cooking method whether charcoal grilling or using a hot air oven was significantly linked to

variations in PAH levels, with charcoal grilling leading to higher concentrations of these harmful compounds. Beef burgers cooked over charcoal presented a persistent risk of human PAH exposure, raising concerns about the potential health risks associated with regular consumption. In contrast, burgers prepared in a hot air oven demonstrated significantly lower PAH levels, indicating that this method is a safer alternative for processing meat.

Based on these findings, it is strongly recommended to reduce the use of charcoal grills and to limit the consumption of grilled meat products. Instead, cooking at lower temperatures in hot air ovens should be encouraged to minimize PAH formation and enhance food safe.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest

TABLE 1. Statistical analytical results of PAHs µg/kg in examined samples of Charcoal Grilled beef burger (*n=10).

	Range			
PHAS	**Min.**	**Max.**	**Mean**	**SD**
Chrysene (ch)	UDL	27.26	18.15	7.79
Acenaphthene(Ac)	UDL	27.6	18.1	8.16
Fluorene (F)	38.18	234.88	86.25	60.95
Phenanthrene (Pa)	113.44	328	228.4	80.30
Anthracene (A)	16.11	82.91	49.34	23.73
Fluoranthene	89.09	171.80	109.26	23.12
Naphthalene (Na)	UDL	UDL	UDL	UDL
Benz[a]anthracene (BaA)	UDL	19.07	13.92	4.26
Benzo[g,h,l]perylene (BghlP)	UDL	8.27	2.36	3.01
Benzo[b]fluoranthene (BbF)	19.03	51.06	32.27	9.45
Benzo[k]fluoranthene (BkF)	UDL	UDL	UDL	UDL
Benzo[a]pyrene (BaP)	4.36	16.23	9.22	5.91
Indo[1,2,3-cd]pyrene (IcdP)	1.61	15.23	6.71	5.93
Dibenzo[a,h]anthracene (DBahA)	UDL	UDL	UDL	UDL
Acenaphthylene (Ace)	11.11	32.66	18.52	6.10
Pyrene (P)	14.33	69.11	39.37	17.83
PAH4	57.9	92.12	73.56	13.64
PAH8	107.35	65.22	82.64	14.42

* PAH4: BaA, Chr, BbF, and BaP PAHs. PAH8: BaA, Chr, BbF, BkF, BaP, IcdP, DBahA and BghlP PAHs. UDL= under detected limits. SD = standard deviation n= Number of samples.

TABLE 2. Statistical analytical results of PAHs µg/kg in examined samples of beef burger cooked in hot air oven at temperature 200°c (*n=10).

	Range			
PHAS	**Min.**	**Max.**	**Mean**	**SD**
Chrysene (ch)	UDL	4.5	1.26	1.60
Acenaphthene(Ac)	UDL	7.6	2.08	2.45
Fluorene (F)	UDL	UDL	UDL	UDL
Phenanthrene (Pa)	9.1	21.30	16.13	3.98
Anthracene (A)	UDL	UDL	UDL	UDL
Fluoranthene	UDL	UDL	UDL	UDL
Naphthalene (Na)	UDL	UDL	UDL	UDL
Benz[a]anthracene (BaA)	UDL	8.05	4.02	3.19
Benzo[g,h,l]perylene (BghlP)	UDL	UDL	UDL	UDL
Benzo[b]fluoranthene (BbF)	UDL	UDL	UDL	UDL
Benzo[k]fluoranthene (BkF)	UDL	UDL	UDL	UDL
Benzo[a]pyrene (BaP)	3.81	10.56	10.10	4.65
Indo[1,2,3-cd]pyrene (IcdP)	UDL	2.5	1.05	0.99
Dibenzo[a,h]anthracene (DBahA)	UDL	UDL	UDL	UDL
Acenaphthylene (Ace)	3.18	8.14	5.88	1.93
Pyrene (P)	4.53	10.66	8.11	2.36
PAH4	8.31	22.85	15.38	5.13
PAH8	8.31	24.81	16.42	5.68

* PAH4: BaA, Chr, BbF, and BaP PAHs. PAH8: BaA, Chr, BbF, BkF, BaP, IcdP, DBahA and BghlP PAHs. UDL= under detected limits. SD = standard deviation, n= Number of samples.

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تقيم طرق طهي مختلفة للسيطرة علي تكوين المركبات الهيدروكربونات العطرية متعددة الحلقات (PAHs) اثناء المعاملة الحرارية

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الملخص

تعتبر الهيدروكربونات العطرية متعددة الحلقات مركبات عضوية ضارة ومسببة للسرطان يمكن أن تنتج هذة المركبات أثناء المعالجة الحرارية للحوم، وخاصة الشواء على الفحم. تهدف هذه الدراسة إلى تقييم مستوى تكون ستة عشر مكونًا من الهيدروكربونات العطرية متعددة الحلقات (الكريسين، الأسينافثين، الفلورين، الفينانثرين، الأنثراسين، الفلورانثين، النفتالين، بنز[أ]أنثراسين، بنزو[ج،ح،ل]بيريلين، بنزو[ب]فلورانثين، بنزو[ك]فلورانثين، بنزو[أ]بيرين. إندو[3،2،1cd]-بيرين، دي بنزو[أ،ح]أنثراسين، الأسينافثيلين، بيرين) على 20 عينة لحوم معالجة حرارياً بطرق طهي مختلفة (الشوي علي الفحم، فرن الهواء الساخن). تم قياس نسبه المركبات الهيدروكربونات العطرية متعددة الحلقات المتكونه باستخدام جهاز الغاز الكروماتوغرافي (GCMS) في برجر لحم البقر المشوي على الفحم وبرجر لحم البقر في فرن الهواء الساخن (10 من كل منهما). كانت القيم المتوسطة لـ PAH4 و PAH8 هي 73.56±13.64 و82.64±14.42 و15.83±5.13 و16.42±5.68 ميكروجرام/كجم على التوالي. اظهرت النتائج ان طريقة الطهي تؤثر علي نسبه انتاج مركبات الهيدروكربونات العطرية متعددة الحلقات حيث وجد نسب عاليه من هذة المركبات في اللحوم المشوية علي الفحم بعكس اللحوم المطبوخة في أفران الهواء الساخن.

الكلمات الدالة: الهيدروكربونات العطرية متعددة الحلقات، البرجر, الفحم ،غاز كوماتوغرافي (GC-MS), الشوي علي الفحم, فرن الهواء الساخن.