

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN GRILLED MEAT AND FISH PRODUCTS AND ITS POTENTIAL HEALTH RISK

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ABSTRACT

The study highlights the investigation of the presence of polycyclic aromatic hydrocarbons (PAHs) in grilled meat and fish products. Specifically, the levels of benzo(a)pyrene, fluorine, pyrene and chrysene were determined for 30 samples of chicken satay, beef satay and grilled fish. The method was based on a Soxhlet extraction step, followed by solid phase extraction (SPE) clean-up and finally, the determination of PAHs using high performance liquid chromatography (HPLC) with fluorescence detector. The highest concentration of the total three studied PAHs compounds was 35.43 µg/kg found in chicken satay, while the intermediate concentration was 16.52 µg/kg in grilled fish and the lowest was 21.02 µg/kg in beef satay. The result shows that no significant difference ($p>0.05$) in the concentration of PAHs between stalls. In addition, there were significant differences ($p<0.05$) in the concentration of benzo(a)pyrene, fluorine and pyrene among chicken satay, beef satay and grilled fish. Finally, Hazard quotient (HQ) and incremental lifetime cancer risk (ILCR) approaches were used to estimate health risk. The HQ and ILCR calculations showed no non-carcinogenic and carcinogenic risk, respectively, towards Malaysian population who consumed chicken satay, beef satay and grilled fish.

Keywords: Polycyclic aromatic hydrocarbons, chicken and beef satay, grilled fish, high performance liquid chromatography, risk assessment

1.0 INTRODUCTION

Increasing food safety issues are becoming a global challenge and some researches have been conducted regarding the hazards caused by the consumption of food contaminated with various pollutants. In recent time, there has been increased interest in the topic of the toxicity of polycyclic aromatic hydrocarbons (PAHs) among international regulatory bodies according to the degree of occurrence and toxicity (Onyedikachi et al., 2019). PAHs are a group of compounds that are formed during the incomplete combustion of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat (Agency for Toxic Substances & Disease Registry, 2015). PAHs are pollutants ubiquitous found in water, air, soil, sediments, and food and consist of two or more fused aromatic rings (Wang et al., 2020). PAHs are compounds that are high in molecular weight, non-polar which are soluble in water, lipophilic, hydrophobic, and can mix with organic solvents (Sojinu et al., 2019). Besides, PAHs are known to have toxic properties, are low bio-degradable, and can bio-accumulate (United Nations Economic Commission for Europe, 1999).

The Environmental Protection Agency (EPA) have been listed 16 PAHs as priority pollutants, namely naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorine (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHRY), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-c,d]pyrene (IND), and dibenz[a,h]anthracene (DahA). Those 16 listed PAHs are known as environmental concerns as they have potential toxicity in humans as well as other organisms and, its prevalence and persistence in the environment (Hussar et al., 2012). Some of them are

of considerable interest due to their carcinogenic and genotoxic effects in laboratory animals and shown to cause several types of human cancers, such as breast, lung, and colon (Ishizaki et al., 2010).

Humans are exposed to PAHs mainly through the consumption of food, apart from smoking and occupational exposure (Li et al., 2016). Various food items and beverages may contain PAHs as contaminants, including drinking water, vegetable, fruits, cereals, oils, fish, and meat (Jahurul et al., 2013). Due to the high solubility in lipids, PAHs can move through the food chain into hydrophobic compartments and can accumulate in food rich in fats (Naccari et al., 2011). Besides, a few sources have been associated with the contamination of PAHs in food, which are environmental contamination from the soil, water and atmosphere, and contamination of packaging materials and thermal treatment used for the preparation and manufacturing of food (Jahurul et al., 2013). In addition, food processing methods like curing, drying, smoking, roasting, grilling, barbecuing and refining are known to produce and increase the level of PAHs in food (Tongo et al., 2017).

There has been extensive research into the detection of PAHs in grilled meat and fish products and it was found that almost all grilled products contain PAHs. Mohammadi et al. (2018) analyzed the levels of naphthalene, fluoranthene, phenanthrene, anthracene, pyrene, and benzo[a]pyrene in Iranian popular grilled beef and chicken dishes. It was found that the concentration of total PAHs in grilled beef (21.95 ng/g) was higher than in grilled chicken (0.29 ng/g). Besides, Sahin et al. (2020) in their study evaluated grilled fish samples in terms of PAH4 (benz(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene and chrysene), and it was reported that total PAH4 concentrations in grilled fish were 3.30 µg/kg.

Furthermore, some Malaysian popular grilled dishes that are prepared at high temperature might produce PAHs, such as satay, grilled chicken and grilled fish (Farhadian, et al., 2010). Due to their good flavour and high nutritional values, grilled meat products have gained an interest in homes and restaurants (Jiang et al., 2018). In order to protect public health, it is necessary to assess the health risk of PAHs in food. However, there is a limited literature data on human health risk assessment due to dietary exposure of PAHs in grilled foods in Malaysia. Therefore, the objective of the present study was firstly to identify the concentration of PAHs in Malaysian popular grilled meat and fish products. The second objective was to compare the concentration of PAHs and the last objective was to investigate the health risk associated with consumption of the selected grilled foods.

2.0 MATERIALS AND METHOD

2.1 Sampling Design and Sample Collection

The sampling method used in this study was purposive sampling. The objective of this sampling method is to select only samples which are relevant to this study. In this study, 30 samples of ready to eat grilled meat and fish products were purchased from five local food stalls in the Puncak Alam and Saujana Utama, Selangor area. The selected grilled meat and fish products for this study were beef satay (10), chicken satay (10), and grilled fish (10). Once the food items were purchased, they were kept in the cool box with a temperature of approximately 4°C. The samples were transported to the laboratory within 24 hours.

