UNIVERSITI PUTRA MALAYSIA

OCCURENCE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SELECTED SMOKED FOODS AND THEIR ASSOCIATED HEALTH RISK TO CONSUMERS IN SELANGOR

NUR SAHIRA BINTI ROZAIMAN ZAIDI

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BY

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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

ACKNOWLEDGEMENTS

Praise to Allah S.W.T, the Most Gracious and Most Merciful for enabling mand give me strength and His blessing to complete my thesis. Thank you so much to numerous people who advice and support me from start until the end of my final year project. I couldn't finish this work without their help. Special appreciation and deep gratitude to my supervisor, Assoc. Prof. Dr. Ho Yu Bin and Dr. Samer for their constant support and guide me throughout this project. I can't imagine how my project will be finished without their help. Their guidance, constructive comments and support have contributed a lot to finish the project. To my supervisor, thank you for your generous help, insightful advice during my thesis. Your help and recommendations have been helpful and appreciated in my life.

Not forgetting to my backbone which are parents and my family member for their endless support and praying me from beginning of my studies until now. They keep motivate me and always be my listeners keep me up all the time. Thanks to all my fellow mates Hanna, Sarah, Athirah, Amira and others for their supports and assistance along the journey.

Finally, I would like to thank my close friends who always be my side and I received their endless support and believe with me that I could finish this thesis. So, I would like to dedicate this thesis to my mother and father because with their unconditional loves and prayers I am here right now.

ABSTRACT

OCCURENCE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SELECTED SMOKED FOODS AND THEIR ASSOCIATED HEALTH RISK TO CONSUMERS IN SELANGOR

NUR SAHIRA BINTI ROZAIMAN ZAIDI

Introduction: Polycyclic Aromatic Hydrocarbons (PAHs) are persistent contaminants where presence in abundance throughout the environment and food that is the primary route of contamination. The prevalence of PAHs in foods has been attributed due to the cooking process and practices including smoking. Consequently, the formation of PAHs in foods have shown to cause negative impacts on human health such as cancer. **Objectives:** This study aimed to measure the concentration of 13 PAHs compounds present in the six different types of local smoked foods (beef, quail, duck, catfish, chicken, and lamb), and estimate the health risk among Selangor population. **Methodology:** Smoked food sample was extracted using ultrasonication, cleaned-up with RP-18 solid phase extraction (SPE) silica column, quantified by gas-chromatography mass spectrometry (GCMS). Incremental lifetime cancer risk (ILCR) was used to estimate the carcinogenic health risk of consuming PAHs contaminated smoked food**. Results and Discussion:** The findings showed that thirteen PAHs were identified in all smoked food samples. The mean concentration of benzo(a)pyrene (B(a)P) of four out of six samples exceeded the maximum limit of European Union (5 µg/kg for B(a)P). Amongst six groups of smoked food samples, smoked beef (354.02 µg/kg) appeared to have greater Σ13 PAHs contamination, followed by smoked quail (261.11 µg/kg), smoked duck (168.10 µg/kg), smoked chicken (64.15 µg/kg), smoked catfish (33.60 µg/kg) and the least was smoked lamb (27.48 µg/kg). The concentration of PAH4 of four samples were above the acceptable limit European Union (30 µg/kg) except for smoked chicken, smoked catfish and smoked lamb. The results of incremental lifetime cancer risk (ILCR) for the smoked foods consumption were between 10^{-8} and 10^{-5} which indicate an acceptable or inconsequential carcinogenic health risk among adult consumer in Selangor. **Conclusion:** The results showed that the thermal cooking process could lead to PAHs formation. Increase in consumption rate of smoked food must be cautious as it increases the carcinogenic health risk.

Keywords: Polycyclic aromatic hydrocarbons, smoked foods, smoked meats, GC-MS, carcinogenic health risk assessment

ABSTRAK

KEJADIAN POLISIKLIK HIDROKARBON AROMATIK (PAHs) DALAM MAKANAN SALAI TERPILIH DAN RISIKO KESIHATAN BERKAITANNYA KEPADA PENGGUNA DI SELANGOR

NUR SAHIRA BINTI ROZAIMAN ZAIDI

Pengenalan: Polisiklik Hidrokarbon Aromatik (PAHs) adalah bahan pencemar yang berterusan di mana boleh didapati dengan banyaknya di seluruh alam sekitar dan juga makanan yang menjadi laluan utama pencemaran. Kelaziman PAHs dalam makanan telah dikaitkan dengan proses memasak dan amalan termasuk salai. Akibatnya, pembentukan PAHs dalam makanan telah terbukti menyebabkan kesan negatif terhadap kesihatan manusia seperti kanser. **Objektif:** Kajian ini bertujuan untuk menentukan kepekatan individu dan jumlah 13 sebatian PAHs yang terdapat dalam enam jenis makanan local salai (daging salai, itik salai, puyuh salai, ikan keli salai, ayam salai dan kambing salai), dan juga menganggarkan risiko kesihatan dalam kalangan penduduk Selangor**. Metodologi:** Sampel makanan salai diekstrak menggunakan ultrasonik, dibersihkan dengan lajur silika pengekstrakan fasa pepejal (SPE) RP-18, dianalisis dengan spektrometri jisim kromatografi gas (GCMS). Risiko kanser seumur hidup tambahan (ILCR) digunakan untuk menganggarkan risiko kesihatan karsinogenik memakan makanan salai yang tercemar PAH. **Keputusan dan Perbincangan:** Penemuan menunjukkan bahawa tiga belas PAH telah dikenal pasti dalam semua sampel makanan salai. Purata kepekatan benzo(a)pyrena (B(a)P) daripada empat daripada enam sampel melebihi had maksimum Kesatuan Eropah (5 µg/kg untuk B(a)P). Di antara enam kumpulan sampel makanan salai, daging salai (354.02 µg/kg) nampaknya mempunyai pencemaran Σ13 PAH yang lebih besar, diikuti oleh puyuh salai (261.11 µg/kg), itik salai (168.77 µg/kg), ayam salai (64.15 µg/kg), ikan keli salai (33.60 µg/kg) dan yang paling sedikit ialah kambing salai (27.48 µg/kg). Kepekatan PAH4 bagi empat sampel adalah melebihi had yang boleh diterima Kesatuan Eropah (30 µg/kg) kecuali ayam salai, ikan keli salai dan kambing salai. Keputusan peningkatan risiko kanser seumur hidup (ILCR) untuk penggunaan makanan salai adalah antara 10^{-8} dan 10^{-5} yang menunjukkan risiko kesihatan karsinogenik yang boleh diterima atau diabaikan dalam kalangan pengguna dewasa di Selangor. **Kesimpulan:** Keputusan menunjukkan bahawa proses memasak terma boleh menyebabkan pembentukan PAH. Peningkatan penggunaan
kadar makanan salai mesti berhati-hati kerana ia meningkatkan risiko kesihatan karsinogenik.

Kata kunci: Polisiklik aromatik hidrokarbon, makanan bersalai, daging salai, GC-MS, penilaian risiko karsinogenik kesihatan

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LIST OF ABBREVATIONS

PAHs	Polycyclic Aromatic Hydrocarbons
US EPA	United State Enviromental Protection Agency
IARC	International Agency for Research on Cancer
NHMS	National Health Malaysian Statistic
ILCR	Incremental Life Cancer Risk
EFSA	European Food Safety Authority
GC-MS	Gas Chromatography-Mass Spectrometry
LOD	Limit Of Detection
LOQ	Limit Of Quantification
RSD	Relative Standard Deviation
TEQ	Toxicity Equivalency Quotient
EU	European Union
TEF	Toxicity Equivalency Factors
IR	Ingestion Rate
EF	Exposure Frequency
SPE	Solid-phase extraction
ED	Exposure Duration
SF	Slope Factor
BW	Body Weight
CF	Conversion Factor
DCM	Dichloromethane

CHAPTER 1

INTRODUCTION

1.1 Study Background

Over the past decade, food smoke was among the most well-known food preservation, particularly for meat and its products. The techniques used for food smoking have been brought down through the years and are now in use today across the world. Foodstuffs that are exposed to smoke have a longer shelf life before deteriorating. Furthermore, food smoking also will cause dehydrating qualities and the existence of organic compounds which have anti-bacterial characteristics. Apart from that, smoking foodstuffs are also known to improve their flavour, colour, and scent. They can also reduce microbiological contamination while preventing lipid oxidation. The smoke-drying technique, as per Codex Alimentarius (2013), allows them to create dried products with a water activity of less than or equal to 0.75, enabling us to maintain the end product at ambient temperature and protect it from bacterial and fungal modification. Even though the smoking provides extra flavour and scent among the food stuffs, but as an unfavourable consequence of smoking will generate polycyclic aromatic hydrocarbons (PAHS) during incomplete combustion of wood. Despite these benefits, the intake of smoked fish or meat products poses serious risks due to pollutants introduced during the manufacturing process. Consumption of polluted food and water are a common way for people to be exposed to PAHs. Smoked foods are a dominant contributor of PAHs in the food. However nowadays, people still

choose to consume smoked food as their choice of dishes, this is because smoked meat, fish, and chicken has grown in popularity towards this day.

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic molecules with two or more aromatic rings. They can pollute meat and its products both directly through pyrolysis of dietary components and indirectly through smoke generated by incomplete combustion of organic substances (Hamidi et al., 2016). As a result, smoked or barbequed foods often contain significant levels of PAHs. Although PAHs are found in abundance throughout the environment, the diet is the primary route of contamination. The prevalence of PAHs in foodstuffs has been attributed mostly to the processes of smoking, drying, roasting, grilling, and barbecuing. Therefore, smoke production parameters have been shown to have a significant effect on the level of PAHs in the food consequently, in smoked foods. Mostly in smoked types of food, such as meat, rice, and fish, polycyclic aromatic compounds have been discovered as hazardous chemicals (Fazeli et al., 2021). Thus, smoke flavourings are made from smoke condensates, which could also be a contributor to PAHs.

Apart from that, PAHs have already been extensively analysed in countries all around the world for their occurrence and toxicity. They are found in complicated combinations containing hundreds of different chemicals. In addition, a study from Parada et al. (2017) stated that high intake of grilled/barbecued and smoked meat among US women could be linked to prevalence of breast cancer. This is because high prevalence sources of PAH were generated in the cooking process that lead to certain

chronic disease. Usually, combustion reaction or pyrolysis of organic material produces PAHs. These toxic PAHs are created when the typical smoking procedure is applied to any food product and the food and smoke come into close contact. The physical or chemical features of PAHs, including their solubility in water and fats/oils, volatility, chemical reactivity, and biotic and abiotic degradability, all influence food contamination from environmental PAHs (Baali & Yahyaoui, 2020).

