
**UNIVERSITI PUTRA MALAYSIA**

***DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN GRILLED CHICKEN AND BEEF AND THEIR BIOACCESSIBILITY USING IN VITRO HUMAN DIGESTION MODEL***

**ELLIYANA NADIA BT HAMIDI**

**FSTM 2015 33**

# DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN GRILLED CHICKEN AND BEEF AND THEIR BIOACCESSIBILITY USING *IN VITRO* HUMAN DIGESTION MODEL

**By**

**ELLIYANA NADIA BT HAMIDI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science**

**November 2015**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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**November 2015**

**Chairman : Ahmad Faizal Abdull Razis, PhD**
**Faculty : Food Science and Technology**

Bioaccessibility of contaminant in food is defined as the fraction of the contaminant that is released from food matrix in the gastrointestinal tract and thus become available for intestinal absorption. As an important human exposure pathway of contaminants, dietary intake of polycyclic aromatic hydrocarbons (PAHs) is of increasing concern for assessing health risk in human body. Therefore, the present study aimed to evaluate the bioaccessibility of PAHs in different parts of digestive system and to determine the effect of degree of doneness and fat content on the bioaccessibility of PAHs in grilled meat. In this study, the bioaccessibility of PAHs was assessed using *in vitro* human digestion model of grilled beef and chicken (satay). The samples were prepared by charcoal grilling and the levels of 15 priority-controlled PAHs in the samples were determined using high performance liquid chromatography (HPLC) equipped with photodiode array detector and fluorescence detector. The level of PAHs in the liquid portion of the digested samples were compared to the level in the undigested samples to determine the percentage of PAHs released i.e. its bioaccessibility. Limit of detection (LOD) of PAHs compounds were ranged from 0.025 to 5.0 ng/g, while limit of quantification (LOQ) were ranged from 0.075 to 15.0 ng/g. Recovery of PAHs was varied in the range 13.68 to154.6% and 43.37 to 144.79% for beef and chicken samples, respectively. In general, there were significant differences ($p<0.05$) in PAHs bioaccessibility in different parts of digestive system with higher in stomach part as compared in mouth and small intestine parts of both grilled samples. The bioaccessibility of PAHs were ranged from 3.4% to 96.79% in stomach, 3.18% to 60.44% in mouth and 2.38% to 81.02% in small intestine of grilled meat; while 7.36% to 83.67% in stomach, 0.67% to 51.13% in mouth and 1.26% to 63.55% in small intestine of grilled chicken. There were

also significant differences ($p<0.05$) in bioaccessibility of PAHs with increased degree of doneness in both grilled beef and chicken. Bioaccessibility of PAHs was higher in well done cooked than in rare and medium cooked meat. Results indicated that the bioaccessibility of PAHs ranged from 2.38% to 43.75% in rare, 5.27 to 79.02%in medium, and 6.51% to 84.83% in well done cooked grilled beef; 0.67% to 8.93% in rare, 1.3% to 21.28% in medium and 2.95% to 21.87% in well done cooked grilled chicken. In addition, there was also a positive correlation ($R^2$ = 0.923) between fat content and bioaccessibility of PAHs in both meat samples. In sum, this study pointed out the importance of food matrix (beef and chicken meat), elemental chemical properties (pH, enzyme) in different part of digestive system, physicochemical (polarity, water solubility) and cooking practices (rare, medium and well done) in the bioaccessibility of PAHs.

Abstraktesis yang dikemukakankepadaSenatUniversiti Putra Malaysia
sebagaimemenuhikeperluanuntukIjazahMaster Sains

**PENENTUAN POLISIKLIK AROMATIK HIDROKARBON
DI DALAM AYAM DAN DAGING PANGGANG
DAN BIOAKSES MENGGUNAKAN
MODEL PENGHADAMAN MANUSIA SECARA *IN VITRO***

