



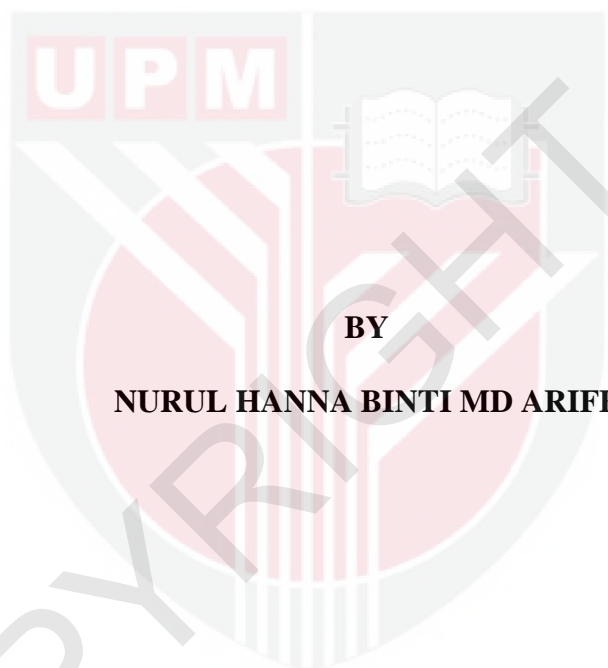
**UNIVERSITI PUTRA MALAYSIA**

***OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBON  
(PAHS) IN SELECTED STREET FOODS IN SERI KEMBANGAN,  
SELANGOR, AND THEIR ASSOCIATED HEALTH RISK TO  
CONSUMER***

**NURUL HANNA BINTI MD ARIFF**

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AND THEIR ASSOCIATED HEALTH RISK TO CONSUMER**



**BY  
NURUL HANNA BINTI MD ARIFF**

**This thesis submitted in fulfilment of the requirement for the degree of Bachelor of  
Science in Environmental and Occupational Health with Honours from the  
Faculty of Medicine and Health Sciences, Universiti Putra Malaysia**

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## ABSTRACT

### OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBON (PAHS) IN SELECTED STREET FOODS IN SERI KEMBANGAN, SELANGOR AND THEIR ASSOCIATED HEALTH RISK

NURUL HANNA BINTI MD ARIFF

**Introduction:** Polycyclic aromatic hydrocarbon (PAHs) is produced when organic matter is burned in incomplete combustion, which occurs whenever fossil fuels or plants are ignited. Carcinogenic PAHs are found in foods and are primarily generated during the grilling, frying and smoking procedures. **Objectives:** The study aimed to determine the occurrence of PAHs in selected street foods (chicken satay, lamb satay, beef satay, grilled chicken wing, fried fish cake (*keropok lekor*), and fried banana) in Seri Kembangan, Selangor and their associated health risk to consumers. **Methodology:** Street foods were sampled using Convenient Sampling. Then, PAHs were extracted using ultrasonication, cleaned up by solid-phase extraction (SPE) RP-18 silica cartridges and analyzed using gas-chromatography and mass spectrometry (GC-MS). The incremental lifetime cancer risk (ILCR) was applied to assess the potential carcinogenic health consumption risk in selected PAHs contaminated street foods. **Results and Discussion:** Beef satay showed the highest concentration in PAH4, PAH8 and  $\Sigma$ PAHs with the concentration of 580.93  $\mu\text{g}/\text{kg}$ , 1451.51  $\mu\text{g}/\text{kg}$ , and 2501.70  $\mu\text{g}/\text{kg}$ , respectively. The concentration of PAH4 of all samples within the acceptable limit of European Union (EU) (30  $\mu\text{g}/\text{kg}$ ) except for the beef satay. The concentration of benzo( $\alpha$ )pyrene (B( $\alpha$ )p) in all samples were within the acceptable limit of European Union (EU) legislation (5  $\mu\text{g}/\text{kg}$ ). The health risk assessment showed that dietary consumption to grilled and fried street foods using ILCR is in an acceptable limit of carcinogenic risk among the adults' population in Malaysia ( $9.24 \times 10^{-6}$  to  $9.86 \times 10^{-8}$ ) for all samples. **Conclusion:** The occurrence of 16 PAHs in a variety of street foods in Seri Kembangan, Selangor, have been quantified and the health risks associated with them. The ILCR values indicate that the exposure of PAHs through dietary consumption posed a low carcinogenic risk to the adult population.

**Keywords:** Polycyclic Aromatic Hydrocarbon (PAHs), street foods, carcinogenic health risk assessment, moisture content, solid phase extraction, GC-MS.

## ABSTRAK

### KEJADIAN POLISIKLIK AROMATIK HIDROKARBON (PAHS) DALAM MAKANAN JALANAN TERPILIH DI SERI KEMBANGAN, SELANGOR DAN RISIKO KESIHATAN BERKAITANNYA

NURUL HANNA BINTI MD ARIFF

**Pengenalan:** Polisiklik aromatik hidrokarbon (PAH) terhasil apabila bahan organik dibakar dalam pembakaran yang tidak lengkap, yang berlaku apabila bahan api fosil atau tumbuhan dinyalakan. PAH karsinogenik terdapat dalam makanan dan dijana terutamanya semasa prosedur memanggang, menggoreng dan menyalai. **Objektif:** Kajian ini bertujuan untuk menentukan kejadian polisiklik aromatik hidrokarbon dalam makanan jalanan terpilih (sate ayam, sate kambing, sate daging, kepak ayam panggang, keropok lekor, dan pisang goreng) di Seri Kembangan, Selangor dan kesihatan yang berkaitan. risiko kepada pengguna. **Metodologi:** Makanan jalanan telah diambil sampel menggunakan Persampelan Mudah (*Convenient Sampling*). Kemudian, PAH diekstrak menggunakan ultrasonik, dibersihkan dengan kartirij silika RP-18 pengekstrakan fasa pepejal (SPE) dan dianalisis menggunakan kromatografi gas dan spektrometri jisim (GC-MS). Risiko kanser seumur hidup tambahan (ILCR) telah digunakan untuk menilai potensi risiko penggunaan kesihatan karsinogenik dalam makanan jalanan tercemar PAH terpilih. **Keputusan dan Perbincangan:** Sate daging menunjukkan kepekatan tertinggi dalam PAH<sub>4</sub>, PAH<sub>8</sub> dan ΣPAH dengan kepekatan 580.93 µg/kg, 1451.51 µg/kg, dan 2501.70 µg/kg, masing-masing. Kepekatan PAH<sub>4</sub> semua sampel dalam had yang boleh diterima Kesatuan Eropah (EU) (30 µg/kg) kecuali sate daging. Kepekatan benzo(α)pyrene (B(α)p) dalam semua sampel adalah dalam had yang boleh diterima oleh perundangan Kesatuan Eropah (EU) (5 µg/kg). Penilaian risiko kesihatan menunjukkan bahawa penggunaan diet kepada makanan jalanan panggang dan goreng menggunakan ILCR adalah dalam had risiko karsinogenik yang boleh diterima dalam kalangan populasi dewasa di Malaysia ( $9.24 \times 10^{-6}$  hingga  $9.86 \times 10^{-8}$ ) untuk semua sampel. **Kesimpulan:** Kehadiran 16 PAHs dalam pelbagai makanan jalanan di Seri Kembangan, Selangor, telah dikira dan risiko kesihatan yang berkaitan dengannya. Nilai ILCR menunjukkan bahawa pendedahan PAH melalui penggunaan diet menimbulkan risiko karsinogenik yang rendah kepada populasi dewasa.

**Kata kunci:** Polisiklik Aromatik Hidrokarbon, makanan jalanan, penilaian risiko karsinogenik kesihatan, kandungan lembapan, pengekstrakan fasa pepejal, GC-MS.

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## LIST OF ABBREVIATIONS

PAH	Polycyclic Aromatic Hydrocarbon
US EPA	United State Environmental Protection Agency
IARC	International Agency for Research on Cancer
EFSA	European Food Safety Authority
EU	European Union
LMW	Low Molecular Weight
MMW	Moderate Molecular Weight
HMW	High Molecular Weight
SPE	Solid-phase extraction
WHO	World Health Organization
SCF	Scientific Committee on Food
EC	European Commission
HPLC	High performance or pressure liquid chromatography
DCM	Dichloromethane
GC-MS	Gas chromatography-mass spectroscopy
TEF	Toxic equivalency factors
TEQB( $\alpha$ )P	Toxic equivalence quotients
ILCR	Incremental life cancer risk
MANS	Malaysia Adult Nutrition Survey
LOD	Limit of detection
LOQ	Limit of quantification

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

Polycyclic aromatic hydrocarbon (PAHs) produced when organic matter is burned in incomplete combustion, which occurs whenever fossil fuels or plants are ignited (Abdel-Shafy & Mansour, 2016; Jamhari et al., 2014). PAHs were detected in groundwater, soils, air, tobacco smoke, and foodstuffs (Kafouris et al., 2020; Zelinkova & Wenzl, 2015). Foods can be contaminated with PAHs from the environment through transfer from the air, sediment deposition, and water transfer (Hamidi et al., 2016; Zelinkova & Wenzl, 2015). Their occurrence is commonly associated with cooking techniques including grilling, smoking, frying, roasting, and baking as well as industries that are exposed to extreme heat procedures such as cement manufacturing, iron production, waste incinerator, and power plant (Kim et al., 2021; Patel et al., 2020; Tran-Lam et al., 2018; Zhang et al., 2021).