PAHs can be ingested, inhaled, or encounter the skin. They can also be ingested through contaminated food (EFSA,2008). Similarly, PAHs are polluted in foods whether by the environment (vehicle exhaust fumes, etc) or through traditional food preparation including smoking, grilling, etc (EFSA, 2008; Silva et al., 2011). Apart from that, 16 PAHs have been added to the European Union's (EU) list of priorities (SCF) because of their mutagenicity (Scientific Committee on Food). Meanwhile, benzo[a]anthracene (B(a)A), chrysene (CHR), benzo[a]pyrene (B(a)P), and benzo[b]fluoranthene (B(b)F) are PAH4 that are significant in food because of their toxicity and incidence (EFSA,2008). Hence, CHR, B(a)A, and B(b)F are categorised as possible human carcinogens (Group 2B) by the International Agency for Research on Cancer (IARC) (International Agency for Research on Cancer). If smoked and grilled foods form a big part of a person's regular diet, they may significantly increase their PAHs intake (Kafeelah et al., 2015). Consequently, there is a continuous need for more research into PAH contamination levels in foods, particularly smoked foods, which are highly valued by many customers.

1.2 Problem Statement

Concern about polycyclic aromatic hydrocarbons (PAHs) in foods began in the 1960s and 1970s, when researchers concentrated on two types of dietary toxicants that cause tumours which are polycyclic aromatic hydrocarbons (PAHs) and n-nitroso compounds. Smoking is a great way as preservation method, however it also a root of contamination of foodstuffs with carcinogenic PAHs (Hitzel et al., 2013). Some of these substances have also been shown to be carcinogenic and genotoxic. The most hazardous of PAHs toxicity is cancer. Thus, the problem of smoked food can cause major health problems if consumed in large quantities because a few methods of smoking are produced by incomplete pyrolysis of the organic fuel (wood) which creates the smoke.

The European Food Safety Authority (EFSA), the Food Scientific Committee (SCF), and the Committee of Food Additives Experts (JECFA) analyse PAH substances Among these, 16 PAH chemicals are deemed to be of particular concern (Singh & Agarwal, 2018). However, it is hard to determine the PAHs compound quantitatively, therefore the most major PAH compound and known as the most potent carcinogen is B(a)P Group 1 carcinogen has been chosen for analysis. Moreover, EFSA has indicated that B(a)P compound alone are insufficient but PAH4 or PAH8 should be used (EFSA,2008).

Basically, PAHs are found in both dietary and non-dietary factors in humans (e.g., inhalation and skin contact). PAHs are transmitted to food in two different ways

where the first contamination of PAHs generated by air, water, and soil while the second way is contamination through food during processing and cooking. So, this processing food such as smoking and dyring while frying, grilling and roasting known as cooking at high temperature which these factors are mainly cause of PAHs formation (Bansal & Kim, 2015; Jiang, 2018).

PAHs also can be found in food due to contamination in the surroundings and manufacturing methods such as smoking, drying, roasting, baking, barbecuing, and frying. Importantly, they have a variety of detrimental consequences on people's health, the most are linked to carcinogenesis and mutagenesis, as well as immunosuppressive effects as stated by (Rengarajan et al., 2015). PAHs found in smoked, grilled, or grilled meat over open flame can harm DNA and raise cancer risk. Even though not all 16 polycyclic aromatic hydrocarbons (PAHs) are considered as carcinogenic, their function as free radicals and bioaccumulation which leads to cellular damage, can have a detrimental effect on human health. Similarly, other epidemiological data have shown that a significant fraction of cancer cases can be attributed to dietary variables, including dietary exposure to PAHs (Kim et al., 2013). As a result, the existence of these chemical compounds in foods is alarming and necessitates ongoing surveillance. According to European Union Commission (EC) Regulations No 1881/2006 and (EU) No 835/ (2011), B(a)P is the present indicator for the prevalence and toxicity of PAHs in meat products.

B(a)P is the most thoroughly investigated PAHs, mainly to its genotoxic action because of direct intercalation in DNA, which causes structural alterations and mutations. The highest concentrations are found in traditionally smoked meat, along

with smoked fish and derived products, were revised by the Commission Regulation 1255/2020 in 2020. It's worth mentioning that PAHs are not harmful in and of themselves, however that they are initiated by the organism's attempt to remove these xenobiotics with elevating polarity through the addition of polar groups.

Another study analysed the amounts of PAHs in foodstuffs accessible in Beijing, China and found that intake of meat (beef, pork, chicken, and lamb) was the primary source of PAHs (Yu et al., 2011). Thus, the higher consumption of meat and its products could significantly affect formation of harmful compounds such as PAH in the body and give health impact to the consumers. Therefore, consumption of meat and animal products, has been relate to the risk of some chronic conditions like colorectal cancer (CRC)(Alexander et al., 2010). Moreover, instead of the technological heating methods such as grilling and smoking which involve direct contact with combustion gases are a significant source of contamination, it is also determined by a variety of factors that lead to the production of PAHs from foodstuffs. Likely, these are the factors that leads to level of contamination such as time and temperature of sorting (higher temperatures and longer times produce more PAHs), distance from source of heat (the greater the distance, the lower the contamination level in foods), type of process (grilling, roasting, smoking, drying), especially if food comes into contact with combustion products, type of fuel used (carbon burning produces fewer PAHs than wood burning), and fat intake in processed food (fat is the major precursor of PAHs) (Purcaro et al., 2013).

In foodstuffs, PAHs are always found as a complex mixture. This must be considered while deciding on the best risk assessment method for assessing PAH

toxicity. In addition, if people adopt cooking methods that raise the quantities of contaminants and their bio accessibility, the concerns that come from eating smoked meat may arise. In terms of harmful PAHs, a few studies have been conducted to assess PAHs in processed and prepared foods, particularly grilled and smoked foods. To the best of our knowledge, there are only a few studies that tested the association between PAHs in smoked foodstuffs among the population in Malaysia. These individuals are at a high health risk due to the contamination to the PAHs by intake of smoked foods in frequent and high quantities.

1.3 Study Justification

Food intake of PAHs could increase relevance for assessing cancer risk in the human body because it is a major human exposure pathway for contamination. Bio accessibility of PAHs is recommended over total PAHs because it's always exceed the exact amount accessible for absorption by the body. Despite that, processed foods were recognised as a human carcinogen by the International Agency for Research on Cancer (IARC) where various chemical compounds contained in processed beef may pose a health risk to people including PAHs (IARC,2015). As a result, the European Union and the US Environmental Protection Agency have designated them as priority pollutants (US EPA) (Farhadian et al., 2011). While, some organizations, such as the International Programme on Chemical Safety, the Scientific Committee on Food and the Joint FAO/WHO Expert Committee on Food Additives, concluded that 15 PAHs are possibly harmful to humans and should be a primary concern in the determining of the long-term health impacts from dietary intake (Alexander et al., 2010).

In addition, the main mode of exposure has been found as contaminated foods consumption, which accounts for 88 to 98% of all cases. Several studies have found that dietary exposure to grilled and smoked food contains PAHs are linked to an elevated risk of certain human cancers (Ledesma et al., 2016; Lingbeck et al., 2014; Olmedilla-Alonso et al., 2013). Then, they also found that high levels of PAHs can contribute to the risk of cancer, such as stomach cancer. However, it still be consumed by humans as smoking is only selected to give foodstuffs an improved, specific organoleptic character, such as better flavour, colour, and smell, because these are qualities that consumers want. Thus, due to the obvious contamination of foodstuffs by PAHs, significant attention has been paid to the smoking process. Other than that, very few studies have assessed PAHs level of various smoked foods commonly consumed among Malaysia. In fact, PAHs are difficult to detect in food since they only occur in trace amounts. Another big obstacle is the matrix's lipophilicity, which frequently results in the coextraction of various interfering complex chemicals (Ledesma et al., 2016).

A few Malaysian foods were recently measured the PAHs concentration and in grilled meat and fish in that study, but they didn't report on PAH concentrations in smoked foods (Farhadian et al., 2010). Hence, least of studies estimate the carcinogenic health risk due to consumption of these foods. Meat, chicken, and other dishes prepared by smoking are increasingly popular both at home and in restaurants in Malaysia as well as in other countries such as Kuwait, Beninese, Serbia and so on. This suggests the need to investigate PAHs in these food products. However, smoked meat, also known as smoked beef, is the outcome of a way of preparing red meat, white

meat, and seafood that can be stored and utilised for a long time. Furthermore, limited study investigates the smoked food samples especially foods that famous in Selangor, Malaysia such as smoked beef, smoked chicken, smoked duck, smoked catfish, smoked quail and smoked lamb. So, the aim of this study was to investigate the levels of the 13 PAHs in different types of smoked food samples and estimate their health risk assessment among consumers in Selangor. To the best of our knowledge, this is the first study to determine PAHs level in different types of smoked food samples in Malaysia. Moreover, population exposure of PAHs has not been assessed in Malaysia. Therefore, the objective of the present study was to determine 13 PAHs concentrations in six groups of smoked foods and estimate their carcinogenic health risk, which in turn can be used in epidemiologic studies to assess health risk due to dietary PAHs exposures in the future.

1.4 Research Question

I. What is the concentration of 13 Polycyclic Aromatic Hydrocarbons compounds in different types of smoked food samples in Selangor, Malaysia?
II. Is there any carcinogenic health risk due to consumption of PAHs contaminated smoked food samples that collected in Selangor, Malaysia?

1.5 Research Objectives

1.5.1 General Objectives

To determine the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in smoked food samples collected in Selangor and assess the carcinogenic health risk of consumption.

1.5.2 Specific Objectives

I. To determine the concentration of 13 Polycyclic Aromatic Hydrocarbons (PAHs) present in different types of local smoked food samples collected in Selangor, Malaysia.

II. To estimate the carcinogenic health risk of Polycyclic Aromatic Hydrocarbons (PAHs) due to consumption of PAHS contaminated smoked foods collected in Selangor, Malaysia.

