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Determination of Polycyclic Aromatic Hydrocarbon (PAHs) in Grilled Chicken and Health Risk Assessment

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Polycyclic aromatic hydrocarbons (PAHs) are compounds formed during the incomplete combustion of organic materials like wood or fatty meats. Cooking methods, particularly grilling, significantly influence PAH formation in food due to high heat involved. This heat causes fats and oils to drip onto the heat source, producing PAH-laden smoke that can adhere to food upon contact. Considering their harmful effects on health, this study aimed to assess PAH concentrations in chicken prepared using three common grilling methods (charcoal-grilled, gas-grilled, and oven-grilled) and evaluate potential health risks associated with their consumption. The samples were analysis using high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). Results showed that charcoal-grilled chicken had the highest total PAH concentration at 64.41 μg/kg, followed by gas-grilled chicken at 49.08 μg/kg, and oven-grilled chicken at 31.08 μg/kg. Despite these differences, statistical analysis using a nonparametric Kruskal-Wallis test revealed no significant differences in PAH concentrations among the three cooking methods ($p > 0.05$). The study also evaluated health risks using Hazard Quotient (HQ) and Incremental Lifetime Cancer Risk (ILCR) assessments. The low HQ values suggested that PAH levels in grilled chicken, across all cooking methods, do not pose significant non-carcinogenic health risks to the Malaysian adult population. Similarly, ILCR analysis indicated a low carcinogenic risk from consuming grilled chicken within the study parameters, as PAH levels were within acceptable limits. Future research with larger sample sizes and more controlled conditions is recommended to further elucidate factors influencing PAH accumulation in grilled foods and refine risk assessments for public health guidelines.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); grilled chicken; health risk assessment; HPLC-FLD

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Polycyclic aromatic hydrocarbons (PAHs), a class of compounds that have two or more aromatic rings made of carbon and hydrogen, are formed by the pyrolysis, or incomplete burning of organic materials like wood, petroleum, or other organic materials such as greasy meat [1, 2]. The colourless, white, or pale-yellow solid compounds are emitted as a gaseous phase or particulate form, depending on its molecular weight [3]. The characteristics of light-molecular PAHs are that they are more volatile and water-soluble compounds compared to heavy PAHs, which are more stable and hazardous [4]. The United States Environmental Protection Agency (USEPA) has designated 16 of the PAHs as high priority pollutants, considering their potential for human exposure, toxicity, frequency of occurrence at hazardous waste sites, and the available information [4]. These 16 PAHs include acenaphthene, benzo[g,h,i]perylene, chrysene, acenaphthylene, benz[a]anthracene, benzo [b]fluoranthene, anthracene, benzo[k]fluoranthene, benzo[a]pyrene, fluoranthene, indeno [1,2,3-c,d]pyrene, naphthalene, phenanthrene, dibenz[a,h]anthracene, fluorene, and pyrene. Seven of these chemicals listed by the International Agency for Research on Cancer (IARC) are classified as probable or known carcinogens based on animal testing [5].

Grilling or barbecue cooking methods are widely popular, known for the distinctive pleasant woody smell imparted by the smoking process. The Maillard reactions, oxidation, and fat degradation that occur during grilling contribute to the aroma and flavour compounds such as unsaturated aldehydes [6]. However, grilling temperatures, typically exceeding

200 ºC, can also result in the formation of PAHs concurrently with flavour development [7]. The formation of PAHs in meat and meat products is a common side effect of nearly all cooking methods. Nevertheless, higher levels of carcinogens are typically found in grilled foods compared to those prepared using other methods. This means that common direct-flame foods such as grilled chicken can contain high concentrations of PAHs. In fact, consumption of smoked or grilled chicken products has been associated with an increased cancer risk, as the diet serves as a pathway of exposure for non-smoking consumers [7].

