INFLUENCE OF OIL DROPPING AT DIFFERENT HEAT SOURCE TEMPERATURES ON POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SMOKE AND CHICKEN MEAT

MISS CHATCHALERM PANCHATEE

A SPECIAL RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
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Abstract

In the grilling process, oil dropping from food on a hot charcoal may cause PAHs contamination in Yakitori products. The objective of this work was to study the effect of heat source temperature on PAHs in oil smoke and on PAHs deposited on the steamed chicken meat. Chicken oil was prepared by heating chicken skin at 60 °C. The melted oil was dropped on the heat source at different temperatures of 250 and 400 °C. PAHs generated in the smoke from the heated oil were collected by an adsorption tube. To determine PAHs in the chicken meat, the steamed chicken meat was smoked by heating the dropped oil on the heat sources at 250 and 400 °C. 16 PAHs in smoke and chicken samples were determined by solvent extraction followed by Gas Chromatography/Mass Spectrometry (GC/MS). The total concentrations of PAHs in smoke generated by dropping oil at 250 and 400 °C were 15.02 and 91.09 µg/g oil, respectively. Smoke formed by dropping oil at 250 °C contained 3 PAHs, while smoke formed at 400 °C contained 5 PAHs. The steamed chicken sample smoked with the dropping oil at 400 °C contained higher amount of total PAHs than that smoked at 250 °C. Moreover, sample smoked at 400 °C contained benzo[a]anthraxcene, benzo[b]fluoranthene and chrysene, which are carcinogen indicators in food according to the Commission Regulation (EU) No 835/2011. Therefore, this result clearly indicated that oil heated at a higher temperature generates more amounts and types of PAHs than at a lower temperature.

Keywords: Grilling/ Melted chicken oil/ Polycyclic Aromatic Hydrocarbons/ Smoke/ Yakitori

หัวข้อโครงการศึกษาวิจัยพิเศษ	อิทธิพลของการหยดน้ำมันลงบนแหล่งความร้อนที่อุณหภูมิต่างๆ ต่อปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในควันและในเนื้อไก่
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บทคัดย่อ

ในระหว่างขั้นตอนการย่าง การหยดของน้ำมันจากอาหารลงบนถ่านร้อนอาจเป็นสาเหตุของการปนเปื้อนสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในผลิตภัณฑ์ยากิโทริ งานวิจัยนี้มีจุดประสงค์เพื่อศึกษาผลของอุณหภูมิแหล่งความร้อนในการหยดน้ำมันต่อปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในควันทั้งหมดและปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนซึ่งเกาะติดเนื้อไก่นึ่ง ลำดับแรก เตรียมน้ำมันไก่โดยให้ความร้อนกับหนังไก่ที่อุณหภูมิ 60 องศาเซลเซียส จากนั้นหยดน้ำมันไก่ลงบนแหล่งให้ความร้อนที่อุณหภูมิ 250 และ 400 องศาเซลเซียส เก็บควันที่เกิดขึ้นด้วยหลอดที่บรรจุสารดูดซับสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอน นอกจากนี้ยังได้ศึกษาปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในเนื้อไก่ โดยการรมควันเนื้อไก่นึ่ง ซึ่งควันดังกล่าวเกิดจากการให้ความร้อนกับน้ำมันที่หยดลงบนแหล่งความร้อนที่อุณหภูมิ 250 และ 400 องศาเซลเซียส ในลำดับสุดท้าย ทำการวิเคราะห์ปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนทั้ง 16 ชนิดในควันและในตัวอย่างเนื้อไก่โดยใช้เทคนิคการสกัดด้วยตัวทำละลาย ตามด้วยเทคนิคก๊าซโครมาโตกราฟี/แมสสเปกโตรมิทรี (GC/MS) จากผลการทดลองพบโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนทั้งหมดในควัน โดยมีปริมาณเท่ากับ 15.02 และ 91.09 ไมโครกรัมต่อกรัมน้ำมัน ในกรณีการหยดน้ำมันที่อุณหภูมิ 250 และ 400 องศาเซลเซียส ตามลำดับ ทั้งนี้ควันที่เกิดจากการหยดน้ำมันที่อุณหภูมิ 250 องศาเซลเซียส ประกอบด้วยสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอน 3 ชนิด ในขณะที่ควันที่

เกิดขึ้นจากการหยดน้ำมันที่อุณหภูมิ 400 องศาเซลเซียส พบ 5 ชนิด สำหรับในตัวอย่างเนื้อไก่นึ่งรมควันด้วยการหยดน้ำมันที่อุณหภูมิ 400 องศาเซลเซียส พบว่ามีปริมาณสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนสูงกว่าในตัวอย่างเนื้อไก่นึ่งรมควันด้วยการหยดน้ำมันที่อุณหภูมิ 250 องศาเซลเซียส นอกจากนี้ ตัวอย่างเนื้อไก่รมควันที่อุณหภูมิ 400 องศาเซลเซียส ประกอบไปด้วยสารเบนโซเอแอน-ทราซีน เบนโซบีฟลูโอแรนทีน และไครซีน ซึ่งเป็นตัวชี้วัดความเป็นสารก่อมะเร็งในอาหารตามข้อกำหนดของสหภาพยุโรป เลขที่ 835/2011 ดังนั้น ผลการทดลองบ่งชี้อย่างชัดเจนว่าการหยดน้ำมันลงบนแหล่งให้ความร้อนอุณหภูมิสูงทำให้เกิดสารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในปริมาณและชนิดที่มากกว่าการหยดน้ำมันลงบนแหล่งให้ความร้อนอุณหภูมิต่ำ

คำสำคัญ: การย่าง/ ควัน/ น้ำมันไก่/ สารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอน/ ยากิโทริ

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CHAPTER 1 INTRODUCTION

1.1 Research Rationale

As the consumption of poultry meat has increased steadily, the poultry industry has grown rapidly. In 2011, Thailand exported approximately 415,585 tons of processed chicken with the values of 57,045 million Baht. These were 13 percent higher than the exported value of the year 2010 (Office of agricultural economics, 2011). Yakitori is one of the important grilled products for the processed chicken industry. Yakitori is a bit-sized chicken on skewers that is grilled over charcoal, so it has charcoal aroma and good color (Yakitori, 2002).

The charcoal grilling process may cause PAHs contamination in Yakitori. Many reports have demonstrated that carcinogenic PAHs can be formed through grilling of foods**.** Variation in PAHs levels in food is mainly due to the type and fat content of the food, temperature and duration of cooking, type of fuel used (electrical, gas, wood, and charcoal) and proximity and direct contact with heat source (Alomirah et al., 2011). Silvia et al. (2007) reported that foods can be contaminated PAHs from intense thermal process such as the charcoal grilling process. PAHs contamination by grilling processes is due to the direct pyrolysis of food nutrients and the direct deposition of PAHs from smoke produced through incomplete combustion of different thermal agents (Farhadian et al., 2011). The formation of PAHs during charcoal grilling at high temperature may be due to the incomplete combustion of charcoal or transformation of some food components such as triglyceride and cholesterol. Also, during charcoal grilling at high temperature, the fat droppings fall on the hot coals were pyrolyzed, producing benzo[*a*]pyrene and other PAHs which were subsequently deposited onto the surface of the meat (Chen and Lin, 1997). Even through grilling process could induce PAHs formation. However, Yakitori's customer still need charcoal aroma and good color from grilling so it is difficult to avoid grilling processes in Yakitori products.

PAHs in charcoal grilled meat and meat products is mainly due to the fact that these PAHs dominate the smoke arising from the pyrolysis of fat dropping over heat source and incomplete combustion of charcoal (Alomirah et al., 2011). Farhadian et al. (2011)

study effects of wrapping (aluminium and banana leaf) of the meat samples prior to charcoal grilling, on the PAHs contents after charcoal grilling. In comparison between charcoal – grilled samples without wrapping treatment and charcoal – grilled samples with wrapping treatment, there was a 100% reduction of both Benzo[a]pyrene and Benzo[b]fluoranthene in aluminium wrapped samples, and 81% and 85% reductions in banana wrapped samples, respectively. This indicated that the reduction of PAHs concentrations in the meat samples is to be expected as the fat was prevented from dropping onto the fire (by aluminium foil and banana leaf) and the meat was also not indirect contact to the heat source.

As the mention above that PAHs dominate the smoke arising from the pyrolysis of fat dropping over heat source and incomplete combustion of charcoal. There are some researches that concern with the effect of charcoal on PAHs generation. However, there are so few researches that concern with the effect of fat dropping over heat source at various temperatures during grilling process on PAHs generation and the doubts about different PAHs level which deposited on grilled chicken. Thus, study PAHs formation from the pyrolysis of fat dropping over heat source at various temperatures without effect of charcoal combustion is interesting. The main aim of this work is to demonstrate the effect of heat source temperature on PAHs generation from dropping fat over heat source. In this work, PAHs level in whole smoke and PAHs level which deposited on the steamed chicken surface were considered.

1.2 Objectives

1. To study the effect of heat source temperature on PAHs content in smoke from heating of dripping chicken oil

2. To study the effect of heat source temperature on PAHs content that deposited on the steamed chicken from heating of dripping chicken oil

1.3 Scopes

1. Melted oil from chicken skin was used as dripping oil, and steamed chicken was used as sample.
2. Different heat source temperatures consisting of 250 °C and 400 °C were used to heat the dripping chicken oil.
3. Whole smoke generated during heating the dripping chicken oil was collected by adsorption tube.
4. Sixteen carcinogenic PAHs in adsorption tube and chicken sample were determined by solvent extraction followed by Gas Chromatography-Mass spectrometry (GC-MS)

1.4 Expected Benefit

1. To understand the effects of fat drippings onto heat source on PAHs content in smoke and chicken meat
2. To produce Yakitori that has lower PAHs

CHAPTER 2 THEORY AND LITERATURE REVIEW

This chapter includes grilling process, Yakitori, Polycyclic Aromatic Hydrocarbons (PAHs) formation, factors affecting PAHs formation. In addition, Gas Chromatography and Gas Chromatography – Mass Spectrometry are also described in this chapter.

2.1 Grilling Process

Grilling process usually involves a significant amount of direct, radiant heat, and tends to be used for cooking meat quickly and meat that has already been sliced (or other pieces). Food to be grilled is cooked on a grill (an open wire grid with a heat source above or below), a grill pan (similar to a frying pan, but with raised ridges to mimic the wires of an open grill), or griddle (a flat plate heated from below). Heat transfer to the food when using a grill is primarily via thermal radiation. Heat transfer when using a grill pan or griddle is by direct conduction. In the United States and Canada, when the heat source for grilling comes from above, grilling is termed broiling. In this case, the pan that holds the food is called a broiler pan, and heat transfer is by thermal convection. Direct heat grilling can expose food to temperatures often in excess of 260 °C. Grilled meat acquires a distinctive roast aroma from a chemical process called the Maillard reaction (Grilling, 2014).

