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Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Beef and Chicken Satay and their Potential Health Risk

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Polycyclic aromatic hydrocarbons (PAHs), known for their carcinogenic and mutagenic properties, are byproducts of the incomplete combustion of various organic sources. Our investigation examined forty beef and chicken satay samples prepared using two distinct grilling methods (charcoal and gas). These samples were analyzed for concentrations of four PAHs: fluoranthene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene using high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). The median and interquartile range (IQR) concentrations for each PAH were: fluoranthene 0.0 (19.5) µg/kg, benzo[b] fluoranthene 8.0 (9.0) µg/kg, chrysene 17.5 (65.8) µg/kg, and benzo[a]pyrene 1.0 (1.0) µg/kg, with chrysene exhibiting the highest and most variable concentrations. There were no statistically significant differences ($p>0.05$) in the concentrations of these PAHs between beef and chicken satay, although chicken satay showed greater variability in fluoranthene levels. Similarly, no significant difference in PAH concentrations was found between foods grilled with charcoal and gas ($p>0.05$), although gas grilling tends to result in higher overall PAH content. Our analysis concludes that the health risk associated with consuming the investigated beef and chicken satay, due to their PAH content, is negligible, providing reassurance regarding the safety of these food items.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); beef satay; chicken satay; health risk assessment

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Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that are generally colourless, white, or light-yellow solid substances made of two or more fused aromatic rings of carbon and hydrogen [1] . PAHs have low water solubility, low vapour pressure, and high melting and boiling points determined by their chemical structure [1]. PAHs with higher molecular weights tend to be less soluble in water and more likely to accumulate in fatty tissues, making them more breakdown-resistant [1]. In 1983, the United States Environmental Protection Agency (USEPA) designated 16 PAHs as high priority pollutants due to their high concentration, widespread exposure, resistance to degradation, and toxicity [2]. The 16 PAHs listed are 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 [2].

PAHs can be transported through air and water, eventually accumulating in marine environments seafood consumed by humans [3]. Their persistence in the environment is influenced by factors such as chemical composition, bioavailability, concentration, dispersal capacity, temperature, pH, oxygen levels, soil characteristics, and microbial nutrient availability for pollutant degradation [3]. PAHs enter food chain via two primary pathways: (i) environmental contamination from soil, water, and air pollution, and (ii) through food processing and cooking. High-temperature food processing methods such as frying, grilling, and roasting are significant contributors to PAHs in food [4]. During cooking at temperature exceeding 200ºC, oil from the food can drip onto the flame, producing fumes through a process called pyrolysis, which can contaminate the food with PAHs. The formation of PAHs during charcoal grilling depends on several factors such as fat content in the meat, the cooking temperature, and the duration of cooking [4].

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In Malaysia, 'satay' is a popular dish consisting of skewered and grilled meat, typically beef or chicken. It is prepared by marinating the meat in a blend of seasonings such as lemongrass, garlic, and turmeric, before grilling it over an open flame. Satay is frequently consumed in large quantities as an appetizer or snack and is widely available at various establishments, from street vendors and hawker stalls to restaurants across the country. It is typically served with rice cakes, cucumbers, and onions. Nonetheless, the prolonged grilling and high temperatures used in cooking process of satay may lead to the formation of PAHs. PAHs are recognized for their carcinogenic and mutagenic effects, capable of altering human genome and contributing to cancer development. Due to the higher level of carcinogens found in grilled foods compared those prepared using other methods, food like satay may contain significant PAH concentrations. Exposure to PAHs has been associated with the development of cancers such as breast, lung, and stomach cancer in humans. The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) have classified benzo[a]pyrene and benz[a]anthracene as carcinogenic to humans [5].

Consumers' health may be at risk from consuming satay with excessive concentration of PAHs [6]. Aside from that, the lack of regulations or guidelines regarding the permissible levels of PAHs in food sold at food premises in Malaysia is a notable food safety concern for consumers. Therefore, this study aimed to assess the concentrations of four types of PAH in beef and chicken satay grilled using charcoal and gas methods sourced from selected restaurants in Selangor, and to evaluate potential health risks associated with their consumption.