1.6 Conceptual framework

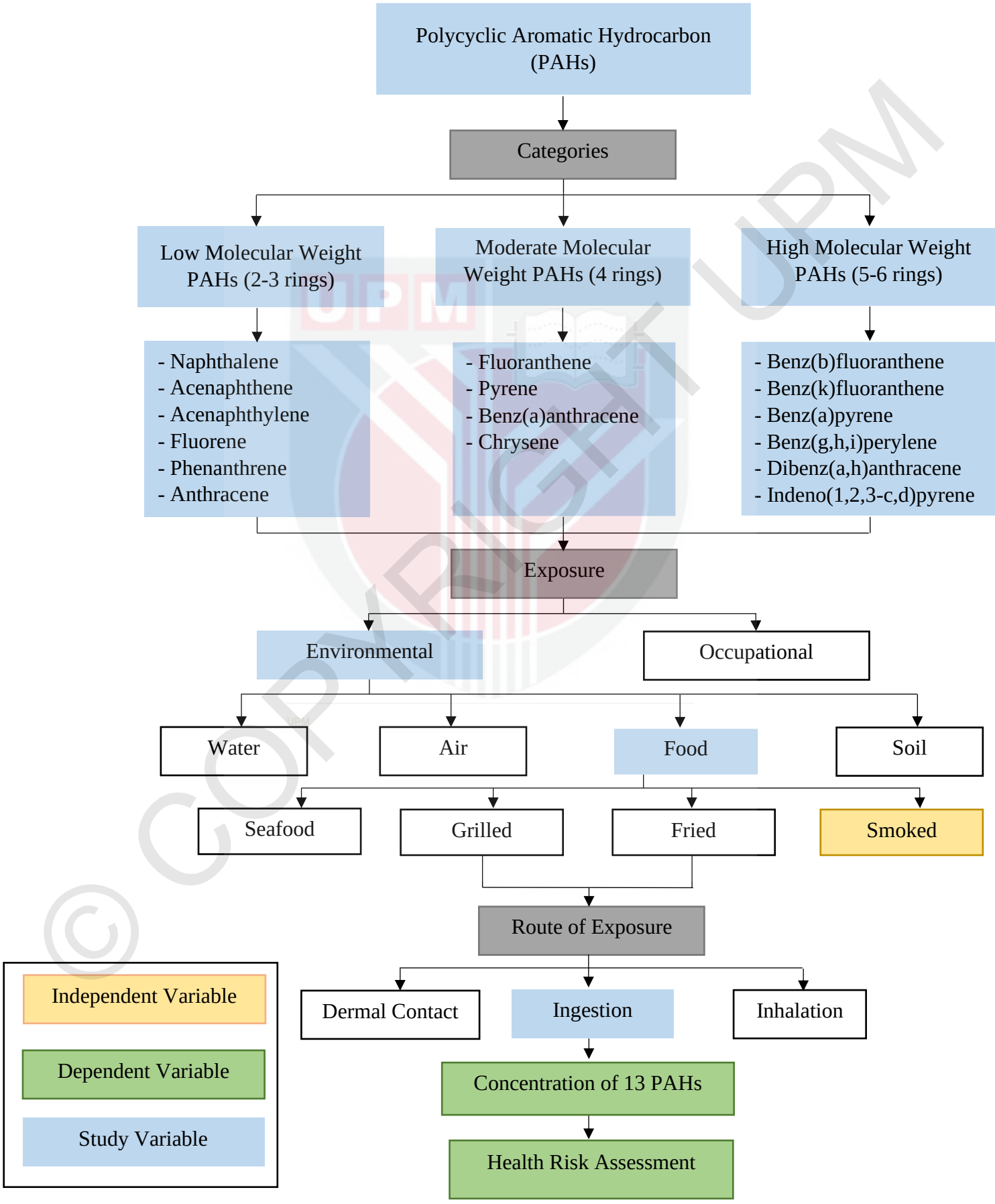

Figure 1.1

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

This chapter will discuss the characterization of PAHs compounds, formation of PAHs in food, method of cooking practises, health risk PAHs to humans and more. However, this chapter also will include some other researchers' studies and previous study which will be used as evidence and explanation in more detail.

2.1 Origin of Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are known as a group of complex compounds that are generally produced during the manufacturing process and other human activities, such as uncontrolled combustion process (burning) of organic substances. They are persistent organic pollutants (POPs) that can have an impact on human health and the environment which have different structures and toxicity. These compounds are colourless, white, or pale-yellow solids (Abdel-Shafy & Mansour, 2016). Unfortunately, PAHs exhibit high carcinogenicity, teratogenicity, and mutagenicity, and most of them were included in the International Agency for Research on Cancer's list of verified human carcinogens (IARC,2010).

Basically, these PAHs compounds can be classed as toxic chemicals which have two or more fused aromatic rings, and they are typical major pollutants that might

result from the during the food preparation process (Alomirah et al., 2011). They can be divided into two groups which are Low Molecular Weight (LMW) PAHs (2-3 rings) and High Molecular Weight (HMW) PAH (4 rings and more) (Sánchez-Arévalo et al., 2020). A study from Farhadian et al. (2010) and Singh et al. (2016) stated that the light PAHs are more volatile, less lipophilic, and hazardous, whereas the heavier PAHs are much more persistent and hazardous to humans than light PAHs.

In fact, PAHs can be found as a group of more than hundreds of chemicals generated by partial combustion of coal, oil, and gas, or they can be formed during certain cooking practices such as grilling, smoking, frying, and barbecuing. Thus, the differences between these cooking practices are frying in which the food is half submerged in oil, and deep frying, or the foodstuffs are completely submerged in hot oil where it has to control the temperature to avoid over hot or over cooked. Next, barbecuing is cooked at a low temperature for a longer period of time, resulting in more soft and tasty meat. Meanwhile, smoking is known as a technique for preparing meat and other foods over an open flame, but it also depends on several factors of temperature (very low heat which is 68 to 176 degrees F) and duration of smoking which this could generate PAHs in food. Furthermore, grilling method employs direct high heat and cooks the food in an open environment for only a few minutes hence it utilizes very high heat (400 to 550 degrees F) (ThermoPro,2019). Breathing outdoor and indoor air, PAHs intake-containing foods, smoking cigarettes are the most common ways for the public to be exposed to PAHs(Kim et al., 2013). Hence, in a simple way, people may be exposed by ingesting food containing PAHs.

According to the Agency for Toxic Substances and Disease Registry (ATSDR) (2015), PAHs are listed as top 10 ranked of Priority Hazardous Substances (PLHS) due to their toxicity and the possibility of human exposure. Over the hundreds of polycyclic aromatic hydrocarbons found in the environment, there are sixteen PAHS that have been concerned by the Environmental Protection Agency (EPA) because they are able to cause toxicity towards humans and other organisms or are known as carcinogenic. The European Commission (EC) has issued various measures in recent years to safeguard consumers against PAH dietary intake. Thus, PAH4 (benzo[a]pyrene, chrysene, benz[a]anthracene, and benzo[b]fluoranthene) and PAH3 (benzo[a]pyrene, chrysene, benz[a]anthracene, and benzo[b]fluoranthene) have been chosen as appropriate markers for PAH presence and risk in food (Husseini et al., 2018).

Similarly, the Department of Health and Human Services (DHHS) has identified most PAHs compounds may be carcinogenic when breathing or in contact with PAHs mixture for long durations that able to cause cancer like lung cancer and skin cancer (Polycyclic Aromatic Hydrocarbons (PAHs) | ToxFAQsTM | ATSDR, 2014).

2.2 Formation of PAH in food

Polycyclic Aromatic Hydrocarbons (PAHs) can be found in the environmental and food pollutants that produce improper pyrolysis of organic matter. These pollutants can be reached and pollute food like meats and products, fish, fruits, vegetables, and shellfish. In addition, having different cooking practices like grilling,

roasting, frying, smoking, and barbecuing at home or any food processing place may contribute to the formation of PAHs in the food products (Racovita et al., 2021).

Furthermore, a study by Singh et al. (2016) stated that the number of PAHs produced is affected by factors such as the distance from the source of heat, the type of fuel used, the degree of processing, cooking times, and techniques. PAHs also can be formed and accumulated on a wide range of food products such as meat products, dairy products, fish, poultry, and more. Thus, when there are food processing and practices applied to food products, they contribute significantly to PAHs formation (Ledesma et al., 2016). In fact, free radicals produced from increasing food combustion recombine to make light PAHs, which then migrate to hydrophobic food chain compartments before being maintained in fat-rich foods (Luzardo et al., 2013). So, it has to focus on the cooking methods as the presence of PAHs may draw from various kinds of food products.

Grilled, smoked, and roasted foods are becoming more popular at home and in restaurants; yet several studies show that these foods provide a greater health risk to consumers because they contain higher levels of PAHs than foods prepared using other ways (J. G. Lee et al., 2016). Even though the actual method of PAHs generation in grilled/smoked foods is unknown, but at least three different methods are widely believed to exist. Pyrolysis of organic compounds like fat, protein, and carbohydrates at high temperatures (< 200 °C) is the initial mechanism, and the formation of PAHs is favoured at ranges between 500–900 °C (Alomirah et al., 2011). Thus, people believe that PAHs will generate more from the pyrolysis of fat. Secondly is the result of direct contact of lipids dripping at high temperatures precisely on the flame where

this may produce volatile PAHs when attached to the surface of food as the smoke rises(Farhadian et al., 2010).

The third method is poor charcoal combustion might result in PAHs being released onto the food product. So, this would cause the formation of genotoxic and carcinogenic PAHs on the food products especially when they are applying cooking food practices/processes like grilling, smoking, frying, and more (Wu et al., 1997). Moreover, several previous studies also discussed on the formation of PAHs from food which they can contaminate food in a variety of ways, including accumulation from pollutants in the environment, contamination from packaging materials, and contamination from food processing techniques (Bansal & Kim, 2015; Chung et al., 2011; Reinik et al., 2007).

Furthermore, another previous study has found PAHs contamination in a wide range of foods, including smoked food particularly fish and pork, fruits and vegetables, cereals, etc. They also stated that the highest PAHs formation has been recorded in grilled/barbecued meat and products, and in smoked fish by traditional methods (Bogdanović et al., 2019; Gorji et al., 2016). Following that, a previous study by Alomirah et al. (2011) stated that grilling and smoked food had the greatest number of PAHs to be found. Next, a study by Ahmed et al. (2015) had detected the PAHs in smoked and canned smoked fish samples in Egypt, concluding that PAHs concentrations are substantially greater in smoked fish samples than in canned smoked fish samples.

2.3 Method of Cooking Practices

Smoking is a great food preservation way, but it can also contaminate food products with carcinogenic polycyclic aromatic hydrocarbons (PAHs). It's mostly utilized to give foods the distinct flavour and look, as well as antibacterial and antioxidant properties, hence increasing the levels of carcinogenic substances in the products, like as PAH compounds, especially when the smoking process is not carefully managed (Stumpe-Viksna et al., 2008). However, some smoked items have historically attracted significant attention due to the highest amounts of PAH have been identified by them (Abbas et al., 2018; Karl & Leinemann, 1996). Moreover, ingestion through contaminated food and water is a major route of human exposure to PAHs, so those who consumed food with PAHs are dangerous and should be avoidable.

Smoking process, fuel type, and smoking circumstances (humidity, temperature, time, and airflow) all affect PAHs contamination and level in smoked goods. Apart from drying and salting, smoking also is a third preservation method that has been employed for meat and fish for over 90 00 years. Thus, it has been linked to extra organoleptic benefits, such as getting a clear appearance of meats by overcooking reactions between meat amino acids and a distinctive smoky odour and flavour. Another study also mentioned that food smoking is the application of heat created by firewood or charcoal smoke to lower the moisture content of food components. Then this process is normally used to give a product a distinct flavour and texture that differs from its natural form. However, due to uncontrolled heat application on the fire or

smoke could give a result of the high level of PAHs formation on the food products (Silva et al., 2011).