In addition, street foods among a significant source of PAHs exposure (Khongtong et al., 2014). Street food means ready-to-eat foods prepared or sold by sellers and hawkers in public locations such as schools, hospitals, and train stations (Khongtong et al., 2014). The food preparation environment usually exposes foods (frequently left open) to atmospheric PAHs in airborne particles, exhaust gases, and

burning emissions, such as outdoor garbage burned due to inadequate solid waste management facilities. Additionally, food can be affected through direct contact with mineral oil that is not food grade or through contaminated packaging. Thus, the combination of exposure from street food compounds, homemade foods, environmental contaminants, and behaviour (tobacco smoke) contributes to the general population daily exposure (Ekhtor et al., 2018).

Generally, food preparation methods such as smoking, grilling, and frying are recognized as the main contributor to PAHs contamination via food consumption. Multiple significant factors, such as food preparation techniques, temperature, distance from a source of heat, and fat dripping, regularly influence the production of PAHs in food. Thus, it is well established that high-temperature cooking and food processing contribute significantly to the formation of harmful carcinogens to human health (Jahurul et al., 2013; Tran-Lam et al., 2018; Chong Wang et al., 2019). Numerous studies have established that carcinogenic PAHs are found in foods and are primarily generated during the grilling, frying and smoking procedures (Adesina, 2020; Alshaarawy et al., 2016; Kamankesh et al., 2015; Kim et al., 2021; Li, Wu, Wang, et al., 2016; Zelinkova & Wenzl, 2015)

PAHs exposure can have acute and chronic effects; however, earlier research has found that chronic effects are more common than acute effects (Abdel-Shafy & Mansour, 2016; Kim et al., 2013). Examples of acute or short-term impacts frequently occur at the workplace. Workplace exposure to excessive concentrations

of pollutant mixes containing PAHs has been linked to eye discomfort, dizziness, nausea, and diarrhoea. PAH mixtures have also been associated with skin redness and inflammation (Abdel-Shafy & Mansour, 2016). PAHs have previously proven associated with an increased chance of cancer in the epidermis, lungs, kidney, and gastrointestinal when in a chronic or long-term effect. PAHs are classed as disruptive endocrine chemicals because of their ability to bind endogenous receptors (Rajpara et al., 2017). According to Kim et al. (2013) and Rajpara et al. (2017), long-term exposure to PAHs can cause stomach, lung, and breast cancer. Several studies were conducted in Malaysia only limited to determining PAHs in food samples without reporting the health risk to the consumers (Farhadian et al., 2010; Jahurul et al., 2013). Thus, this study aimed to determine the occurrence of polycyclic aromatic hydrocarbon (PAHs) in selected street foods in Seri Kembangan, Selangor and their associated health risk to consumers.

## 1.2 Problem Statement

PAHs was generated due to pyrolytic processes when organic molecules are exposed to severe temperatures in the presence of minimal or no oxygen (Abdel-Shafy & Mansour, 2016). Meanwhile, PAHs can be emitted from natural and anthropogenic sources in the unintentional process due to the incomplete combustion of organic materials during manufacturing and other anthropogenic activities. (Jamhari et al., 2014). In terms of food consumption, PAHs can be generated when organic matter, such as fat, is pyrolyzed at temperatures greater than 200 °C, or when smoke is released from the incomplete combustion of charcoal or direct fires, which can lead to PAHs adhering to the surface of food (Cheng et al., 2019; Lee et al., 2016; Chong Wang et al., 2019). Grilling over charcoal is among the most intensive thermal sources. This intensive heating source was used directly or indirectly to the flame in the food products. (Samet Babaoglu et al., 2017).

Humans are likely exposed to the PAHs every day via various main routes of exposure, such as direct contact, ingestion, and inhalation. The most common routes of human exposure to PAHs are inhalation and ingestion (Abdel-Shafy & Mansour, 2016; Gachanja, 2019). Even though humans are exposed to PAHs through inhalation and drinking water, dietary consumption as the significant contribution to PAHs as it represents 88 to 98 % of such contamination (Farhadian et al., 2011; Jahurul et al., 2013; Onwukeme & Okafor, 2015; Zhang Xiaoqian Chen Yu Zhang & Yu Zhang, 2021). Foods can be polluted by ambient PAHs found in the air, soil, and

water, while their primary occurrence comes from high-temperature thermal activities and cooking techniques (Farhadian et al., 2011). A. Paris et al. (2018) stated that consumption of meat, oils, and fats is the primary PAH consumption source in adults, accounting for 54 to 71 % and 8-12 %, respectively.

PAHs have been classified as toxic substances by the United States Environmental Protection Agency (USEPA) and the European Community due to their carcinogenic, mutagenic, and toxic properties (Abdel-Shafy & Mansour, 2016; Patel et al., 2020; Zhang Xiaoqian Chen Yu Zhang & Yu Zhang, 2021; Zhang et al., 2021). Some PAHs have been classified as known, possible, or probable carcinogens in humans (Groups 1, 2A, and 2B, respectively) by the International Agency for Research on Cancer (IARC); for example, B( $\alpha$ )P (Group 1), D( $\alpha$ h)A (Group 2A), and NAP, CHR, B( $\alpha$ )A, B(k)F, and B( $\beta$ )F (Group 2B) (IARC, 2010; Zhang et al., 2021). Based on the European Food Safety Authority (EFSA), four main PAHs (benzo( $\alpha$ )pyrene, benzo( $\alpha$ )anthracene, benzo( $\beta$ )fluoranthene, and chrysene) could be used as the indicator for the presence and effect of carcinogenic PAHs in food (Babaoglu et al., 2017; EFSA, 2008; Lee et al., 2016). In some studies, the consumption of high level of PAHs have been linked to the increased risk of pancreatic, esophageal, and prostate cancer (Hamidi et al., 2016; Onopiuk et al., 2021)

### 1.3 Study Justification

In developing countries such as Malaysia, people rely heavily on street food vendors, which play a significant part in their livelihoods and provide a vital nutrition source. It supplies millions of urban residents and workers in developing countries with affordable, nutrient-rich meals (Wei, 2021). However, high health risks are exacerbated by dietary exposure due to unsafe cooking methods used in vendors' preparation of street food. Several unsafe cooking methods used by the vendors such as reusing the dark oil for frying, grilled foods over open flames or enabling food to contact directly with coal materials during smoking, and exposed food to fumes from exhaust pipes of motor vehicles (Ekhtor et al., 2018). Thus, the occurrence of PAHs in Malaysian street foods and their associated health risk is our concern.

In addition, many studies were focusing only on carcinogenic PAHs with a few compounds. Farhadian et al. (2010) investigated three PAH (Fluoranthene, Benzo( $\beta$ )fluoranthene, and Benzo( $\alpha$ )pyrene) in grilled meat, while Jahurul et al. (2013) reported the same three PAHs in meat and fish products in Malaysia. However, limited studies reported the 16 PAHs dietary intake suggested by the USEPA in Malaysia. Moreover, many studies were conducted of 16 compounds of PAHs in foods from other countries, but their amount of dietary intake was different from dietary intake in Malaysia as the food consumption among the population were different (Alomirah et al., 2011; Kim et al., 2021; Li et al., 2016; H. Li et al., 2018; Rocha et al., 2020; Zelinkova & Wenzl, 2015).

The study by Hamidi et al. (2016) has indicated a high risk of colorectal, prostate, pancreas, and stomach cancer in people who consume complex PAHs compounds, which is the predominant route of PAHs exposure for non-smoking people. Additionally, a limited study is conducted on health risk assessment from the consumption of foods with the occurrence of PAHs compound. Thus, the occurrence result of PAHs compound and their health risk assessment in this study gave the benefits in terms of public understanding and concerns on the hazardous health effect from PAHs compound as well as practising the good cooking method.

#### **1.4 Research Question**

- i. What is the concentration of PAHs in different street foods collected in Seri Kembangan, Selangor?
- ii. Is there any carcinogenic health risk assessment due to consumption of PAHs contaminated in street foods that collected in Seri Kembangan, Selangor?

#### **1.5 Objectives**

##### **1.5.1 General objectives**

To determine the occurrence of PAHs in selected street foods (chicken satay, lamb satay, beef satay, grilled chicken wing, fried fish cake, and fried banana) in Seri Kembangan, Selangor and their associated health risk to consumers.