EXPERIMENTAL

Chemicals and Materials

HPLC grade solvents were used for the extraction process. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Carrez I and Carrez II solvents and the analytical PAHs standard (benzo[a]pyrene, fluoranthene, benzo[b]fluoranthene, and chrysene) were purchased from Sigma Aldrich (Supelco, USA).

Sample Collection

A total of 40 beef and chicken satay samples were selected randomly from various restaurants in Selangor. These samples were grilled using two different methods: charcoal and gas. Each sample was purchased randomly from different restaurants, immediately placed in zip-lock bags, transported to the laboratory in an ice box to maintain a cold chain at 4ºC, and stored at -20ºC in the freezer until extraction and analysis [4].

Sample Preparation

Prior to HPLC analysis, sample preparation was carried out following a method adopted from previous study [4]. Approximately 50.0 g of each samples was homogenized, and 3.0 g was weighted and transferred 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 added, followed by sealing and vigorous mixing. Tubes underwent 10 minutes sonication in an ultrasonic water bath at 40ºC. After sonication, the tubes were shaken at 120 rpm for 30 minutes on an orbital shaker to transfer the organic content into the solution phase. The tubeswere then centrifuged at 4,200×g for 5 minutes. The liquid phase was then transferred to another tube, and 1.3 mL 6 M hydrogen chloride (HCl) was added to adjust pH to 6. Following that, 1 mL of Carrez I and 1 mL of Carrez II solutions were added, followedby thorough shaking. The tubes were centrifuged again at 4,200×g for another 5 minutes. Finally, the 1.5 mL sample from the upper phase underwent filtration using 0.45 µm syringe tip filters and was transferred to HPLC vials.

Sample Analysis

The HPLC-FLD instrument (Shimadzu Prominence, Japan) was used to analyse the PAHs in all chicken and beef satay. A reverse phase µ-Bondapak C18 HPLC column (4.6 mm x 250 mm, 10.0 µm, Waters Co.) featuring polymeric C18 bonding specifically tailored for efficient PAH separation was employed. Before analysis, eluents underwent filtration using a 0.45 µm microporous membrane and were degassed via ultrasound for 15 minutes. The injection volume for all PAH compounds was set at 10 µl, with a flow rate of 1.8 ml/min maintained throughout the analysis. PAH separation occurred under isocratic conditions using a mixture of ultra-pure water (A) and acetonitrile (B) in a 65:35 ratio. The 65:35 isocratic elution program was selected after observing and testing various mobile phase compositions. Each PAH was detected using optimal excitation and emission wavelengths (Ex/Em λ) set at 260/440 nm, with a runtime of 10 min for each analysis. Chromatogram peaks were identified by comparing retention times with those of known PAH standards, and quantification was based on peak area.

Data Analysis

All data were analysed using IBM SPSS Statistical software version 26 (IBM SPSS Statistics, Inc., Chicago, IL, USA). Descriptive statistics analyses were calculated using range and mean PAH concentration. The significant differences between mean PAH values for various satay and cooking methods were assessed using the Mann-Whitney non-parametric test.

Health Risk Assessment

Health risks associated with beef and chicken satay consumption were calculated using similar equations used in previous study [7]. Toxicity equivalence factors (TEFs) [8] were applied to express the relative potency of different PAHs compared to the Benzo[a]pyrene equivalent factor (Equation 1). Ci represents the median concentrations of individual PAH compounds, each assigned with their respective TEFi. TEQB(a)P was determined by converting concentrations of all PAH components into Benzo[a]pyrene equivalent concentrations using their respective TEFs, as detailed in Table 1 [9].

$$TEQ\ B(a)P = TEFi \times Ci \qquad (1)$$

Equation 2 calculated the estimated chronic daily intake (CDI) of PAHs by calculating the ingestion rate for an adult resident over a lifetime.

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

IR represents the ingestion rate derived from the estimated intake of beef and chicken obtained from the Food Consumption Statistics of Malaysia 2014 [10]. The IR for meat and chicken was determined to be 0.00534 kg/day and 0.03301 kg/day, respectively. The adult population's exposure duration (ED) was 30 years. The adult population's exposure frequency (EF) was assumed to be 30.44 days/year, equivalent to once-a-month consumption. Malaysia's average adult body weight (BW) was 62.65 kg [10]. AT, which is the average lifespan for cancer risk, is set at 70 years (25,550 days), while the lifespan for non-cancer risk is set at 30 years (10,950 days), all based on WHO guidelines [11].