Yakitori

Yakitori is commonly a Japanese type of grilled chicken skewered on sticks. All different parts of the chicken, thighs, skin, liver, etc. can be used for yakitori (Yakitori, 2002). Yakitori process in industry was shown in Figure 2.1

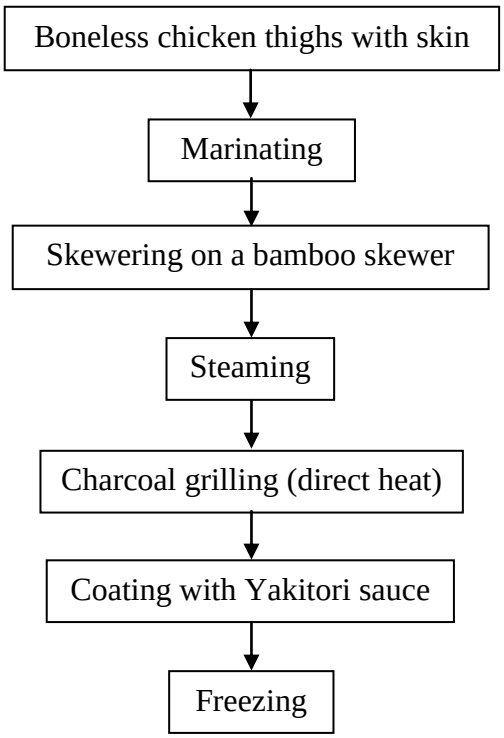

Figure 2.1 Yakitori Process in industry **(**B. Foods Products International, 2012)

2.2 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) commonly refers to a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. PAHs are formed and released during incomplete combustion or pyrolysis (burning) of organic matter such as waste or food, during industrial processes and other human activities. PAHs are also formed in natural processes, such as carbonisation. The general characteristics common to the class are high melting and boiling points, low vapour pressure, and very low water solubility which tends to decrease with increasing molecular mass. PAHs are soluble in many organic solvents and are therefore lipophilic (Risk Assessment Section, 2004)

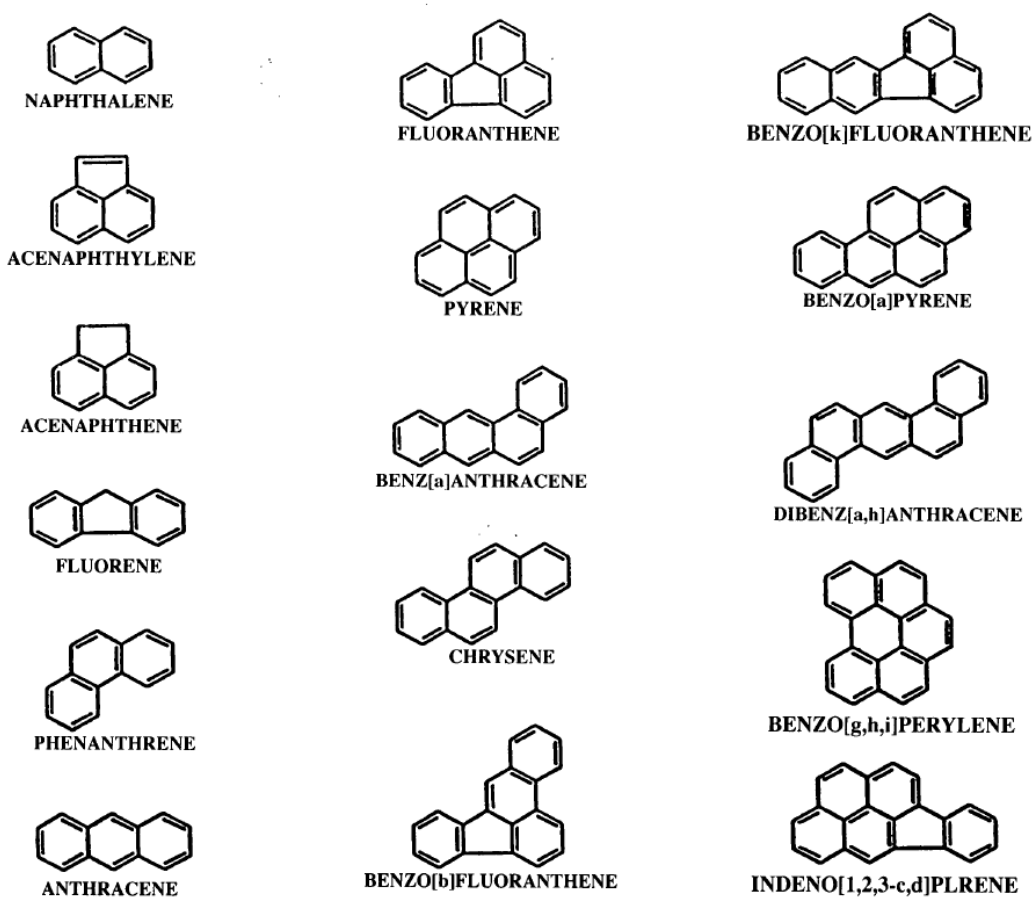

Figure 2.2 Structure of the 16 PAHs identified as priority pollutants by U.S. Environmental Protection Agency (Chen, 1997)

To date more than 100 PAHs have been characterized in nature, 16 of which were classified as the U.S. Environmental Protection Agency (EPA) (Figure 2.2). Of these 16 PAHs, benzo (a) pyrene and dibenz (a,h) anthracene was reported to be the most carcinogenic by The International Agency for Research into Cancer (IARC). Since environmental pollution is becoming a serious problem throughout the word, it is possible that PAHs may be widely distributed within the environment and thus contaminate processed food. As many PAHs have been proven to be carcinogenic and mutagenic, the development of a fast and accurate method foe determination of PAHs in food is necessary (Chen, 1997).

Maximum limits have been set for PAHs in key foodstuffs, e.g. meat and meat products, fish and fishery products, milk and milk products, oils and fats, infant formulae and follow-on formulae and processed cereal based foods and baby foods for infants and young children via Commission Regulation (EU) No 835/2011 which came from amending Regulation (EC) No 1881/2006. The limits laid down in the Regulation are reproduced in Table 2.1 and Table 2.2

Table 2.1 Maximum level in Regulation No. 835/2011 for polycyclic aromatic hydrocarbons (PAHs) in certain foodstuffs

Foodstuffs	Maximum levels (μg/kg)	
6.1Benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene	Benzo(a)pyrene	Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene
6.1.1 Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2.0	10.0
6.1.2 Cocoa beans and derived products	5.0 μg/kg fat as from 1.4.2013	35.0 μg/kg fat as from 1.4.2013 until 31.3.2015 30,0 μg/kg fat as from 1.4.2015
6.1.3 Coconut oil intended for direct human consumption or use as an ingredient in food	2.0	20.0
6.1.4 Smoked meat and smoked meat products	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014

Source: Commission regulation (EU) No 835/2011 (2011)

Table 2.2 Maximum levels in Regulation No. 835/2011 for polycyclic aromatic hydrocarbons (PAHs) in certain foodstuffs (to be continued)

Foodstuffs	Maximum levels (μg/kg)	
6.1.5 Muscle meat of smoked fish and smoked fishery products, excluding fishery products listed in points 6.1.6 and 6.1.7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014
6.1.6 Smoked sprats and canned smoked sprats (*sprattus sprattus*); bivalve molluscs (fresh, chilled or frozen); heat treated meat and heat treated meat products sold to the final consumer	5.0	30.0
6.1.7 Bivalve molluscs (smoked)	6.0	35.0
6.1.8 Processed cereal-based foods and baby foods for infants and young children	1.0	1.0
6.1.9 Infant formulae and follow-on formulae, including infant milk and follow-on milk	1.0	1.0
6.1.10 Dietary foods for special medical purposes intended specifically for infants	1.0	1.0

Source: Commission regulation (EU) No 835/2011 (2011)

2.2.1 PAHs formation

PAHs can be formed by thermal decomposition of any organic material containing carbon and hydrogen. The formation is based on two major mechanism: (1) pyrolysis or incomplete combustion, and (2) carbonization processes (Bjorseth and Becher, 1986).

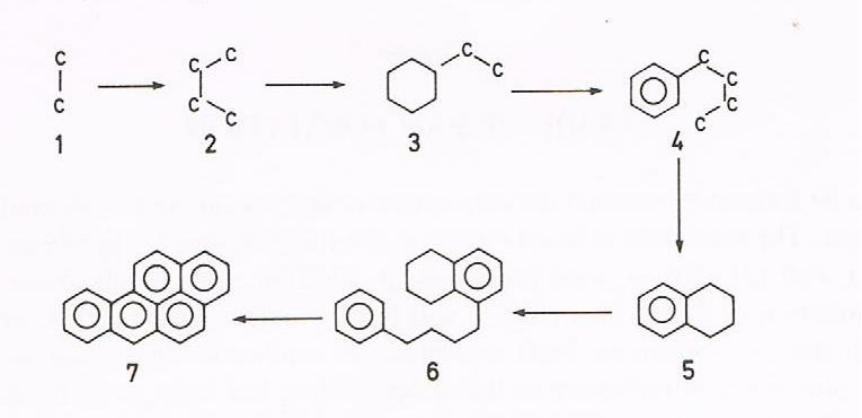

Figure 2.3 Pyrolytic formation of benzo[a]pyrene after Badger (Bjorseth and Becher, 1986)

Although the mechanism of PAHs formation in combustion processes is complex and variable, a pioneering contribution to the understanding has been given by Badger and coworker. The chemical reactions in flames proceed by free radical paths, and a synthetic route based on this concept is postulated for the formation of PAHs also. Based upon the results of a series of pyrolysis experiments, Badger suggested the stepwise synthesis of PAHs from C_2 species during hydrocarbon pyrolysis as outlined in Figure 2.3 for benzo[a]pyrene (BaP) as a example. These pyrolysis studies were conducted by passing the hydrocarbon vapor in nitrogen through a silica tube at 700 °C. Although the use of nitrogen atmospheres has been criticized as lacking relevance to actual combustion, the reducing conditions are similar to those of the oxygen-deficient environments common in the center of flamed and the data are in good qualitative agreement with the PAHs combustion products formed. For example, Boubel and Ripperton found that BaP is produced during combustion even at high percentages of excess air, although the amount of BaP is larger at lower percentages of excess air (Bjorseth and Becher, 1986).

Lendding support to the postulated route to PAHs, Badger and Spotswood pyrolyzed toluene, ethlbenzene, propylbenzene, and butylbenzene and obtained the highest yields of BaP with butylbenzene, a potential intermediate in Badger's reaction scheme. Obviously it is unnecessary to break the starting material down completely to a two-carbon radical in order to form BaP. Any component of the combustion reaction that can contribute intermediate pyrolysis products of the structure required for BaP synthesis would be expected also to lead to increased yields of BaP. It was also found that, when 1,3-butadiene was pyrolyzed with pyrene, no increase in the yield of BaP was observed, indication that Diels-Alder type reactions are probably not important (Bjorseth and Becher, 1986).