### 1.5.1 Specific objectives

- i. To determine the concentration of PAHs in a different types of street foods collected in Seri Kembangan, Selangor.
- ii. To estimate the carcinogenic health risk due to consumption of PAHs contaminated in street foods that collected in Seri Kembangan, Selangor



## 1.6 Conceptual Framework

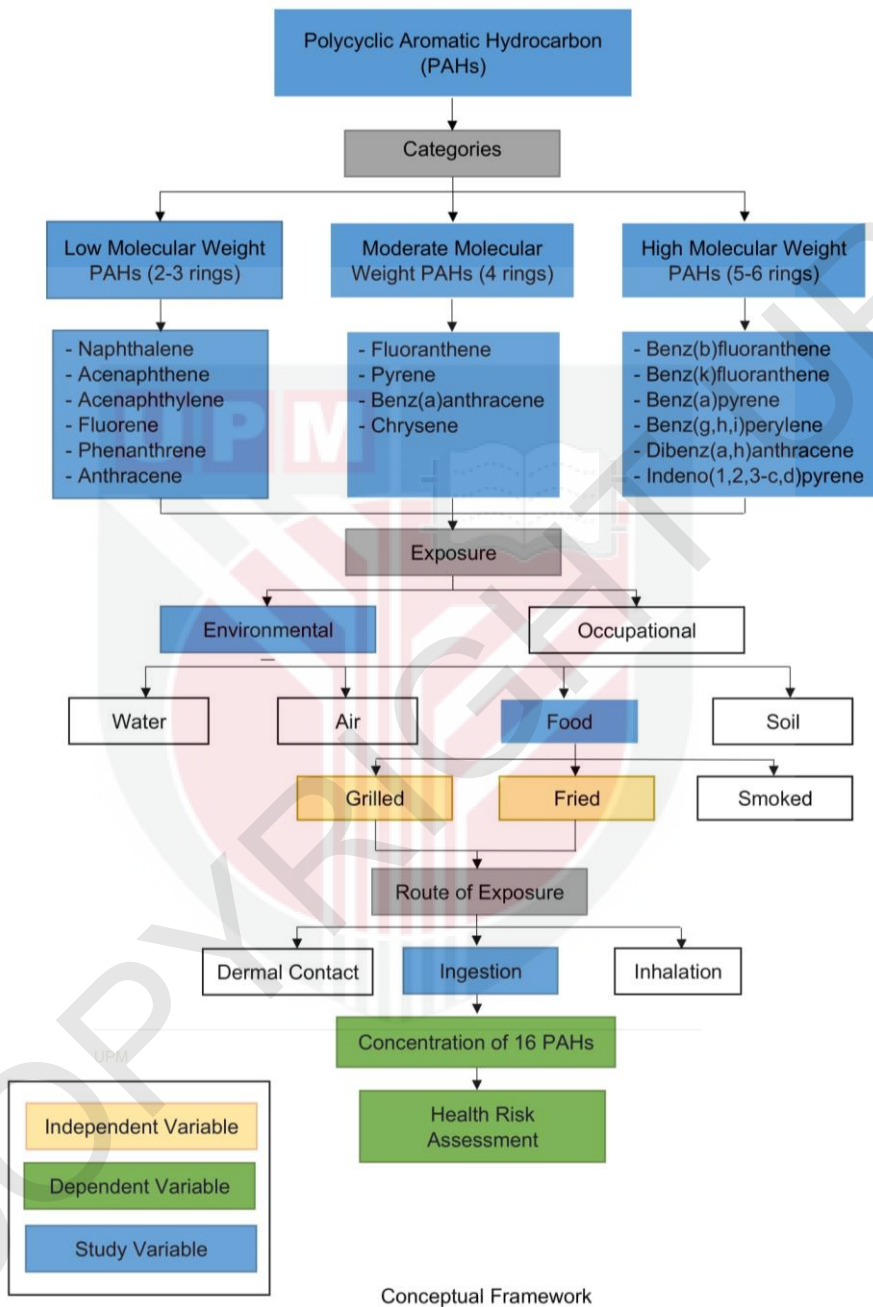


Figure 1.1 Conceptual Framework

## CHAPTER 2

### LITERATURE REVIEW




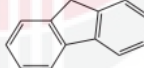

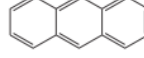
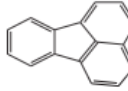
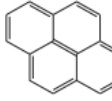
#### 2.1 Polycyclic Aromatic Hydrocarbon



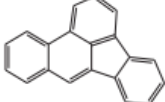
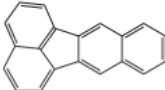




Polycyclic Aromatic Hydrocarbons (PAHs) are composed entirely of carbon and hydrogen atoms arranged in aromatic ring structures that are likely colourless, white, or pale yellow solid. PAHs are a large group of organic compounds consisting of two or more fused aromatic rings that are highly lipophilic despite having poor solubility in water (WHO, 2000). Moreover, PAHs are ubiquitous groups with hundreds of chemically related compounds that are semi-volatile, persistent in the environment, and have varying toxicity (Khan et al., 2015). Therefore, PAHs with a high molecular weight, such as benzo( $\alpha$ )pyrene, indeno(1,2,3-c,d)pyrene, dibenzo( $\alpha$ ,h)anthracene, and benzo(g,h,i)perylene, are more persistent and hazardous than PAHs with a low molecular weight (Ekhtor et al., 2018).

PAHs are considered to be carcinogenic and genotoxic to humans. Moreover, Plaza-Bolaos et al. (2010) reported that in 2005, the European Union (EU) listed 15 compounds of PAHs in its monitoring programs. Within this listed compound, benzo( $\alpha$ )anthracene, chrysene, benzo( $\beta$ )fluoranthene, benzo(k)fluoranthene, benzo( $\alpha$ )pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)pyrene, and dibenzo( $\alpha$ ,h)anthracene were chosen as the best indicators of the prevalence and possible

carcinogenicity of the whole class of PAHs (Hamidi et al., 2016). In addition, the United States Environmental Protection Agency (USEPA) established regulations for 16 PAHs, which were selected based on their relative toxicity, abundance, risk of exposure, and quantities in environmental samples presented in Table 2.1 (USEPA, 2011).

**Table 2.1: The 16 PAHs according to US Environmental Protection Agency**

PAH	CAS No.	No. of Rings	Chemical Formula	Structural Formula	Molecular Weight <sup>a</sup>	Water Solubility (mg L <sup>-1</sup> water) <sup>a</sup>	Boiling Point (°C) <sup>a</sup>	IARC Group <sup>b</sup>
<b>Naphthalene</b>	91-20-3	2	C <sub>10</sub> H <sub>12</sub>		128.18	3.17	218	2B
<b>Acenaphthylene</b>	208-96-8	3	C <sub>12</sub> H <sub>8</sub>		152.20	3.93	265–280	-
<b>Acenaphthene</b>	83-29-9	3	C <sub>12</sub> H <sub>10</sub>		154.20	1.93	278–279	3
<b>Fluorene</b>	86-73-7	3	C <sub>13</sub> H <sub>10</sub>		166.23	1.68–1.98	293–295	3
<b>Phenanthrene</b>	85-01-8	3	C <sub>14</sub> H <sub>10</sub>		178.24	1.2	339–340	3
<b>Anthracene</b>	120-12-7	3	C <sub>14</sub> H <sub>10</sub>		178.24	0.076	340	3
<b>Fluoranthene</b>	206-44-0	4	C <sub>16</sub> H <sub>10</sub>		202.26	0.2–2.6	375–393	3
<b>Pyrene</b>	129-00-0	4	C <sub>16</sub> H <sub>10</sub>		202.26	0.077	360–404	3

<b>Benzo(<math>\alpha</math>)anthracene</b>	56-55-3	4	C <sub>18</sub> H <sub>12</sub>		228.30	0.01	435	2B
<b>Chrysene</b>	218-01-9	4	C <sub>18</sub> H <sub>12</sub>		228.30	0.0028	441–448	2B
<b>Benzo(<math>\beta</math>)fluoranthene</b>	205-99-2	5	C <sub>20</sub> H <sub>12</sub>		252.32	0.0012	481	2B
<b>Benzo(k)fluoranthene</b>	207-08-9	5	C <sub>20</sub> H <sub>12</sub>		252.32	0.00076	480–471	2B
<b>Benzo(<math>\alpha</math>)pyrene</b>	50-32-8	5	C <sub>20</sub> H <sub>12</sub>		252.32	0.0023	493–496	1
<b>Dibenzo(<math>\alpha,h</math>)anthracene</b>	53-70-3	6	C <sub>22</sub> H <sub>14</sub>		278.35	0.0005	524	2A
<b>Benzo(g,h,i)perylene</b>	191-24-2	6	C <sub>22</sub> H <sub>12</sub>		276.34	0.00026	525	3
<b>Indeno(1,2,3-cd)pyrene</b>	193-39-5	6	C <sub>22</sub> H <sub>12</sub>		276.34	0.062	530	2B

<sup>a</sup> List by Hussain et. al, (2019).

<sup>b</sup> Group 1: carcinogenic to humans; group 2A: probably carcinogenic to humans; group 2B: possibly carcinogenic to humans; group 3: not classifiable as to its carcinogenicity to humans

### 2.1.1 Categories of Molecular Weight of PAHs

According to the number of rings that consist in the compounds, PAHs are classified into low molecular weight (LMW PAHs), moderate molecular weight (MMW PAHs), and high molecular weight (HMW PAHs). These PAHs' boiling

temperatures and melting points generally increase as their molecular weight increases, but their vapour pressure and water solubility steadily decrease. LMW PAHs, such as naphthalene (Nap), acenaphthylene (Ace), acenaphthene that have 2 to 3 aromatic rings, are mainly present in the gas phase. While HMW PAHs have five to six aromatic rings, such as benzo( $\alpha$ )pyrene (B( $\alpha$ )p), indeno(1,2,3-cd)pyrene (I(cd)p), and benzo(g,h,i)perylene (B(ghi)p), are primarily present in particulate (Chimjarn et al., 2021; Sun et al., 2021; Cui Wang et al., 2017). Based on US EPA (2002), PAHs' aqueous solubility and vapour pressure decrease as their molecular weight increases; however, PAHs are resistant to oxidation. Their reduction increases as their molecular weight increases (Hussain et al., 2019).