After calculating the CDI, the carcinogenic risk compounds were obtained by calculating the incremental life cancer risk (ILCR) using Equation 3. The carcinogenic risk associated with eating beef and chicken satay over 70 years of average time of cancer risks. Each PAH compound's slope factor (SF), shown in Table 2, was calculated individually.

$$ILCR = CDI \times SF \qquad (3)$$

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

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

Table 2 Cancer slope factor (CSf).

PAHs	**Code**	**CSf mg/kg/day**
Benzo[b]fluoranthene	BbF	7.3×10^{-1}
Chrysene	Chr	7.3×10^{-3}
Benzo[a]pyrene	BaP	7.30×10^{0}

Table 3. LOD, LOQ and regression coefficient of the standard solution of PAHs.

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 determined the presence of PAHs in beef and chicken satay and the potential health risks associated with their consumption. Four PAHs were identified by comparing HPLC retention times with PAH standards. Table 3 showcases the PAH standards' LOD, LOQ, and regression coefficients. Each analyte's correlation coefficient (R^2) ranged from 0.9877 to 0.9982. The R^2 and detection limits indicated that the method was suitable for investigating the selected PAHs. The LOD for the four PAH compounds in Table 3 ranged from 0.006 to 0.014 µg/kg, while the LOQ ranged from 0.017 to 0.045 µg/kg.

PAH Concentration in Satay Samples

Table 4 highlights the median concentration and interquartile range (IQR) of fluoranthene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene in 40 chicken satay samples. Fluoranthene shows a median concentration of 0.0 µg/kg, indicating that at least half of the samples have concentrations below the detection limit. However, the IQR of 19.5 µg/kg suggests variability among the samples, with some having higher concentrations. Benzo[b]fluoranthene has a median concentration of 8.0 µg/kg, meaning half of the samples have concentrations above this value and half below, with an IQR of 9.0 µg/kg indicating moderate variability. Chrysene exhibits the highest median concentration at 17.5 µg/kg and the greatest variability, with an IQR of 65.8 µg/kg, suggesting significant differences in chrysene levels among the samples. Benzo[a]pyrene shows a median concentration of 1.0 µg/kg and an IQR of 1.0 µg/kg, indicating low concentrations with little variability across the samples.

In general, chrysene has the highest and most variable concentrations among the PAHs, while fluoranthene has low median concentrations but some variability. Benzo[b]fluoranthene and benzo[a]pyrene show moderate and low concentrations with corresponding variabilities. The samples' higher concentrations of chrysene are due to their chemical stability and formation during grilling. Their multiple fused aromatic rings make them resistant to thermal degradation and oxidation [1]. Grilling at high temperature promotes the pyrolysis of organic matter, favouring the formation of these stable PAHs. Incomplete combustion, especially with charcoal, generates PAHs [1], with chrysene being prominent. High-fat foods like chicken satay drip fats onto the heat source, causing flare-ups and smoke that deposit PAHs onto the food [12]. Prolonged smoke exposure increases PAHs accumulation, leading to higher concentrations of these compounds in the samples [13].