Concerns about food safety and human health have increased, particularly regarding foods containing contaminants such as PAHs. Consuming unsafe food containing harmful chemical substances can lead to various diseases, ranging from diarrhea to cancer. Globally, cancer is the leading cause of death, accounting for one in six deaths in the population [8]. Although no definitive link between PAHs exposure from grilled chicken and cancer in humans has been established through population studies, exposure to PAHs can cause changes in DNA, which may increase a person's risk of cancer. Consequently, to address the knowledge gaps, this study aims to determine the concentration of PAHs compounds such as benzo[a]pyrene, fluoranthene, benzo[b]fluoranthene, and chrysene in grilled chicken. Samples were obtained from restaurants in Kuala Selangor that were prepared using charcoal, gas, and oven grilling methods. Additionally, a health risk assessment for adults in Malaysia was conducted, focusing on hazard quotient associated with PAH exposure from grilled chicken consumption, chronic daily intake, and incremental lifetime cancer risk. The results are intended to contribute to the formulation of evidence-based cooking and dietary guidelines, aimed at safeguarding the safety of grilled chicken consumption and advancing public health initiatives.

EXPERIMENTAL

Chemicals and Materials

Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). The reagent kit for sample preparation in food analysis consists of potassium hexacyanoferrate (II) trihydrate (Carrez I) and zinc sulfate heptahydrate (Carrez II) solvents were bought from Sigma-Aldrich (Supelco, USA). The analytical PAHs standard, such as benzo(a)pyrene, fluoranthene, benzo(b)fluoranthene, and chrysene, were purchased from Sigma-Aldrich (Supelco, USA).

Sample Collection

A total of 36 samples of grilled chicken were selected randomly from various food establishments in Kuala Selangor, Selangor, Malaysia. These samples were prepared using three distinct cooking methods: charcoal grilling, gas grilling (direct heat), and oven grilling (indirect heat). Each dish was purchased randomly from different local premises and restaurants, then promptly placed in zip-lock bags, transported to the laboratory in an ice box maintaining a cold chain at 4 ºC, and stored at -20 ºC in the freezer until extraction and analysis.

Samples Preparation

Sample Extraction of PAHs

Prior to HPLC analysis, sample preparation was carried out following a method adopted from Sahin et al. [9]. Initially, approximately 50.0 g of each sample was homogenized, and 3.0 g was weighted and placed into 50 mL centrifuge tubes. A mixture of 10 mL of 1 M potassium hydroxide (KOH) and 10 mL of methanol/acetonitrile (50:50) was then added to the samples, which were then closed and mixed vigorously. Then, the tubes were sonicated for 10 minutes in an ultrasonic water bath (Cole-Parmer, USA) with a water temperature of 40 ºC, followed by shaking at 120 rpm for 30 minutes in an orbital shaker to transfer the organic content into the solution phase. After waiting for 30 minutes, the tubes were centrifuged at 4,200×g (Centurion Scientific, UK) for 5 minutes. The liquid phase was transferred to another tube, where 1.3 mL of 6 M hydrogen chloride (HCl) was added to adjust the pH to 6. Following that, 1 mL of Carrez I and 1 mL of Carrez II solutions were added, followed by thorough shaking. The tubes were centrifuged again at 4,200×g for 5 minutes. After centrifugation, 1.5 mL of the upper phase sample was filtered through 0.45 μm syringe tip filters and transferred to HPLC vials. Samples in the vial were degassed for 2 minutes in an ultrasonic water bath.

