DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN GRILLED CHICKEN SATAY

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THIS DISSERTATION IS SUBMITTED IN PARTIAL FULLFILLMENT FOR BACHELOR DEGREE OF FOOD SCIENCE WITH HONOURS IN FOOD SCIENCE AND NUTRITION

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ZAH: SARJANA MUDA SAINS DENGAN KEPUJIAN (SAINS MAKANAN DAN PEMAKANAN)

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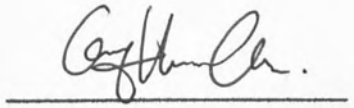

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ACKNOWLEDGEMENT

First and foremost, I would like to express deep sense of gratitude and acknowledgement for the help, advice and guidance of my project supervisor, Dr. Muhammad Iqbal Hashmi. Dr. Muhammad Iqbal Hashmi has guided me through the whole project and kindly gives me many useful ideas and examples. Those examples and ideas lead me to finish the whole project. Besides, Dr. Muhammad Iqbal Hashmi also provides me with the correction when I did some mistakes in the project. He uses his valuable time to help me to make my project become better.

Secondly, I want to show my gratitude to my school, School of Food Science and Nutrition (SSMP). It gives me a very good environment to study and do my experiment.

Thirdly, I would like to show my appreciation to Assoc. Prof. Dr. Mohd. Ismail Abdullah who gives me his comments and suggestions for improving my project, his innumerable suggestions improve the completeness of this project.

Finally, I would also like to express my gratitude to those who had helped me in my project that I did not mention above. Thanks.

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ABSTRACT

The polycyclic aromatic hydrocarbons (PAH) in grilled chicken satay have been qualitative and quantitative determined using reversed phase column Supleco 516 C_{18} high performance liquid chromatography (HPLC). Acetonitrile-water gradient (75:25) was used as mobile phase, and the PAH compounds were detected at wavelength 254 nm by UV Detector. Testing samples were prepared by diluting the sample extraction with mobile phase followed by filtration. The figures showed that 5 types of PAH compounds, which are benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene were present in grilled chicken satay. The results showed that grilled chicken meat contains the highest amount of indeno(1,2,3-cd)pyrene with concentration of 5.16×10^{-3} $mgkg^{-1}$ when grilled under 200°C. Grilled chicken meat contains the least amount of benzo(k)fluoranthene with concentration of 1.54×10^{-3} $mgkg^{-1}$ and 2.02×10^{-3} $mgkg^{-1}$ when grilled under 180°C and 200°C, respectively. Higher temperature was found to directly affect the concentration of PAH compounds generated during grilling process.

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ABSTRAK

PENENTUAN POLISIKLIK AROMATIK HIDROKARBON (PAH) DALAM SATAY AYAM PANGGANG

Polisikilk aromatik hidrokarbon dalam satay ayam panggang telah ditentukan secara kualitatif dan kuantitatif oleh kromatografi cecair tekanan tinggi (HPLC) turus Supleco 516 C_{18}. Penimbal asetonitril-air telah digunakan sebagai fasa bergerak, dan kompaun-kompaun PAH dikesan oleh Pengesan UV pada panjang gelombang 254 nm. Sampel ujikaji disediakan secara pencairan ekstrak sample dengan fasa bergerak diikuti dengan penurasan. Rajah-rajah menunjukkan 5 jenis kompaun PAH telah dikesan dalam satay ayam panggang, iaitu benzo(b)fluorantena, benzo(k)fluorantena, benzo(a)pirena, benzo(g,h,i)perilena, and indeno(1,2,3-cd)pirena. Keputusan menunjukkan bahawa satay ayam panggang mengandungi kanduangan indeno(1,2,3-cd)pirena paling tinggi dengan kepekatan 5.16 x 10^{-3} $mgkg^{-1}$ apabila dipanggang dalam suhu 200°C. Satay ayam panggang mengandungi kandungan benzo(k)fluoranthene paling rendah dengan kepekatan 1.54 x 10^{-3} $mgkg^{-1}$ dan 2.02 x 10^{-3} $mgkg^{-1}$ apabila dipanggang dalam suhu 180°C dan 200°C masing-masing. Diketahui bahawa penigkatan suhu semasa panggang akan secara langsungnya menpengaruhi kepekatan kompaun PAH yang dihasilkan dalam satay ayam.