2.4 Health risk of PAH exposure to human

Some harmful and carcinogenic substances have been discovered in food and the environment in recent years, one of it is PAHs that appeared in the air, water, and food. So, PAHs can affect humans through a variety of routes such as ingestion, inhalation or absorbed through skin exposure. They can also be ingested through food contamination. Consumption of food contamination has been known as a major cause of human exposure to PAHs, accounting up to 98% of such food contamination (Onwukeme et al., 2015; Plaza-Bolaños et al., 2010; Silva et al., 2011). Meanwhile, majority of PAHs toxicity investigations have focused on dermal, subcutaneous, and inhalation exposure, mostly as a work-related concern.

Thus, these dangerous chemicals are carcinogenic and mutagenic which can give a health impact on humans who consume food with the presence of PAHs. According to Wang et al. (2019), stated that when humans are exposed to PAHs, they would develop breast cancer, lung cancer, and stomach cancer. This is because PAHs contain activated diol epoxides causing DNA mutation, replication, and cancer (Xue & Warshawsky, 2005). Besides that, information regarding the dose and duration of exposure is necessary to evaluate the degree of risk and to determine sensitive receptors within a population. Furthermore, because of these PAHs, epidemiological studies have indicated that regular eating of smoked and grilled meat is substantially linked with the prevalence of colon cancer (Kim et al., 2013).

Hence, the acute effects of PAHs on people's health will be determined primarily by the prolonged exposure (e.g., time), the amount of PAHs throughout the exposure, the PAHs' toxicity, and the mode of exposure (e.g., inhalation, ingestion, or skin contact). Several other aspects may influence health outcomes. This would include things like age and pre-existing health issues (Kim et al., 2013). Based on Marques et al. (2011), mentioned the size of PAHs, the lipophilicity of the compounds, and the fat content of food could all affect dietary PAHs uptake. So, PAHs are taken and transported through the bloodstream to a variety of organs, particularly those with a high-fat content, where some of them are converted into DNA-active mutagens or genotoxic carcinogenic chemicals (diol epoxides). For non-smokers, they will be exposed to PAHs by dietary intake from food containing, it also gives significant health risk for human cancer development like cancer of prostate, colorectal, and pancreatic. Furthermore, according to Chung et al. (2011) food samples of grilled, smoked, and roasted provide a greater health risk to consumers because they contain higher quantities of PAHs than foods made using other cooking methods. They also conclude that 16 PAHs compounds have demonstrated mutagenicity/genotoxicity in somatic cells in vivo in animal experiments. As an overall, PAHs compounds contained in the smoked foodstuffs could be carcinogenic to the human as they consumed regularly and in large quantities. Figure 2.1 show the health effect of short terms and long terms exposure to PAHs.

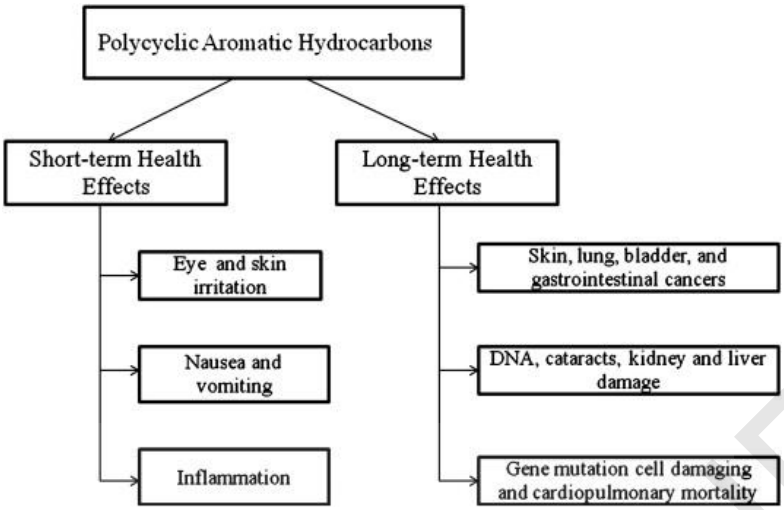

Figure 2.1: Health effect of exposure to PAHs.

2.5 Legislation related to PAHs in food

In terms of regulation, since mixture of PAHs in food samples would cause harmful effects to human health, this should impose and regulate standards to manage the PAHs present in food. In Malaysia, there is absence of specified standards on PAHs concentration in the grilled foods, smoked foods and seafoods. Furthermore, regulatory laws do not specify a approaches for qualitative and quantitative PAH analysis; rather, they specify the requirements for the procedures used.

Many previous studies concluded that PAHs give potential health impacts to humans. So, several organizations, including the United States Environmental Protection Agency (US EPA), the International Agency for Research on Cancer (IACR), the Scientific Committee on Food (SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the International Programme on Chemical Safety (IPCS), and the European Food Safety Authority (EFSA), have assessed the presence and PAHs carcinogenic (EFSA). In the European Union, SCF measured the

toxicity of PAHs. Other than that, SCF also stated that among 33 PAHs compounds, 16 PAHs are clearly shown evidence of mutagenic/carcinogenic (Zelinkova & Wenzl, 2015).

Food processing (drying, smoking) and high-temperature cooking (grilling, frying, roasting, baking) are usually thought to be the main causes of PAHs exposure in diet. Thus, The European Commission adopted Regulations No. 835/2011 and No. 2015/1125 (EC, 2011; EU, 2015) revising Regulation No. 1881/2006 (EC, 2006) by establish new limit levels for PAHs in foodstuffs based on this view. The maximum possible level for B(a)P in smoked fish meat and smoked fish products was 5.0 µg/kg of wet weight while the limit for the sum of PAH4 was set at 30.0 µg/kg of wet weight (Sampaio et al., 2021). In addition, B(a)P is the most researched PAHs, attributed to its genotoxic activity caused by direct intercalation in DNA, which causes structural alterations that lead to mutations.

Several nations have established legislation to set permissible limits for PAHs in foods, food items, and drinks, as well as to implement monitoring strategies for the most significant chemicals, due to their toxicity. Moreover, health organizations such as the World Health Organization and the European Commission have begun initiatives to reduce PAH levels in food, particularly through attempts to handle the processes that cause them to develop (Abdel-Shafy & Mansour, 2016). Because of that, until 2008 the EFSA relied solely on B(a)P as an indicator for PAHs in food. The permissible levels of B(a)P and PAH4 in various foods were reduced because of these instructions. Moreover, to control the PAHs contents in the foodstuffs, the food

industry is based on Hazard Analysis Critical Control Point (HACCP) principles, which can be used by the food sector as a guide to decrease the pollution during smoking and direct drying processes. The European society also released guideline publications, such as the European Salmon Smokers Association European Guide, which outlines best practices for smoking, salting, and marinating fish (ESSA). The incidence and biological effects of PAHs, as well as monitoring their levels, regulation, and measures to minimize their formation in food products, were all thoroughly investigated. So, effective procedures including various food processing strategies are required to avoid and limit PAH contamination, resulting in lower human exposure and negative health impacts.

CHAPTER 3

METHODOLOGY

3.1 Research Design

Local smoked food samples were collected to assess the occurrence of concentration Polycyclic Aromatic Hydrocarbons (PAHs) in different smoked food samples and estimate their health risk for the consumers.

3.2 Duration of Study

This study was performed in September 2021 until December 2021 to complete the laboratory sampling.

3.3 Sampling Method

3.3.1 Reagents and chemicals

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(a)anthracene (B(a)a), chrysene (Chy), benzo(b)fluoranthene (B(b)f), benzo(k)fluoranthene (B(k)f), benzo(a)pyrene (B(a)p), indeno(1,2,3-cd)pyrene (I(c)p), dibenzo(a,h)anthracene (D(a,h)a), and benzo(g,h,i)perylene (B(g,h,i)p). Chrysene-d12 (Supelco, USA) and perylene-d12 (Supelco, USA) were used as surrogate internal standards at the beginning of the extraction steps.

3.3.2 Sample collection

Six different types of local smoked foods such as smoked chicken, smoked beef, smoked quail, smoked catfish, smoked duck and smoked lamb were purchased randomly from three different famous food restaurants spots in Selangor. Food samples were kept in a zip lock bag before being brought to the laboratory (Figure 3.1). Then, these samples were refrigerated at the temperature of at least−20 °C and homogenised in the next day.

Figure 3.1: Example of smoked quail *(puyuh salai)* placed in zip lock after purchased.

3.3.3 Sample preparation

Before extraction, the samples were homogenized completely using pestle and mortar and laboratory blender Model 38BL40 Waring (New Hartford, Connecticut, USA) to obtain representative bulk sample. Thus, the pestle and mortar were properly cleaned using solvents before and after each sample were placed to avoid cross-contamination. The homogenize samples were kept in zip lock bag and stored in freezer at least -20°C until analysis (Table 3.1).

Table 3.1. Homogenised of samples

The samples were homogenised using pastel and mortal, and laboratory blender.	
The homogenize samples were placed in the zip lock and kept it in the freezer before extraction steps.	

3.3.4 Dry weight determination

The moisture content of the samples was determined using a gravimetric method following the USEPA Method 8275A (USEPA,1996). Ten grams of a portion of each sample were weighed to determine the percent (%) of dry weight of the smoked

food samples. The samples were placed using cleaned spatula or tweezers in the crucible wrapped up with aluminium foil first before starting weighing. After weighing, the samples were dried overnight (24 hours) in the drying oven at 105 °C. The % of dry weight and % of moisture of each sample was calculated using was calculated using Eq.3.1 and Eq.3.2.

$$\% \text{ dry weight } = \left(\frac{\text{gram of dry weight}}{\text{gram of sample}}\right) \text{x } 100 \qquad \text{Eq.3.1}$$

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

3.4 Extraction of PAHs using Solid Phase Extraction-silica column

The samples were extracted using Khan et al. (2015) with several adjustments. Before extraction, five grams of homogenized food sample were weighed using analytical balance and placed into 50 ml centrifuge tubes. The samples were spiked with internal standards, followed by added of twenty millimetres of dichloromethane (DCM) (R & M Chemicals, UK) of HPLC grade in the centrifuge tube. The samples were sonicated for 20 min in an ultrasonic bath (Elmasonic S70H, Elma, Germany) with water temperature in the bath less than 30°C. Next, the samples were centrifudge at 2500 rpm (Hettich Rotina 46R centrifuge, Germany) for 10 min and mixed vigorously using a vortex mixer (ZX3 Advanced Vortex Mixer, Italy) for 10 min. Before filtering the extract using glass microfiber filters (Whatman™, UK), the sonication, centrifugation, and vortexing steps were performed three times. The solution volume was decreased to approximately 200 µl under a gentle stream of nitrogen gas (N_2), and the residual was reconstituted with 800 µl of n-hexane. Then,

the volume reduction steps were carried out duplicates. After that, the sample was cleaned up and pre-concentrated using silica SPE cartridges (Lichrolut® RP-18, Merck, Germany). The extraction mixtures were added and passed through the RP-18 SPE cartridges under a moderate vacuum after being conditioned with 10 ml of n-hexane. Ten millilitres of DCM: n-hexane (1:9) was then applied to elute the RP-18 SPE cartridges at a 1ml/min flow rate by vacuum. The eluate was placed into a 20ml test tube and further reduced to 500 µl under a moderate stream of N_2 gas into 2ml amber glass vial up to 1.5ml. The samples were sent for GC-MS analysis as soon as possible.