Sample Analysis

Analysis of PAHs using HPLC-FLD

The analysis of four PAHs in all grilled chicken was conducted using the high-performance liquid chromatography equipped with fluorescence detector (HPLC-FLD) (Shimadzu Prominence, Japan). μ-Bondapak C18 HPLC column (4.6 mm x 250 mm, 10.0 μm, Waters Co.) was used to separate the analytes. Eluents were filtered through 0.45 μm microporous membrane and degassed under ultrasound for 15 minutes before use. The injection volume was 10 μL, with a flow rate of 1.8 mL/min for all PAH compounds throughout the analysis. PAH separation was performed under isocratic conditions using a mobile of ultra-pure water (A) and acetonitrile (B). A 65(A):35(B) isocratic elution program was selected after evaluating various mobile phase compositions. Each PAH compound was detected using the optimal excitation and emission wavelengths (Ex/Em λ) of 260/440 nm, with a runtime of 10 minutes per analysis. Peaks in the chromatograms were identified by comparing retention times with those of PAH standards, and quantification was based on peak area.

Data Analysis

Statistical Analysis

All data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) version 27.0. Prior to analysis, normality testing of continuous variables was conducted using the Shapiro-Wilk test. Descriptive statistics were calculated for the mean PAH concentrations. The differences in mean PAH values across various cooking methods were assessed using the Kruskal-Wallis non-parametric test.

Health Risk Assessment

The potential health risks associated with the consumption of grilled chicken were evaluated based on the guidelines set by the USEPA [10]. Each PAH concentration (Ci) is multiplied by its corresponding toxic equivalency factors (TEF) from Table 1 to convert all PAH concentrations into toxic equivalent quotient (TEQ), using Equation 1. The carcinogenic potentials of the detected PAHs were determined by adding up each TEQ value. TEQ values help in assessing the combined toxicological impact of PAH mixtures, providing a standardized measure to compare and regulate their health risks.

$$TEQ = TEF \times Ci \quad (1)$$

The chronic daily intake (CDI) of PAHs was calculated using equation 2. IR represents the ingestion rate derived from the estimated chicken intake from Malaysia's Food Consumption Statistics 2014, specifically 0.03301 kg/day [10]. ED denotes the exposure duration for the adult population, set at 30 years [11]. EF stands for the exposure frequency assumed for the adult population, equivalent to 52 days per year (once-a-week consumption) [12]. BW represents the average adult body weight in Malaysia, recorded as 62.65 kg. AT corresponds to the average lifespan used to assess cancer risk (70 years, 25,550 days) and non-cancer risk (30 years, 10,950 days) [13].

$$CDI = \frac{Ci \times IR \times ED \times EF}{BW \times AT} \quad (2)$$

The non-carcinogenic risk associated with fluoranthene was assessed using the hazard quotient (HQ), calculated by dividing the chronic daily intake (CDI) by the reference dose (RfD) (Table 2) as per Equation 3.

$$HQ = \frac{CDI}{RfD} \times CF \quad (3)$$

Meanwhile, the carcinogenic risk posed by three other PAH compounds (Benzo(a)pyrene, Benzo(b)fluoranthene, and Chrysene) was assessed by calculating the Incremental Lifetime Cancer Risk (ILCR) using Equation 4. The Cancer Slope Factor (CSF) for each PAH compound, as specified in Table 2, was employed to determine their respective ILCRs.

$$ILCR = CDI \times CSF \quad (4)$$

Table 1. Benzo[a]pyrene Equivalent Factor for Carcinogenicity (TEF).

PAH Compound	TEF
Benzo[b]fluoranthene	0.1
Chrysene	0.001
Fluoranthene	0.001
Benzo[a]pyrene	1.0

Table 2. Cancer slope factor (CSF) and reference dose (RfD).

PAHs	CSF/*RfD (mg/kg/d)
Benzo(a)pyrene	0.73×10^{-1}
Benzo(b)fluoranthene	7.30×10^{-1}
Chrysene	7.30×10^{-3}
*Fluoranthene	4.00×10^{-2}

Table 3. Limit of detection (LOD), limit of quantification (LOQ), and regression coefficient (R^2) of standard solution of PAHs.