More recent studies tend to confirm most of the mechanism proposed by Badger. Crittenden and Long determined the chemical species formed in rich oxy-acetylene and oxy-ethylene flames. Compounds identified suggest that the C_2 species react to form C_4, C_6, and C_8 species, and that reactions involving styrene and phenylacetylene are probably important in the formation of PAHs. Also, a $C_{10}H_{10}$ species was detected in the gases of both flames, which corresponds to the C_4 substituted benzene postulated by Badger (Structure 4 in Figure 2.3) (Bjorseth and Becher, 1986).

The mechanism in Figure 2.3 is a possible pathway to BaP formation, but similar routes could be devised with different intermediates, to lead to most of the known PAHs produced in combustion processes. Badger's work, with its reliance on calculated C-C and C-H bond energies to predict favored pathways and the experimental confirmation of these steps with radioisotopic labeling, provides a plausible mechanism for PAHs formation in combustion or pyrolytic processes (Bjorseth and Becher, 1986).

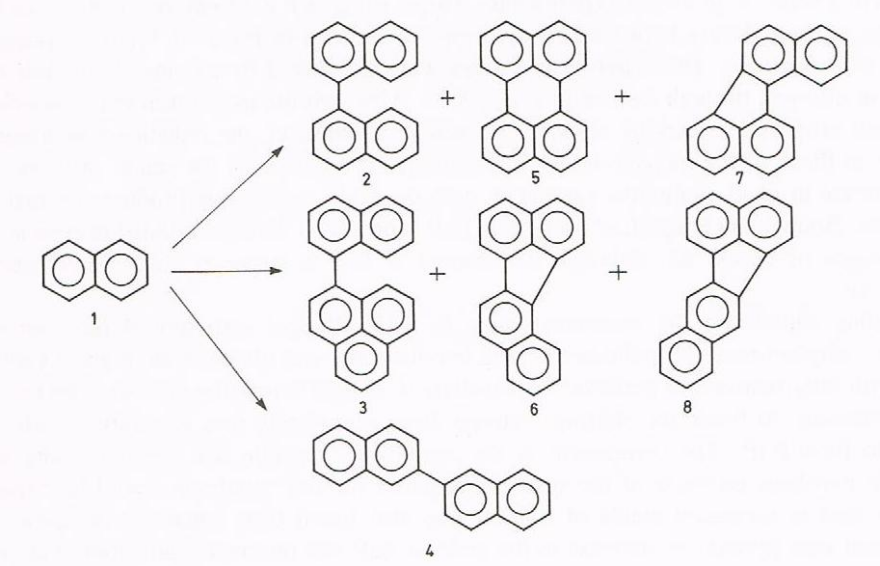

Figure 2.4 Pyrolytic formation of larger PAH from smaller ones (Bjorseth and Becher, 1986)

Once formed, PAHs might undergo further pyrolytic reactions to form larger PAHs by intermolecular condensation and cyclization. This has been extensively studied by Zander and co-workers. With nonsubstituted PAHs, polyaryls are formed, to be followed by ring closure to highly condensed hydrocarbons. Figure 2.4 shows the pyrolytic formation of 5-ring PAHs from naphthalene as an example. The three possible biaryls 2 to 4 are converted to the pericondensed system 5 to 8. Cyclization products from 4 are benzologs of the relatively unstable biphenylene and are not observed (Bjorseth and Becher, 1986).

In spite of the tremendous number of different PAHs which might be formed during the primary reactions, only a limited number of PAHs enters the environment. Many of the primarily formed PAHs will have short half-lives under the pyrolysis conditions and will stabilize in the following reactions. At high temperature the thermodynamically most stable compounds will be formed in corresponding quantitative ratios. These are mainly the unsubstituted parent PAHs. Irrespective of the type of material to be burned, surprisingly similar ratios of PAHs are formed at a defined temperature. For example, thermal decomposition of pit coal, cellulose, tobacco, and also of polyethylene and polyvinylchloride which is carried out at 1000 °C yields very similar PAHs profiles.

Consequently, PAHs profiles seem to depend more on the combustion conditions rather than on the type of organic material burned (Bjorseth and Becher, 1986).

Although the exact mechanism of formation of PAHs in grilled/smoked foods is not precisely known, it is generally considered that at least three possible mechanisms exist. Alomirah et al. (2011) reported that the first mechanism is the pyrolysis of organic matter such as fat, protein and carbohydrates at temperatures above 200 °C, and PAH formation is favored at a temperature range of 500 – 900 °C. The greatest concentrations of PAHs have been shown to arise from pyrolysis of fat. The second mechanism is the yield of direct contact of lipids dripping at intense heat directly over the flame. This condition can generate volatile PAHs that in turn be adhered to the surface of the food as the smoke rises. The third mechanism is the incomplete combustion of charcoal which can generate PAHs that are brought onto the surface of the food. It has been suggested that low molecular weight PAHs (containing 2 – 3 aromatic rings) arise from smoke generated during meat grilling as these PAHs are more volatile than high molecular weight PAHs (containing more than 3 aromatic rings).

In 1988, Schwab et al. proposed a mechanism (Figure 2.5) to account for the formation of alkanes, alkenes, alkadienes, aromatics and carboxylic acids from the pyrolysis of unsaturated triglycerides. The cracking of triglyceride produces free radical followed by decarboxylation. The alkanes and alkenes are formed by decarboxylation of Radical. If the oil also contains unsaturated fatty acid, unsaturated sites enhance cleavage at the C–C double bond, at a position α, β to the unsaturation and this cleavage is a dominant reaction. The formation of aromatics is supported by Diels–Alder ethylene addition of a conjugated diene and carboxylic acids are most likely formed through cleavage of the glycerol moiety as suggested by Nawar (1969).

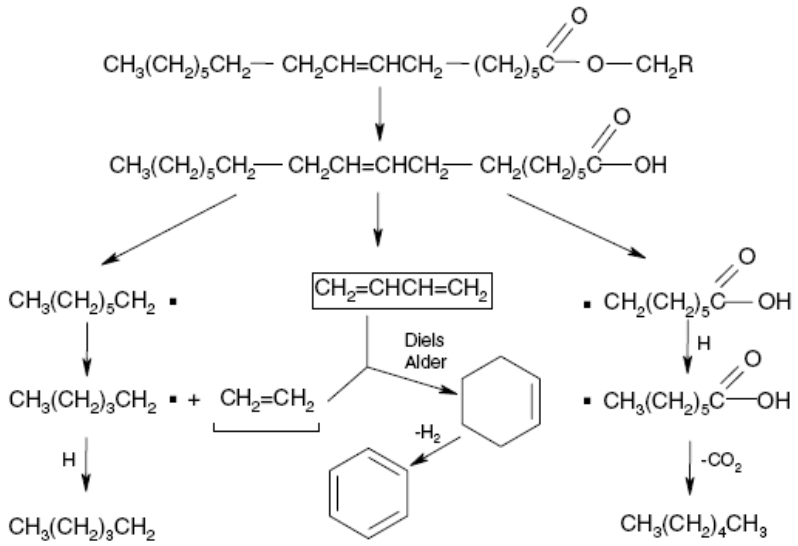

Figure 2.5 Reaction mechanism for the pyrolysis of triglycerides (Schwab et al., 1988)

Chen and Chen (2001) reports that benzene is a probable precursor for PAH formation. According to a study by Mestres and Sola (1998), the benzene-containing compound may react with conjugated-diene-containing degradation products such as 1,3-butadiene to form PAH through Diels-Alder cycloaddition.

2.2.2 Factors affecting PAHs formation in food processing

Since formation of PAHs occurs through pyrolysis of fat at temperature of above 200 °C (Scientific Committee on Foods of EC, 2002). Factors affecting PAHs formation in food processing has been identified which were divided into food and food processing.

1. Food

Variation in PAHs levels in foods is mainly due to the type and fat content of the food (Alomirah et al., 2011). Chen and Chen (2001) reported that soybean oil produced a higher amount of smoke than sunflower oil or canola oil because of the presence of a large amount of linolenic acid (8.79%) and high degree of unsaturation. Although canola oil also contained a high amount of linolenic acid (10.69%), the iodine value was significantly lower than soybean oil, and thus a smaller amount of smoke was formed.

This result clearly indicated that the degree of unsaturation of fatty acids could affect the variety and amount of PAHs formed in the smoke.

Chen and Lin (1997) reported that a comparison of PAHs levels in duck breast steaks undergoing various processing and cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 μg/kg), followed by charcoal grilling with skin (300 μg/kg). This result seems to be contradictory to some reports by Engst and Fritz (1977), and Doremire, et al. (1979) who reported that the amount of benzo[*a*]pyrene is directly proportional to fat content during charcoal grilling.

2. Food Processing

Apart from type and fat content of the food, Factors of food processing also affect PAHs formation.

2.1 Melted fat from food dropping onto the charcoal

Aluminium wrapped chicken samples did not contain Benzo(a) Pyrene and Benzene(b) Fluoranthene, while banana wrapped chicken samples contained 1.48 and 3.29 ng/g, respectively. This indicated that compared to the charcoal-grilled samples without wrapping treatment, there was a 100% reduction of both compounds in the aluminium wrapped, and 65% and 39% reductions in the banana wrapped samples, respectively (Farhadian et al., 2011).

2.2 Processing method temperature and duration of cooking

Chen and Lin (1997) concluded that with processing time from 0.5 to 1.5 h, charcoal grilling of duck samples with skin contained the highest amount of total PAHs, followed by charcoal grilling of duck without skin, smoking, roasting, steaming, and liquid smoke flavoring (LSF).

In general, the higher grilling temperature, the greater the formation of PAHs.

In recent study Chen and Lin (1997) report that eleven PAHs were detected after roasting of duct breast at 200 °C for 40 min, and in most cases the amounts of PAHs increased along with increasing roasting time. Bjorseth et al. (1986) reported that under comparable conditions, 1 g of tobacco yields 44 ng of benzo[a]pyrene at 400 °C and

183,500 ng of benzo[a]pyrene at 1000 °C. The absolute amount of PAHs formed during incomplete combustion is dependent on temperature.

2.3 Type of fuel used (electrical, gas, wood and charcoal)

Farhadian et al. (2010) concluded that the PAHs concentrations between the three groups of grilled dishes were found to be significantly different ($p < 0.05$). The highest concentration of PAHs was detected in charcoal grilled followed by flame-gas grilled and oven grilled dishes. PAHs concentrations of flame-gas grilled dishes was found to be low when the gas-flame source was vertical.

2.3 Gas Chromatography (GC)

GC is a physical separation technique in which components of a mixture are separated using a mobile phase of inert carrier gas and a solid or liquid stationary phase contained in a column. The separation is based on the interactions of the vaporized components in a mixture with the stationary phase as they are moved along by the mobile phase. Since GC is a gas-based separation technique, it is limited to components that have sufficient volatility and thermal stability (Kupiec, 2004).