Oleh

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**November 2015**

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Bioakses pada kontaminan dalam makanan ditakrifkan sebagai pecahan kontaminan yang telah dibebaskan daripada matrik makanan di dalam saluran gastro-usus dan berupaya untuk penyerapan usus. Sebagai laluan penting pendedahan manusia kepada kontaminan, pengambilan polisiklik aromatik hidrokarbon (PAHs) dalam makanan adalah masalah yang semakin meruncing dalam menilai risiko kesihatan di dalam badan manusia. Oleh itu, kajian ini bertujuan untuk menilai bioakses PAHs pada bahagian yang berbeza dalam sistem penghadaman dan untuk menentukan kesan tahap kemasakan dan kandungan lemak terhadap bioakses PAHs dalam daging panggang. Dalam kajian ini, bioakses PAHs dinilai menggunakan model penghadaman manusia secara *in vitro* ke atas daging lembu dan ayam panggang (sate). Sampel telah disediakan melalui kaedah memanggang menggunakan arang dan aras 15 PAHs utama yang dikawal dalam sampel ditentukan menggunakan kromatografi cecair berprestasi tinggi (HPLC) dengan bantuan pengesan fotodiod pelbagai dan pengesan pendarfluor. Aras PAHs dalam bentuk cecair pada bahagian sampel yang telah dihadam dibandingkan dengan aras dalam sampel yang tidak dihadamkan untuk menentukan peratusan PAHs yang dibebaskan (pecahan boleh diakses), iaitu bioaksesnya. Had pengesanan (LOD) sebatian PAHs telah dianggarkan dari 0.025 kepada 5.0 ng/g, manakala had pengkuantifan (LOQ) telah dianggarkan dari 0.075 kepada 15.0 ng/g. Pemulihan PAHs masing-masing dianggarkan dalam julat 13.68hingga154.6% dan 43.37hingga144.79% untuk sampel daging lembu dan ayam. Secara umumnya, terdapat perbezaan yang ketara ($p<0.05$) pada bioakses PAHs di bahagian yang berbeza dalam sistem pencernaan dengan bioakses didapati tinggi dalam bahagian perut berbanding di bahagian mulut dan usus

ke atas kedua-dua sampel panggang. Bioakses PAHs telah dianggarkan dari 3.4% hingga 96.79% dalamperut, 3.18% hingga 60.44% dalammulutdan 2.38% hingga 81.02% dalamusus di dalamdagingpanggang; manakala 7.36% hingga 83.67% dalamperut, 0.67% hingga 51.13% dalammulutdan 1.26% hingga 63.55% dalamusus di dalamayampanggang. Terdapat juga perbezaan yang ketara ($p<0.05$) dalam bioakses PAHs dengan penambahan tahap kadar kemasakan dalam kedua-dua daging lembu dan ayam panggang. Bioakses PAHs didapati meningkat dalam keadaan masak berbanding kurang dan sederhana masak daging panggang. Keputusan menunjukkan bioakses PAHs dianggarkan dari 2.38% hingga 43.75% dalam kurang masak, 5.27 hingga 79.02% dalam sederhana masak dan 6.51% hingga 84.83% dalam daging barbeku yang masak; manakala 0.67% hingga 8.93% dalam kurang masak, 1.3% hingga 21.28% dalam sederhana masak dan 2.95% hingga 21.87% dalam ayam barbeku yang masak. Tambahan pula, terdapat juga perkaitan positif ($R^2 = 0.923$) antara kandungan lemak dan bioakses PAHs dalam kedua-dua sampel daging. Kesimpulannya, kajian ini menunjukkan kepentingan matriks makanan (daging lembu dan daging ayam), sifat-sifat unsur kimia (pH, enzim) di bahagian yang berbeza dalam sistem pencernaan, fizikokimia (kekutuban, keterlarutan air) dan amalan memasak (kurang masak, sederhana masak dan masak) dalam bioakses PAHs .

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I certify that a Thesis Examination Committee has met on 11 November 2015 to conduct the final examination of Elliyana Nadia bt Hamidi on her thesis entitled "Determination of Polycyclic Aromatic Hyrocarbons in Grilled Chicken and Beef and their Bioaccessibility using *In Vitro* Human Digestion Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| µg | Microgram |
| µm | Micrometer |
| µL | Microliter |
| λex | Excitation wavelength |
| λem | Emission wavelength |
| A | Anthracene |
| Ace | Acenaphthene |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Analytical Chemists |
| B(a)A | Benzo(a)anthracene |
| B(a)P | Benzo(a)pyrene |
| B(b)F | Benzo(b)fluoranthene |
| B(c)F | Benzo(c)fluorene |
| B(g)P | Benzo(ghi)perylene |
| B(j)F | Benzo(j)fluoranthene |
| B(k)F | Benzo(k)fluoranthene |
| BSE | Bovine spongiform encephalopathy |
| Ch | Chrysene |
| $CH_2Cl_2$ | Dichloromethane |
| D(ah)A | Dibenzo(a,h)anthracene |
| EFSA | European Food Safety Authority |
| F | Fluorene |
| Fl | Fluoranthene |
| FAO | Food and Agriculture Organization |