Analysed PAHs	LOD (µg/kg)	LOQ (µg/kg)	Regression coefficient (R^2)
Fluoranthene	0.006	0.017	0.9982
Benzo(b)fluoranthene	0.010	0.032	0.9877
Chrysene	0.014	0.045	0.9939
Benzo(a)pyrene	0.006	0.017	0.9981

RESULTS AND DISCUSSION

This study assessed the presence of PAHs in grilled chicken and evaluated the potential health risks associated with their consumption. Four PAH concentrations were determined by comparing HPLC retention times with PAH standards. Table 3 presents the limit of detection (LOD), limit of quantification (LOQ), and correlation coefficient (R^2) for these PAH standards, with correlation coefficients ranging from 0.9877 to 0.9982 for each analyte. The regression coefficient and detection limits confirm the suitability of the method for analyzing the selected PAHs. The LOD for the four PAH compounds ranged from 0.006 to 0.014 µg/kg, while the LOQ ranged from 0.017 to 0.045 µg/kg.

The Concentration of PAHs in Grilled Chicken

The concentrations of PAHs (fluoranthene, benzo[b] fluoranthene, chrysene, benzo[a]pyrene) in grilled chicken samples were meticulously analyzed and are presented in Table 4, illustrating notable variability among the samples. Charcoal-grilled chicken showed the highest PAH concentration, averaging 81 µg/kg, which is consistent with previous research attributing elevated PAH levels to the incomplete combustion and high temperatures associated with charcoal grilling [2]. Conversely, oven-grilled chicken exhibited the lowest PAH concentration at 7.33 µg/kg, highlighting the effectiveness of its controlled combustion process in minimizing PAH formation compared to charcoal grilling [2].

Furthermore, chrysene stood out with the highest mean concentration among individual PAHs. The concentration of chrysene spanned from 2.33 µg/kg to 51.33 µg/kg across the samples, highlighting the necessity of analyzing specific PAHs to comprehensively assess the health implications associated with the consumption of grilled foods [14]. This underscores the importance of considering cooking methods in understanding the impact of PAH exposure from grilled meats on human health.

Table 4. Mean concentrations of four PAHs in the samples of grilled chicken, n=36.

Food samples	n	Fluoranthene (µg/kg)	Benzo(b)-fluoranthene (µg/kg)	Chrysene (µg/kg)	Benzo(a) pyrene (µg/kg)	Σ PAHs
Charcoal chicken satay	3	0.33	21.00	19.33	1.33	42.00
Honey Chicken	3	3.33	21.33	48.00	2.00	74.67
Chicken wings	3	1.00	10.67	47.67	0.67	60.00
Charcoal grilled chicken	3	BDL	BDL	51.33	5.33	81.00
Gas chicken satay	3	0.33	10.33	41.33	2.00	54.00
Chicken kebab	3	BDL	21.00	26.33	1.33	48.67
Chicken patty	3	BDL	21.33	40.67	0.67	62.67
Gas grilled chicken	3	BDL	12.00	16.67	2.33	31.00
Oven chicken satay	3	2.00	3.00	2.33	BDL	7.33
Tandoori chicken	3	1.00	7.67	18.00	1.33	28.00
Roasted chicken	3	0.33	12.33	BDL	1.33	21.67
Oven grilled chicken	3	BDL	30.00	35.67	1.67	67.33

*BDL: Below Detection Limit

Concentration of PAHs in Different Grilling Methods

The concentrations of four PAH compounds across three grilling methods (charcoal-grilled, gas-grilled, oven-grilled) are presented in Table 5. Charcoal grilling exhibited the highest PAH content, with ΣPAH measured at 64.41 µg/kg. This charcoal grilling method involves incomplete combustion, resulting in elevated temperatures that promote increased PAH production. This phenomenon is consistent with previous studies indicating that open flame cooking, such as charcoal grilling, enhances PAH levels due to direct exposure of food to combustion byproducts released into the cooking environment [2]. Ali et al. found elevated PAH levels in charcoal-grilled samples, including benzo[a]pyrene, PAH4, PAH8, and total PAHs. Additionally, Abdel-Shafy & Mansour [3] suggested that the porous nature of charcoal facilitates prolonged combustion, further contributing to PAH formation.