2.2 *Sample Preparation for PAHs Determination*

The samples were homogenized using a blender. Then, the samples were pooled and mixed together, and stored at -18°C prior to extraction and analysis. After that, the extraction procedure was performed according to the method described by Mohammadi & Valizadeh-kakhki (2018) with some modifications. Briefly, the samples of chicken satay, beef satay and grilled fish (5 g) were weighed and mixed with anhydrous sodium sulphate (5 g) in a cellulose thimble. The samples were then extracted with methanol (250 mL) using Soxhlet apparatus for 8 hours. The extracted samples were collected for further clean-up.

The solid phase extraction (SPE) clean-up procedure was carried out based on the method described by Pan & Cao (2010) with some modifications. Firstly, the C-18 cartridge was pre-treated by rinsing with dichloromethane (5 mL), methanol (5 mL) and water (5 mL). Next, extracted samples (2 mL) were loaded and passed through the C-18 cartridge. After that, the cartridge was washed with water (5 mL) and vaporized to dryness. Then, the PAHs fraction that retained in the C-18 cartridge were eluted using dichloromethane (6 mL). The dichloromethane extract, which contained PAHs, was evaporated to dryness by rotary evaporator. The residue was dissolved in acetonitrile (250 µL) for further HPLC analysis.

2.3 *Preparation of Standard Solution*

Standards for PAHs (benzo(a)pyrene, fluorene, pyrene and chrysene) were purchased in powder form (100 mg). Four stock standards of 100 ppm were prepared by diluting the standards in acetonitrile and stored in the refrigerator at -20°C.

2.4 Preparation of Calibration Curve

The quantification of PAHs compounds in grilled meat and fish was carried out by the use of an external calibration curve method. The quantification of PAHs was performed using five diluted standard calibration solutions in the range of 0.01-1.00 ppm (Jahurul et al., 2013). After that, the calibration curve was constructed by plotting the mean peak area against PAHs standard concentration. Table 2.1 showed the linearity equations, with R^2 higher than 0.99 for all the four PAHs standards.

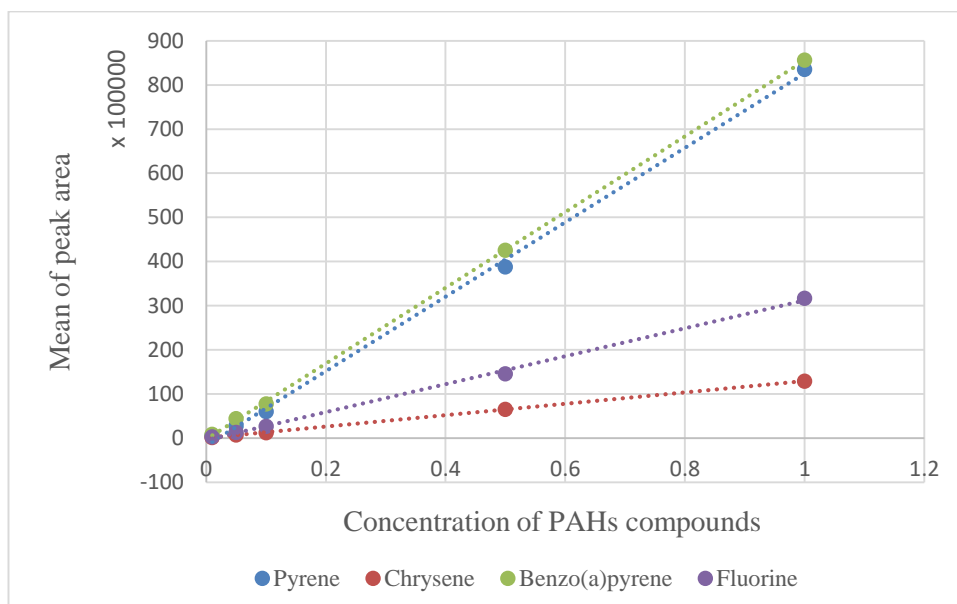


Figure 2.1 Calibration curve.

Table 2.1 Linearity equations and correlation coefficient (R^2) of four PAHs standards.

PAHs compounds	Linearity equations	Correlation coefficient (R^2)
Benzo(a)pyrene	$Y = 9E+07x - 218425$	0.999
Pyrene	$Y = 8E+07x - 2E+06$	0.999
Fluorine	$Y = 3E+07x - 411652$	0.999
Chrysene	$Y = 1E+07x + 15458$	0.999

2.5 HPLC Analysis

All samples were analysed using high-performance liquid chromatography (HPLC) apparatus coupled with a fluorescence detector. The mobile phase is composed of acetonitrile and water (80:20) and a flow rate of 1 mL/min. The injection volume of 20 µl was used to inject the sample into the HPLC column (Jahurul et al., 2013). The detection of fluorescence was carried out by adjusting the time of emission and excitation wavelengths (Table 2.2).

Table 2.2 The wavelength program for the fluorescence detection of individual PAHs.

PAHs compounds	Retention time (min)	Excitation (nm)	Emission (nm)
Benzo(a)pyrene	20.0	280	410
Chrysene	18.0	270	385
Fluorene	14.6	250	420
Pyrene	14.6	250	420

2.6 Estimation of Health Risk Assessment

In this study, both non-cancer and cancer risk were evaluated for PAHs through the consumption of chicken satay, beef satay and grilled fish. The health risk were estimated by calculating the chronic daily intake (CDI) first, then hazard quotient (HQ), for non-carcinogenic risk calculation, and incremental lifetime cancer risk (ILCR), for carcinogenic risk calculation. All formulas that have been used in this study were developed by USEPA (2019). The CDI value was calculated based on the following equation:

$$CDI = C \times EFr \times ED \times IR \times CF / BW \times AT \quad (1)$$

Where C = concentration of individual PAHs compounds in each sample; EFr = exposure frequency (365 days/year); ED = exposure duration (year) (for adults: ED

= 43; for children: ED = 7); IR = ingestion rate (for chicken satay: IR = 2.16 g/day; for beef satay; IR = 0.76 g/day; for grilled fish: IR = 0.38 g/day) (Jahurul et al., 2013); BW = average body weight (kg) (for men: BW = 66.56 kg; for women: BW = 58.44 kg; for children: BW = 32.30 kg) (Azmi et al., 2009 & Yang et al., 2017); AT = average time exposure (days) (for cancer risk: AT = 70 years x 365 days/year; for non-cancer risk: AT = ED x 365 days/year); CF = conversion factor (10^{-3} mg/ng).