The high molecular weight of PAHs is more persistent and hazardous than LMW PAHs. Compared to HMW PAHs, LMW PAHs are more volatile, soluble in water, and less lipophilic (Afsaneh Farhadian et al., 2010; Hamidi et al., 2016). Due to the toxicity of HMW, PAH4 and PAH8 were used to calculate the health risk assessment of human consumption as PAH4 and PAH8 are the most appropriate markers of the prevalence and potential carcinogenicity of the whole group of PAHs (Babaoglu et al., 2017; Lee et al., 2016). In the immune response, certain HMW produced genotoxic effects that were more significant than among their parent compounds. However, LMW was considered less hazardous than HMW since the risk of metabolizing a mutative metabolite is significantly smaller than HMW. (Cui Wang et al., 2017)

## 2.2 Source of PAHs

The source of PAHs is very complex and broad. PAHs can impact the environment through two different methods: natural and anthropogenic sources. Combustion processes such as forest and prairie fires, volcanic eruptions, and plant and microbe biosynthesis are all-natural sources of PAHs (Sun et al., 2021). In general, PAHs are a group of environmental pollutants formed when organic matter such as wood, charcoal, oil, gas, or waste is not completely combusted (incomplete combustion) or pyrolyzed. They contaminate food via soil, water, and air (Kafouris et al., 2020; Zelinkova & Wenzl, 2015).

Additionally, mutagenic and carcinogenic compounds of PAHs can be generated due to the cooking method with heat treatments such as drying, smoking, and grilling, which are particularly common at home and in restaurants. PAHs are more frequently found in grilled or smoked meat products due to fat pyrolysis at temperatures more than 200 °C and smoke released by incomplete combustion of charcoal (Alomirah et al., 2011; Kafouris et al., 2020). Then, it is crucial to emphasize that incomplete combustion, whether natural or anthropogenic, has been recognized as the single primary source of PAHs in the environment (Abdel-Shafy & Mansour, 2016). Typically, once the combustion temperature is low and the environment is anaerobic, the combustion process is shown to be incomplete. Inside the flame, free radicals commence the chemical production of hydrocarbon. Free

radicals with a high molecular weight often generate large PAH particles, but methane can also generate PAH particles (Hussain et al., 2019).

### 2.3 Reaction of PAHs

PAHs may provide a concern to human health once they are metabolized in the liver, stomach, as well as other extrahepatic tissues to form reactive metabolites that impair target organ activity, resulting in mortality and morbidity. The amount of the PAHs, their lipophilicity, and the fat content of the foods can significantly lead to dietary PAH absorption. After absorption, PAHs are transported through the bloodstream to various organs, particularly those with a high lipid content, and some are converted to DNA-active mutagens or genotoxic carcinogens (diol epoxides). It has been found that absorption of <sup>14</sup>C-BaP in Wistar rats was enhanced when fed a diet high in lipids and inhibited when fed a high-fibre diet. (Hamidi et al., 2016; Marques et al., 2011).

The Scientific Committee on Food (2002) concluded that 15 PAHs demonstrated significant carcinogenicity in somatic cells of animal experiments *in vivo*. They may be considered possibly genotoxic and carcinogenic to humans due to their metabolic change in mammalian cells to diol epoxides that covalently attach to cellular macromolecules, including DNA, resulting in replication failures and mutations (Afsaneh Farhadian et al., 2010; Kim et al., 2021; Tran-Lam et al., 2018).

In addition, the latest research recently revealed that the intestinal microbiota regulates the action of xenobiotics like PAHs. The host-microbiota may aggravate PAHs' harmful action through routes including the enterohepatic cycle and alterations in gene expression by liver enzymes like P450. However, the microbiota's precise processes behind this modulation are unknown (Sampaio et al., 2021).

#### **2.4 Exposure Pathway of PAHs**

In the current situation, PAH exposure is unavoidable. PAHs can be found in various sources, including water, food, tobacco smoke, and pharmaceuticals. Airborne PAHs are present in both gas and particle phases, making ambient air one of the essential sources of PAH intake (Samburova et al., 2017). There are several pathways of humans to expose to PAHs, such as dermal contact, inhalation, and ingestion (Patel et al., 2020). Inhalation usually relates to active and second-hand smoking and inhaling from polluted indoor and outdoor environments. After inhalation of B( $\alpha$ )P containing smoke by humans, this has been proved that B( $\alpha$ )P can penetrate the epithelial barrier and enter the pulmonary capillaries (Sun et al., 2021). For the general population, inhalation of PAH-containing vehicle exhaust and road dust is the most common source of exposure, especially in the high traffic volume area (Abdel-Shafy & Mansour, 2016).

Besides occupational exposure of PAHs and smoke, food exposure is the primary route of PAH consumption in people (Alomirah et al., 2011; Bansal & Kim, 2015; Ekhtator et al., 2018; Kafouris et al., 2020; Xia et al., 2010). A previous study demonstrated that the longer the ham was smoked, the more amount of PAHs it contains because most traditional recipes need months of smoke duration. Furthermore, smoked bacon was reported to have the highest PAH levels due to its high lipid content (Kafouris et al., 2020). Humans are most likely to be exposed to PAHs through their diets when they consume meat and associated products that have been cooked using various methods such as grilling, roasting, smoking, or frying. Despite this, PAH levels in food continue to be low. However, regular and excess consumption of these foods over an extended period can be extremely hazardous to human health. (Chong Wang et al., 2019).

## **2.5 Method of cooking**

Food cooking methods such as smoking, grilling, and frying are typically regarded as the primary cause of PAH contamination through ingestion. The development of PAHs in food is frequently influenced by several essential aspects, including food preparation procedures, temperature, distance from a heat source, and fat dripping. Thus, high-temperature cooking and processing of food are known to be significant factors in the production of hazardous toxicants for human health. (Tran-Lam et al., 2018; Chong Wang et al., 2019)

According to its dehydrating characteristics and the existence of organic compounds of antibacterial and antioxidant capabilities, food smoking is among the oldest ways of preservation. Additionally, smoking enhances meals' flavours, colour, and scent (Kaljac et al., 2014). Generally, smoke is produced when the wood is partially burned through the smoking process. Direct or indirect smoking is applied in food preparation, and so is traditional cold or hot smoking, based on the smoke heat, the site of the smoke formation, and the smoke generators (Kafouris et al., 2020)

Charcoal grilling is a popular method of severe heat grilling that leads to the formation of PAHs in food. Across several Asian nations, traditional wood charcoal utilized as a heat source was categorized based on the manufacturing process, either white or black charcoals. Black charcoal is generated at temperatures between 600 and 800 °C and thus is relatively easy to burn. Therefore, white charcoal is generated at 1000 °C and is more difficult to burn but retains good thermal energy for an extended period. White and black charcoals are both produced from genuine wood. However, artificial extrusion charcoal is frequently used in conjunction with wood charcoal or on its own for cost and effectiveness reasons (Kim et al., 2021). Grilling procedures can result in significant changes in carcinogen levels. Grilling meat, fish, or other foods over an open flame leads to fat dripping, creating flames containing a variety of PAHs and releasing smoke. Then, PAHs compounds from present smoke will attach to the food surface. Thus, the presence of PAHs increases with the intensity of the heat (Afsaneh Farhadian et al., 2010).

Hamidi et al. (2016) and Alomirah et al. (2011) showed that even though the specific method for developing PAHs in grilled and smoked foods is unknown, there are at least three probable mechanisms. The mechanisms that could be used are: First, the pyrolysis of organic matter such as protein, fat, and carbohydrate at temperatures more than 200 °C, with the optimal temperature range for PAH production being 500-900 °C. The largest concentration of PAHs is found to be produced during fast pyrolysis. Second, at high temperatures, the yield of lipids drops in direct contact with the flame. This environment is conducive to creating volatile PAHs, which are then coated on the surface of the meat. Third, incomplete combustion of charcoal can result in PAHs, which then attach to the food's surface.

## **2.6 Health effects from PAHs exposure**

PAHs are among the most hazardous pollutants in the environment due to their genotoxicity and the fact that they are immunotoxin, carcinogenic, mutagenic, and teratogenic to a variety of organisms. However, the health effects of each PAHs compounds are different. Several PAHs are categorized by the International Agency for Research on Cancer (IARC) as known, potentially, or probably carcinogenic to people (Group 1, 2A or 2B). These compounds include benzo( $\alpha$ )pyrene as Group 1, which is carcinogenic to humans and naphthalene, chrysene, benzo( $\alpha$ )anthracene, benzo(k)fluoranthene, and benzo( $\beta$ )fluoranthene as Group 2B, which is possibly carcinogenic to human. Some PAHs are well-known carcinogenic, mutagens, and

teratogens, presenting a significant risk to human health and well-being (IARC, 2010).

The severity of the harmful effects from PAHs was determined from some key factors such as the route of exposure, frequency of PAHs exposure, and the amount of PAHs exposure (Patel et al., 2020). PAHs exposure could be acute effect and chronic effect; however, the previous studies reported chronic effect study more than the acute effect (Abdel-Shafy & Mansour, 2016; K. H. Kim et al., 2013). In acute or short-term effects, the cases usually happen at the workplace. Eyes irritation, dizziness, vomit, and diarrhoea have all been reported due to workplace exposure to high concentrations of contaminant mixes containing PAHs. Skin sensitivity and inflammation have also been linked to PAH mixtures (Abdel-Shafy & Mansour, 2016). While in chronic or prolonged-term effect, PAHs have previously been related to an increased risk of cancer in the skin, lungs, bladder, and stomach. Because of their potential to bind endogenous receptors, PAHs are also classified as endocrine-disrupting compounds (Rajpara et al., 2017). Kim et al. (2013) and Rajpara et al. (2017) stated that exposure to prolonged PAHs can lead to several cancer such as stomach cancer, lung cancer and breast cancer.