Instrument component

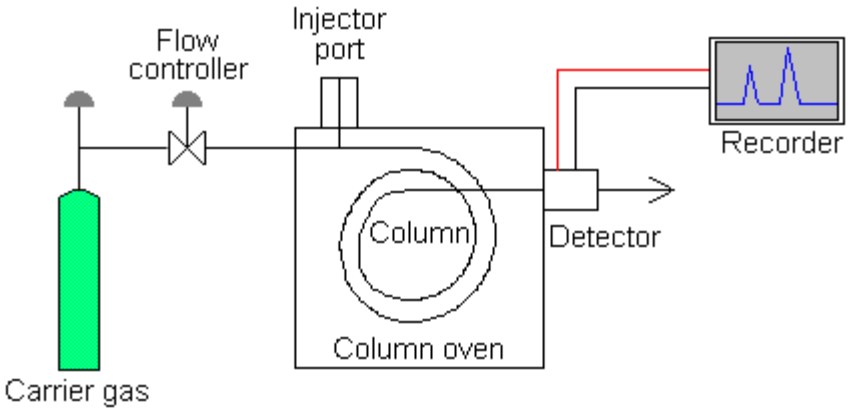

Figure 2.6 Schematic of a gas chromatography (Kupiec, 2004)

The hardware components used in typical GC systems include an injector, a carrier gas, a column (stationary phase), an oven, a detector and a recorder or information processor (Figure 2.6). Several components have variable settings that can be used to optimize the analysis of different sample types.

Injectors: Introduction of the sample into a GC system is a critical step in separation. The reproducibility of the amount of sample injected is important to ensure the reproducibility of results. A sample can be injected manually into the system or by using an auto sampler system. A major source of precision errors in GC is poor injection technique. Auto samplers are very effective and help ensure that precisely the same sample volume is injected every time, thereby eliminating injection errors. The injector temperature is also important for separation. The temperature of the injector is used to rapidly vaporize the liquid sample into a gaseous phase that can be carried to the column for separation. The temperature of the injector site can be varied to help optimize separation. Different sample components will dictate what temperature is necessary for vaporization (Kupiec, 2004).

Carrier Gas: In the early days of GC experiments, the carrier gas was seen merely as the mass transport system. However, it is becoming clearer that carrier gas is integral to the chromatographic process. Several inert gases can be used as the carrier gas or mobile phase of GC. Hydrogen, helium and nitrogen are all common carrier gases. Each carrier gas has its benefits and systems for which it is best suited. For example, helium is the most common gas used with GC/mass spectrometry systems. Before the carrier gas can be used, it is important to ensure that it contains no oxygen because oxygen can have detrimental effects on the stationary phase of GC. Also, the chemical nature of the carrier gas has an effect on the efficiency of the GC column. The pressure at which the carrier gas is moving influences the retention time of samples on the column. Increasing the pressure decreases the retention time. Varying both the carrier gas and the pressure at which the gas is exerted on the column can ensure that the sample has ample time to interact with the stationary phase and improve the separation (Kupiec, 2004).

Column/Stationary Phase: The column and stationary phase are responsible for the majority of the separation of sample components. Interaction between the mobile phase, stationary phase and sample components determines how components are separated, so

selection of columns and stationary packing material is critical. There is a great deal of variation in commercially available columns and packing material. Depending on the components to be separated, the mobile phase being used and the desired degree of separation, different combinations of column type, column length and packing material can be used to achieve optimal results. Columns can be classified by column diameter and packing material. The three main types of GC columns are (1) conventional, (2) preparative and (3) capillary. Columns can be either packed or open. Packed columns can contain either a porous or nonporous stationary phase. A multitude of different materials are used to pack columns. Each material has its own properties, limitations and effective separation parameters. The capillary column is the most frequently used column for GC separations. Both conventional and capillary columns have advantages and disadvantages. For example, more packing materials are available for conventional columns, but capillary columns give improved sensitivity. When selecting a column, a major choice is among nonpolar, moderately polar or polar columns. Special-phase columns such as chiral columns can also be utilized to separate isomers. Other important factors to consider are packing material, column length and column diameter. Columns can be the most expensive component of a chromatographic system, so proper maintenance and use can help control cost. For proper column care it is best to consult the manufacturer's guidelines (Kupiec, 2004).

Oven: The column resides in an oven, and temperature, which greatly affects the effectiveness of the chromatographic separation, is an extremely important factor used in controlling GC. In many cases, isothermal (constant temperature) is not the most effective temperature mode for sample separation; in such cases, a temperature program can be used. Most GC temperature programs have an initial temperature, a ramp (degree increase per minute) and a final temperature (Kupiec, 2004)**.**

Detector: The detector is used to sense the presence of a compound passing through and to provide an electronic signal to an integrator. A variety of detectors are commercially available to be used with GC, each having its own limitations and advantages:

- Electron capture (ECD). The ECD is used with organic compounds and has many environmental applications.

- Flame ionization (FID). The most commonly used detector in GC, FID is typically used with organic compounds and is widely used in quality-control analysis of pharmaceutical compounds.
- Mass spectrometry (MSD). The MSD can be coupled with GC as a powerful qualitative component for the identification of compounds.
- Nitrogen phosphorous (NID). This detector is used most commonly for drug analysis in tissues and bodily fluids.
- Thermal conductivity (TCD). This detector is considered a universal detector and is nondestructive to analyses.

Recorder: The recorder in a GC system serves to convert the information collected by the detector into a format that is understandable. Since the detector signal is electronic, the use of modern data acquisition can aid in signal analysis. The most common data-acquisition technique is through use of a computer, which integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret. Other more advanced features can also be applied to a chromatographic system. These include computer-controlled automatic injectors, oven temperature programs and carrier gas pressure (Kupiec, 2004).

Quantitative Analysis

The basic theory for quantitation of sample components involves the measurement of peak height or peak area. For peaks that are well resolved, both peak height and area are proportional to the concentration. Three different calibration methods, each with its own benefits and limitations, can be used in quantitative analysis: (1) the external standard (std), (2) the internal standard (IS) and (3) the standard addition method. For GC, the most commonly used quantitative methods are the IS and standard addition methods. The IS is an effective method because it tends to yield the most accurate and precise results of all the quantitative methods. With this method, an equal amount of an IS, a component that is not present in the sample, is added to both the sample and calibrator solutions. The IS selected should be chemically similar to the analysis and have a similar retention time and similar derivatization. Additionally, it is important to ensure that the IS is stable and does not interfere with any of the sample components. The IS should be added before any preparation of the sample so that extraction efficiency can

be evaluated. Quantitation is achieved by using ratios of peak height or area of the component to the internal standard, as shown in the following formula:

$$\text{Conc.sample} = \frac{(\text{Area}_{\text{ISCalibrator}})}{\text{Area}_{\text{ISsample}}} \times \frac{(\text{Area}_{\text{sample}})}{\text{Area}_{\text{calibrator}}} \times (\text{Conc.}_{\text{calibrator}})$$

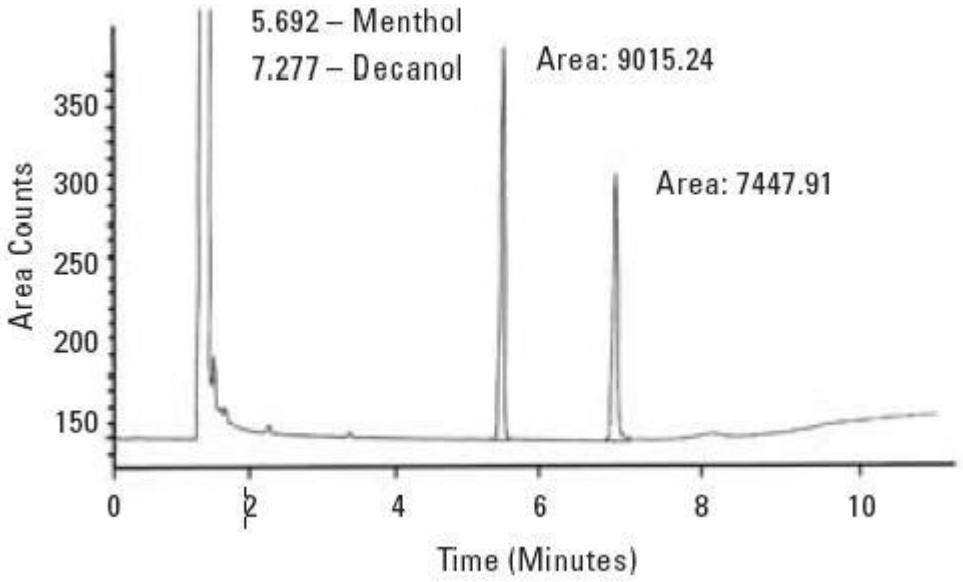

Figure 2.7 Example chromatogram illustrating gas chromatography analysis of menthol with decanol used as an internal standard (Kupiec, 2004)

Note: The area ratio of menthol/decanol was used in determining the concentration of the sample. The sample was a 110 ppm (μg/mL) solution.

Figure 2.7 is an example chromatogram produced in the GC analysis of menthol, with decanol employed as an IS. Alternatively, a calibration curve is established by plotting the response ratios of calibrator/IS versus concentration of the calibrators. Sample concentration is then extrapolated from the regression equation of the calibration curve based on the response ratio of sample/IS (Kupiec, 2004).

Gas Chromatography – Mass Spectrometry (GC-MS)

In GC/MS, the mass spectrometer ionizes the gas-phase elute from the GC column as it enters the mass spectrometer. It is important to remember that this gas-phase elute contains mobile phase, analysis molecules, volatile matrix components that elute with the analysis, and molecules that have bled off of the stationary phase of the GC column

formed by decomposition of the stationary phase. Depending on the ionization technique, these ions representing the intact molecule may have sufficient energy to undergo fragmentation to ions that have a smaller mass. In GC/MS, the vast majority of the ions formed have only a single charge. Primarily, only ions of aromatic hydrocarbons subjected to electron ionization (EI) will form double-charge ions; and these ions are of low abundances compared to single-charge ions of the same mass. Mass spectrometers separate ions according to their mass-to-charge ration (m/z) values; therefore, because virtually all of the ions have a single charge, the m/z value of ions formed in GC/MS is considered to be also the mass of the ion (Sparkman et al., 2011)

After the initial formation of ions representing the intact molecule and their subsequent fragmentation (ion of the intact molecule that are going to fragment will do so within less than a microsecond of their formation), they are accelerated out of the ion source with constant energy into the m/z analyzer. GC/MS uses EI, chemical ionization (CI), electron capture negative ionization (ECNI), field ionization (FI), and, to a much lesser extent, atmospheric pressure chemical ionization (APCI) (Sparkman et al., 2011).