PAH Concentration Between Beef and Chicken Satay

Table 5 compared the concentrations of four types of polycyclic aromatic hydrocarbons (PAHs) in beef and chicken satay using the non-parametric Mann-Whitney test. Both types of satays exhibit similar median concentrations for fluoranthene (0.00 µg/kg), but chicken satay shows a wider IQR of 61.25 µg/kg compared to beef at 11.00 µg/kg, indicating greater variability in chicken samples. Benzo[b]fluoranthene concentrations are 8.50 µg/kg in beef and 6.50 µg/kg in chicken, with comparable IQRs (10.00 µg/kg and 9.00 µg/kg, respectively). Chrysene levels are similar in beef (15.5 µg/kg) and chicken (16.0 µg/kg) satay, with similar IQRs (60.25 µg/kg and 71.25 µg/kg, respectively). Benzo[a]pyrene is present at median concentration of 1.00 µg/kg in both beef and chicken satay, with IQRs of 1.00 µg/kg and 1.75 µg/kg, respectively. Overall, there are no statistically significant differences in the concentrations of fluoranthene, benzo[b]fluoranthene, chrysene, and benzo[a]pyrene between beef and chicken satay. The concentration of PAHs in satay could be attributed to several factors, including variability in grilling methods, differences in fat content and composition, and the type of marinades or seasonings used, which can all influence PAH formation [14]. 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 satays [15]. Moreover, the higher levels of Chrysene in both chicken and beef satay could raise concerns about potential health risks, as these compounds are known carcinogens [16]. Although statistical insignificance suggests that the observed differences could be due to random variation, it is essential to consider the cumulative exposure to PAHs from various dietary sources. The findings from our study reveal median PAH concentrations that contrast with those reported by Haiba et al. [17], who found higher levels of total PAHs in chicken meat compared to beef. Similarly, Gorji et al. [12] observed higher PAH levels in grilled chicken than in beef, attributing this disparity to the higher fat content typically found in chicken.

Table 4. Median and IQR concentration of each PAH (µg/kg).

Types of PAHs	n	Concentration (µg/kg)	
		Median	IQR
Fluoranthene	40	0.0	19.5
Benzo[b]fluoranthene	40	8.0	9.0
Chrysene	40	17.5	65.8
Benzo[a]pyrene	40	1.0	1.0

Table 5. Concentration of each PAH in beef and chicken satay (µg/kg).

Type of PAHs	Median (IQR)		*P*-value
	Beef satay	Chicken satay	
Fluoranthene	0.00 (11.00)	0.00 (61.25)	0.071
Benzo[b]fluoranthene	8.50 (10.00)	6.50 (9.00)	0.356
Chrysene	15.5 (60.25)	16.00 (71.25)	0.855
Benzo[a]pyrene	1.00 (1.00)	1.00 (1.75)	0.954

PAHs Concentration Between Charcoal and Gas Grilling Method

Based on Table 6, the study compared PAH levels in food cooked using charcoal and gas grilling, using the Mann-Whitney non-parametric test. For both methods, Fluoranthene had median concentrations of 0.00 with high variability (IQR 21.00 for charcoal, 14.40 for gas). Benzo[b]fluoranthene was slightly higher for meat cooked using gas (median 8.50, IQR 9.75) compared to charcoal (median 8.00, IQR 9.00), while chrysene was significantly higher in meat cooked in gas (median 14.50, IQR 65.75) than charcoal (median 3.10, IQR 65.75). Benzo[a]pyrene was consistent across both cooking methods (median 1.00) but slightly more variable for gas (IQR 2.00) compared to charcoal (IQR 1.00). Despite these differences, the p-values were above 0.05, indicating no significant difference in PAH concentrations between the grilling methods. Thus, the type of grilling does not significantly affect PAH levels in the food. Although there is no significant difference between the charcoal and gas grilling methods, gas grilling method tends to exhibit a relatively higher overall PAHs content. The variability can be attributed to several factors. One possible cause is the difference in grilling temperatures and exposure times [4]. Gas grills typically allow for more consistent and controlled heating than charcoal grills, which can have fluctuating temperatures. Higher and more consistent temperatures in gas grilling could lead to increased PAH formation. Fat drippings from the meat onto the gas flames can produce more smoke and PAHs, which adhere to the meat [12]. In contrast, charcoal grilling, while does promote the formation of PAHs, might have less consistent heat, leading to lower levels of PAH. Related studies have shown that different cooking methods significantly affect PAH levels in food. For instance, a study by Sahin et al. [4] found that grilling meat over direct flames from gas or charcoal generally increases PAH levels compared to other cooking methods, such as baking or boiling. Another study by Öz [18] highlighted that the distance between the meat and the heat source and the presence of marinades can also affect PAH formation. These findings align with our observation that benzo[a]pyrene levels are higher in gas-grilled satay, suggesting that direct exposure to intense heat and smoke plays a critical role.

Table 6. Mean concentration of each PAH in charcoal and gas grilling method (µg/kg).