In addition, the cancer risk from the consumption of food can be carried out through risk assessment (Li et al., 2016; Wu et al., 2016). Based on the Scientific Committee Food (SCF) on the December 2002 finding, B( $\alpha$ )P was chosen to indicate carcinogenic PAHs' occurrence and effect in food. Moreover, according to the SCF,

more research is needed to determine whether maintaining benzo( $\alpha$ )pyrene as an indicator is suitable (Ledesma et al., 2015; Li et al., 2016). However, in 2008, the European Food Safety Authority (EFSA) issued a report on PAHs, finding that B( $\alpha$ )P alone would not be a great overall indicator for PAHs in food. As a result, a set of four PAHs (PAH4) and eight PAHs (PAH8) were more accurate indicators to calculate the risk of food consumption (EFSA, 2008; Rose et al., 2015)

## **2.7 Legislation related to PAHs exposure**

In terms of legislation related to PAHs exposure, government agencies should implement and regulate the acceptable standard of PAHs in food consumption because dietary intake contaminated with PAHs would cause harmful effects to humans. Furthermore, there are no specific criteria or standards for PAH concentrations in the foodstuffs in Malaysia. Nevertheless, several research on the occurrence of PAHs in foodstuff were conducted to evaluate the risk to human health.

Moreover, the federal government of the United States established regulatory guidelines for protecting people from the effect of PAHs, but no maximum limitations for PAHs concentration in foods have been enacted. In 2005, the European Commission issued Regulation (EC) No 208/2005, modifying Regulation (EC) No 466/2001, which defined limit values for benzo( $\alpha$ )pyrene for the first time. Subsequently, the maximum limits of benzo( $\alpha$ )pyrene were established in Regulation

(EC) No 1881/2006 and Regulation (EU) No 835/2011 amending previous regulation by added a limit level for the sum of the PAH4 which are benzo( $\alpha$ )pyrene, benzo( $\alpha$ )anthracene, benzo( $\beta$ )fluoranthene, and chrysene (Zelinkova & Wenzl, 2015). The maximum level of benzo( $\alpha$ )pyrene and PAH4 in meat product that have been exposed to heat are 5.0  $\mu\text{g}/\text{kg}$  and 30  $\mu\text{g}/\text{kg}$ , respectively (European Commission, 2015).



## CHAPTER 3

### METHODOLOGY

#### 3.1 Sampling

Street foods were sampled using convenient sampling. The street foods samples were bought in Seri Kembangan, Selangor, Malaysia. The grilled foods such as chicken satay, beef satay, grilled chicken wings, and lamb satay and fried foods such as fried fish cake and fried banana were purchased from the roadside stall. All food samples were grilled and fried at the roadside stall when we purchased. The samples were placed carefully in the zip-lock bag, transported back to the laboratory and stored in -20 °C freezer.

#### 3.3 Standards and solvents

Analytical grade solvents were used for glassware cleaning, and HPLC grade solvents were used for the extraction process. Analytical grade methanol and acetone were sourced from R & M Chemicals (UK), while dichloromethane (DCM) was purchased from Merck (Darmstadt, Germany). HPLC grade dichloromethane (DCM) and n-hexane were bought from Merck (Darmstadt, Germany). The PAHs standard (SS EPA 610 PAH Mix, Supelco, USA) consists of the United States Environmental Protection Agency (USEPA) 16 PAHs which includes naphthalene (Nap), acenaphthene (Acp), acenaphthylene (Acy), anthracene (Ant), fluorene (Flr),

phenanthrene (Phe), fluoranthene (Flt), pyrene (Phy), benzo( $\alpha$ )anthracene (B( $\alpha$ )a), chrysene (Chy), benzo( $\beta$ )fluoranthene (B( $\beta$ )f), benzo(k)fluoranthene (B(k)f), benzo( $\alpha$ )pyrene (B( $\alpha$ )p), indeno(1,2,3-cd)pyrene (I(c)p), dibenzo( $\alpha$ ,h)anthracene (D( $\alpha$ ,h)a), and benzo(g,h,i)perylene (B(g,h,i)p). At the beginning of the extraction process, chrysene-d12 (Supelco, USA) and perylene-d12 (Supelco, USA) were used as surrogate internal standards.

### **3.4 Sample preparation**

The grilled chicken wings samples were deboned and took only the edible part. Because the chicken wing was usually cooked and eaten with its skin in Malaysia, the skin was not removed and included in the analysis. Before extraction, all the samples were sliced into small pieces and then homogenized using pestle and mortar. The pestle and mortar were properly cleaned using solvents before and after each sample to avoid cross-contamination. The homogenized samples were placed in separate zip-lock bags and kept at -20 °C freezer before clean-up extraction and analysis.

### **3.5 Moisture content of food samples**

The moisture content of the samples was determined using a gravimetric method following the USEPA Method 8275A (USEPA, 1996). The crucible wrapped with aluminum foil was weighted and tared prior weight the samples. Ten grams of a

portion of the street foods samples were weighed using analytical balance into tared crucible aluminum foil. In the drying oven, the samples were dried at 105 °C for 24 hours to determine the % dry weight of the samples. The drying oven should be hooded or vented as a precautionary measure, and the crucible aluminum foil should be capped with another aluminum. The samples needed to be cold before weighting to calculate the dry weight. Thus, the calculation for % of dry weight and % of moisture contents is as follows:

$$\% \text{ dry weight} = (\text{g of dry sample} / \text{g of initial sample}) \times 100 \quad (\text{Eq 3.1})$$

$$\% \text{ moisture content} = 100 - (\% \text{ dry weight}) \quad (\text{Eq 3.2})$$

### **3.5 Quality control**

#### **3.5.1 Glassware cleaning**

All glassware was washed using analytical grade solvents to ensure that it was free of contaminants, based on USEPA Method TO-13A (USEPA, 1999). First, all glassware was soaked in Decon 90 overnight and rinsed with tap water. Then, the glassware was immediately cleaned, first with methanol, acetone, and then dichloromethane and repeated three times. After the cleaning process, the glassware was dried at 60°C in the drying oven and sealed with aluminium foil to avoid dust or cross-contamination.

### **3.5.2. Calibration of GC-MS**

The calibration curves were used to determine the linearity. The instrument was calibrated with each analyte at the five-point calibration curve at the range of 1 to 2000 ng/mL. The peak area ratio of PAHs vs nominal concentrations of the analytes was used to create calibration standards for each concentration.

### **3.5.3. Preparation of blank**

The internal standard was spiked into blank sample (boiled chicken) and ran through the entire sample preparation and extraction procedure for every batch of sample analysis to check for any possible background contamination in the sample. Internal standards (chrysene-d12 and perylene-d12) were utilized to correct the concentration of the target compound in this study.

### **3.5.4. Method performance**

The method's sensitivity was evaluated by looking at the limits of detection (LOD) and limit of quantification (LOQ) values. LOD derived using the analyte concentration that produces a peak three times the height of the noise from a blank sample (ratio of signal to noise (S/N)=3) while LOQ derived by replicating the lowest calibration standard three times (S/N=10) (Afsaneh Farhadian et al., 2010).

The recovery procedure of extraction was conducted by spiking the blank samples PAHs standard (500 µg/kg). Triplicate of both spiked and unspiked samples (control samples) were extracted using the same method. The recovery rates were estimated using equation (Eq 3.3) by evaluating the total amounts of every PAH in spiked and unspiked samples (Agus et al., 2020; EURACHEM, 2014).

$$\text{Recovery (\%)} = (X1 - X2) / X3 \times 100 \quad (\text{Eq 3.3})$$

Where X1 = Mean value of spiked sample, X2 = Mean value of unspiked sample and X3 = Concentration's known value

### **3.6 Extraction of PAHs using solid phase extraction (SPE)**

The extraction method was adapted from Khan et al. (2015) with some modifications. Five grams of homogenized food samples were weighted and placed into 50 mL glass centrifuge tubes. The samples were spiked with internal standard, followed by adding 20 mL of DCM after that. Then, the samples were sonicated for 20 min in an ultrasonic bath (Witeg Wisd WiseClean, Germany) with water temperature in the bath less than 30 °C. Next, the samples were centrifuged at 2500 rpm (Hettich Rotina 46R Centrifuge, Germany) for 10 min and mixed vigorously with a vortex mixer (ZX3 Advanced Vortex Mixer, VELP Scientifica Srl, Italy) for 10 min. Before filtering the extract using glass microfibre filters (Whatman™, UK),

the sonication, centrifugation, and vortexing steps were performed three times. The solution volume was decreased to approximately 200  $\mu\text{L}$  using a gentle flow of nitrogen gas ( $\text{N}_2$ ), and the residue was reconstituted with 800  $\mu\text{L}$  of n-hexane. Then the volume suppression procedure was carried out twice. After that, the sample was cleaned up and pre-concentrated using silica SPE cartridges (Lichrolut® RP-18, Merck, Germany). The extraction solutions were added and passed through the RP-18 SPE cartridges under a gentle vacuum after being conditioned with 10 mL of n-hexane. Ten millilitres of DCM: n-hexane (1:9) was then used to elute the RP-18 SPE cartridges at a 1 mL/min flow rate by vacuum. The eluate was collected into a 20 mL test tube and further decreased to 500  $\mu\text{L}$  under a moderate stream of  $\text{N}_2$  gas. The eluate was then reconstituted with n-hexane into a 2 mL amber glass vial up to 1.5 mL. The samples were sent for GC-MS analysis as soon as possible.