CI, ECNI, FI, and APCI do not transfer much energy to the molecule during the ionization process and are called soft ionization techniques. EI produces very energetic molecular ions with only a positive charge ($M^{+\cdot}$). Depending on the structure of these molecular ions, a significant number will undergo fragmentation. Different molecular ions can produce fragment ions with different m/z values and different elemental compositions. The formation and subsequent detection of these fragment ions and their abundances produce the characteristic EI mass spectrum, sometimes referred to as a characteristic “EI fingerprint” for the compound. Molecular ions formed by EI are sometimes so energetic that their mass spectra do not exhibit a $M^{+\cdot}$ peak. This is why the soft ionization techniques can be considered complementary to EI because they usually provide the molecular mass of the analysis. The fragmentation of the ions representing the intact molecule is used to determine the structure of an analysis. Both the m/z values of the fragments and the dark matter (the elemental compositions implied by the difference in the m/z values of two mass spectral peaks) represented by these m/z values and that of the molecular ion are crucial in structure determinations. The fragments produced by EI are what are necessary to determine the structure of the

original molecule. Soft ionization techniques have limited use in deterring that identity of the analysis because of the lack of fragment-ion formation (Sparkman et al., 2011).

The data resulting from a GC/MS analysis are known as “mass spectra.” Mass spectra are acquired one after another at a consistent rate. The coordinates for each mass spectral peak represent the m/z value of an ion and the abundance of the ion with that m/z value. More often than not in GC/MS, these coordinates are presented by dropping a vertical line from their position on a two-dimensional Cartesian coordinate system to the *x* axis (the value of x represents the m/z value of the ion and the value for *y* represents the abundance of the ion, usually a relative intensity value). The data are displayed in this presentation because most of the mass spectral peaks that differ by an integer m/z unit (Figure 2.8) (Sparkman et al., 2011).

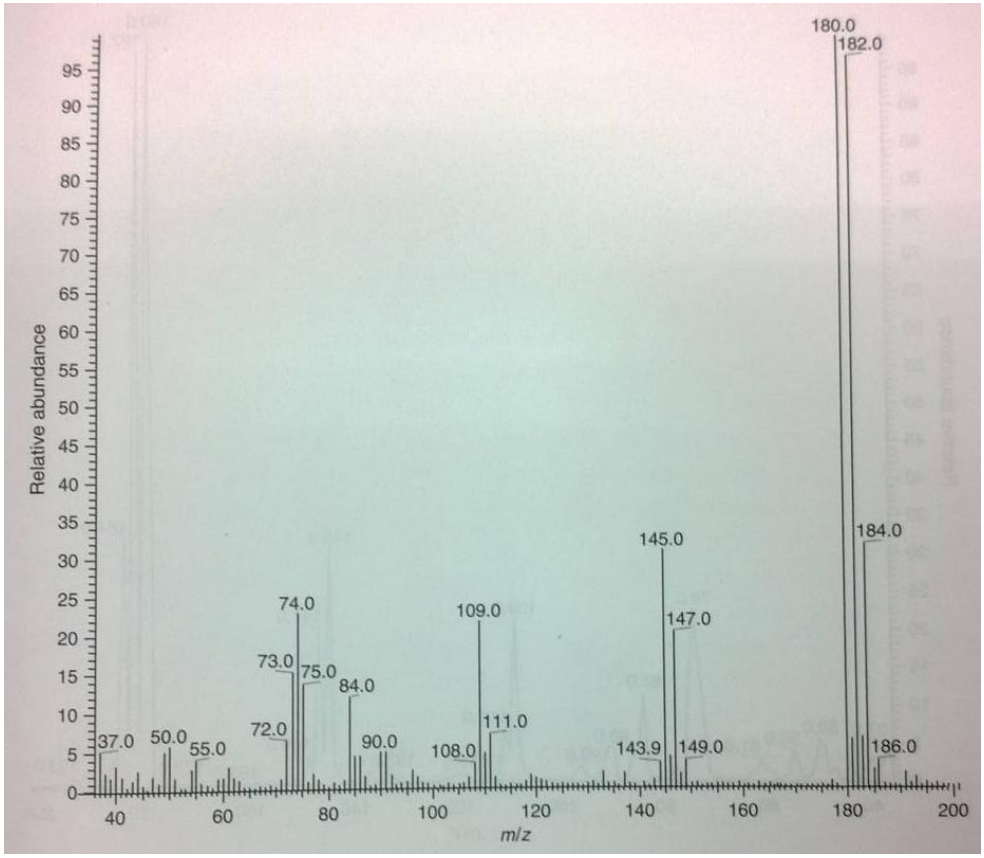

Figure 2.8 A typical mass spectrum acquired using EI on a GC-MS that is capable of separating ions that differ by only 1 m/z unit. This is the most common presentation of mass spectra in GC-MS (Sparkman et al., 2011)

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

Chicken thighs and chicken skin were obtained from B. Foods Product International Co. Ltd., and stored at -20°C in a freezer before using in each experiment.

3.2 Apparatus and Reagents

3.2.1 Equipments

1. Digital thermometer, Yokogawa, model TX1002, Japan
2. Balance 4 digits, Satorious, model RC250S, GÖttingen, Germany
3. Balance 2 digits, Satorious, model BA4100, GÖttingen, Germany
4. Rotary Evaporator, Resona, model Labo Rota 300, Gossau, Switzerland
5. Heater from Protein Analyzer, Tecator, model 1002, Hoganas, Sweden
6. Heater, FALC Instruments, model MM 500 ml, Italy
7. Heater, Progress Technical, model MM 500 ml, Thailand
8. Heating plate, IKA, model C-MAG HS 7, Selangor, Malaysia
9. Water bath, Memmert, model W600, Schwabach, Germany
10. Soxhlet Extraction Apparatus with Allihn Condenser, Pyrex Quickeit, model EX 5/83, United Kingdom
11. Auto pipette, Rainin Instrument, model Classic, Oakland, California
12. Nylon syringe filter, 0.2 μm
13. Air Pump, Resun, model AP – 40, China
14. Sep-Pak Florisil cartridge containing 1000 mg of packing material, Waters , Milford, USA
15. Glass reaction tube (29x4.0 cm. I.D.)
16. ORBO-1500 Small PUF/XAD-2/PUF absorption tube (size PUF: 22 mm OD × 30 mm length, XAD – 2: 1.5 g, PUF: 22 mm OD × 30 mm length), Supelco, Bellefonte Pennsylvania, USA
17. Gas Chromatography Mass Spectrometry system (GC – MS), Agilent Technologies, model 7890A GC, Canada.
18. DB-5MS column, size 30 m length x 0.25 mm I.D., Agilent Technologies, USA

3.2.2 Chemical Reagents

1. Sixteen PAH mixed standards solution in cyclohexane concentration 10 ng/μL, including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo -[*b*] fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo [*g,h,i*] perylene and indeno[1,2,3-*c,d*] pyrene , Sigma, Germany
2. Surrogate standard D_{10}-Pyrene, Cambridge Isotope Laboratories, Andover Massachusetts, USA
3. Internal standard D_{10}-Phenanthrene, Cambridge Isotope Laboratories, Andover Massachusetts, USA
4. Potassium hydroxide, Merck, Darmstadt, Germany
5. Anhydrous sodium sulfate, Carlo Erba, Rodano, Italy
6. Hexane, RCI Labscan, Bangkok, Thailand
7. Diethyl ether, RCI Labscan, Bangkok, Thailand
8. Dichloromethane, RCI Labscan, Bangkok, Thailand
9. Methanol, RCI Labscan, Bangkok, Thailand

3.3 Preparation of standard curve

3.3.1 Mixed stock PAHs standard including surrogate compound

Prepare a mixed stock PAH standard including D_{10}-Pyrene by mixing 500μL of the stock PAH standard(s) and 500 μL of 10 μg/mL D_{10}-Pyrene and diluting to mark with cyclohexane in a 5 mL volumetric flask. The concentration of the mixed stock PAH standard(s) including surrogate compound is 2.0 ng/μL.

3.3.2 Calibration PAHs standards including surrogate compound

Calibration PAH standards can be generated from mixed stock PAH standard(s) which include surrogate compound using serial dilution. A series of calibration PAH standards are 0.1ppm, 0.5ppm, 1ppm, 1.5ppm and 2.0ppm in cyclohexane. Five concentrations of 0.1 to 2.0ppm of PAHs standard solutions were injected into the GC/MS to determine the standard curve. Prior to GC/MS analysis, each 1 mL aliquot of the five calibration standards is spiked with 10 μL of 50 ng/μL internal standard to a final concentration of 0.5 ng/μL.

3.3.3 PAHs analysis by using GC-MS

GC-MS Instrument Operating Conditions

Gas Chromatography

- Carrier Gas: Helium
- Injection volume: 2 µl
- Injector Temperature: 290 °C
- Initial Column Temperature: 70 °C
- Initial Hold Time: 4 min
- Program: 10^{o}C /min to 300 °C and hold 10 min
- Final Temperature: 300 °C

Mass Spectrometer

- MS transfer line temperature: 290 °C
- Electron ionization mass spectra: 70 eV
- Mass Range: 35 to 500 atomic mass units (SIM mode)