3.5 Analysis of PAHs in food sample using GC-MS

The sample were analysed using GC-MS (Agilent, 5975C, USA). The instrument was equipped with a capillary column (HP-5MS) with an internal diameter (id) of 0.25 mm, a length of 30 m, and a thickness of 0.2 5 mm. The findings were made using the selective ion monitoring (SIM) mode, which is more sensitivity than the full scan function. External calibration was conducted to assess the concentrations of individual the 13 PAHs using standard mixtures of PAHs (SS EPA 610 PAH Mix, Supelco, USA). Additionally, 500 ppb of chrysene-d12 (Supelco, USA) and perylene-d12 (Supelco, USA) were added as surrogate internal standards at the beginning of the extraction. For chrysene-d12 and perylene-d12 were 92% and 84% for the average recoveries. Chrysene-d12 had a recovery range of 76-106 %, and perylene-d12 had a recovery range of 76-90 %, respectively. The concentrations of each 13 PAHs were corrected using the total 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 13 PAHs (SS EPA 610 PAH Mix, Supelco, USA).

3.6 Analytical quality assurance and quality control

3.6.1 Calibration of GC-MS

The calibration curves were used to determine the linearity. The equipment was calibrated for each analyte at five-point calibration curve at the range of 1 to 2000 ng/mL. Moreover, the calibration curve is measured by considering the target ion's integrated peak area ratio to the internal standard, and it is considered acceptable when the correlation coefficient (r) is > 0.9995. The peak area ratio of PAHs vs nominal concentration of the analytes was used to create calibration standards for each concentration. The correlation, y-intercept, and slope of the linear regression were used to assess the linear relationship.

3.6.2 Method of performance

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

The recovery of extraction was obtained 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 Eq.3.4 by comparing the entire amounts of every PAH in spiked and unspiked samples (Agus et al., 2020; EURACHEM, 2014).

Recovery (%) = (X1-X2) / X3 x 100 Eq. 3.4

X1 = Mean value of spiked sample

X2 = Mean value of unspiked sample

X3 = Known value of added concentration

3.6.3 Cleaning of glassware

According to USEPA Method TO-13A USEPA (1999), all the glassware such as pestle and mortar, tweezers, test tubes and so on were cleaned up by acid washed techniques to guarantee they were free from contaminants. First, all glassware was soaked in Decon 90 as decontaminating agents for about overnight before rinsed with tap water. Then, the glassware was immediately cleaned with analytical grade reagents such as methanol, followed by acetone, and then dichloromethane (DCM). These steps need to repeat for three times. Subsequently, after cleaning process, the glassware was dried at 60°C in drying oven and were sealed with aluminium foil to prevent airborne contamination such as dust.

3.6.4 Preparation of blank

The internal standard was spiked into blank sample (boiled chicken) and each batch of samples testing was run throughout the whole sample preparation and extraction process to test for any potential background contamination in the sample. Internal standards (chrysene-d12 and perylene-d12) were applied to correct the concentration of the target compound in this study.

3.7 Carcinogenic health risk estimation

The toxicity equivalency factors (TEFs) was used to create the toxic equivalence quotients (TEQBaP). Each PAH was estimated to be equivalent to B(a)P to highlight its relative hazardous potential in relation to this compound (Jiang et al.,2015; Wu et al.,2016). According to the report from Nisbet & Logay (1992), the sum of each TEQ_{BaP} was used to calculate the carcinogenic potentials of 13 PAHs according to equation 3.5.

$$TEQ_{BaP}=Ci \times TEFi \qquad \text{Eq.3.5}$$

Where, C_i= The concentration of each PAHs compound in food (µg/kg), TEF_i= The toxicity equivalency factor of individual PAHs.

Table 3.2. Toxicity equivalency factors (TEFs) of each individual PAHs

PAHs compound	TEFs
Naphthalene	0.001
Acenaphthylene	0.001
Acenaphthene	0.001
Fluorene	0.001
Phenanthrene	0.001
Anthracene	0.01
Fluoranthene	0.001
Pyrene	0.001
Benz(a)anthracene	0.1
Chrysene	0.01

Benzo(b)fluoranthene	0.1
Benzo(k)fluoranthene	0.1
Benzo(a)pyrene	1

The incremental lifetime cancer risk (ILCR) of smoked food to consumers based on the mean intake of smoked food was calculated by Equation 3.6 which based on other's reported methods (Jiang et al., 2018). The variables used in the ILCR are presented in Table 3.3.

$$\text{ILCR} = \frac{\sum \text{BaPeq x IR x EFx EDx SFx CF}}{\text{BW x AT}} \qquad \text{Eq.3.6}$$

Table 3.3. Variables used in risk assessment

Name	Acronyms	Unit	Value	Reference
Ingestion rate	IR	kg/day	Meat: 0.00534 Duck: 0.00049 Quail: 0.00016 Chicken: 0.00457 Fish: 0.00871 Lamb: 0.00105	National Health and Morbidity Survey 2014: Malaysia Nutrition Survey (MANS): Vol. III: Food Consumption Statistics of Malaysia NHMS (2014)
Oral cancer slope factor of	SF	mg kg^{-1} day^{-1}	7.3	USEPA (2017)

B(a)P for ingestion				
Exposure duration	ED	years	30	USEPA (2011)
Body weight of adult population	BW	kg	65.4	Y. Y. Lee & Muda (2019)
Average lifespan for carcinogens	AT	years days	70 25,550	WHO
Exposure frequency	EF	Days/year	365	Jiang et al. (2018)
Conversion factor	CF	mg/µg	10^{-3}	-

CHAPTER 4

RESULTS

4.1 Moisture content determination in different types of smoked foods.

The level of PAHs obtained in smoked food might be different because the moisture content in the foods may affect the level of PAHs generated during smoking food methods. The moisture content of smoked beef, smoked duck, smoked quail, smoked chicken, smoked catfish, and smoked lamb were presented in Table 4.1 Among six different types of smoked food, smoked beef (64.83%) has the highest % of moisture followed by smoked chicken (60.59%), while smoked lamb has the lowest % of moisture.

Table 4.1. Percentage of dry weight and moisture in different smoked foods (initial sample weight=10g).

Smoked food samples	Dry weight (g)	% of dry weight	% of moisture
Smoked beef	3.52	35.17%	64.83%
Smoked duck	4.40	43.97%	56.03%
Smoked quail	4.07	40.66%	59.34%
Smoked chicken	3.94	39.41%	60.59%
Smoked catfish	4.02	40.17%	59.83%
Smoked lamb	5.05	50.49%	49.51%

4.2 Quality control of PAHs extraction from different types of smoked food using SPE

Prior to the analysis of six different types of smoked foodstuffs, GC-MS method performance parameters and analyte characteristics, such as coefficient of determination (R2), limits of detection (LOD), and limits of quantification (LOQ), linear range and relative standard deviation (RSD) were determined.

The calibration curves were constructed to test linearity range for each PAHs compound. Five-point internal calibration curve were built in the range of 1-2000 µg/mL with the correlation coefficients (R^2) ranging from 0.9952-0.9999 detected for all analytes (R^2) as shown in Table 4.2. However, a study by Farhadian et al. (2010) stated that a high R^2 value which >0.97 shows the satisfactory linearity for the calibration curve. LOD derived using the concentration of analyte that creates 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). For LOD results show in this study was within ranged from 0.07 to 0.43 µg/kg, meanwhile the LOQ for all compounds presented were ranged from 0.32-1.28 µg/kg, respectively. The method performance was comparable to a previous study where they reported LOD results (0.15-0.30 µg/kg), and LOQ was within 0.50-1.00 µg/kg (Kamankesh et al.,2015). The recoveries were between 77.4% and 118.9% as recommended by European Union, (2011) (50% - 120%). Details information of linear range, R^2, LOD and LOQ, and percentage of recovery for PAHs compound in smoked food samples were summarized in Table 4.2.

Table 4.2. Linear range, coefficient of determination (R^2), limits of detection (LOD), and limits of quantification (LOQ), linear range and recoveries and relative standard deviation (RSD).

Target compounds	Linear range (ng/mL)	R^2	LOD (µg/kg)	LOQ (µg/kg)	Recovery ± RSD (%) (n=3)
Naphthalene	3-2000	0.9992	0.18	0.94	89.1 ± 1.8
Acenaphthylene	2-2000	0.9989	0.15	0.61	114.0 ± 4.3
Acenaphthene	2-2000	0.9985	0.20	0.62	102.4 ± 4.1
Fluorene	3-2000	0.9994	0.31	0.91	77.4 ± 2.2
Phenanthrene	1-2000	0.9990	0.06	0.33	102.8 ± 3.5
Anthracene	1-2000	0.9971	0.07	0.38	107.7 ± 2.9
Fluoranthene	2-2000	0.9993	0.13	0.67	103.3 ± 2.3
Pyrene	4-2000	0.9981	0.41	1.24	90.1 ± 1.5
Benz(a)anthracene	1-2000	0.9998	0.13	0.30	96.8 ± 1.7
Chrysene	1-2000	0.9974	0.09	0.35	90.3 ± 1.5
Benzo(b)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(a)pyrene	1-2000	0.9999	0.17	0.34	108.4 ± 2.9

R^2: Coefficient of determination

LOD: Limit of detection

LOQ: Limit of quantification

Recovery %: Percentage of the concentration of each analyte spiked before sample extraction compared to its concentration spiked after sample extraction

RSD: Relative standard deviation

4.3 Determination of PAHs concentration in selected smoked foods

Table 4.3 presents the results of the mean concentration of 13 PAHs in six different types of smoked food collected in Selangor. The highest PAHs compound found across B(a)P were ranged within 6.83±0.50 µg/kg in smoked chicken to 126.12±10.09 µg/kg in smoked beef. These range showed that the B(a)P mean concentration were exceed the maximum limit of 5 µg/kg based on European Union (European Union, 2011). While the other three food samples were detected in the lowest B(a)P mean concentration.

Among 13 PAHs compounds, B(a)P was found the highest concentration detected in three types of smoked foods (beef, duck, and quail), followed by naphthalene was the second highest mean concentration in smoked quail (53.45±3.21 µg/kg) respectively. Similarly, acenaphthene and benzo(a)fluorene were also having the highest mean concentration detected in smoked quail (37.42±2.47µg/kg) and smoked beef (43.68±2.62µg/kg). Nevertheless, the mean concentration of Phenanthrene was detected as the lowest concentration (0.49 ±0.33 µg/kg) in smoked lamb across 13 PAHs. However, some of the concentrations were not detected in several samples due to below limit of quantification (LOQ).