### **3.7 Analysis of PAHs using GCMS**

Gas chromatography-mass spectroscopy (GC-MS) (Agilent, 5975C, USA) was used to analyze the samples. A capillary column (HP-5MS) with an internal diameter (id) of 0.25 mm, a length of 30 m, and a thickness of 0.25  $\mu\text{m}$  was used with the GC-MS. The results were obtained using the selective ion monitoring (SIM) mode, which is more sensitive than the full scan mode option. External calibration was utilized to determine the concentrations of each compound 16 PAHs using standard mixtures of PAHs (SS EPA 610 PAH Mix, Supelco, USA). The following are the US EPA's 16 PAHs: naphthalene, acenaphthene, acenaphthylene, anthracene, fluorene,

phenanthrene, fluoranthene, pyrene, benzo( $\alpha$ )anthracene, chrycene, benzo( $\beta$ )fluoranthene, benzo(k)fluoranthene, benzo( $\alpha$ )pyrene, indeno(1,2,3-cd)pyrene, dibenzo(h)anthracene, and benzo(g,h,i)perylene. Additionally, 500 ppb of chrysene-d12 (Supelco, USA) and perylene-d12 (Supelco, USA) were added as surrogate internal standards in the early steps of the extraction. The recoveries' average for chrysene-d12 and perylene-d12 were 92% and 84%, respectively. Chrysene-d12 had a recovery range of 76-106 %, and perylene-d12 had a recovery range of 76-90 %, respectively. The concentrations of each 16 PAHs were corrected using the overall average recovery (%) of surrogate internal standards of chrysene-d12 and perylene-d12. The overall recovery of each PAH varies between 77.4 % and 120.5 %, as measured by external calibration using standard mixtures of 16 PAHs (SS EPA 610 PAH Mix, Supelco, USA).

### **3.8 Carcinogenic health risk assessment**

To quantify the carcinogenicities of 16 PAHs compared to benzo( $\alpha$ )pyrene and estimate the BaP-equivalent quantities of the 16 PAHs in grilled and fried streets foods, toxic equivalency factors (TEF) are depicted in Table 3.8 (Nisbet & LaGoy, 1992). TEF were used to create toxic equivalence quotients (TEQB( $\alpha$ )P) based on equation (Eq 3.4) (Cheng et al., 2019; Rocha et al., 2020). Each PAH was estimated to be equivalent to B( $\alpha$ )p to highlight its relative hazardous potential in relation to this compound, which is established and shown to be toxic and carcinogenic to humans (Lee et al., 2018).

$$\text{TEQB}(\alpha)\text{P} = \sum C_i \times \text{TEF}_i \quad (\text{Eq 3.4})$$

Where  $C_i$  represents the concentration of each PAHs compound in food, and  $\text{TEF}_i$  is the toxicity equivalency factor of individual PAHs, according to the report from Nisbet & Logay (1992). The sum of each  $\text{TEQB}(\alpha)\text{P}$  was used to calculate the carcinogenic potentials of 16 PAHs. The calculation of  $\text{TEQB}(\alpha)\text{P}$  was calculated from  $\Sigma\text{PAH}_4$  as European Food Safety Authority (EFSA, 2008) recommended that the used of  $\Sigma\text{PAH}_4$  or the  $\Sigma\text{PAH}_8$  systems in determining health risk because  $\text{B}(\alpha)\text{P}$  analyzed alone are insufficient (Sahin et al., 2020).

**Table 3.8 The toxicity equivalency factor (TEF) of 16 PAHs**

PAHs	TEF
Napthalene	0.001
Acenaphthylene	0.001
Acenaphthene	0.001
Fluorene	0.001
Phanthrene	0.01
Anthracene	0.001
Fluoranthene	0.001
Pyrene	0.001
Benz(a)anthracene	0.1
Chrysene	0.01
Benzo( $\beta$ )fluoranthene	0.1

Benzo( $\alpha$ )pyrene	1
Benzo(k)fluoranthene	0.1
Dibenz( $\alpha$ ,h)anthracene	1
Benzo(ghi)perylene	0.01
Indeno(1,2,3-C,D)pyrene	0.01

The incremental life cancer risk (ILCR) was obtained using equation (Eq 3.5) based on the result in this study and the previously reported method (Duan et al., 2016; Ekhtor et al., 2018; GAO et al., 2021).

$$ILCR = TEQB(\alpha)P \times IR \times EF \times ED \times SF \times CF / (BW \times AT) \quad (\text{Eq 3.5})$$

Where IR = ingestion rate from estimated intake of selected street foods obtained from Food Consumption Statistics of Malaysia, Malaysia Adult Nutrition Survey in 2014 (NHMS, 2014). The IR of food products were chicken: 0.00457 kg/day, lamb: 0.00105 kg/day, beef: 0.00534 kg/day, fried fish cake: 0.00279 kg/day, and fried banana: 0.00237 kg/day. SF = oral cancer slope factor of benzo ( $\alpha$ ) pyrene (7.3 mg/kg per day) (USEPA, 2017). ED = exposure duration for the adult population is 30 years (USEPA, 2011). EF = exposure frequency for 365 days/year and CF = conversion factor of  $10^{-3}$ . BW = average adult body weight in Malaysia is 65.4 kg (Lee & Muda, 2019). AT = average lifespan for carcinogens (70 years, 25550 days) from World Health Organization (WHO).

## CHAPTER 4

### RESULT

#### 4.1 Moisture content of foods samples

The moisture content of street food samples was presented in Table 4.1. The result showed that lamb satay had the highest moisture content (53.76%), followed by grilled chicken wings (49.24%), chicken satay (48.04%), fried banana (41.16%), beef satay (40.74%), and fried fish cake (27.97%). Previous study reported that during the cooking process over the open flame, the moisture content of food products was reduced. The decrease in moisture level is critical because it could inhibit microbiological growth and increasing its lifespan (Ledesma et al., 2015).

**Table 4.1 Determination of moisture content (%) in street food sample (initial sample weight = 10g)**

<b>Samples</b>	<b>Dry weight (%)</b>	<b>Moisture content (%)</b>	<b>Dry sample (g)</b>
<b>Chicken Satay</b>	51.96	48.04	5.20
<b>Lamb Satay</b>	46.24	53.76	4.62
<b>Beef Satay</b>	59.26	40.74	5.93
<b>Grilled Chicken Wing</b>	50.76	49.24	5.08

<b>Fried fish cake</b>	72.03	27.97	7.20
<b>Fried Banana</b>	58.84	41.16	5.89

#### 4.2 Quality control

Table 4.2 shows the linear range, correlation coefficients ( $R^2$ ), the limit of detection (LOD), the limit of quantification (LOQ), recovery and RSD values of 16 PAHs standards. The instrument was calibrated with each analyte at a five-point calibration curve at the range of 1 to 2000 ng/mL. For all analytes, the correlation coefficients of measurement ( $R^2$ ) were between (0.9952 and 0.999). Based on evidence reported by some authors, high  $R^2$  value close to unity ( $r=1$ ) reflects a calibration curve with adequate linearity (Moosavi & Ghassabian, 2018).

**Table 4.2. Linear range, correlation coefficients ( $R^2$ ), limit of detection (LOD), limit of quantification (LOQ), and recovery of 16 PAHs compounds (n=3)**

Target Compound	Linear range (ng/mL)	$R^2$	LOD ( $\mu\text{g}/\text{kg}$ )	LOQ ( $\mu\text{g}/\text{kg}$ )	Recovery $\pm$ RSD (%) (n=3)
Naphthalene	3-2000	0.9992	0.18	0.94	89.1 $\pm$ 1.8
Acenaphthylene	2-2000	0.9989	0.15	0.61	114.0 $\pm$ 4.3
Acenaphthene	2-2000	0.9985	0.20	0.62	102.4 $\pm$ 4.1
Fluorene	3-2000	0.9994	0.31	0.91	77.4 $\pm$ 2.2
Phenanthrene	1-2000	0.9990	0.06	0.33	102.8 $\pm$ 3.5
Anthracene	1-2000	0.9971	0.07	0.38	107.7 $\pm$ 2.9

Fluoranthene	2-2000	0.9993	0.13	0.67	103.3 ± 2.3
Benzo(α)anthracene	1-2000	0.9981	0.08	0.30	90.1 ± 1.5
Pyrene	2-2000	0.9998	0.13	0.66	96.8 ± 1.7
Chrysene	1-2000	0.9974	0.09	0.35	90.3 ± 1.5
Benzo(β)fluoranthene	1-2000	0.9997	0.11	0.32	112.7 ± 2.0
Benzo(k)fluoranthene	4-2000	0.9952	0.43	1.28	118.9 ± 3.6
Benzo(α)pyrene	1-2000	0.9999	0.17	0.34	108.4 ± 2.9
Dibenzo(α,h)anthracene	5-2000	0.9957	0.76	1.52	120.5 ± 3.9
Benzo(ghi)perylene	1-2000	0.9988	0.07	0.36	87.9 ± 1.8
Indeno(1,2,3-C,D)pyrene	3-2000	0.9996	0.46	0.93	102.7 ± 2.4

Table 4.2 indicated that the LOD between 16 compounds of PAHs ranged from 0.06 to 0.76 µg/kg and LOQ between 0.30 to 1.52 µg/kg. These limitations correspond to the study that demonstrated a similar range when using GC-MS for the comparable samples with LOD ranged from 0.15 to 0.3 µg/kg, and LOD ranged from 0.47 to 1.00 µg/kg (Kamankesh et al., 2015). The data indicated that the LOD and LOQ remained sufficiently low to recognize the target PAHs compounds in the samples. Based on Table 4.4, the recovery of 16 PAHs samples varied from 77.4 % to 120.5 %. These ranges are comparable to the recovery (87% to 116%) reported by Chiang et al. (2020). In addition, this study is also similar to Chiang et al. (2020) as they used boiled chicken drumstick to test for extraction recovery by spiking with the EU

priority of PAHs. The recoveries data stated in this study was within the acceptable range of 50% to 120% suggested by European Union (2011).