| | |
|---|---|
| GI | Gastrointestinal tract |
| HPLC | High Performance Liquid Chromatography |
| HPLC/UV-FLD | High Performance Liquid Chromatography/Ultraviolet-Fluorescence detector |
| HCl | Hydrocloride acid |
| IARC | International Agency Research on Cancer |
| i.e | “id est”, that is |
| IP | Indeno(1,2,3-cd)pyrene |
| KCl | Potassium chloride |
| kg | Kilogram |
| KSCN | Potassium thiocyanate |
| ml | Mililiter |
| Na | Naphthalene |
| NaCl | Sodium chloride |
| ng | Nanogram |
| NaOH | Sodium hydroxide |
| Na2SO4 | Sodium sulfate |
| NaHCO3 | Sodium bicarbonate |
| NaH2PO4 | Sodium dihydrogen phosphate |
| NH4Cl | Ammonium chloride |
| P | Pyrene |
| PAH | Polycyclic aromatic hydrocarbon |
| pbb | Parts per billion |
| PCB | Polychlorinated biphenyls |
| Phe | Phenanthrene |
| PRS SPE | Propyl Sulfonic Acid Solid Phase Extraction |

| | |
|---|---|
| US-EPA | US Environmental Protection Agency |
| v/v | Volume/volume |
| WHO | World Health Organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 Overview

Diet contains variety of carcinogens which consists of chemicals present naturally, synthetic compounds and compounds produced during cooking (Viegas *et al*., 2012). Worldwide, health authorities and consumers are increasingly concerned with the exposure to toxic chemical contaminants in the diet, such as heavy metals, persistent organic pollutants (POPs), veterinary drugs, mycotoxins as well as heat-generated contaminants (Marques *et al*., 2011). Last decades, much attention is being paid to these heat-generated toxicants for examples polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HCAs) and acrylamide; concerning their development, occurrence, reduction and effect on human health (Viegas *et al*., 2012). Avoiding exposure to these toxicants is difficult; however, our eating habits, as well as cooking preparation and composition can limit the exposure to these food contaminants (Krul *et al.*, 2000).

Polycyclic aromatic hydrocarbons (PAHs) have been the subject of much concern as they are ubiquitous environmental contaminants (Phillips, 1999). PAHs are commonly found in meat, fish or other foods which cooked at high temperature. PAHs are formed from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities (Onwukeme *et al*., 2015). Factors responsible for the formation of PAHs include the temperature, time/level of doneness, method of cooking, fat content, type of heat source as well as direct contact with the heat source.

PAHs were believed to make a substantial contribution to the burden of cancers in humans as many of them are carcinogenic in experimental animals (Phillips, 1999). It has been reported that 35% of breast, pancreas, stomach and colon cancer incidences may be related to dietary factors (Krul *et al*., 2000). Stomach, breast, colorectal, lung and prostate cancer have been demonstrated to have close relationship to exposure of significant levels of dietary and environmental PAHs which generally generated from well-done cooked meat consumption (Sinha *et al*., 1999). Based on Malaysian Cancer Statistics in 2006, ten frequent cancers among population of Peninsular Malaysia are breast cancer (16.5%), colorectal cancer (13.2%), lung cancer (9.4%), cervical cancer (4.9%), nasopharyngeal cancer (4.5%), thyroid gland cancer (4.1%), liver cancer (3.6%), stomach cancer (3.6%), prostate gland cancer (3.4%) and lymphoma cancer (3.2%).

Nowadays, cooking procedure is one of the aspects not taken into account in food risk assessment of foodstuff, despite it strongly affects the availability of toxic elements in foodstuff. It was well-explained by Afzaninawati *et al*. (2013) who has pointed out that based on Malaysian diet, taste of cooking such as spicy, salty and sour as well as type of cooking for examples frying, grilling and smoking are the main factors contributing to colorectal cancer. According to Jahurul *et al.* (2010), one's daily diet may contain PAHs and HCAs resulting from meat and fish being cooked at high temperature. These heat-induced food toxicants are normally found in grilled and fried meat, fish and poultry (Jahurul *et al*., 2010).