Table 4.3. Mean concentration of 13 PAHs in six different types of smoked food samples (µg/kg wet wt.) (n=3)

Target Compound	Smoked beef	Smoked duck	Smoked quail	Smoked chicken	Smoked catfish	Smoked lamb
Naphthalene	36.31±2.18	19.41±0.97	53.45±3.21	18.46±1.29	17.50±1.05	12.53±0.75
Acenaphthylene	6.59±0.53	11.29±0.90	35.32±2.47	13.45±1.08	5.50±0.33	6.56±0.46
Acenaphthene	41.59±2.91	4.90±0.39	37.42±2.99	6.12±0.37	0.80±0.05	ND
Fluorene	8.79± 0.79	4.34±0.39	15.33±0.92	8.11±0.49	1.10±0.08	2.29±0.16
Phenanthrene	13.83±0.69	6.15±0.37	23.08±1.35	7.34±0.44	4.31±0.30	3.23±0.26
Anthracene	3.16±0.25	6.15±0.43	23.08±1.85	ND	ND	0.49±0.03
Fluoranthene	12.91±1.03	2.85±0.20	15.98±0.96	ND	1.01±0.07	ND
Pyrene	13.04±0.91	ND	2.69±0.13	ND	ND	ND
Benz(a)anthracene	3.11±0.25	0.40±0.03	0.97±0.07	0.97±0.08	ND	0.51±0.02
Chrysene	2.87±0.20	0.37±0.03	ND	ND	ND	0.51±0.03

Benzo(b)fluoranthene	43.68±2.62	21.33±1.49	10.22±0.51	2.84±0.20	0.81±0.06	0.83±0.07
Benzo(k)fluoranthene	42.03±2.52	20.53±1.03	ND	ND	ND	ND
Benzo(a)pyrene	126.12±10.09*	70.38±4.22*	43.57±2.61*	6.83±0.50*	2.57±0.13	0.53±0.04

*B(a)P concentration exceed the maximum limit of 5 µg/kg (European Union,2011)

ND: not detected (<LOQ)

As shown in Table 4.3 and 4.4, among six different types of smoked food analysed in this study, the highest concentration of benzo(a)pyrene was found in the smoked beef (126.12±10.09 µg/kg), followed by smoked duck (70.38±4.22 µg/kg) and smoked quail (43.57±2.61µg/kg). The mean concentration of ∑13 PAHs in the smoked beef, smoked duck, smoked quail, smoked chicken, smoked catfish, and smoked lamb were 354.02±24.78 µg/kg, 168.10±10.10 µg/kg, 261.11±20.94 µg/kg, 64.15±5.13 µg/kg, 33.60±2.35 µg/kg and 27.48±1.65 µg/kg. The highest mean concentration of ∑13 PAHs was detected in smoked beef, while smoked lamb was the lowest mean concentration of ∑13 PAHs. Then, for ∑PAH4 concentration, three smoked food samples (smoked beef, smoked duck, and smoked quail) were detected higher and exceed the limit of ∑PAH4 in European Union which was 30 µg/kg, while ∑PAH4 concentration in smoked lamb, smoked catfish, and smoked chicken were within range from 2.38± 0.17 µg/kg to 10.64± 0.75 µg/kg

respectively However, as mentioned in the EFSA instead of B(a)P alone, PAH4 compounds would be the best indicators of detecting PAHs in food (EFSA, 2008).

Table 4.4. Mean concentration of B(a)P, ∑PAH4, ∑13 PAH (µg/kg) of smoked foods sample.

Smoked foods	Mean concentration µg/kg wet wt		
	B(a)P	∑PAH4	∑13 PAH
Smoked beef	126.12±10.09*	175.77±12.03*	354.02±24.78
Smoked duck	70.38±4.22*	92.48 ±7.40*	168.10±10.10
Smoked quail	43.57±2.61*	54.42±3.81*	261.11±20.94
Smoked chicken	6.83±0.55*	10.64± 0.75	64.15±5.13
Smoked catfish	2.57±0.13	3.38 ±0.22	33.60±2.35
Smoked lamb	0.53±0.04	2.38± 0.17	27.48±1.65

4 PAH: Benzo[a]anthracene + Chrysene + Benzo[b]fluoranthene + Benzo[a]pyrene

*B(a)P concentration exceeded the maximum limit of 5 µg/kg (European Union, 2011).

*PAH4 exceeded the maximum limit of 30 µg/kg (European Union, 2011).

4.4 Estimation health risk assessment

As based on USEPA (2001), the ILCR model was used to analyse the health risks posed by dietary PAH exposure to the Selangor population as presented in Table 4.5. In general, one-in-a-million increased human cancer risk over a 70-year life span (ILCR = 10^{-6}) is an acceptable or insignificant level whereas a one-in-ten-thousand possibility (ILCR = 10^{-4}) or above is thought to be a dangerous level (Arinze Udowelle et al., 2017). Therefore, it is necessary to calculate the carcinogenic health risk of PAHs, as previous studies also reporting on determining PAHs concentration in food samples and assessing their health risk assessment (Jiang et al., 2018; J. Li et al., 2016; Y. Wang et al., 2021). Table below show the estimation daily intake, B(a)P, PAH4, and total of 13 PAHs concentrations, meanwhile ∑B(a)Peq and ILCR values were used to estimate the carcinogenic health risk.

Table 4.5 Estimated daily intake of six selected smoked foods, ∑B(a)Peq and ILCR

Types of smoked food samples	Estimation means daily intake (g/day)	∑B(a)Peq (µg/kg)	ILCR
Smoked beef	0.00534	130.83	3.34×10^{-5}
Smoked duck	0.00049	73.16	1.71×10^{-6}
Smoked quail	0.00016	44.69	3.42×10^{-7}
Smoked chicken	0.00457	7.21	1.58×10^{-6}

Smoked catfish	0.00871	2.65	1.10×10^{-6}
Smoked lamb	0.00105	0.67	3.37×10^{-8}

In the present study, the values of ILCR were estimated according to TEQ_{BaP} from the concentration of PAH4 with its TEF (USEPA & IRIS,2017). The ILCR reported in this study were within range 3.34×10^{-5} to 3.37×10^{-8} detected in all samples, where the values of ILCR expected to 3.34×10^{-5}, 1.71×10^{-6}, 3.42×10^{-7}, 1.58×10^{-6}, 1.10×10^{-6} and 3.37×10^{-8}, for smoked beef, smoked duck, smoked quail, smoked chicken, smoked catfish and smoked lamb. Among six different types of smoked food samples, smoked beef was categorized as the highest carcinogenic health risk to the consumers, followed by smoked duck, smoked chicken, smoked catfish, smoked quail and smoked lamb. However, the ILCR values evaluated in this study were in the acceptable carcinogenic risk among consumer in Selangor as within the value of 10^{-4} to 10^{-6} according to USEPA.

CHAPTER 5

DISCUSSION

5.1 Moisture content in six groups of smoked foods

Based on the result shown in Table 4.1, the highest % of moisture was detected in smoked beef (64.83%), followed by smoked chicken (60.59%) while the lowest % of moisture was present in smoked lamb (49.51%). Hence, by comparing to previous study investigated % of moisture in smoked meat products where their findings show the % moisture detected in smoked chorizo samples ranged from 15.13% to 38.74% which was lower than present study (Ledesma et al., 2015). Next, another study detected that dried kitoza had a lower moisture content than smoked where the moisture in smoked foods ranged from 30.0-60.8 g/100g. They also suggesting that the increased protein content of dried kitoza was most likely attributable to a higher protein concentration because of reduced moisture level (Ratsimba et al., 2019). Thus, this study is in contrast with those of Ledesma et al. (2015) whereby smoked foods have higher moisture content.

5.2 Concentration of PAHs in the smoked foods

An overview of the determined concentrations of individual PAHs in the analyses from different types of smoked food samples is shown in Table 4.3. According to table 4.3 & 4.4, smoked beef contained the highest B(a)P concentration (126.12±10.09 µg/kg) while smoked lamb contained the lowest B(a)P concentration (0.53±0.04 µg/kg). These findings are in agreement with those of Hokkanen et al.

(2018) where they stated that direct smoking showed significantly greater B(a)P and PAH4 total concentrations in smoked fish samples than indirect smoking. Furthermore, compared to indirect smoking, direct smoking produced significantly greater PAH levels in smoked beef samples. In addition, it is well known that direct exposure to smoke can result in the build-up of particles and hazardous compounds in the sample such as PAHs. Moreover, the cooking method of the present study was direct smoking and compatible with the previous study. We agree that the concentration level of B(a)P was higher compared to other PAHs compounds. These results were in agreement with those mentioned by Kafouris et al. (2020) showing that the majority of the samples evaluated (>90%) found at least one PAH and 12% of the smoked samples found in contaminated meat were above the EU legislation's maximum values which were comparable with the present study. Hence, they also stated that the samples with higher fat content and prolonged smoking or cooking times had the greatest PAH contents.

Meanwhile, similar research investigated PAHs concentration in smoked chicken where the result showed that among all the PAHs compounds been analysed, naphthalene had the greatest concentration. As compared to this study naphthalene also detected the highest mean concentration in smoked chicken and smoked quail (53.45±3.21 μg/kg) (Rekanovic, 2015). Next, Alomirah et al. (2011) investigated the PAHs level in grilled and smoked food items at Kuwait. They found that the mean B(a)P content in smoked beef (1.09 μg/kg) and smoked fish (0.50 μg/kg) samples were below the exceeded the permissible limit of 5 μg/kg, while the mean value of the genotoxic PAH8 was above 5 μg/kg. In contrast to the findings in this study, four out

of six smoked samples were exceeding the limit of the European Union (5 µg/kg) except for smoked catfish and smoked lamb.

Meanwhile, the highest concentration of ∑13 PAHs were found in smoked beef, while the lowest was found in smoked lamb. The concentration of ∑PAH4 in smoked beef, smoked duck and smoked quail were above the European Union maximum (30 µg/kg) (EU,2011). Thus, similar findings detected B(a)P levels in smoked meats ranged from 0.05 µg/kg to 166 µg/kg, while the PAH4 content ranged from 0.42 µg/kg to 628 µg/kg (Rozentale et al., 2018).

5.3 Estimation Health Risk Assessment

According to the result of the incremental lifetime cancer risk (ILCR) of PAHs presented in Table 4.5, the present data indicate that the carcinogenic risk levels of this study are considered acceptable or inconsequential. The ILCR reported in this study were within range 3.34×10^{-5} to 3.37×10^{-8} detected in all samples. Among six different types of smoked food samples, smoked beef was categorised as the highest carcinogenic health risk to the consumers, followed by smoked duck, smoked chicken, smoked catfish, smoked quail and smoked lamb.