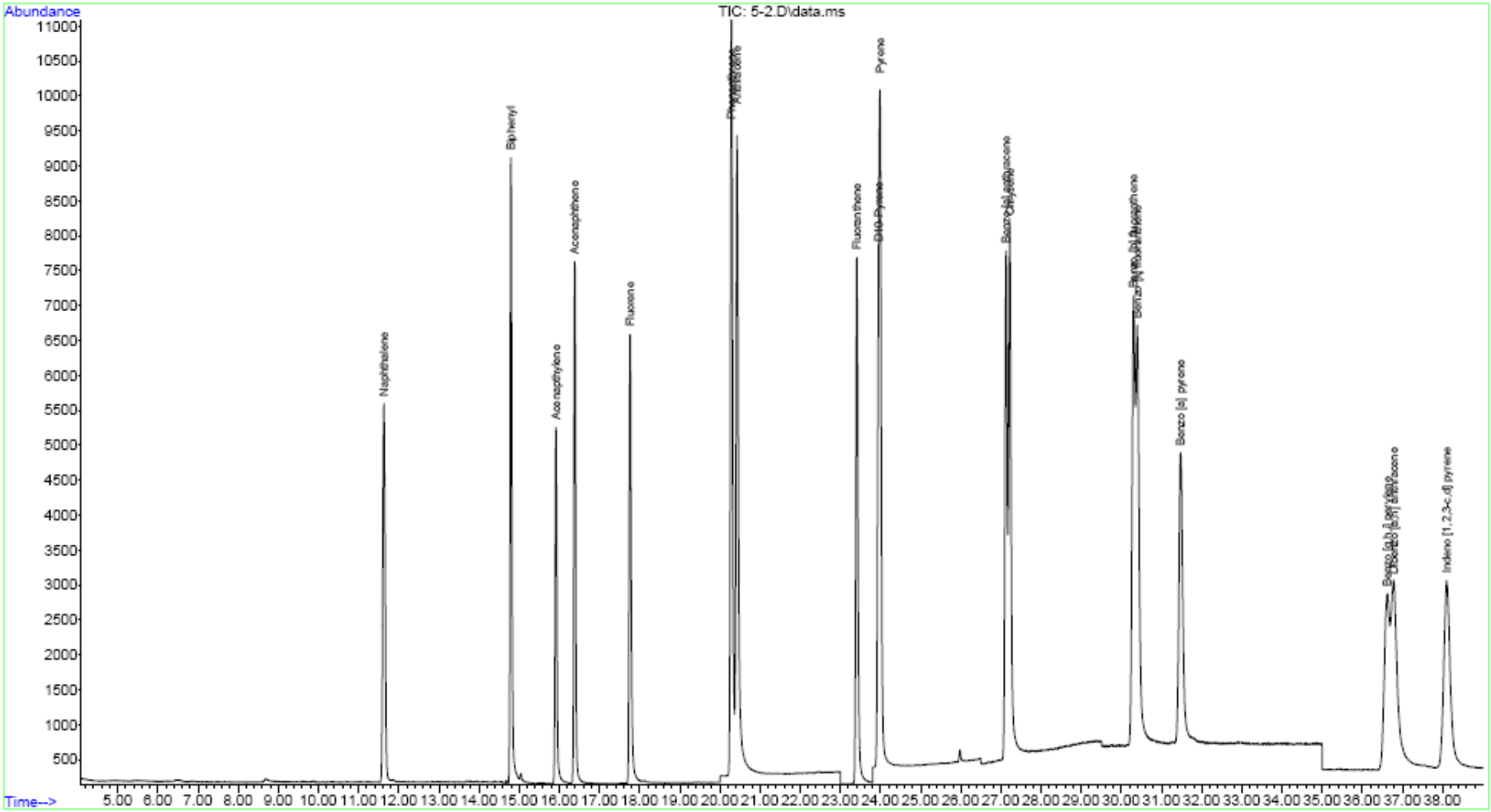

Figure 3.1 GC-MS chromatogram of 16 PAHs

Table 3.1 Characteristic ions and molecular weight for 16 PAHs

PAHs	Molecular weight	Linear equation
Naphthalene	128.18	$Y = 1.721\times10^{2}(X) + 3.814\times10^{2}$
Acenaphthylene	152.20	$Y = 1.451\times10^{2}(X) + 1.339\times10^{3}$
Acenaphthene	154.20	$Y = 1.115\times10^{2}(X) + 3.310\times10^{3}$
Fluorene	166.23	$Y = 1.270\times10^{2}(X) + 4.172\times10^{3}$
Phenanthene	178.24	$Y = 1.950\times10^{2}(X) + 1.359\times10^{4}$
Anthracene	178.24	$Y = 1.908\times10^{2}(X) + 8.111\times10^{3}$
Fluoranthene	202.26	$Y = 2.127\times10^{2}(X) + 9.439\times10^{3}$
Pyrene	202.26	$Y = 2.131\times10^{2}(X) + 4.339\times10^{3}$
Benzo[a]anthracene	228.30	$Y = 1.974\times10^{2}(X) + 5.521\times10^{3}$
Chrysene	228.30	$Y = 2.653\times10^{2}(X) - 8.043\times10^{2}$
Benzo[b]fluoranthene	252.32	$Y = 2.301\times10^{2}(X) + 2.233\times10^{3}$
Benzo[k]fluoranthene	252.32	$Y = 2.772\times10^{2}(X) + 3.502\times10^{3}$
Benzo[a]pyrene	252.32	$Y = 2.380\times10^{2}(X) + 2.748\times10^{3}$
Benzo[g,h,i]perylene	276.34	$Y = 2.118\times10^{2}(X) + 3.572\times10^{3}$
Dibenzo[a,h]anthracene	278.35	$Y = 1.691\times10^{2}(X) + 2.200\times10^{3}$
Indeno[1,2,3-c,d]pyrene	276.34	$Y = 2.121\times10^{2}(X) - 1.157\times10^{3}$

Y is abundance.

X is concentration (ppm).

3.4 PAHs in whole smoke from dropping chicken oil at different heat source temperatures

3.4.1. Preparation of chicken oil

Fresh chicken skin was obtained from B. Foods Product International Co. Ltd. and stored at -20 °C in a freezer. The chicken oil used in this study was melted oil. Chicken skin was sliced and rendered fat down by heating at 60°C with hot plate. Melted oil was collected to use in further experiment. The melted oil was not kept more than one day after heating. The melted oil was determined free fatty acid composition by using gas chromatographic method.

3.4.2. Dropping oil combustion and smoke collection

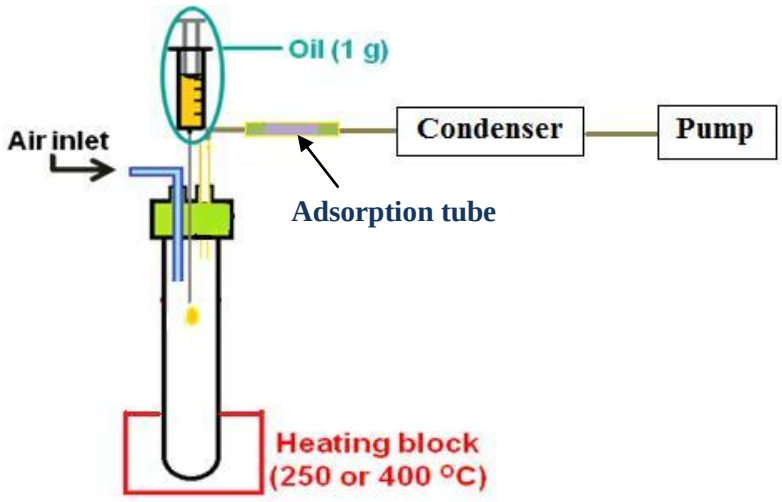

Figure 3.2 Dropping oil combustion and smoke collection system model

The top of reaction tube was closed with rubber. The front of adsorption tube was connected to the reaction tube with glass tube and the back of adsorption tube was connected to the condenser unit which was connected to air pump. The rubber was drilled for air inlet. The reaction tube was heated at 250 °C. After the reaction tube temperature reached to 250 °C, the melted oil was dropped in to the reaction tube one droplet by one droplet with syringe. The syringe and melted oil was weighed prior to use. Smoke in the reaction tube was pumped through the adsorption tube, and air flow rate was controlled at 2 L/min. One gram of melted oil was heated. The experiment was

conducted 2 replications. The experiment above was repeated with 400 °C and using two grams of melted oil.

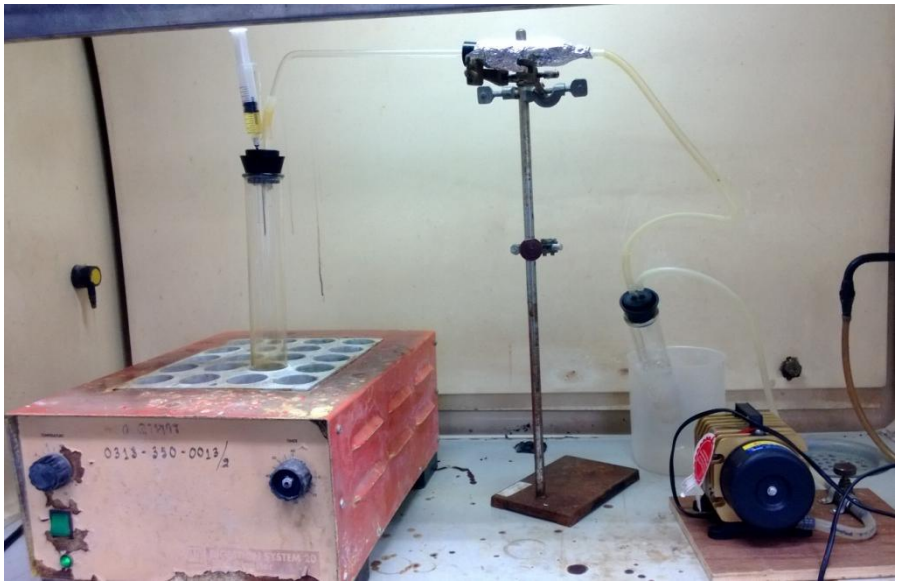

Figure 3.3 Dropping oil combustion and smoke collection system

3.4.3 PAHs extraction from adsorption tube (Method TO – 13A)

Figure 3.4 Soxhlet extraction apparatus

Extraction procedures were performed according by method TO – 13A. Prior to extraction, add appropriate laboratory surrogate standards to the PUF cartridge. A surrogate standard (i.e., a chemically compound not expected to occur in an environmental sample). D_{10} – pyrene was used as a surrogate standard in this study. The recovery of the laboratory surrogate standard is used to monitor for unusual matrix effects, gross sample processing errors, etc. Surrogate recovery is evaluated for acceptance by determining whether the measure concentration falls within the acceptance limits. Spike 20 µL of a 50 µg/mL solution of the surrogates onto the PUF cartridge, prior to Soxhlet extraction, to yield a final concentration of 1 µg. After adding the laboratory surrogate compounds to the PUF cartridge, add 700 mL of 10 percent diethyl ether in hexane to the apparatus and reflux for 18 hours at a rate of at least 3 cycles per hour. Allow to cool, then disassemble the apparatus. Dry the extract from the Soxhlet extraction by passing it though a filter paper containing about 10 grams of anhydrous sodium sulfate. Collect the dried extract in a evaporation flask. Wash the extractor flask and sodium sulfate with 100-125 mL of 10 percent diethyl ether/hexane to complete the quantitative transfer. The extract was evaporated to dryness and the residue was re-dissolved in cyclohexane 2 mL. Collect the extract from PUF cartridge and XAD-2 in another evaporation flask. The extract was concentrated to 2 mL using a rotary evaporator and transferred to Teflon®-sealed screw-cap amber vial, label the vial, and store at -20°C. The extract is now ready for GC/MS analysis. Spike the extract with internal standards before analysis. D10-Phenanthene was used as an internal standard in this study.

Note: If PUF is the sorbent, the extraction solvent is 10 percent diethyl ether in hexane. If XAD-2 is the sorbent, the extraction solvent is Dichloromethane.

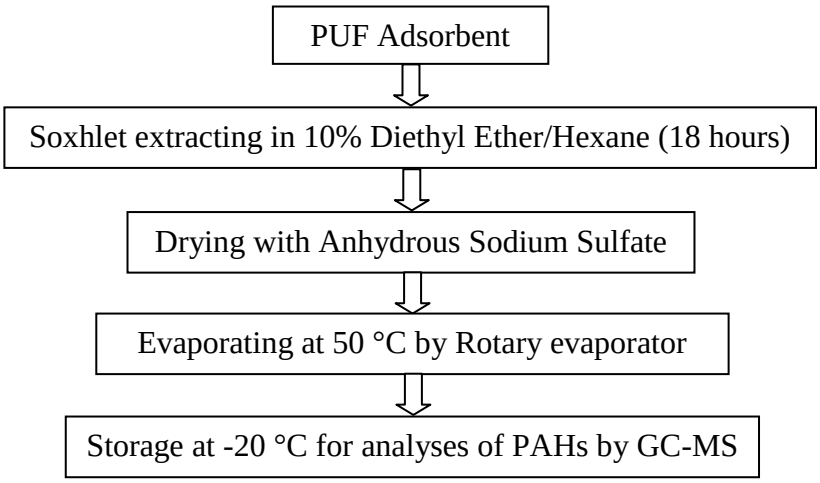

Figure 3.5 Flow diagram of PAHs extraction from adsorption tube

3.4.4. PAHs analysis by using GC-MS

The extract was analyzed PAHs by GC-MS following the GC-MS instrument operating conditions in 3.3.3.