### 4.3 Occurrence of PAHs in street foods

The occurrence of 16 PAHs concentrations in street food samples is summarized in Table 4.3.1. The concentration of 16 PAHs ranged from less than LOQ to the maximum level of 710.60  $\mu\text{g}/\text{kg}$  across all samples. The total mean concentration of 16 PAHs in chicken satay, lamb satay, beef satay, grilled chicken wings, fried fish cake, and fried banana was 262.11  $\mu\text{g}/\text{kg}$ , 713.35  $\mu\text{g}/\text{kg}$ , 2501.70  $\mu\text{g}/\text{kg}$ , 595.05  $\mu\text{g}/\text{kg}$ , 432.55  $\mu\text{g}/\text{kg}$ , and 282.74  $\mu\text{g}/\text{kg}$ , respectively. The beef satay showed the highest mean concentration of 16 PAHs (2501.70  $\mu\text{g}/\text{kg}$ ) compared to other samples, while the chicken satay showed the lowest mean concentration of  $\Sigma 16$  PAHs (262.11  $\mu\text{g}/\text{kg}$ ). The highest mean concentration of PAHs found across all samples was dibenzo( $\alpha$ ,h)anthracene, which ranged from 146.58  $\mu\text{g}/\text{kg}$  (chicken satay) to 710.60  $\mu\text{g}/\text{kg}$  (beef satay). In contrast, the lowest PAHs concentration across all samples was benzo(g,h,i)perylene, only detected in beef satay at 0.90  $\mu\text{g}/\text{kg}$ . Amongst all PAHs compounds, dibenzo( $\alpha$ ,h)anthracene and indeno(1,2,3-cd)pyrene were detected in all street food samples.

**Table 4.3.1 Occurrence of PAHs concentration (mean  $\pm$  standard deviation, n=3) in different type of street foods.**

Target Compound	Chicken Satay ( $\mu\text{g}/\text{kg}$ )	Lamb Satay ( $\mu\text{g}/\text{kg}$ )	Beef Satay ( $\mu\text{g}/\text{kg}$ )	Grilled Chicken Wing ( $\mu\text{g}/\text{kg}$ )	Fried fish cake ( $\mu\text{g}/\text{kg}$ )	Fried Banana ( $\mu\text{g}/\text{kg}$ )
Naphtalene	25.17 $\pm$ 2.39	20.49 $\pm$ 1.15	159.14 $\pm$ 10.98	13.86 $\pm$ 1.14	6.21 $\pm$ 0.51	ND
Acenaphthylene	14.48 $\pm$ 1.35	7.01 $\pm$ 0.48	93.36 $\pm$ 7.38	1.40 $\pm$ 0.09	ND	ND
Acenaphtene	5.20 $\pm$ 0.42	7.03 $\pm$ 0.45	100.26 $\pm$ 6.78	ND	ND	ND
Fluorene	25.68 $\pm$ 2.18	25.57 $\pm$ 2.48	156.45 $\pm$ 15.18	6.56 $\pm$ 0.44	ND	ND
Phenanthrene	18.92 $\pm$ 1.57	28.59 $\pm$ 2.43	139.37 $\pm$ 11.71	6.48 $\pm$ 0.47	ND	0.51 $\pm$ 0.04
Anthracene	4.76 $\pm$ 0.38	28.58 $\pm$ 2.17	140.22 $\pm$ 11.92	ND	ND	0.51 $\pm$ 0.04
Fluoranthene	ND	10.57 $\pm$ 0.67	133.45 $\pm$ 10.14	ND	ND	ND
Benzo( $\alpha$ )anthracene	11.01 $\pm$ 0.86	0.74 $\pm$ 0.06	127.94 $\pm$ 9.72	0.86 $\pm$ 0.07	ND	ND
Pyrene	1.67 $\pm$ 0.11	0.58 $\pm$ 0.05	93.97 $\pm$ 6.48	2.12 $\pm$ 0.18	1.10 $\pm$ 0.08	ND
Chrysene	ND	ND	253.45 $\pm$ 19.77	ND	ND	ND
Benzo( $\beta$ )fluoranthene	ND	1.19 $\pm$ 0.12	232.52 $\pm$ 16.04	ND	ND	1.33 $\pm$ 0.09

Benzo(k)fluoranthene	$0.77 \pm 0.04$	$1.76 \pm 0.11$	$0.99 \pm 0.08$	$0.70 \pm 0.06$	$1.65 \pm 0.11$	$3.28 \pm 0.22$
Benzo( $\alpha$ )pyrene	$3.08 \pm 0.21$	$2.12 \pm 0.17$	ND	$3.11 \pm 0.28$	$2.06 \pm 0.14$	ND
Dibenza( $\alpha$ ,h)anthracene	$146.58 \pm 13.49$	$508.93 \pm 29.52$	$710.60 \pm 48.32$	$543.60 \pm 46.21$	$419.85 \pm 23.51$	$275.80 \pm 23.99$
Benzo(ghi)perylene	ND	ND	$0.90 \pm 0.05$	ND	ND	ND
Indeno(1,2,3-C,D)pyrene	$4.79 \pm 0.39$	$69.92 \pm 4.68$	$159.08 \pm 12.09$	$16.36 \pm 1.13$	$1.68 \pm 0.10$	$1.31 \pm 0.12$
Total 16 PAHs	$262.11 \pm 22.36$	$713.35 \pm 44.23$	$2501.70 \pm 172.69$	$595.05 \pm 51.88$	$432.55 \pm 35.63$	$282.74 \pm 26.51$

ND = Not detected (< LOQ)

**Table 4.3.2 Mean concentration of B( $\alpha$ )p,  $\Sigma$ PAH4,  $\Sigma$ PAH8, and  $\Sigma$ PAHs ( $\mu\text{g}/\text{kg}$ ) of street foods sample**

Street foods	Mean concentration ( $\mu\text{g}/\text{kg}$ wet wt)			
	B( $\alpha$ )p	$\Sigma$ PAH4	$\Sigma$ PAH8	$\Sigma$ PAHs
Chicken satay	0.77	2.44	156.89	262.11
Lamb satay	1.76	3.8	584.77	713.35
Beef satay	0.99	580.93*	1451.51	2501.70
Grilled chicken wings	0.70	2.82	565.89	595.05
Fried fish cake	1.65	2.75	426.34	432.55
Fried banana	3.28	4.61	281.72	282.74

\* Exceed European Union limit: 30  $\mu\text{g}/\text{kg}$

$\Sigma$ PAH4: Total of benzo( $\alpha$ )anthracene, chrysene, benzo( $\beta$ )fluoranthene, and benzo( $\alpha$ )pyrene

$\Sigma$ PAH8: Total of benzo( $\alpha$ )anthracene, chrysene, benzo( $\beta$ )fluoranthene, benzo( $\alpha$ )pyrene, benzo(k)fluoranthene, dibenzo( $\alpha$ ,h)anthracene, benzo(ghi)perylene, and indeno(1,2,3-C,D)pyrene

Table 4.3.2 shows the mean concentration of benzo( $\alpha$ )pyrene,  $\Sigma$ PAH4,  $\Sigma$ PAH8, and  $\Sigma$ PAHs ( $\mu\text{g}/\text{kg}$ ) of street foods sample. Based on the benzo( $\alpha$ )pyrene concentration stated in Table 5, all the samples were reported below the maximum limit of 5.0  $\mu\text{g}/\text{kg}$  from European Union (Ekhaton et al., 2018; EU, 2011). Beef satay contained the highest concentration of PAH4 (580.93  $\mu\text{g}/\text{kg}$ ), and chicken satay contained the lowest concentration of PAH4 (2.44  $\mu\text{g}/\text{kg}$ ). Then, the sum of eight PAHs showed the concentration ranged from 156.89 to 1451.51  $\mu\text{g}/\text{kg}$ . Beef satay contained the highest concentration in PAH4, PAH8 and  $\Sigma$ PAHs with the concentration of 580.93  $\mu\text{g}/\text{kg}$ , 1451.51  $\mu\text{g}/\text{kg}$ , and 2501.70  $\mu\text{g}/\text{kg}$ , respectively.

#### 4.4. Potential carcinogenic health risk of PAHs

In assessing carcinogenic health risk, an incremental lifetime cancer risk (ILCR) value between  $10^{-6}$  and  $10^{-4}$  suggests the potential risk, while a value larger than  $10^{-4}$  indicates severe risks to human health and needs public health concern. An ILCR value of  $10^{-6}$  or less indicates an acceptable degree of carcinogenic risk (Duan et al., 2016; Jiang et al., 2018; USEPA, 2005). Therefore, it is necessary to calculate the carcinogenic health risk of PAHs from foods samples as previous studies only reported PAHs' occurrence without estimating the health risk assessment (Afsaneh Farhadian et al., 2010; Jahurul et al., 2013).