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LIST OF SYMBOLS AND ABBREVIATIONS

PAH	polycyclic aromatic hydrocarbon
BaP	benzo(a)pyrene
HCA	heterocyclic amines
AGE	advanced glycation end-product
C_{18}	Carbon *n*-octidecil
M	concentration of solution
V	volume of solution
R^2	correlation coefficient
MFG	monofructoseglycine
RAGE	receptor for advanced glycation endproducts
HPLC	High Performance Liquid Chromatography
CYP1A1	cytochrome P450 1A1
CYP1B1	cytochrome P450 1B1

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CHAPTER 1

INTRODUCTION

1.1 MEAT PRODUCTS

Meat, in its broadest definition, is animal tissue used as food. Most often it references to skeletal muscle and associated fat, but it may also refer to non-muscle organs, including lungs, livers, skin, brains, bone marrow and kidneys. The word *meat* is also used by the meat packing and butchering industry in a more restrictive sense - the flesh of mammalian species (pigs, cattle, etc.) raised and butchered for human consumption, to the exclusion of seafood, fish, poultry, game, and insects. Eggs are rarely referred to as *meat* even though they consist of animal tissue. Animals that consume only meat are carnivores (Hedrick, 1994).

1.1.1 Methods of Preparation

Meat is prepared in many ways, as steaks, in stews, fondue, or as dried meat. It may be ground then formed into patties (as burgers or croquettes), loaves, or sausages, or used in loose form (as in "sloppy joe" or Bolognese sauce). Some meats are cured, by smoking, pickling, preserving in salt or brine (see salted meat and curing) (Kierant,

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1964). Others are marinated and barbecued, or simply boiled, roasted, or fried. Meat is generally eaten cooked, but there are many traditional recipes that call for raw beef, veal or fish. Meat is often spiced or seasoned, as in most sausages. Meat dishes are usually described by their source (animal and part of body) and method of preparation.

Meat is a typical base for making sandwiches. Popular sandwich meats include turkey, chicken, ham, pork, bacon, salami and other sausages, and beef, such as steak, roast beef, corned beef, and pastrami. Meat can also be molded or pressed (common for products that include offal, such as haggis and scrapple) and canned.

1.1.2 Nutritional Benefits and Concern

All muscle tissue is very high in protein, containing all of the essential amino acids. Muscle tissue is very low in carbohydrates. The fat content of meat can vary widely depending on the species and breed of animal, the way in which the animal was raised including what it was fed, the anatomical part of its body, and the methods of butchering and cooking (Jay, 1986). Wild animals such as deer are typically leaner than farm animals, leading to the increasing popularity of game such as venison; however, centuries of breeding meat animals for size and fatness is being reversed by consumer demand for meat with less fat (Boyer, 1993). Animal fat is relatively high in saturated fat and cholesterol, which have been linked to various health problems, including heart disease and arteriosclerosis.

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1.2 OBJECTIVES OF RESEARCH

Quantitative analysis is carried out to analyse a type of grilled chicken satay sample. The objectives of this research are:

i. to isolate the polycyclic aromatic hydrocarbon (PAH) compounds generated in grilled meat product, which is grilled chicken satay.
ii. to determine the concentration of polycyclic aromatic hydrocarbons (PAHs) in grilled chicken satay.
iii. to compare the different concentrations of PAH compounds generated in chicken satay when grilled using different temperature.

1.3 SCOPES OF RESEARCH

This research was carried out to isolate and compare the heat-formed compounds, which are polycyclic aromatic hydrocarbons (PAHs) in grilled chicken satay by using quantitative analysis. Quantitative analysis was the determination of the absolute or relative abundance of one or several particular substances present in a sample. Once the presence of certain substances in a sample was ascertained, the study of their absolute or relative abundance could help in determining specific properties. High performance liquid chromatography (HPLC) was used to determine the polycyclic aromatic hydrocarbons (PAHs) contents. The data obtained from the experiment was then interpreted by Microsoft Excel and SPSS compared with each other.

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CHAPTER 2

LITERATURE REVIEW

2.1 SATAY

Satay (also written saté) is a dish that may have originated in Sumatra or Java, Indonesia, but which is popular in many other Southeast Asian countries such as Malaysia, Singapore, Philippines, and Thailand, as well as in Holland which was influenced through its former colonies. In Malaysia, satay is a very popular dish especially during celebrations and it can be found throughout the country. A close analog in Japan is yakitori. Additionally, shish kebab is also very similar to satay.

Although recipes and ingredients vary from country to country, satay generally consists of chunks or slices of meat on bamboo or coconut leaf spine skewers, which are grilled over a wood or charcoal fire. Turmeric is often used to marinate satay and gives it a characteristic yellow color. Meats used include beef, pork, venison, fish, shrimp and chicken. Some have also used more exotic meats, such as crocodile and snake meat. It may be served with a spicy peanut sauce dip, or peanut gravy, slivers of onions and cucumbers, and ketupat. Pork satay can be served in a pineapple based satay sauce. An Indonesian version uses a soy-based dip (Hedrick, 1994).