Gas grilling, in contrast, showed moderate PAH levels at 49.08 µg/kg, positioning it between charcoal and oven grilling methods. This aligns with studies indicating that gas grilling generally produces lower PAH levels than charcoal grilling. However, gas grilling can still contribute to PAH formation, particularly in the presence of fat drippings that cause flare-ups. Research indicates that meats grilled horizontally, where oils directly drip into the flames, release higher amounts of PAHs compared to meats cooked vertically [16]. Among the three methods examined, oven grilling yielded the lowest PAH concentration at 31.08 µg/kg. The controlled and enclosed environment of an oven minimizes direct exposure to flames and ensures more uniform heat distribution, resulting in reduced PAH production compared to open flame grilling techniques. This finding corroborates previous research indicating lower PAH concentrations associated with oven cooking compared to direct flame grilling [9]. Although heating does not completely eliminate PAHs, oven grilling provides a safer alternative for individuals worried about PAH exposure, leading to reduced levels of these compounds.

A nonparametric Kruskal-Wallis test was employed to assess the mean differences in PAH concentrations among different cooking methods. While PAH concentrations showed some variability across the three grilling methods, the p-values for all PAH compounds exceeded 0.05, indicating no statistically significant differences between the types of cooking methods. This suggests that, despite observed variations, the choice of grilling method did not significantly influence PAH levels in the samples analyzed. The lack of significant difference might indicate that the sample size was not large enough to detect more minor, yet potentially meaningful, differences. Further studies with larger sample sizes and more controlled grilling conditions are recommended to understand better the factors contributing to PAH accumulation in different cooking methods.

Health Risk Assessment

The toxic equivalent quotient (TEQ) of PAH compounds in grilled chicken was assessed and is detailed in Table 6. Chronic daily intake (CDI) and Incremental Lifetime Cancer Risk (ILCR) for carcinogens over a 70-year period in adults were also calculated alongside TEQ. The ΣTEQ values for carcinogens were 6.51 µg/kg for charcoal-grilled, 2.69 µg/kg for gas-grilled, and 4.73 µg/kg for oven-grilled chicken. These concentrations fall below the maximum allowable limit of 30 µg/kg set by the European Commission in 2014, indicating that the associated carcinogenic risk from consuming these foods is within safe thresholds [17]. Moreover, the ILCR outcomes from this study indicate minimal to acceptable risk levels, ranging from 10^{-4} to 10^{-6}. The US Environmental Protection Agency (EPA) considers ILCR values below 1×10^{-6} as negligible and values above 1×10^{-4} as potentially posing health risks [18]. ILCR values within the range of 1×10^{-6} to 1×10^{-4} suggest a tolerable risk level [18].

Table 5. Occurrence of PAHs concentration (mean ± standard deviation, n=12) in different types of chicken grilled methods.

PAHs Compounds	Mean ± Standard deviation			p-value
	Charcoal-grilled (µg/kg)	Gas-grilled (µg/kg)	Oven-grilled (µg/kg)	
Fluoranthene	1.17 ± 2.92	0.08 ± 0.29	0.83 ± 1.27	0.170
Benzo(b)fluoranthene	19.33 ± 16.04	16.17 ±14.41	13.25 ± 13.82	0.938
Chrysene	41.58 ± 32.12	31.25 ± 27.47	15.92 ± 21.48	0.082
Benzo(a)pyrene	2.33 ± 3.87	1.58 ± 1.38	1.08 ± 1.31	0.601
ΣPAH	64.41	49.08	31.08	

Table 6. Risk Assessment for Carcinogenic Risk based on Toxicity Carcinogenicity Equivalency Quotient (TEQ), Chronic Daily Intake (CDI), and Incremental Life Cancer Risk (ILCR) values of benzo(b)fluoranthene, chrysene, benzo(a)pyrene in grilled chickens.