However, according to the existing data from previous study, all smoked meat in their study was acceptable to consume since the data shows carcinogenic risk for benzo(a)Pyrene (5.74×10^{-5}), benzo(a)anthracene (1.65×10^{-6}), chrysene (2.77×10^{-11}) (Tareq et al.,2020). Thus, their study was comparable with this study because the ILCR in smoked meat was similar our study. Moreover, a study by Jiang. (2018) presented in their findings of estimating the health risk assessment of PAHs in grilled and fried

meats at China, the ILCR values were all less than 10^{-4} and indicate a potential cancer risk potency. So, their study has carcinogenic risk to consumers, while this study shows that the smoked food samples were less than the USEPA acceptable limit and safe for consumption. Other than that, a comparison with previous studies by Li et al. (2016) shows that, the ILCR values related to PAHs exposure from consumption of smoked meat ranged from 4.46 10^{-7} to 4.64 10^{-6} for eight groups in southwest China which were also comparable with present study. In addition, the body weight of adults was not significantly lower than others such as children which did not cause a relatively high-risk value for adults who consumed smoked food products. Therefore, it should be emphasised that adults were not the most vulnerable to PAHs compound as can see the carcinogenic risk levels indicate negligible and special consideration should not be given for their health in comparison to other previous study (Zhang et al., 2021).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study aimed to determine the PAHs concentration in the six different types of smoked foods in Selangor. The results showed that all PAHs were detected in different types smoked food. The concentrations of B(a)P for smoked beef (126.12±10.09 µg/kg), smoked duck (70.38±4.22 µg/kg), smoked quail (43.57±2.61 µg/kg) and smoked chicken (6.83±0.55 µg/kg) were exceeded the maximum levels for B(a)P in smoked meat products as proposed by European Union at 5 µg/kg (Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011. Furthermore, the concentrations of ∑PAH4 in smoked beef (175.77 µg/kg), smoked duck (92.48 µg/kg), and smoked quail (54.42 µg/kg) were exceeded the maximum limit by European Union at 30 µg/kg). The results of ILCR indicate that the carcinogenic health risks of consuming the smoked food samples investigated in this study were all acceptable (ILCR: 10^{-8} - 10^{-5}). The results of this study indicated that the process and method of smoking has a significant effect on the concentration of PAHs formation in the food samples. Hence, the duration and temperature of smoking method, distance of source heat and foods could be the factors that may lead to formation of PAHs.

6.2 Study limitations

The ILCR in this study was calculated based on the recommended values by previously published report and journals, such as ingestion rate, body weight, exposure duration and exposure frequency. Therefore, the estimated carcinogenic health risk could be different if we use the data collected among the targeted smoked food consumers. Hence, we manage to analyze 13 PAHs instead of 16 PAHs compound due to insufficient internal standard. Then, this study was not able to consider duration and temperature of preparation of smoking process, distance from source of heat and fat intake in processed food as it is limited time. Moreover, as undergraduate students were not allowed to handle GC-MS machine themselves due to lack of experiences and skill.

6.3 Recommendations

In order to obtain accurate result on carcinogenic health risk, future research have to conduct community survey to gain the information of ingestion rate of smoked foods, exposure frequency and average body wieght of adult population via questionnaire instead of obtain from NHMS. To increase the accuracy of the research, future study have to analyze 16 PAHs compound and consider the factors that lead to formation of PAHs from smoked food as it influence the amount of PAHs contaminating the food after smoking. This study provides the information of individual mean concentration of PAHs, B(a)P, ∑PAH4, ∑13 PAHs in six different types of smoked foods which most consumed in Malaysia. Although, there are no serious and requires close surveillance from ILCR values, but there might be risk for

those who consumed frequently in large amount. Thus, there are necessary to conduct further studies and research in future to establish the guidelines and limitation for PAHs contamination in foods. To date, more research and investigation are required to create the standards and limitation for PAHs contamination as currently no legislative restrictions for this category of food contamination issues. Moreover, establishing safety measures for preparing and preparation smoking food and manufacturing also should be highlighted such as control the duration of smoking process and reduce the food fat content may decrease the formation of PAHs in food.

REFERENCES

Abbas, I., Badran, G., Verdin, A., Ledoux, F., Roumié, M., Courcot, D., & Garçon, G. (2018). Polycyclic aromatic hydrocarbon derivatives in airborne particulate matter: sources, analysis and toxicity. *Environmental Chemistry Letters*, *16*(2), 439–475. https://doi.org/10.1007/S10311-017-0697-0

Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*(1), 107–123. https://doi.org/10.1016/J.EJPE.2015.03.011

Ahmed, M. T., Malhat, F., & Loutfy, N. (2015). Residue Levels, Profiles, Emission Source and Daily Intake of Polycyclic Aromatic Hydrocarbons Based on Smoked Fish Consumption, An Egyptian Pilot Study. *Http://Dx.Doi.Org/10.1080/10406638.2014.957405*, *36*(3), 183–196. https://doi.org/10.1080/10406638.2014.957405

Alexander, D. D., Miller, A. J., Cushing, C. A., & Lowe, K. A. (2010). Processed meat and colorectal cancer: a quantitative review of prospective epidemiologic studies. *European Journal of Cancer Prevention : The Official Journal of the European Cancer Prevention Organisation (ECP)*, *19*(5), 328–341. https://doi.org/10.1097/CEJ.0B013E32833B48FA

Ali Zainuddin, A., Husna Zainudeen, A., Mahadir Naidu, B., Cheow Keat, C., Rabaah Ahmad Suhaimi, L., Hasnan Ahmad, M., Hidayah Jamaluddin, N., Saari Editors Tahir Aris, R., Ani Ahmad, N., & Yoep, N. (n.d.). *NATIONAL HEALTH AND MORBIDITY SURVEY 2014: MALAYSIA ADULT NUTRITION SURVEY (NMRR-12-815-13100) VOLUME III FOOD CONSUMPTION STATISTICS OF MALAYSIA CONTRIBUTORS*.

Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., & Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control*, *22*(12), 2028–2035. https://doi.org/10.1016/J.FOODCONT.2011.05.024

Arinze Udowelle, N., Nkeiruka Igweze, Z., Asomugha, R. N., & Orisakwe, O. E. (2017). HEALTH RISK ASSESSMENT AND DIETARY EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHs), LEAD AND CADMIUM FROM BREAD CONSUMED IN NIGERIA. *Rocz Panstw Zakl Hig*, *68*(3), 269–280. http://wydawnictwa.pzh.gov.pl/roczniki_pzh/

as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs (Text with EEA relevance). (n.d.). Retrieved December 24, 2021, from http://ec.europa.eu/food/fs/sc/scf/out153_en.pdf

Baali, A., & Yahyaoui, A. (2020). Polycyclic Aromatic Hydrocarbons (PAHs) and Their Influence to Some Aquatic Species. *Biochemical Toxicology - Heavy Metals and Nanomaterials*. https://doi.org/10.5772/INTECHOPEN.86213

Bansal, V., & Kim, K. H. (2015). Review of PAH contamination in food products and their health hazards. *Environment International*, *84*, 26–38. https://doi.org/10.1016/J.ENVINT.2015.06.016

Bogdanović, T., Petričević, S., Listeš, E., & Pleadin, J. (2019). POLYCYCLIC AROMATIC HYDROCARBONS IN SELECTED FOOD ITEMS COMING FROM THE CROATIAN MARKET. *Italian Journal of Food Science*, *31*(3), 661–680. https://doi.org/10.14674/IJFS-1444

Chung, S. Y., Yettella, R. R., Kim, J. S., Kwon, K., Kim, M. C., & Min, D. B. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, *129*(4), 1420–1426. https://doi.org/10.1016/J.FOODCHEM.2011.05.092

Commission, T. H. E. E. (2014). *COMMISSION REGULATION (EU) No 1327/2014 of 12 December 2014*. *1327*, 13–14.

el Husseini, M., Makkouk, R., Rabaa, A., al Omar, F., & Jaber, F. (2018). Determination of Polycyclic Aromatic Hydrocarbons (PAH4) in the Traditional Lebanese Grilled Chicken: Implementation of New, Rapid and Economic Analysis Method. *Food Analytical Methods*, *11*(1), 201–214. https://doi.org/10.1007/S12161-017-0990-3/FIGURES/12

Epa, U. (1999). *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air Second Edition Compendium Method TO-13A Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS)*.

European Food Safety Authority [EFSA] Polycyclic aromatic hydrocarbons in food scientific opinion of the panel on contaminants in the food chain. EFSA J. 2008; 724:1–114. doi: 10.2903/j.efsa.2008.724

European Union [EU] Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. L215/4-L215/8. Off J Eur Union. 2011; 215:4–8.

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

Farhadian, A., Jinap, S., Hanifah, H. N., & Zaidul, I. S. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chemistry*, *124*(1), 141–146. https://doi.org/10.1016/J.FOODCHEM.2010.05.116

Fazeli, F., Seyedain Ardabili, S. M., Piravivanak, Z., Honarvar, M., & Mooraki, N. (2021). The effect of the smoking process on polycyclic aromatic hydrocarbons contents in Hashemi and Domsiah rice cultivars. *Journal of Food Processing and Preservation*, *45*(2). https://doi.org/10.1111/JFPP.15172

Gorji, M. E. haghi, Ahmadkhaniha, R., Moazzen, M., Yunesian, M., Azari, A., & Rastkari, N. (2016). Polycyclic aromatic hydrocarbons in Iranian Kebabs. *Food Control*, *60*, 57–63. https://doi.org/10.1016/J.FOODCONT.2015.07.022

Hamidi, E. N., Hajeb, P., Selamat, J., & Razis, A. F. A. (2016). Polycyclic aromatic hydrocarbons (PAHs) and their bioaccessibility in meat: A tool for assessing human cancer risk. In *Asian Pacific Journal of Cancer Prevention* (Vol. 17, Issue 1, pp. 15–23). Asian Pacific Organization for Cancer Prevention. https://doi.org/10.7314/APJCP.2016.17.1.15

Hitzel, A., Pöhlmann, M., Schwägele, F., Speer, K., & Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in meat products smoked with different types of wood and smoking spices. *Food Chemistry*, *139*(1–4), 955–962. https://doi.org/10.1016/J.FOODCHEM.2013.02.011

Hokkanen, M., Luhtasela, U., Kostamo, P., Ritvanen, T., Peltonen, K., & Jestoi, M. (2018). Critical Effects of Smoking Parameters on the Levels of Polycyclic Aromatic Hydrocarbons in Traditionally Smoked Fish and Meat Products in Finland. Journal of Chemistry, 2018. https://doi.org/10.1155/2018/2160958

IARC. (2010). Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. In IARC Working Group (Vol. 92). https://doi.org/10.1136/jcp.48.7.691-a

Jiang, D. (2018). *Occurrence , dietary exposure , and health risk estimation of polycyclic aromatic hydrocarbons in grilled and fried meats in Shandong of China*. *September*, 2431–2439. https://doi.org/10.1002/fsn3.843

Jiang, D., Wang, G., Li, L., Wang, X., Li, W., Li, X., Shao, L., & Li, F. (2018). Occurrence, dietary exposure, and health risk estimation of polycyclic aromatic hydrocarbons in grilled and fried meats in Shandong of China. *Food Science & Nutrition*, *6*(8), 2431. https://doi.org/10.1002/FSN3.843