2.6.1 Non-cancer Risk

Non-cancer risk for individual PAHs compounds was calculated based on the following equation:

$$HQ = CDI / RfD \quad (2)$$

Where HQ = hazard quotient; CDI = chronic daily intake; RfD = reference dose (mg/kg/day) (for benzo(a)pyrene: RfD = 3×10^{-4} ; for fluorine: RfD = 6×10^{-2} ; for pyrene: RfD = 3×10^{-2}) (USEPA, 1987, 1990 & 2017).

2.6.2 Cancer Risk

Cancer risk for benzo(a)pyrene was calculated based on the following formula:

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

Where ILCR = incremental lifetime cancer risk; CDI = chronic daily intake; SF = slope factor for benzo(a)pyrene (1 mg/kg/day) (USEPA, 2017).

2.7 Statistical Analysis

The data obtained was analysed using Statistical Package for Social Science (SPSS) software version 21.0. The results on PAHs concentration were analysed using

analysis of variance (ANOVA) to compare the mean of PAHs concentration between different types of stall and between different types of food samples.

3.0 RESULT AND DISCUSSION

3.1 Mean of PAHs Concentration in Chicken Satay, Beef Satay and Grilled Fish

Figure 3.1(a) showed the mean concentration of benzo(a)pyrene, fluorine, pyrene chrysene in chicken satay. Based on the bar graph, the highest and the lowest individual total levels of PAHs detected in chicken satay were pyrene (30.48 $\mu\text{g}/\text{kg}$) and fluorine (0.8 $\mu\text{g}/\text{kg}$), respectively. The PAHs levels observed in the present study were compared with those reported in the previous studies. Hamzawy et al. (2016) reported that pyrene and fluorene were found at concentrations of 66.48 $\mu\text{g}/\text{kg}$ and 51.83 $\mu\text{g}/\text{kg}$, respectively, in charcoal grilled chicken. However, their results were higher to be compared with the present study. In another study by Sahin et al., (2020), fluorine was detected at concentration of 0.24 $\mu\text{g}/\text{kg}$, which was lower than current study. While pyrene was not detected in grilled chicken. On the other hand, accumulation of PAHs in chicken satay can be the result of fat dripping onto the flame or hot coals that are carried back on the food surface during the grilling process (Wang et al., 2019).

Figure 3.1(b) presented the mean concentration of benzo(a)pyrene, fluorine, pyrene and chrysene in beef satay. Among beef satay, it was found that fluorine (7.7 $\mu\text{g}/\text{kg}$) was the highest PAHs compound present in beef satay samples. On the contrary, chrysene (3.23 $\mu\text{g}/\text{kg}$) showed the lowest concentration in beef satay. In a study by Hasyimah et al. (2020), fluorine and chrysene were found at concentrations

of 8.47 ng/g and 5.77 ng/g respectively, and these readings were higher to be compared with present study. Based on the findings of Jiang et al., (2018), fluorine and pyrene were detected in grilled meat at levels of 11.4 µg/kg and 0.98 µg/kg, respectively. Their findings were a bit higher for fluorine, while lower for chrysene than the present study. In addition, pyrolysis combined with smoke deposition during open flame grilling facilitates the creation of PAHs in beef satay.

Figure 3.1(c) displayed the mean of PAHs concentration in grilled fish. Among grilled fish, the highest mean of PAHs was fluorine (9.5 µg/kg), whereas the lowest mean of PAHs was benzo(a)pyrene (1.14 µg/kg). Racovita et al. (2021) in their study showed, the concentration of fluorine and benzo(a)pyrene in smoked fish were 10.6 ng/g and 0.5 ng/g, respectively. The level of benzo(a)pyrene in current study was a bit higher than in the previous study. However, the level of benzo(a)pyrene in the present study was lower than the level established by the European Food Safety Authority (2011). Furthermore, Asamoah et al., (2021) revealed that benzo(a)pyrene and fluorine were detected in smoked fish with concentrations of 15.51 µg/kg and 34.77 µg/kg, respectively. Their results were slightly higher than the current study. The presence of PAHs in grilled fish might be because of the direct deposition of smoke generated during incomplete combustion of thermal source (Wang et al., 2019).