Carcinogenic equivalency	Charcoal-grilled (µg/kg)			Gas-grilled (µg/kg)			Oven-grilled (µg/kg)		
	TEQ	CDI (µg/kg/day)	ILCR	TEQ	CDI (µg/kg/day)	ILCR	TEQ	CDI (µg/kg/day)	ILCR
Benzo(b)fluoranthene	0.0193	6.2×10^{-4}	4.5×10^{-4}	0.0133	4.3×10^{-4}	3.1×10^{-4}	0.0162	5.2×10^{-4}	3.8×10^{-4}
Chrysene	4.1583	1.3×10^{-3}	9.8×10^{-6}	1.5917	5.1×10^{-4}	3.7×10^{-6}	3.1250	1.0×10^{-3}	7.3×10^{-6}
Benzo(a)pyrene	2.3330	7.5×10^{-5}	5.5×10^{-4}	1.0833	3.5×10^{-5}	2.5×10^{-4}	1.5833	5.1×10^{-5}	3.7×10^{-4}
Σ TEQ	6.51			2.69			4.73		

Table 7. Risk Assessment for Non-Carcinogenic Risk based on Toxicity Carcinogenicity Equivalency Quotient (TEQ), Chronic Daily Intake (CDI), and Hazard Quotient (HQ) values of fluoranthene in grilled chickens.

Carcinogenic equivalency	Charcoal-grilled (µg/kg)			Gas-grilled (µg/kg)			Oven-grilled (µg/kg)		
	TEQ	CDI (µg/kg/day)	HQ	TEQ	CDI (µg/kg/day)	HQ	TEQ	CDI (µg/kg/day)	HQ
Fluoranthene	0.0012	8.8×10^{-5}	2.9×10^{-2}	0.0008	6.3×10^{-5}	2.1×10^{-2}	0.0001	6.3×10^{-6}	2.1×10^{-3}
Σ TEQ	0.0012			0.0008			0.0001		

Additionally, Table 7 presents the Hazard Quotient (HQ) for non-carcinogenic effects from consuming grilled chicken over 70 years in adults. Fluoranthene, classified as non-carcinogenic by the US EPA, exhibited HQ values of 2.9×10^{-2} for charcoal-grilled, 2.1×10^{-2} for gas-grilled, and 2.1×10^{-3} for oven-grilled chicken respectively. HQ values for all cooking methods were below 1, indicating minimal risk to human health from non-carcinogenic PAH exposure [19].

CONCLUSION

In conclusion, this study extensively examined the concentrations of polycyclic aromatic hydrocarbons (PAHs) in chicken prepared using charcoal-grilled, gas-grilled, and oven-grilled methods. It was found that charcoal-grilled chicken had the highest total PAH concentration, followed by gas-grilled and oven-grilled chicken, highlighting the significant impact of cooking methods on PAH levels. Despite these variations, statistical analysis using the nonparametric Kruskal-Wallis test did not reveal significant differences in PAH concentrations among the three cooking methods ($p > 0.05$). Health risk assessments employing Hazard Quotient (HQ) and Incremental Lifetime Cancer Risk (ILCR) analyses indicated minimal non-carcinogenic and carcinogenic risks associated with consuming grilled chicken under the study conditions, suggesting that current consumption practices of grilled chicken are within acceptable safety limits for the Malaysian adult population. Future research should focus on expanding sample sizes and implementing more controlled experimental conditions to further understand the factors influencing PAH accumulation in grilled foods. This will help refine risk assessments and develop targeted public health guidelines to mitigate potential health risks associated with PAH exposure from grilled chicken consumption. Advancements in detection technologies and cooking methodologies should also be pursued to enhance food safety practices and promote healthier dietary habits among consumers, ensuring continued safety in food consumption practices.

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