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Some allege that satay was invented by Chinese immigrants who sold the skewered barbecue meat on the street. Their argument is that the word *satay* means "triple stacked" in Amoy dialect, and indeed, satay is often made with three flat lozenges of meat. Credence is lent to this view from the fact that *satay* in Malay has an older meaning of 'submission' as a verb, and there exists several dishes in typical Malay Cuisine that are heavily influenced by Chinese cuisine. Furthermore, satay is found in Malaysia, Singapore, Indonesia, Thailand and the Philippines, all countries with significant Chinese populations.

On the other hand, it is also possible that it was invented by Malay or Javanese street vendors influenced by the Arabian kebab. The explanation draws on the fact that satay only became popular after the early 19th Century, also the time of the arrival of a major influx Arab immigrants in the region. The satay meats popularly used by Malays and Indonesians, mutton and beef, are also traditionally favoured by Arabs and are not very popular among Chinese, which mostly prefer pork or chicken meat (Hedrick, 1994).

Known as *sate* in Indonesian (and pronounced similar to the English), satay is a widely renowned dish in almost all regions of Indonesia. As a result, many variations have been developed.

- *Satay Madura,* originating in the island of Madura, near Java, is certainly the most famous variant known among Indonesians. Most often made from mutton or chicken, the distinctive characteristic of the recipe is the black sauce made from soy sauce mixed with palm sugar, garlic, shallots, peanut paste, fermented

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shrimp paste (petis), pecans, and salt. It is mainly eaten with rice and venison curry.

- *Satay Lilit* is a satay variant from Bali, a famous tourist destination. Unlike most varieties of satay, it is made from minced beef, chicken, fish, pork, or even turtle meat, which is then mixed with grated coconut, thick coconut milk, lemon juice, shallots, and pepper. Wound around bamboo, sugar cane or lemon grass sticks, it is then grilled on charcoal.
- *Satay Padang*, a dish from Padang city and surrounding area in West Sumatra, made from cow or goat offal boiled in spicy broth, which is then grilled. Its main characteristic is yellow sauce made from rice flour mixed with spicy offal broth, turmeric, ginger, garlic, coriander, galanga root, cumin, curry powder and salt. It is further separated into two sub-variants, the Pariaman and the Padang Panjang, which differ according to taste and the composition of their yellow sauces.
- *Saté Susu*, or *Milky Satay*, a tasty dish commonly found in Java and Bali, grilled spicy cow breast with distinctive 'milky' taste, served with hot chili sauce.
- *Satay Makassar*, from a region in Southern Sulawesi, is made from beef and cow offal marinated in sour carambola sauce. It has a unique sour and spicy taste. Unlike most satays, it is served without sauce.
- *Satay Meranggi*, commonly found in Purwakarta and Bandung, two towns in Java, is made from beef marinated in a special paste. The two most important elements of the paste are *kecombrang* (*Nicolaia speciosa*) flower buds and *ketan* (sweet rice) flour. Nicola buds brings a unique smell and liquorice-like taste. It is served with ketan cake (*juadah*).

Satay Kulit found in Sumatra is a crisp satay made from marinated chicken skin.

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Satay is a popular dish in Malaysia. Satay in Malaysia can be found throughout every state in the country. Besides restaurants that serve satays, one can find hawkers selling satay in food courts and Pasar malam. While the popular type of satay are usually beef and chicken satays, different regions of Malaysia have developed their own unique variations of satay.

- Kajang town in Selangor is famous for its *Sate Kajang* (*sate* is satay in Malay) and it has earned a reputation over the years as the "Satay Town" of Malaysia.
- In Johor, especially in Muar, satay is served for breakfast in the morning.
- A unique pork satay can be found in Malacca and Sarawak. Instead of the traditional peanut sauce it features a pineapple-based spicy sauce.

Satay celup or steamboat satay, which is also unique to Malacca, is a variation of satay. It consists of raw meat, seafood or vegetables on skewers that are dipped into a boiling satay sauce to cook during the meal.

As one of Malaysia's national dishes, Malaysia Airlines serves satay to its First and Business Class passengers as an appetizer on many of its long-haul flights. AirAsia, Malaysia's second carrier as well as premier budget carrier also serves satay on its flights (Kierant, 1964).

Satay was one of the earliest foods to be associated with Singapore since the 1940s. They were moved to the Esplanade Park in the 1960s, where they grew to the point of being constantly listed in tourism guides. Open only after dark with an al fresco concept, the Satay Club was to define the way satay is popularly served in Singapore

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since then, although they are also commonly found across the island in most hawker stalls, modern food courts, and upscale restaurants at any time of the day. Moved several times around the vicinity of Esplanade Park due to development and land reclamation, the outlets finally left the area permanently to Clarke Quay in the late 1990s to make way for the building of the Esplanade - Theatres on the Bay.