Each target compound is characterized by one quantitation ion and two qualifier ions. The abundance ratios between qualifiers and the quantitation ions allow a check of the identity of the suspected allergen, according to the following formula:

$$Q = 100 - \frac{\sum_{i=1}^{i=n} \left(100 * \left|r_1 - r_1'\right|\right)\left(In\left[100 r_1 + 1\right]\right)^2}{21.3 * \sum_{i=1}^{i=n} r_1} \qquad (3.1)$$

where n is the number of ions per compound, r_i is the reference peak area ratio, and r_i is the observed peak area ratio. A Q-value between 90 and 100 indicates a positive recognition of the target peak. A lower value indicates that either the quantitation ion belongs to another compound or coelutes with another analyte (Debonneville et al., 2004). Therefore, PAHs with Q-value below 90 was rejected.

3.5 PAHs deposited on the steamed chicken surface from dropping chicken oil at different heat source temperatures

3.5.1 Preparation of steamed chicken

Fresh chicken thigh was obtained from B. Foods Product International Co. Ltd. and stored at -20 °C in a freezer. The visible fat and skin of chicken thigh were trimmed off. Then the sample was trimmed with size 3 cm. of width, 3 cm. of length and 2 cm. of thickness and steamed until its core temperature reached 75 °C.

3.5.2 Dropping oil combustion and adherence of smoke to meat surface

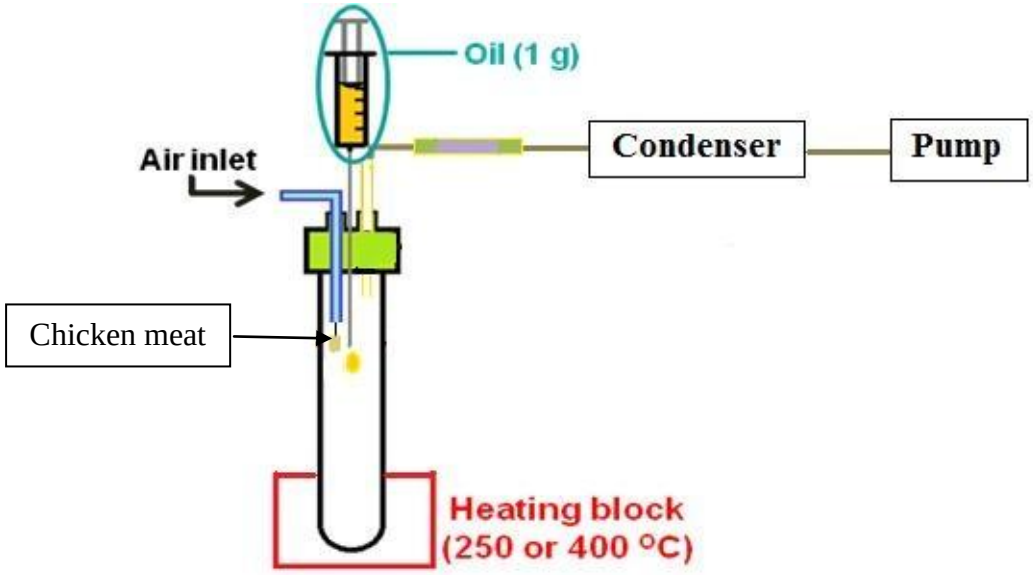

Figure 3.6 Dropping chicken oil for Adherence of PAHs to meat surface system model

The steamed chicken was weighted before it was hung in the reaction tube which was set as the experiment 1. The distance between steamed chicken and the bottom of reaction tube was about 23 cm. The reaction tube was heated at 250 °C. After the reaction tube temperature reached to 250 °C, the melted oil was dropped in to the reaction tube one droplet by one droplet with syringe. The syringe and melted oil was weighed prior to use. Some smoke was deposited on chicken surface and some smoke in the reaction tube was pumped through the adsorption tube, and air flow rate was controlled at 1 L/min. One gram of melted oil was heated. The experiment was

conducted 2 replications. The experiment above was repeated with 400 °C and using two grams of melted oil.

3.5.3 Extraction of PAHs in chicken meat

A method based on that described by Chen et al. (1997) was used. Chicken meat from the above experiment was cut, freeze-dried and ground before placement in a round filter paper. The paper was placed in the center of a Soxhlet extractor. A 500-mL round bottom flask, to which 200 mL of methanol and 25 mL of 50% aqueous potassium hydroxide were added for extraction of PAHs and saponification of lipid, was connected to the bottom of the Soxhlet extractor. After reflux for 3 h, the alkaline mixture was cooled to 40 °C, and 150 mL of *n*-hexane was added with occasional swirling. Then the solution was poured into a 500-mL separatory funnel containing 150 mL of water. The flask was rinsed twice with 10 mL of methanol, and the rinses were added to the separatory funnel, which was then shaken vigorously and allowed to stand to form aqueous and organic layers. The aqueous layer was extracted with 100 mL of hexane, and the hexane extracts were all combined, washed with 100 mL of water three times, and dried over anhydrous sodium sulfate. The dried hexane extract was poured into a 500-mL flask and concentrated to 1 mL by a rotary evaporator. The 1 mL concentrate was poured into a Sep-Pak Florisil cartridge, which had been previously conditioned with 10 mL dichloromethane and 20 mL of cyclohexane. 10 mL of cyclohexane followed by 8 mL of cyclohexane/dichloromethane (1:1, v/v) were passed through the cartridge. The eluate was collected, evaporated to dryness, and the residue was dissolved in 2 mL of methanol/dichloromethane (1:1, v/v). The solution was filtered through a 0.2-μm membrane filter and stored in a vial filled with nitrogen gas for GC-MS analysis.

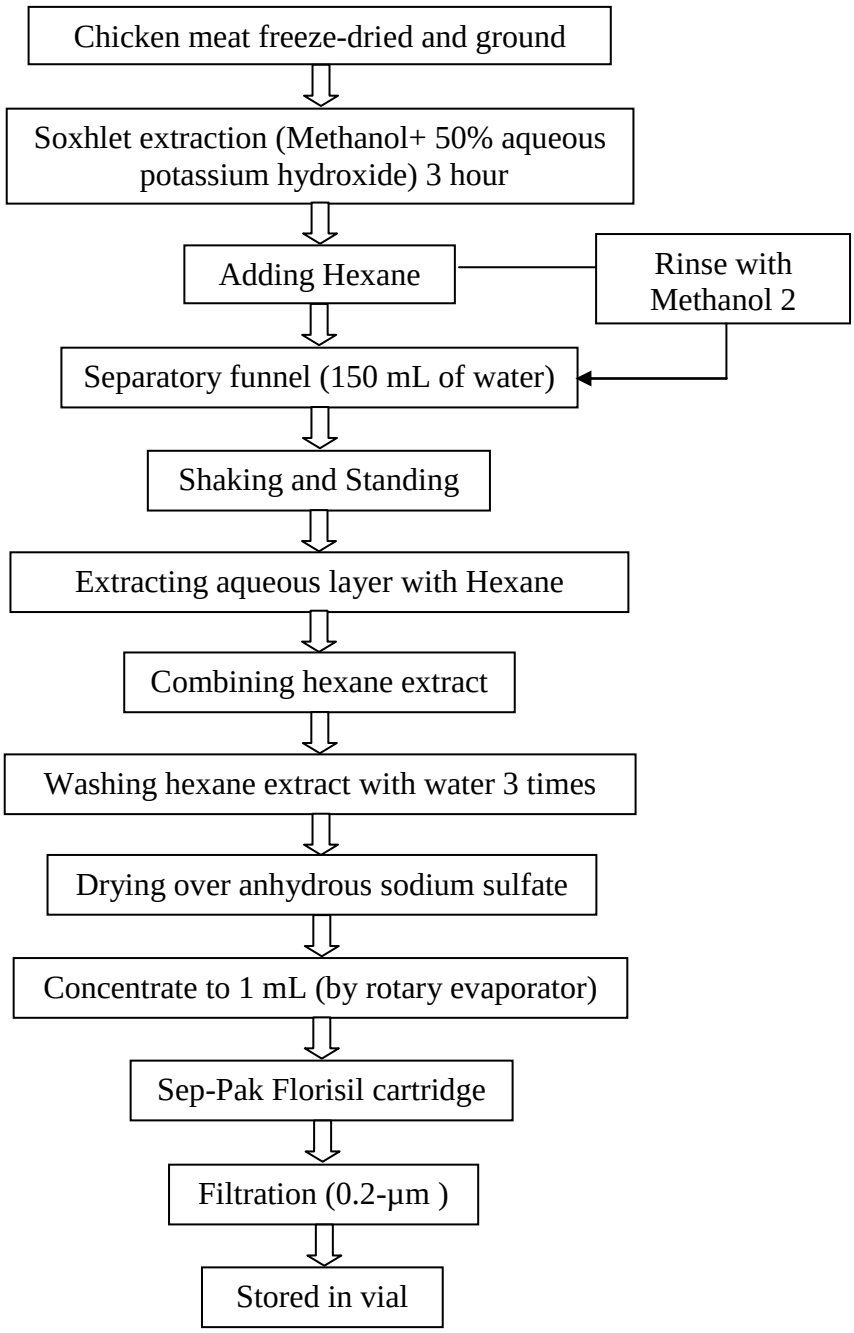

Figure 3.7 Flow diagram of PAHs extraction in chicken meat

3.5.4 PAHs analysis by using GC-MS

The extract was analyzed PAHs by GC-MS following the GC-MS instrument operating conditions in 3.3.3.

A Q-value between 90 and 100 indicates a positive recognition of the target peak. A lower value indicates that either the quantitation ion belongs to another compound or coelutes with another analyte. Therefore, PAHs with Q-value below 90 was rejected.

CHAPTER 4 RESULTS AND DISCUSSION

This work investigated the influence of oil dropping at different heat source temperatures on PAHs concentrations in smoke and chicken meat. This work was divided into two major parts consisting of the determination of PAHs in whole smoke and determination of PAHs in smoke that deposited on the steamed chicken surface.

4.1 PAHs in whole smoke from dropping chicken oil at different heat source temperatures

The melted chicken oil from heating chicken skin at 60 °C was dropped one by one drop on heat source in order to determine PAHs in whole smoke. In this study, 250 °C and 400 °C of heat source temperature were used as low and high grilling temperature, respectively. One gram and two grams of melted chicken oil were dropped at 250^{o}C and 400^{o}C respectively. Fatty acid compositions in melted chicken oil were determined since degree of unsaturated of fatty acids could affect variety and amount of PAHs formed in smoke (Chen and Chen, 2001). Fatty acid composition was shown in Table 4.1. Not only, oleic acid was the main fatty acid of the melted chicken oil but it also was the unsaturated fatty acid.