PAHs	Median (IQR) (µg/kg)		*P*-value
	Charcoal-grilled	Gas-grilled	
Fluoranthene	0.00 (21.00)	0.00 (14.50)	0.881
Benzo[b]fluoranthene	8.00 (9.00)	8.50 (9.75)	0.773
Chrysene	3.10 (65.75)	14.50 (65.75)	0.955
Benzo[a]pyrene	1.00 (1.00)	1.00 (2.00)	0.495

Health Risk Assessment

The potential health risk associated with consuming beef and chicken satay was assessed by comparing their carcinogenic toxicity TEQ with Benzo(a)pyrene, as detailed in Table 7. Chronic daily intake (CDI) and incremental lifetime cancer risk (ILCR) from consuming these foods over a 70-year period for adults were also presented alongside TEQ in the same table. The ΣTEQ B(a)P values for carcinogens were 1.80 µg/kg for beef and 1.67 µg/kg for chicken satay when prepared using the charcoal-grilled method. Conversely, using the gas-grilled method, these values were slightly higher at 1.86 µg/kg for both beef and chicken satay. These findings indicate that the levels of these carcinogens are well below the maximum allowable limit of 30 µg/kg set by the European Commission in 2014, confirming that the carcinogenic risk associated with consuming these foods is within safe limits [19]. Additionally, the ILCR results for this study indicate a negligible health risk, reinforcing the safety of consuming beef and chicken satay prepared using these methods. The US Environmental Protection Agency (USEPA) recognizes ILCR values below 1×10^{-6} as negligible and considers values above 1×10^{-4} to potentially pose health risks to individuals [20]. Furthermore, ILCR values falling within the range of 1×10^{-6} to 1×10^{-4} indicate a tolerable risk level [20]. These findings are consistent with reviews indicating that the carcinogenic risks associated with PAHs in food samples worldwide generally adhere to acceptable limits. However, diagnostic ratio analysis has shown that certain cooking methods, such as grilling or smoking, can significantly influence PAH levels in cooked foods [21].

Table 7. Risk Assessment for Carcinogenic Risk based on TEQ, CDI, and ILCR values of PAH in beef and chicken satay.

Type of Samples	Carcinogenic equivalency	Charcoal-grilled			Gas-grilled		
		TEQ	CDI	ILCR	TEQ	CDI	ILCR
Beef satay	Benzo[b]fluoranthene	0.80	1.03×10^{-7}	7.53×10^{-8}	0.85	8.04×10^{-8}	5.87×10^{-8}
	Chrysene	0.003	1.48×10^{-6}	1.08×10^{-8}	0.015	1.93×10^{-6}	1.41×10^{-8}
	Benzo[a]pyrene	1.00	7.71×10^{-9}	5.63×10^{-9}	1.0	7.71×10^{-9}	5.63×10^{-9}
	Σ TEQB(a)P	1.80			1.86		
Chicken satay	Benzo[b]fluoranthene	0.65	1.99×10^{-7}	1.45×10^{-7}	0.85	1.96×10^{-7}	1.43×10^{-7}
	Chrysene	0.02	4.86×10^{-6}	3.55×10^{-8}	0.015	4.79×10^{-5}	3.50×10^{-7}
	Benzo[a]pyrene	1.00	3.18×10^{-8}	2.32×10^{-8}	1.0	6.24×10^{-6}	4.56×10^{-6}
	Σ TEQB(a)P	1.67			1.86		

CONCLUSION

In conclusion, our comprehensive investigation into the concentrations of four PAHs in beef and chicken satay prepared using charcoal and gas grilling methods revealed crucial insights into the safety of these popular dishes. Despite variations in PAH concentrations, our analysis found no significant differences between the two types of meat or among the grilling methods. The negligible health risk associated with consuming beef and chicken satay, as indicated by the PAH content, provides reassurance regarding the safety of these food items. Future research could expand upon these findings by exploring additional factors influencing PAH formation during grilling, such as marinating techniques and cooking temperatures. Longitudinal studies tracking PAH levels and assessing dietary intake patterns could also provide valuable insights into the long-term health implications of consuming grilled foods. Additionally, investigating alternative cooking methods and their effectiveness in reducing PAH formation could inform strategies for minimising health risks associated with PAH exposure. These avenues for future research could contribute to a more comprehensive understanding of PAH contamination in grilled foods and support efforts to enhance food safety and public health measures.

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