Several competing satay hotspots have since emerged, with no one being able to lay claim to the reputation the Satay Club had at the Esplanade. While the name has been transferred to the Clarke Quay site, several stalls has been noted to have moved to Sembawang in the north of the city. Equally famous are the satay stalls which opened at Lau Pa Sat, particularly popular with tourists. Served only at night when Boon Tat Street is closed from vehicular traffic and the stalls and tables occupy the street, it mimics the open-air dining style of previous establishments. Other notable outlets include the ones at Newton Food Centre, East Coast Park Seafood Centre and Toa Payoh Central.

The common types of satay sold in Singapore include Satay Ayam (chicken satay), Satay Lembu (beef satay), Satay Kambing (mutton satay), Satay Perut (Goat's stomach), and Satay Babat (Goat's skin).

Like Malaysia Airlines, Singapore's national carrier, Singapore Airlines also serves satay to its First and Raffles Class passengers as an appetizer (Newbold, 1966).

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2.2 MAILLARD REACTION

The reaction between sugars and amino groups was first described in 1908 by two Englishmen, Ling & Malting, who considered color formation in beer. In 1912 Louis-Camille Maillard described a browning reaction between reducing sugars and amino groups. Despite not being the first to report the reaction, Maillard was the first to realize the significance of the reaction in areas as diverse as plant pathology, geology and medicine. It is interesting to note that despite there having been six international symposiums on the Maillard reaction, not one of the papers was a direct study on the Maillard reaction in confectionery (Waller & Feather, 1983; Finot *et al.*, 1990; Labuza *et al.*, 1994). In fact, it seems that recent symposia were as much concerned with the medical aspects as food chemistry of the Maillard reaction. The medical world has begun to realize that there may be a role for the Maillard reaction in the formation of complications of diabetes and aging, in which the blood glucose becomes bound to proteins in the body in a similar way to food proteins and glucose. Despite the lack of direct research on the Maillard reaction in confectionery, it can be seen that the Maillard reaction will play an important role in the formation of flavors and colors of some confectionery products since the main Maillard reactants are present. This paper is particularly concerned with the Maillard reaction in non-chocolate confectionery.

2.3 NON-ENZYMATIC BROWNING REACTIONS PATHWAYS

The Maillard reaction is one of four non-enzymatic browning reactions which occur in food. The other three are:

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1) the degradation of ascorbic acid,

2) lipid peroxidation

3) sugar-sugar caramelization.

The chemistry of these reactions is related to the Maillard reaction. Ascorbic acid (AsA) undergoes a reaction chemically similar to that of sugars except that amino acids are not necessary for browning. Since AsA is very reactive, it degrades by two pathways, both of which lead to the formation of dicarbonyl intermediates and subsequently to form browning compounds (Davies *et al.*, 1992). Lipid peroxidation occurs by the action of oxygen and reactive oxygen species on the fatty acids, especially unsaturated fatty acids. These are oxidized to form aldehydes and ketones which then react with amino acids to form brown pigments, as in the Maillard reaction. It is possible that peroxidation products induce the browning reaction of the Amadori products (Hermosin *et al.*, 1992). At high temperatures (> 80°C) sugar-sugar interactions or the caramelization reaction occurs. This is a complex series of reactions but many of the intermediate flavor compounds and products are similar to those observed for the Maillard reaction.

2.3.1 Mechanism of the Maillard reaction

The chemistry of the Maillard reaction is known as a complex series of reactions leading to the formation of a variety of products, including the flavors, aromas and colors considered important in food science today. The classical scheme of the chemical reaction is that of Hodge as shown in Figure 1 (Hodge, 1953). This is still used today to describe the reaction. The Maillard reaction was first described as between reducing

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sugars with amino acids, but now is extended to include many other carbohydrate and amine groups. Sugar sources include dextrose, fructose, high fructose corn syrup, sucrose, corn starches and maltodextrins. Protein (-NH2) sources for candy may include milk solids, cream, egg solids, nuts and nut fragments, cocoa solids, butter (small source of nitrogen), fruits and fruit juices provide free amino acids, gelatin, whey proteins and emulsifiers such as lecithin.

The mechanism of the Maillard reaction as shown in Figure 1 is very complicated. However, it is generally divided into three stages (Lee, 1983; Mauron, 1981):

(1) The first stage involves the sugar-amine condensation and the Amadori rearrangement. The reaction steps have been well-defined and no browning occurs at this stage.

(2) The second stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker reaction especially at high temperatures as used in candy manufacture. At the end of stage two there is a beginning of flavor formation - depending on which flavor is studied.

(3) formation of heterocyclic nitrogen compounds. Browning occurs at this stage.

The Maillard reaction is initiated by a condensation reaction between the carbonyl group of the aldose and the free amino group of an amino acid to give an N-substituted aldosylamine. This is the result of a nucleophilic attack group by the NH2 group of the amino acid on the electrophilic carbonyl groups of sugar. It is basically an amineassisted dehydration reaction of sugar. The condensation product rapidly loses water as a product and is converted into a Schiff base. This reaction is reversible and

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