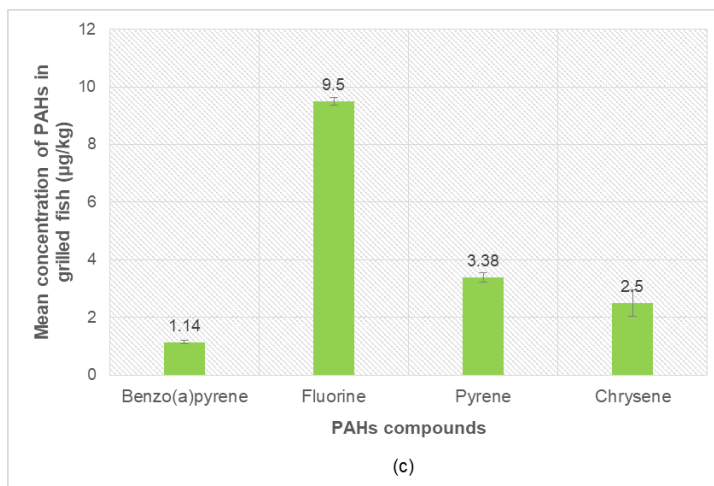
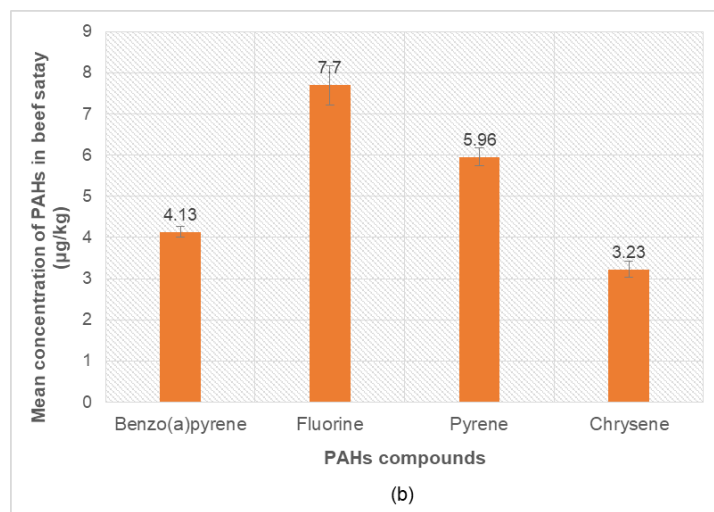
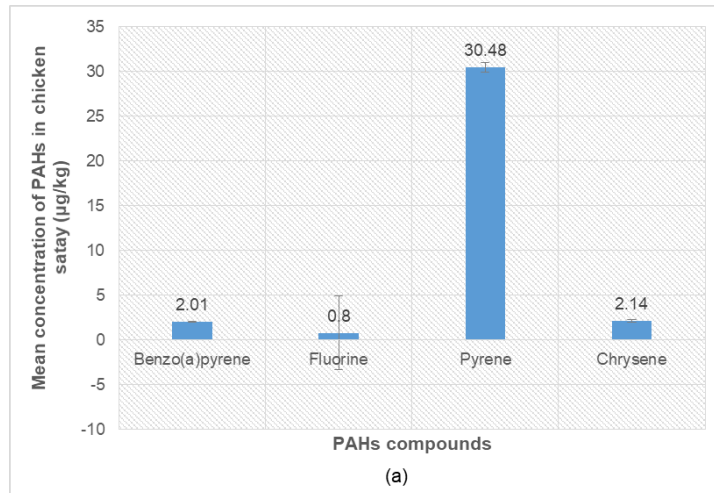


Figure 3.1 Mean of PAHs concentration in (a) chicken satay, (b) beef satay and (c) grilled fish.

3.2 Comparison of Stalls and Type of Grilled Food Based on the Concentration of PAHs

The mean of PAHs concentration based on stall (A, B, C, D and E) were presented in Figure 3.2. The highest and lowest concentrations of benzo(a)pyrene among investigated samples were nominated for beef satay from stall B and grilled fish also from stall B, respectively. While for fluorine, the highest concentration was found in grilled fish from stall C and the lowest concentration was in chicken satay from stall A. Furthermore, the lowest mean of pyrene was grilled fish from stall A, while the highest mean of pyrene was in chicken satay also from Stall A. Moreover, the lowest mean of chrysene was determined in beef satay from Stall A, while the highest mean of pyrene was in chicken satay from stall D. In a local study conducted by Jahurul et al., (2013), the sum of three PAHs (benzo(a)pyrene, benzo(b)fluoranthene and fluoranthene) in chicken satay, beef satay and charcoal grilled fish were 42.31 ng/g, 66.28 and 40.69 ng/g, respectively.

Figure 3.3 displayed the mean of PAHs concentration based on three different types of food samples. The highest level of benzo(a)pyrene was found in beef satay, while the lowest was in grilled fish. In addition, the highest and the lowest mean of fluorine was determined in grilled fish and chicken satay, respectively. Furthermore, for pyrene, the highest level was in chicken satay and the lowest level was in grilled fish. Beef satay contains the highest level of chrysene, whereas chicken satay contains the lowest level of chrysene. Farhadian et al., (2010) reported that the total levels of benzo(a)pyrene, benzo(b)fluoranthene and fluoranthene in chicken satay, beef satay and grilled fish were 25.61 ng/g, 103.32 ng/g and 11.73, respectively.