**Table 4.4 Incremental lifetime cancer risk (ILCR) of PAHs and toxicity equivalency quotient concentrations (TEQB( $\alpha$ )P,  $\mu\text{g}/\text{kg}$ ) in the different types of street foods**

Street foods	TEQB( $\alpha$ )P ( $\mu\text{g}/\text{kg}$ )	Estimated risk
Chicken Satay	0.94	$2.05 \times 10^{-7}$
Lamb Satay	1.96	$9.87 \times 10^{-8}$
Beef Satay	36.17	$9.24 \times 10^{-6}$
Grilled Chicken Wing	0.91	$1.99 \times 10^{-7}$
Fried fish cake	1.76	$2.34 \times 10^{-7}$
Fried Banana	3.41	$3.87 \times 10^{-7}$

The calculated ILCR values from all samples and toxicity equivalency quotient in street foods are shown in Table 4.4. In this study, the value of ILCR was

estimated based on TEQB( $\alpha$ )P from the concentration of PAH4 with the toxicity equivalency factor and oral cancer slope (US EPA & IRIS, 2017). The ILCR reported in this study for chicken satay, lamb satay, beef satay, grilled chicken wings, fried fish cake, and fried banana were  $2.05 \times 10^{-7}$ ,  $9.87 \times 10^{-8}$ ,  $9.24 \times 10^{-6}$ ,  $1.99 \times 10^{-7}$ ,  $2.34 \times 10^{-7}$ , and  $3.87 \times 10^{-7}$  respectively. Thus, based on the estimated risk stated in Table 6, the health risk assessment of dietary consumption of fried and grilling street foods is within the USEPA acceptable degree of carcinogenic risk among Malaysia's adults' population. Beef satay is identified as the highest carcinogenic risk among the six street foods, followed by fried banana, chicken satay, grilled chicken wing, fried fish cake, and lamb satay.

## CHAPTER 5

### DISCUSSION

#### 5.1. Moisture content of food samples

Based on the result shown in Table 2, the highest percentage of moisture content was detected in lamb satay (53.76%), followed by grilled chicken wings (49.24%), while the lowest percentage of moisture content was detected in fried fish cake (27.97%). The result in this study was in agreement with Siddique et al. (2021), as the data showed 43% cooking loss from frying method and 49% from grilling method with equivalent with moisture content of friend banana (41.16%) and chicken satay (49%). The degradation of moisture during thermal processing is determined not only from the different cooking methods, the surface area of foods, duration, and temperature, but also by the meat quality, which includes the amount of lipid, water, protein, and also the pH and thickness of the meat (Siddique et al., 2021).

#### 5.2. Occurrence of PAHs in street foods

The highest mean concentration of PAHs was dibenzo( $\alpha$ ,h)anthracene (710.60  $\mu\text{g}/\text{kg}$ ) and it was detected in all street food samples. The result was in contrast with previous studies, which indicate that dibenzo( $\alpha$ ,h)anthracene was not detected in

various kind of samples such as grilled meat, fried chicken, and boiled chicken (Onwukeme & Okafor, 2015; Sahin et al., 2020). Furthermore, the lowest PAHs compound found across in all samples was benzo(ghi)perylene, in which the concentration was below LOQ except for beef satay (0.90  $\mu\text{g}/\text{kg}$ ). In addition, the concentration of naphthalene in chicken satay reported in this study (25.17  $\mu\text{g}/\text{kg}$ ) was in agreement with previous findings where they had reported the concentration of naphthalene in grilled chicken using black charcoal was 23.87  $\mu\text{g}/\text{kg}$  (Kim et al., 2021). Meanwhile, the concentration of benzo( $\alpha$ )pyrene in chicken satay was 0.77  $\mu\text{g}/\text{kg}$ , which indicated that the concentration was lower than the findings from Farhadian et al. (2010) (1.97  $\mu\text{g}/\text{kg}$ ) and was higher than the findings from Onwukeme et, al (2015) (0.03  $\mu\text{g}/\text{kg}$ ).

The highest mean concentration of  $\Sigma$ PAHs was found in beef satay (2501.70  $\mu\text{g}/\text{kg}$ ) across all samples. The result in this study was comparable to literature that reported  $\Sigma$ PAHs concentration for chicken and beef where the concentration of  $\Sigma$ PAHs in beef was usually higher than chicken meat (Alomirah et al., 2011; Babaoglu et al., 2017; Duedahl-Olesen et al., 2015; Kim et al., 2021; Mohammadi & Valizadeh-kakhki, 2018). The concentration of PAHs concentration was different between samples which may be related to the cooking method, heating temperature, fat content, and duration of cooking time (Ekhatior et al., 2018; Iwegbue, 2016; Kim et al., 2021; Li et al., 2016; Zelinkova & Wenzl, 2015).

The concentrations of benzo( $\alpha$ )pyrene of all samples were below the limit of the

European Union (5 µg/kg) (EU, 2011). Similar to the findings by Wang et al. (2019), the concentration of benzo(α)pyrene in the grilled chicken wing was 1.51 µg/kg, which was lower than the prescribed level. According to the Scientific Committee on Food (SCF), further research on the appropriateness of keeping benzo(α)pyrene as a baseline is required (Ledesma et al., 2015; Li et al., 2016). However, based on evidence of prevalence and toxicity, the European Food Safety Authority (EFSA) stated that benzo(α)pyrene itself would not be an adequate overall indicator for PAHs in food. Thus, compounds of four PAHs (PAH4) and eight PAHs (PAH8) performed as better indicators (EFSA, 2008; Lee et al., 2016; Rose et al., 2015).

Based on the concentration of PAH4, all street foods samples were within the maximum limit of 30.0 µg/kg according to the Commission Regulation (EU) No 835/2011 except for beef satay (EU, 2011). Beef satay was the highest concentration in PAH4 could be linked to the fat content of beef satay. Fat loss from beef satay may happen primarily during the initial stages of grilling, and then PAHs generated on the heated charcoal are coated on the surface of beef satay. Then, the grilling process is re-volatilized or degraded during a longer duration of cooking (Rose et al., 2015).

### 5.3. Potential carcinogenic health risk of PAHs

The ILCR values reported in this study for all street foods were acceptable carcinogenic health risks because the value was reported within  $10^{-6}$  and lower than  $10^{-4}$ . Jiang et al. (2018) stated that the adult population had higher chances of developing cancer risk than children, adolescents, and seniors with the ILCR value of  $3.75 \times 10^{-6}$ . Duan et al. (2016) discovered that the estimated ILCR from dietary intake consumption of PAHs in adults was  $6.65 \times 10^{-5}$ , which is comparable to the result reported in this current study. Li et al. (2016) demonstrated that the ILCR for an adult was  $5.45 \times 10^{-7}$  from Chinese traditional fried food, which is also similar than the ILCR of fried food from this study in fried fish cake ( $2.34 \times 10^{-7}$ ) and fried banana ( $3.87 \times 10^{-7}$ ). According to previous studies, assessing the health risks related to PAHs exposure is a complicated issue. The various ILCR outcomes are explained because these studies used different exposure durations, daily food consumption amounts, and PAH exposure levels (Jiang et al., 2018; Li et al., 2016; Patel et al., 2020). Generally, the higher contamination levels and dietary consumption of PAHs may lead to a higher health risk.

## CHAPTER 6

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

This study aimed to determine the occurrence of PAHs in a variety of street foods in Seri Kembangan, Selangor (chicken satay, lamb satay, beef satay, grilled chicken wing, fried fish cake, and fried banana) as well as the health risks associated with them. The results of this investigation showed that benzo( $\alpha$ )pyrene was detected in all street foods, in the range of 0.70  $\mu\text{g}/\text{kg}$  – 3.28  $\mu\text{g}/\text{kg}$ . However, the concentration of benzo( $\alpha$ )pyrene in all street foods sample were stated in the acceptable limit of 5  $\mu\text{g}/\text{kg}$ . The highest PAH concentration was detected in beef satay followed by lamb satay, grilled chicken wings, fried fish cake, fried banana, and chicken satay. Based on the results of this study, high cooking temperatures and methods have a substantial effect on the formation of PAHs in street foods samples.

Based on the other objective, moisture content from different types of selected street foods was investigated to correlate with the concentration of PAHs. Lamb satay has the highest moisture content (53.76%), followed by grilled chicken wings (49.24%), chicken satay (48.04%), fried banana (41.16%), beef satay

(40.74%), and fried fish cake (27.97%). Moreover, the moisture content of the samples in this study is not correlated with PAHs as the highest total concentration in this study was beef satay.

The carcinogenic health risk assessment conducted in this study showed that the ILCR value for all street foods samples was in the USEPA acceptable level of carcinogenic risk among the adults' population in Malaysia. ILCR values reported from this study are  $2.05 \times 10^{-7}$ ,  $9.87 \times 10^{-8}$ ,  $9.24 \times 10^{-6}$ ,  $1.99 \times 10^{-7}$ ,  $2.34 \times 10^{-7}$ , and  $3.87 \times 10^{-7}$  for chicken satay, lamb satay, beef satay, grilled chicken wings, fried fish cake, and fried banana, respectively. The ILCR values indicate that the exposure of PAHs through dietary consumption posed a low carcinogenic risk to the adult population compared to the other studies.

## **6.2 Study limitation**

Based on the moisture content method, we must dry the sample for 24 hours continuously. However, due to technical issues from the oven as it cannot operate continuously, we can only dry the sample by calculating how long it has taken to complete the required 24 hours.

### 6.3 Recommendations

The occurrence of PAHs in certain selected street foods may indicate a high risk to human health. Furthermore, there are currently no legislative restrictions for this category of food contamination issues. Thus, more research and investigations are required to create the standards and limitations for PAHs contamination in foodstuffs. In addition, the existence of PAHs in food needs more attention by food security strategies from the authorities to conduct guidelines on safe and healthy cooking practices. Then, several awareness programs to increase the knowledge level on the occurrence of PAHs in food and the potential health impacts from prolonged exposure need to be organized. The factors contributing to the occurrence of PAHs need to spread as they can help the Malaysian population practice the safe cooking method from the guideline given. Since the environment is one of the factors that contribute to the presence of PAHs in street foods, thus several precautions shall be practised by the sellers. The precaution practices include choosing the area that is far from heavy road traffic, covering the food all the time, and practising more hygiene during food preparation.

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