Marques *et al*. (2011) has also came out with the statistic that approximately 97% of the total daily intake of PAHs in humans derived from their diet. Although grilled food only contributed a small part of PAHs intake, people who regularly consume roasted, barbecued or grilled and even smoked food may have significant intake of PAHs. Viegas *et al.* (2012) has reported the uptake of grilled food can represent a large portion of PAHs up to ~120g or more per meal.

Grilled foods are popular both at home and even in restaurants, where this type of food may be detrimental to human health due to high concentration of carcinogens found in such products compared to food which prepared by alternative cooking methods (Sundararajan *et al.*, 1999). Some Malaysian popular dishes i.e various grilled meats such as satay (grilled beef), ayam bakar (grilled chicken) and ikan bakar (grilled fish) are prepared at high temperature that can produce marked differences in the concentrations of PAHs. A study by Farhadian *et al*. (2010) has found in Malaysian dishes, the maximum concentration of fluoranthene (Fl), one of frequently detected PAHs was reported to be as high as 106 ng/g in beef satay, 33.2 ng/g in chicken satay, 38.4 ng/g in grilled chicken and 11.8 ng/g in grilled fish. Such high values indicate high probability that Malaysians consumed PAHs in the level that may be harmful and can lead to cancer.

Grilled satay is a good example of food prepared at high temperature cooking method which applied grilling and roasting method. Satay has been used in this study since it is a popular dish in Malaysia as well as being well-known grilled dish in many other Southeast Asia countries like Indonesia, Thailand and Singapore and some Western countries. Similar to shish kebab, it consists of chunks or slices of dice-sized boneless meat for examples chicken, mutton, beef, pork and fish on skewers made from the midrib of coconut leaf or bamboo (Safzan, 2009; Jinap *et al.,* 2013). In general, satay is grilled over charcoal fire, and then served with variety of spiced seasoning depending on the satay recipe. In Southeast Asian countries, satay commonly been prepared by open charcoal grilling method (Safzan, 2009; Jinap *et al.,* 2013).

Excessive consumption of grilled meat might place the regular consumers at risk of cancer. Therefore, it is crucial to determine the level of PAHs present in food products and their bioccessibility after digestion. In this sense, *in vitro* gastrointestinal model which is closely related to the human digestive system was employed. The bioacessibility of PAHs were quantified in grilled beef and chicken satay digested in different part of digestion system at three degree of doneness. The effect of fat content and the bioacessibility of PAHs was studied in order to correlate the amount of PAHs released during digestion and fat content of the meat.

## 1.2 Statement of problem

Dietary style of grilled meat may increase exposure to PAHs and put the consumer at risk of cancer. Diet is one aspect of an individual's lifestyle that may be practically modified. In relation to this, the analysis in some of the most commonly consumed foods in Malaysia such as grilled meat for the occurrence of PAHs is crucial in order to estimate the exposure of the Malaysian population to these toxic compounds by ingestion of food and to identify practices that increase or decrease an individual's risk. Therefore, bioaccessibility of PAHs is needed in order to quantify the release of PAHs naturally formed during cooking and to assess the ingestion of PAHs from grilled meat which becomes available for intestinal absorption after digestion. In addition, previous studies indicated that concentrations of PAHs varies depending on different cooking conditions such as degree of doneness and were significantly correlated with fat content. In that sense, we evaluated to what extent the doneness level and fat content of meat can affects the bioaccessibility of PAHs.

Yet, so far, only few studies addressed the bioaccessibility of PAHs in food. On the other hand, this is the first extensive study on PAHs bioaccessibility in Malaysian food. Thus, the present study was designed to investigate the effect of cooking conditions by varying degree of doneness and different types of meat (chicken and beef) on PAHs formation during cooking and its bioaccessibility to human body. In this concern, together with the role of cooking method in the initiation and promotion of cancer, this present study aimed:

1. To evaluate the bioaccessibility of PAHs from grilled meat in different parts of digestive system.

2. To determine the effect of degree of doneness and fat content on the bioaccessibility of PAHs in grilled meat.

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