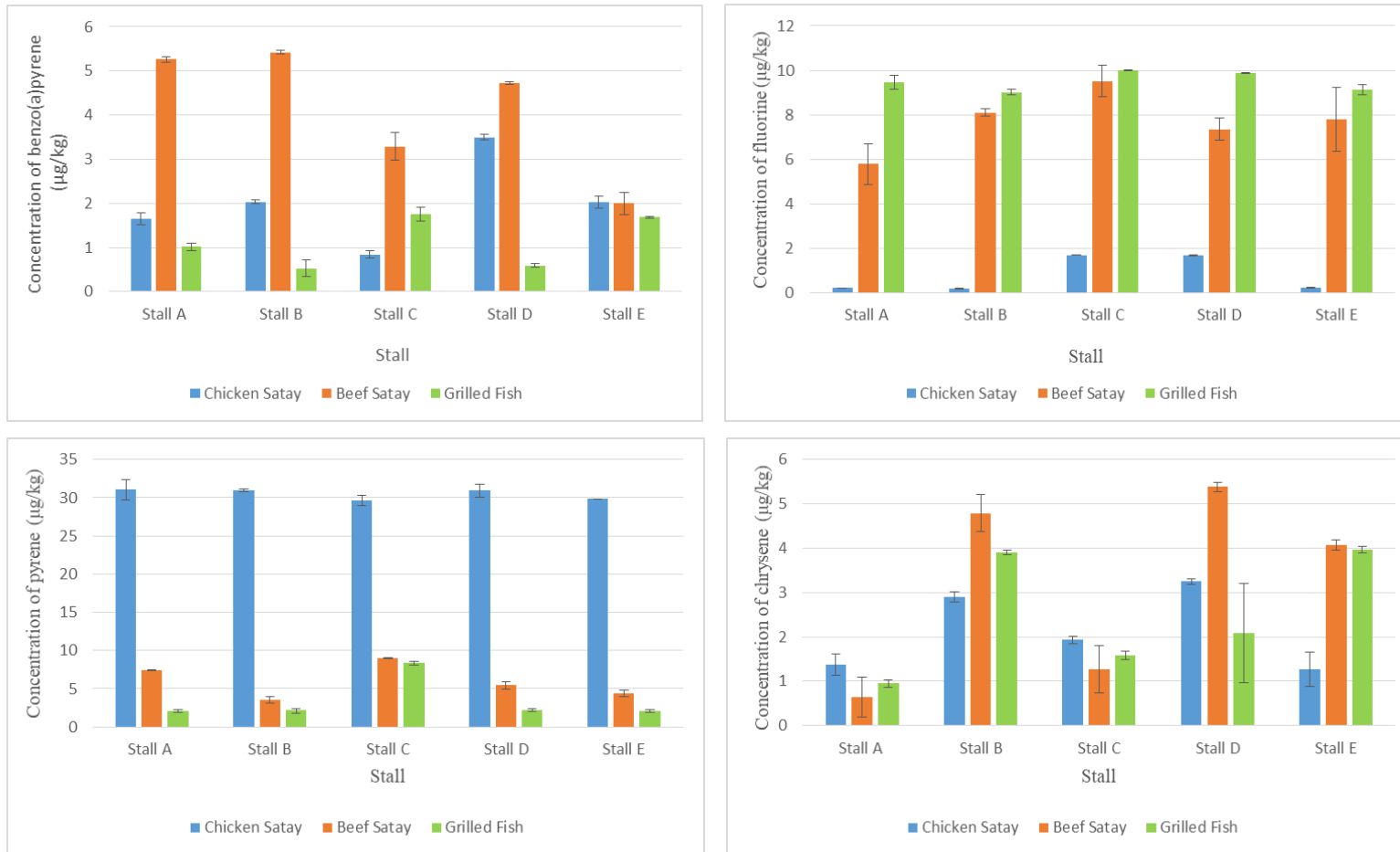


Figure 3.2 Mean concentration of PAHs based on stall according to different type of PAHs compounds.

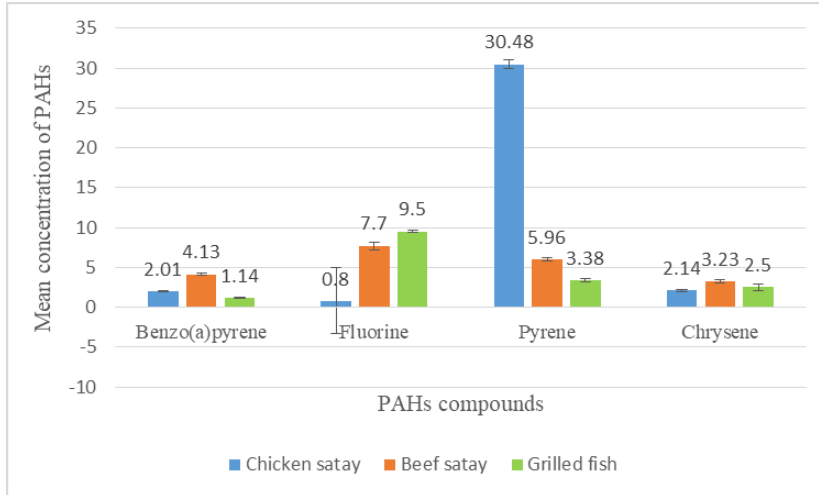


Figure 3.3 Mean concentration of PAHs based on different types of food samples.

A one-way ANOVA test was conducted to compare the mean of PAHs concentration between stalls and between the three types of food samples. The statistical result from Table 3.1 showed no significant difference ($p > 0.05$) in the concentration of benzo(a)pyrene, fluorine, pyrene and chrysene in chicken satay, beef satay and grilled fish among five stalls (Stall A, B, C, D and E) was observed. This can be probably due to the method of grilling, the duration of grilling and the temperature used (Farhadian et al., 2012). Furthermore, based on Table 4.3, there were significant differences ($p < 0.05$) in the concentrations of benzo(a)pyrene, fluorine and pyrene among chicken satay, beef satay and grilled fish. The finding is in agreement with a previous study conducted by Jahurul et al. (2013), where there was also significant difference ($p < 0.05$) in the levels of fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene in meat and fish products. Furthermore, the present study indicates that the ingredients used to marinate the food samples and the variation in fat content has a significant effect on the concentration of PAHs generated in the food samples

(Jiang et al., 2018). Nevertheless, no significant difference ($p>0.05$) in the concentration of chrysene between three different types of food samples was observed.

Table 3.1 Mean concentrations and statistical result of one-way ANOVA test (p-value).