Kamankesh, M., Mohammadi, A., Hosseini, H., & Modarres Tehrani, Z. (2015). Rapid determination of polycyclic aromatic hydrocarbons in grilled meat using microwave-assisted extraction and dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry. Meat Science, 103, 61–67. https://doi.org/10.1016/J.MEATSCI.2015.01.001

Kafeelah, A. Y., Lucy, N. E., Kafayat, A. F., Shehu, L. A., Julius, I. A., & Titus, O. O. (2015). Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health. *African Journal of Food Science*, *9*(3), 126–135. https://doi.org/10.5897/AJFS2014.1227

Karl, H., & Leinemann, M. (1996). Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. *Zeitschrift Fur Lebensmittel -Untersuchung Und -Forschung*, *202*(6), 458–464. https://doi.org/10.1007/BF01197265

Khan, M. F., Latif, M. T., Lim, C. H., Amil, N., Jaafar, S. A., Dominick, D., Mohd Nadzir, M. S., Sahani, M., & Tahir, N. M. (2015). Seasonal effect and source apportionment of polycyclic aromatic hydrocarbons in PM2.5. *Atmospheric Environment*, *106*, 178–190. https://doi.org/10.1016/J.ATMOSENV.2015.01.077

Kim, K. H., Jahan, S. A., Kabir, E., & Brown, R. J. C. (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health

effects. *Environment International*, *60*, 71–80. https://doi.org/10.1016/J.ENVINT.2013.07.019

Ledesma, E., Rendueles, M., & Díaz, M. (2016). Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and prevention. *Food Control*, *60*, 64–87. https://doi.org/10.1016/J.FOODCONT.2015.07.016

Lee, J. G., Kim, S. Y., Moon, J. S., Kim, S. H., Kang, D. H., & Yoon, H. J. (2016). Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats. *Food Chemistry*, *199*, 632–638. https://doi.org/10.1016/J.FOODCHEM.2015.12.017

Lee, Y. Y., & Muda, W. A. M. W. (2019). Dietary intakes and obesity of Malaysian adults. *Nutrition Research and Practice*, *13*(2), 159. https://doi.org/10.4162/NRP.2019.13.2.159

Li, J., Dong, H., Li, X., Han, B., Zhu, C., & Zhang, D. (2016). Quantitatively assessing the health risk of exposure to PAHs from intake of smoked meats. *Ecotoxicology and Environmental Safety*, *124*, 91–95. https://doi.org/10.1016/J.ECOENV.2015.10.007

Lingbeck, J. M., Cordero, P., O'Bryan, C. A., Johnson, M. G., Ricke, S. C., & Crandall, P. G. (2014). Functionality of liquid smoke as an all-natural antimicrobial in food preservation. *Meat Science*, *97*(2), 197–206. https://doi.org/10.1016/J.MEATSCI.2014.02.003

Luzardo, O. P., Rodríguez-Hernández, Á., Quesada-Tacoronte, Y., Ruiz-Suárez, N., Almeida-González, M., Henríquez-Hernández, L. A., Zumbado, M., & Boada, L. D. (2013). Influence of the method of production of eggs on the daily intake of polycyclic aromatic hydrocarbons and organochlorine contaminants: An independent study in the Canary Islands (Spain). *Food and Chemical Toxicology*, *60*, 455–462. https://doi.org/10.1016/J.FCT.2013.08.003

Marques, A., Lourenço, H. M., Nunes, M. L., Roseiro, C., Santos, C., Barranco, A., Rainieri, S., Langerholc, T., & Cencic, A. (2011). New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in meat and seafood. *FRIN*, *44*, 510–522. https://doi.org/10.1016/j.foodres.2010.12.003

NHMS. (2014). NATIONAL HEALTH AND MORBIDITY SURVEY 2014: MALAYSIA ADULT NUTRITION SURVEY (NMRR-12-815-13100). In Institute for Public Health, Ministry of Health.

Nisbet, I. C. T., & LaGoy, P. K. (1992). Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regulatory Toxicology and Pharmacology, 16(3), 290–300. https://doi.org/10.1016/0273-2300(92)90009-X

Olmedilla-Alonso, B., Jiménez-Colmenero, F., & Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, *95*(4), 919–930. https://doi.org/10.1016/J.MEATSCI.2013.03.030

Onwukeme, V. I., Asomugha, O. C., & Okafor, R. N. (2015). Impact of Cooking Methods on the Levels of Polycyclic Aromatic Hydrocarbons

(PAHs) in Chicken Meat. *IOSR Journal of Environmental Science*, *9*(4), 21–27. https://doi.org/10.9790/2402-09412127

Parada, H., Steck, S. E., Bradshaw, P. T., Engel, L. S., Conway, K., Teitelbaum, S. L., Neugut, A. I., Santella, R. M., & Gammon, M. D. (2017). Grilled, Barbecued, and Smoked Meat Intake and Survival Following Breast Cancer. *Journal of the National Cancer Institute*, *109*(6). https://doi.org/10.1093/JNCI/DJW299

Plaza-Bolaños, P., Frenich, A. G., & Vidal, J. L. M. (2010). Polycyclic aromatic hydrocarbons in food and beverages. Analytical methods and trends. *Journal of Chromatography. A*, *1217*(41), 6303–6326. https://doi.org/10.1016/J.CHROMA.2010.07.079

Polycyclic Aromatic Hydrocarbons in Food - Scientific Opinion of the Panel on Contaminants in the Food Chain. (2008). *EFSA Journal*, *6*(8). https://doi.org/10.2903/J.EFSA.2008.724

Purcaro, G., Moret, S., & Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: occurrence, legislation and innovative determination in foods. *Talanta*, *105*, 292–305. https://doi.org/10.1016/J.TALANTA.2012.10.041

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*. https://doi.org/10.1016/J.FOODCONT.2020.107586

Ratsimba, A., Rakoto, D., Jeannoda, V., Andriamampianina, H., Talon, R., Leroy, S., Grabulos, J., & Arnaud, E. (2019). Physicochemical and microbiological characteristics of kitoza, a traditional salted/dried/smoked meat product of Madagascar. *Food Science & Nutrition*, *7*(8), 2666–2673. https://doi.org/10.1002/FSN3.1122

Reinik, M., Tamme, T., Roasto, M., Juhkam, K., Tenno, T., & Kiis, A. (2007). Polycyclic aromatic hydrocarbons (PAHs) in meat products and estimated PAH intake by children and the general population in Estonia. *Food Additives and Contaminants*, *24*(4), 429–437. https://doi.org/10.1080/02652030601182862

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, *5*(3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4

Rozentale, I., Zacs, D., Bartkiene, E., & Bartkevics, V. (2018). Polycyclic aromatic hydrocarbons in traditionally smoked meat products from the Baltic states. *Food Additives and Contaminants: Part B Surveillance*, *11*(2), 138–145. https://doi.org/10.1080/19393210.2018.1440637

Sampaio, G. R., Guizellini, G. M., da Silva, S. A., de Almeida, A. P., Pinaffi-Langley, A. C. C., Rogero, M. M., de Camargo, A. C., & Torres, E. A. F. S. (2021). Polycyclic Aromatic Hydrocarbons in Foods: Biological Effects, Legislation, Occurrence, Analytical Methods, and Strategies to Reduce

Their Formation. *International Journal of Molecular Sciences 2021, Vol. 22, Page 6010*, *22*(11), 6010. https://doi.org/10.3390/IJMS22116010

Sánchez-Arévalo, C. M., Olmo-García, L., Fernández-Sánchez, J. F., & Carrasco-Pancorbo, A. (2020). Polycyclic aromatic hydrocarbons in edible oils: An overview on sample preparation, determination strategies, and relative abundance of prevalent compounds. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3528–3573. https://doi.org/10.1111/1541-4337.12637

Silva, B. O., Adetunde, O. T., Oluseyi, T. O., Olayinka, K. O., & Alo, B. I. (2011). Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. *African Journal of Food Science*, *5*(7), 384–391.

Singh, L., & Agarwal, T. (2018). Polycyclic aromatic hydrocarbons in diet: Concern for public health. *Trends in Food Science and Technology*, *79*, 160–170. https://doi.org/10.1016/J.TIFS.2018.07.017

Singh, L., Varshney, J. G., & Agarwal, T. (2016). Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. *Food Chemistry*, *199*, 768–781. https://doi.org/10.1016/J.FOODCHEM.2015.12.074

Stumpe-Viksna, I., Bartkevičs, V., Kukare, A., & Morozovs, A. (2008). Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. *Food Chemistry*, *110*(3), 794–797. https://doi.org/10.1016/J.FOODCHEM.2008.03.004

Tareq, A. R. M., Afrin, S., Hossen, M. S., Hashi, A. S., Quraishi, S. B., Nahar, Q., Begum, R., & Ullah, A. K. M. A. (2020). Gas Chromatography–Mass Spectrometric (GC-MS) Determination of Polycyclic Aromatic Hydrocarbons in Smoked Meat and Fish Ingested by Bangladeshi People and Human Health Risk Assessment. Https://Doi.Org/10.1080/10406638.2020.1790017, 1–11. https://doi.org/10.1080/10406638.2020.1790017

US EPA, & IRIS. (2017). Toxicological Review of Benzo [a] pyrene [CASRN 50-32-8]. January. www.epa.gov/iris

USEPA. (1999). Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Compendium Method TO-13A: Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS). Center for Environmental Research Information Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268, II(EPA/625/R-96/010b), 78.

USEPA. (2011). EPA-600-R-090-052F, Exposure Factors Handbook, 2011 Edition. September 1466. www.epa.gov

Wang, H., Wang, C., Li, C., Xu, X., & Zhou, G. (2019). Effects of phenolic acid marinades on the formation of polycyclic aromatic hydrocarbons in charcoal-grilled chicken wings. *Journal of Food Protection*, *82*(4), 684–690. https://doi.org/10.4315/0362-028X.JFP-18-420

Wu, J., Wong, M. K., Lee, H. K., Shi, C. Y., & Ong, C. N. (1997). Determination of polycyclic aromatic hydrocarbons in rougan, a traditional Chinese barbecued food, by capillary gas chromatography. *Environmental Monitoring and Assessment*, *44*(1–3), 577–585. https://doi.org/10.1023/A:1005748017025

Xue, W., & Warshawsky, D. (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicology and Applied Pharmacology*, *206*(1), 73–93. https://doi.org/10.1016/J.TAAP.2004.11.006

Yu, Y., Wang, X., Wang, B., Tao, S., Liu, W., Wang, X., Cao, J., Li, B., Lu, X., & Wong, M. H. (2011). Polycyclic aromatic hydrocarbon residues in human milk, placenta, and umbilical cord blood in Beijing, China. *Environmental Science & Technology*, *45*(23), 10235–10242. https://doi.org/10.1021/ES202827G

Zelinkova, Z., & Wenzl, T. (2015). The Occurrence of 16 EPA PAHs in Food - A Review. *Polycyclic Aromatic Compounds*, *35*(2–4), 248–284. https://doi.org/10.1080/10406638.2014.918550