PAHs	Stall	Chicken Satay	Beef Satay	Grilled Fish	p-value
		Mean ($\mu\text{g}/\text{kg}$)	Mean ($\mu\text{g}/\text{kg}$)	Mean ($\mu\text{g}/\text{kg}$)	
Benzo(a)pyrene	Stall A	1.65	5.26	1.02	0.986 ($p>0.05$)
	Stall B	2.03	5.42	0.53	
	Stall C	0.84	3.29	1.76	
	Stall D	3.5	4.72	0.59	
	Stall E	2.03	2	1.69	
	p-value		0.015 ($p<0.05$)		
Fluorene	Stall A	0.21	5.79	9.47	0.764 ($p>0.05$)
	Stall B	0.19	8.1	9.02	
	Stall C	1.69	9.51	10.01	
	Stall D	1.69	7.35	9.87	
	Stall E	0.22	7.79	9.13	
	p-value		0.004 ($p<0.05$)		
Pyrene	Stall A	31.04	7.45	2.04	0.925 ($p>0.05$)
	Stall B	30.95	3.54	2.22	
	Stall C	29.64	8.95	8.42	
	Stall D	30.92	5.47	2.16	
	Stall E	29.83	4.37	2.05	
	p-value		0.004 ($p<0.05$)		
Chrysene	Stall A	1.37	0.65	0.95	0.073 ($p>0.05$)
	Stall B	2.9	4.79	3.91	
	Stall C	1.93	1.27	1.58	
	Stall D	3.25	5.38	2.08	
	Stall E	1.27	4.07	3.96	
	p-value		0.692 ($p>0.05$)		

3.3 Health Risk Assessment

Three approaches have been carried out to estimate the human health risk due to consumption of PAHs in chicken satay, beef satay and grilled fish in the present study, as described in the methodology section.

3.3.1 Non-cancer Risk

Table 3.2 demonstrated the chronic daily intake (CDI) and hazard quotient (HQ) of the investigated PAHs compounds via the consumption of selected grilled foods for three groups of population, including men, women and children. However, HQ estimation for chrysene was unable to calculate due to lack of information data.

The rank order of the population group who consumed chicken satay based on their HQ were children > women > men. Also, the rank order of the population group based on their HQ for beef satay were children > women > men. Furthermore, the trend of the population group according to their HQ for grilled fish were children > women > men. The trend of the population groups based on their HQ were the same for all food samples. As observed from the result, the value of HQ for benzo(a)pyrene, fluorine and pyrene in chicken satay, beef satay and grilled fish were higher for children. This is because children's body weight was slightly lower than both men and women, resulting in significantly high-risk value for children (Jiang et al., 2018). Furthermore, the HQ for all grilled food samples were less than 1 (HQ<1), indicating that no possible health risk to the majority of the population by consuming chicken satay, beef satay and grilled fish.

Table 3.2 Chronic daily intake (CDI) and hazard quotient (HQ) for different population groups.

Food sample	PAHs compound	Population group	Health Risk Assessment		
			CDI	HQ	
Chicken satay	Benzo(a)pyrene	Men	6.52×10^{-5}	0.22	
		Women	7.43×10^{-5}	0.25	
		Children	1.34×10^{-4}	0.45	
	Fluorine	Men	2.60×10^{-5}	4.33×10^{-4}	
		Women	2.96×10^{-5}	4.93×10^{-3}	
		Children	5.35×10^{-5}	8.92×10^{-3}	
	Pyrene	Men	9.89×10^{-4}	0.03	
		Women	1.13×10^{-3}	0.04	
		Children	2.04×10^{-3}	0.07	
		Benzo(a)pyrene	Men	4.72×10^{-5}	0.16
			Women	5.37×10^{-5}	0.18
			Children	9.72×10^{-5}	0.32
Beef satay	Fluorine	Men	8.79×10^{-5}	1.47×10^{-3}	
		Women	1.00×10^{-4}	1.67×10^{-3}	
		Children	1.81×10^{-4}	3.02×10^{-3}	
	Pyrene	Men	6.81×10^{-5}	2.29×10^{-3}	
		Women	7.75×10^{-5}	2.58×10^{-3}	
		Children	1.40×10^{-4}	4.67×10^{-3}	
	Benzo(a)pyrene	Men	6.51×10^{-5}	0.02	
		Women	7.41×10^{-6}	0.02	
		Children	1.34×10^{-5}	0.04	
		Fluorine	Men	5.42×10^{-4}	9.03×10^{-4}
			Women	6.18×10^{-5}	1.03×10^{-3}
			Children	1.12×10^{-4}	1.87×10^{-3}
Pyrene	Men	1.93×10^{-5}	6.43×10^{-4}		
	Women	3.91×10^{-6}	1.30×10^{-4}		
	Children	3.98×10^{-5}	1.33×10^{-3}		

3.3.2 *Cancer Risk*

Incremental lifetime cancer risk (ILCR) equation was used to calculate the possible carcinogenic risk towards the population who consumed the contaminated grilled foods. The acceptable level for cancer risk mentioned by the United States Environmental Protection Agency (USEPA) was within the range of 10^{-6} to 10^{-4} (Sultana et al., 2017). Among four PAHs compounds studied, benzo(a)pyrene was considered as carcinogenic to humans and acts as representative PAHs (Jiang et al., 2018). That is why it is used to calculate ILCR.

The result of carcinogenic health risk due to ingestion of PAHs via consumption of chicken satay, beef satay and grilled fish was presented in Table 3.3. The rank values of ILCR according to the population group for chicken satay were women (4.56×10^{-5}) > men (4.01×10^{-5}) > children (1.34×10^{-5}). Furthermore, the trend values of ILCR due to ingestion of beef satay based on population group were women (3.30×10^{-5}) > men (2.90×10^{-5}) > children (9.72×10^{-6}). Lastly, for grilled fish, the rank values of ILCR according to the population group in decreasing order was women (4.55×10^{-6}) > men (4.00×10^{-6}) > children (1.34×10^{-6}).

According to the result, health risk assessment of dietary exposure to grilled meat and fish products was in the USEPA acceptable level, indicating cancer risk is tolerable for consumers. Among the three population groups, women suffered from highest carcinogenic risk, followed by men and children. The estimated health risk due to consumption of grilled food samples are compared with the data obtained in the published studies. Jiang et al. (2018) reported that ILCR due to consumption of grilled and fried meats in Shandong of China were ranging from 1.02×10^{-6} to 3.75×10^{-6} for different groups, which were slightly lower than the result in this study. Racovita et

al., (2021) presented that the ILCR value associated with consumption of smoked mackerel among adults in Ghana was 1.7×10^{-4} , which was lower than the present result. The difference in value of ILCR can be due to the lower ingestion rate among men, women and children, and also lower concentration of benzo(a)pyrene (Shariatifar et al., 2020).

Table 3.3 Incremental lifetime cancer risk (ILCR) values for different population groups.

PAHs compound	Food sample	Population group	ILCR
Benzo(a)pyrene	Chicken satay	Men	4.01×10^{-5}
		Women	4.56×10^{-5}
		Children	1.34×10^{-5}
	Beef satay	Men	2.90×10^{-5}
		Women	3.30×10^{-5}
		Children	9.72×10^{-6}
	Grilled fish	Men	4.00×10^{-6}
		Women	4.55×10^{-6}
		Children	1.34×10^{-6}

4.0 CONCLUSION

In conclusion, chicken satay, beef satay and grilled fish were analysed using a high performance liquid chromatography with fluorescence detection method for the determination of PAHs. It was found that benzo(a)pyrene, fluorine, pyrene and chrysene were present in all of the samples. However, the level of benzo(a)pyrene was not exceeding the permissible limit set by the EFSA. Moreover, the risk assessment results indicated that by consuming chicken satay, beef satay and grilled fish,

Malaysian population group (men, women and children) will not pose any non-carcinogenic and carcinogenic health risk. Although the risk levels due to PAHs exposure for Malaysian population were at acceptable range, frequent and excessive consumption of such foods for a long duration can be very harmful for human health. Therefore, it is important to conduct more studies to assess the level of PAHs in other popular grilled and fried food in Malaysia. Besides, more studies on the health risk estimation due to consumption of other food products is crucial with the aim to protect public health and to spread awareness on the health effects of PAHs among consumers in Malaysia.

5.0 REFERENCES

- Agency for Toxic Substances & Disease Registry. (n.d.). *Toxic Substances Portal - Polycyclic Aromatic Hydrocarbons (PAHs)*. <https://www.atsdr.cdc.gov/phs/phs.asp?id=120&tid=25>
- Asamoah, E. K., Nunoo, F. K. E., Addo, S., Nyarko, J. O., & Hyldig, G. (2021). Polycyclic aromatic hydrocarbons (PAHs) in fish smoked using traditional and improved kilns: Levels and human health risk implications through dietary exposure in Ghana. *Food Control*, *121*(August 2020), 107576. <https://doi.org/10.1016/j.foodcont.2020.107576>
- Azmi, M. Y., Jr, Junidah, R., Siti Mariam, A., Safiah, M. Y., Fatimah, S., Norimah, A. K., Poh, B. K., Kandiah, M., Zalilah, M. S., Wan Abdul Manan, W., Siti Haslinda, M. D., & Tahir, A. (2009). Body Mass Index (BMI) of Adults: Findings of the Malaysian Adult Nutrition Survey (MANS). *Malaysian journal of nutrition*, *15*(2), 97–119.
- European Food Safety Authority (EFSA) (2011). *Amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs*. Commission Regulation (EU) No 835/2011.
- Farhadian, A., Jinap, S., Abas, F., & Sakar, Z. I. (2010). Determination of polycyclic

- aromatic hydrocarbons in grilled meat. *Food Control*, 21(5), 606–610. <https://doi.org/10.1016/j.foodcont.2009.09.002>
- H. Hamzawy, A., Khorshid, M., Elmarsafy, A. M., & Souaya, E. R. (2016). Estimated Daily Intake and Health Risk of Polycyclic Aromatic Hydrocarbon by Consumption of Grilled Meat and Chicken in Egypt. *International Journal of Current Microbiology and Applied Sciences*, 5(2), 435–448. <https://doi.org/10.20546/ijcmas.2016.502.049>
- Hussar, E., Richards, S., Lin, Z., Dixon, R. P., & Johnson, K. A. (2012). *Human Health Risk Assessment of 16 Priority Polycyclic Aromatic Hydrocarbons in Soils of Chattanooga, Tennessee, USA*. 5535–5548. <https://doi.org/10.1007/s11270-012-1265-7>
- Ishizaki, A., Saito, K., Hanioka, N., Narimatsu, S., & Kataoka, H. (2010). Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection. *Journal of Chromatography A*, 1217(35), 5555–5563. <https://doi.org/10.1016/j.chroma.2010.06.068>
- Jahurul, M. H. A., Jinap, S., Zaidul, I. S. M., Sahena, F., Farhadian, A., & Hajeb, P. (2013). Determination of fluoranthene, benzo[b]fluoranthene and benzo[a]pyrene in meat and fish products and their intake by Malaysian. *Food Bioscience*, 1, 73–80. <https://doi.org/10.1016/j.fbio.2013.03.006>
- Farhadian, A., Jinap, S., Faridah, A., & Zaidul, I. S. M. (2012). Effects of marinating on the formation of polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[b]fluoranthene and fluoranthene) in grilled beef meat. *Food Control*, 28(2), 420–425. <https://doi.org/10.1016/j.foodcont.2012.04.034>
- Li, G., Wu, S., Wang, L., & Akoh, C. C. (2016). Concentration, dietary exposure and health risk estimation of polycyclic aromatic hydrocarbons (PAHs) in youtiao, a Chinese traditional fried food. *Food Control*, 59, 328–336. <https://doi.org/10.1016/j.foodcont.2015.06.003>
- Mohammadi, M., & Valizadeh-kakhki, F. (2018). Polycyclic Aromatic Hydrocarbons Determination in Grilled Beef and Chicken. *Polycyclic Aromatic Compounds*, 38(5), 434–444. <https://doi.org/10.1080/10406638.2016.1236824>
- Naccari, C., Cristani, M., Giofrè, F., Ferrante, M., Siracusa, L., & Trombetta, D.

- (2011). PAHs concentration in heat-treated milk samples. *Food Research International*, 44(3), 716–724. <https://doi.org/10.1016/j.foodres.2010.12.029>
- Nor Hasyimah, A. K., Jinap, S., Sanny, M., Ainaatul, A. I., Sukor, R., Jambari, N. N., Nordin, N., & Jahurul, M. H. A. (2020). Effects of Honey-Spices Marination on Polycyclic Aromatic Hydrocarbons and Heterocyclic Amines Formation in Gas-Grilled Beef Satay. *Polycyclic Aromatic Compounds*, 0(0), 1–29. <https://doi.org/10.1080/10406638.2020.1802302>
- Onyedikachi, U. B., Belonwu, C. D., & Wegwu, M. O. (2019). The determination of polycyclic aromatic hydrocarbons in some foods from industrialized areas in South Eastern Nigeria: human health risk impact. *Ovidius University Annals of Chemistry*, 30(1), 37–43. <https://doi.org/10.2478/auoc-2019-0007>
- Pan, H., & Cao, Y. (2010). Optimization of pretreatment procedures for analysis of polycyclic aromatic hydrocarbons in charcoal-grilled pork. *Analytical Letters*, 43(1), 97–109. <https://doi.org/10.1080/00032710903276497>
- Racovita, R. C., Secuianu, C., & Israel-Roming, F. (2021). Quantification and risk assessment of carcinogenic polycyclic aromatic hydrocarbons in retail smoked fish and smoked cheeses. *Food Control*, 121, 107586. <https://doi.org/10.1016/j.foodcont.2020.107586>
- Sahin, S., Ulusoy, H. I., Alemdar, S., Erdogan, S., & Agoaoglu, S. (2020). Evaluation on Presence of Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Beef, Chicken and Fish by Considering Dietary Exposure and Risk Assessment. *Food Science of Animal Resources*. <https://doi.org/10.5851/kosfa.2020.e43>
- Shariatifar, N., Dadgar, M., Fakhri, Y., Shahsavari, S., Moazzen, M., Ahmadloo, M., Kiani, A., Aeenehvand, S., Nazmara, S., & Mousavi Khanegah, A. (2020). Levels of polycyclic aromatic hydrocarbons in milk and milk powder samples and their likely risk assessment in Iranian population. *Journal of Food Composition and Analysis*, 85(June 2019), 103331. <https://doi.org/10.1016/j.jfca.2019.103331>
- Sojinu, O. S., Idowu, A. O., Mosaku, A. M., & Oguntuase, B. J. (2019). Determination of Polycyclic Aromatic Hydrocarbons (PAHs) In Smoked Fish and Meat Samples In Abeokuta. *J. Chem Soc. Nigeria*, 44(1), 96–106.
- Sultana, M. S., Rana, S., Yamazaki, S., Aono, T., & Yoshida, S. (2017). Health risk assessment for carcinogenic and non-carcinogenic heavy metal exposures from

- vegetables and fruits of Bangladesh. *Cogent Environmental Science*, 3(1).
<https://doi.org/10.1080/23311843.2017.1291107>
- Tongo, I., Ogbeide, O., & Ezemonye, L. (2017). Human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in smoked fish species from markets in Southern Nigeria. *Toxicology Reports*, 4, 55–61.
<https://doi.org/10.1016/j.toxrep.2016.12.006>
- United Nations Economic Commission for Europe. (1999). *Protocol to the 1979 convention on long-range transboundary air pollution on persistent organic pollutants*.
<https://www.unece.org/fileadmin/DAM/env/lrtap/full%20text/1998.POPs.e.pdf>
- United States Environmental Protection Agency. (2019). *Guidelines for human exposure assessment*. (EPA/100/B-19/001). Washington, D.C.
- United States Environmental Protection Agency (USEPA). (1987). *Integrated Risk Information System (IRIS): fluorine*. CASRN 218-01-01.
https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=53
- United States Environmental Protection Agency (USEPA). (1990). *Integrated Risk Information System (IRIS): chrysene*. CASRN 218-01-01.
https://iris.epa.gov/ChemicalLanding/&substance_nmbr=455
- United States Environmental Protection Agency (USEPA). (1990). *Integrated Risk Information System (IRIS): pyrene*. CASRN 7782-41-4.
https://iris.epa.gov/ChemicalLanding/&substance_nmbr=445
- United States Environmental Protection Agency (USEPA). (2017). *Integrated Risk Information System (IRIS): benzo(a)pyrene*. CASRN 50-33-8.
https://iris.epa.gov/ChemicalLanding/&substance_nmbr=136
- Wang, X., Wang, S., Li, F., Li, R., Zhu, J., Chen, J., Li, W., & Jiang, D. (2020). Occurrence of polycyclic aromatic hydrocarbons in youtiao and exposure assessment from Shandong Province, China. *Food Control*, 111(December 2019), 107049. <https://doi.org/10.1016/j.foodcont.2019.107049>
- Yang, W. Y., Burrows, T., MacDonald-Wicks, L., Williams, L. T., Collins, C. E., Chee, W. S., & Colyvas, K. (2017). Body Weight Status and Dietary Intakes of Urban Malay Primary School Children: Evidence from the Family Diet Study. *Children (Basel, Switzerland)*, 4(1), 5.

<https://doi.org/10.3390/children4010005>