Table 4.1 Fatty acid composition in the melted chicken oil 100 gram

Fatty acid		**% of total fatty acid**	**Weight (gram)**
Oleic acid	C18:1	45.89	41.87
Palmitic acid	C16:0	28.89	26.36
Linoleic acid	C18:2, n-6	12.64	11.53
Stearic acid	C18:0	6.60	6.02
Palmitoleic acid	C16:1	4.96	4.52
Myistic acid	C14:0	0.56	0.51
Eicosenoic acid	C20:1	0.46	0.42

Table 4.2 showed the amounts of various PAHs in whole smoke from dropping the melted chicken oil at 250 and 400 °C. Heating temperature at 400 °C was found to generate the largest amount of total PAHs (91.097 µg/ g oil). Smoke from dropping the chicken oil at 400 °C contained 5 PAHs while smoke from dropping chicken oil at 250 °C contained 3 PAHs.

Table 4.2 PAHs concentration (µg/ g oil) in whole smoke from heating chicken oil

PAHs	Molecular weight	PAHs concentration µg/ g oil (ppm)	
		250 °C	400 °C
Naphthalene	128.18	14.287 ± 8.607	64.443 ± 35.724
Acenaphthylene	152.20	N/A	N/A
Acenaphthene	154.20	N/A	N/A
Fluorene	166.23	N/A	N/A
Phenanthene	178.24	0.508 ± 0.062	14.101 ± 10.234
Anthracene	178.24	N/A	10.945 ± 15.214
Fluoranthene	202.26	N/A	N/A
Pyrene	202.26	N/A	1.575 ± 1.221
Benzo[a]anthracene	228.30	0.225 ± 0.319	N/A
Chrysene	228.30	N/A	N/A
Benzo[b]fluoranthene	252.32	N/A	N/A
Benzo[k]fluoranthene	252.32	N/A	N/A
Benzo[a]pyrene	252.32	N/A	N/A
Benzo[g,h,i]perylene	278.35	N/A	N/A
Dibenzo[a,h]anthracene	276.34	N/A	0.033 ± 0.047
Indeno[1,2,3-c,d]pyrene	276.34	N/A	N/A
Total PAHs		15.020 ± 8.987	91.097 ± 62.440

Note: N/A = Not Applicable

This result clearly indicated that the heating temperature could affect the variety and amount of PAHs formed in smoke. When oil was dropped on heat source, thermal cracking or pyrolysis of oil occurred (Maher and Bressler, 2007). Oil pyrolysis causes by PAHs formation. Thus, temperature is known to have significant effects on rates of oil pyrolysis. Because thermal cracking reactions proceed with significant activation energies, temperature has an important effect on rate (Albright et al., 1983). This result seems to be according to report by Bjorseth et al. (1986) which reported that under comparable conditions, 1 g of tobacco yields 44 ng of benzo[a]pyrene at 400 °C and 183,500 ng of benzo[a]pyrene at 1000 °C. The absolute amount of PAHs formed during incomplete combustion is dependent on temperature.

Scientific Committee on Food (2002) reported that it was likely that there were several mechanisms of formation of PAH such as melted oil that undergoes pyrolysis when dripping onto the heat source and pyrolysis of the food due to high temperatures, above 200 °C. However, Hampikyan and Colak (2010) reported that temperature of smoke generally plays an important role, because the amount of PAHs in smoke formed during pyrolysis increases linearly with the smoking temperature between 400-1000 °C. This report revealed that heat source temperature at 250 °C was not possibly sufficient to pyrolyzed oil.

Melted oil from chicken skin contains both saturated and unsaturated triglycerides. There have been studies conducted on the decomposition of both saturated and unsaturated triglycerides during the applications of heat. Chen and Chen (2001) reported that the types of degradation products formed during heating of model lipids varied depending upon degree of unsaturation. Most degradation products belong to short-chain alkanes, alkenes, aldehydes, ketones, acid and fatty acid esters. It is well recognized that at 300 °C the gross pyrolysis of fats results in the formation of fatty acids and acrolein. At higher temperatures, (400–500 °C) cracking occurs, producing short chain hydrocarbons (Maher and Bressler, 2007). Both fatty acid, acrolein and short chain hydrocarbons are initiative products of triglyceride cracking in order to form PAHs. Dripping oil at 400 °C may produce many initiative products to form PAHs.

Maher and Bressler (2007) attempt to better understand the pyrolysis of vegetable oil. They studied the thermodynamics of vegetable oil process by computer simulation.

They found that the main findings were that cleavage of the C–O bond takes place at 288 °C and that scission of C=C takes place at 400 °C. That means heat source temperature at 250 °C may not sufficient to crack carbon double bond. Therefore, products from thermal cracking of triglyceride may be less. Whereas, heat source temperature at 400 °C can be sufficient to crack carbon double bond so products of thermal cracking of triglyceride may be great.

4.2 PAHs deposited on the steamed chicken surface from dropping chicken oil at different heat source temperatures

When melted chicken oil was dropped onto heat source, smoke occurred. PAHs were assumed that it formed and suspended in smoke. In addition, PAHs also were assumed that it can deposit on chicken meat since PAHs are lipophilic and have very low aqueous solubility. So they can accumulate in lipid which is composition of chicken. The aim of this section was to determine PAHs which deposited on the steamed chicken surface.

The steamed chicken was hung in the reaction tube before the melted chicken oil was dropped one by one drop on heat source at 250 °C and 400 °C. This experiment was performed in 2 replications. First replication, the reaction tube was also connected with absorption tube to determine PAHs in smoke. However, second replication was not connected with absorption tube. One gram and two grams of melted chicken oil were dropped at 250 °C and 400 °C respectively. PAHs in Chicken meat were determined following the method in chapter 3.

Table 4.3 showed that PAHs concentration which deposited on the steamed chicken surface (heat source temperature = 250 °C) in 2 replications. Not applicable PAHs both in chicken and smoke in the first replication. However, acenaphthene was detected in chicken in the second replication (0.108 µg/ g oil). Since oil pyrolysis causes by PAHs formation so the results possibly vary. The experiment should be performed more than two replications to confirm the results.

Table 4.3 PAHs concentration (µg/ g oil) which deposited on the steamed chicken surface at 250 °C of heat source temperature

PAHs ug/g oil (ppm)	Rep1			Rep2
	Chicken	Smoke	Chicken + Smoke	Chicken
Naphthalene	N/A	N/A	N/A	N/A
Acenaphthylene	N/A	N/A	N/A	N/A
Acenaphthene	N/A	N/A	N/A	0.108
Fluorene	N/A	N/A	N/A	N/A
Phenanthene	N/A	N/A	N/A	N/A
Anthracene	N/A	N/A	N/A	N/A
Fluoranthene	N/A	N/A	N/A	N/A
Pyrene	N/A	N/A	N/A	N/A
Benzo[a]anthracene	N/A	N/A	N/A	N/A
Chrysene	N/A	N/A	N/A	N/A
Benzo[b]fluoranthene	N/A	N/A	N/A	N/A
Benzo[k]fluoranthene	N/A	N/A	N/A	N/A
Benzo[a]pyrene	N/A	N/A	N/A	N/A
Benzo[g,h,i]perylene	N/A	N/A	N/A	N/A
Dibenzo[a,h]anthracene	N/A	N/A	N/A	N/A
Indeno[1,2,3-c,d]pyrene	N/A	N/A	N/A	N/A
Total PAHs	N/A	N/A	N/A	0.108

Table 4.4 showed that PAHs concentration which deposited on the steamed chicken surface (from heating the chicken oil at 400 °C of heat source temperature in 2 replications. Naphthalene, pyrene, benzo[a]anthracene and chrysene were detected in chicken meat in the first replication. Not only detected PAHs in chicken meat but also detected naphthalene, phenanthene and anthracene in smoke in the first replication. For the second replication, only naphthalene was detected in chicken meat. Naphthalene was detected in both the first replication and the second replication. It is the simplest PAHs and its structure consists of a fused pair of benzene rings. Therefore, napthalene

may form easily. However, the experiment should be performed more than two replications to confirm the results.

Table 4.4 PAHs concentration (µg/ g oil) which deposited on the steamed chicken surface from heating the chicken oil at 400 °C of heat source temperature

PAHs ug/g oil (ppm)	Rep1			Rep2
	Chicken	Smoke	Chicken + Smoke	Chicken
Naphthalene	1.665	42.513	44.178	0.579
Acenaphthylene	N/A	N/A	N/A	N/A
Acenaphthene	N/A	N/A	N/A	N/A
Fluorene	N/A	N/A	N/A	N/A
Phenanthene	N/A	6.192	6.192	N/A
Anthracene	N/A	0.549	0.549	N/A
Fluoranthene	N/A	N/A	N/A	N/A
Pyrene	0.526	N/A	0.526	N/A
Benzo[a]anthracene	0.068	N/A	0.068	N/A
Chrysene	0.073	N/A	0.073	N/A
Benzo[b]fluoranthene	N/A	N/A	N/A	N/A
Benzo[k]fluoranthene	N/A	N/A	N/A	N/A
Benzo[a]pyrene	N/A	N/A	N/A	N/A
Benzo[g,h,i]perylene	N/A	N/A	N/A	N/A
Dibenzo[a,h]anthracene	N/A	N/A	N/A	N/A
Indeno[1,2,3-c,d]pyrene	N/A	N/A	N/A	N/A
Total PAHs	2.333	49.254	51.587	0.579

The comparison of the results between Table 4.3 and Table 4.4 was found that heat source temperature at 400 °C could generate the larger amount of total PAHs in chicken meat and smoke than those at 250 °C. This result also accorded with the result of section 4.1. In addition, generated PAHs could deposit on chicken meat at 400 °C. This

result clearly accorded with the assumption that PAHs can deposit on chicken meat since PAHs are lipophilic and have very low aqueous solubility so they can accumulate in lipid which is composition of chicken. However, the result in Table 4.3 and Table 4.4 could not be calculated as percentage of deposited PAHs on chicken meat since PAHs in smoke in section 4.2 was not exact with PAHs in smoke in section 4.1.

Table 4.5 showed comparison between PAHs concentration which deposited on the steamed chicken surface and PAHs concentration in commercial grilled chicken. The information in Table 4.5 was reported in µg/ g of chicken*g of oil unit. Each data was calculated from data in Table 4.3 and Table 4.4 divided by total gram of chicken.
Acenaphthene was detected in steamed chicken which was smoked from dropping chicken oil at 250 °C. Naphthalene, pyrene, benzo[a]anthracene and chrysene were detected in steamed chicken which was smoked from dropping chicken oil at 400 °C. This result was similar with PAHs in commercial grilled chicken which contained naphthalene, pyrene, benzo[a]anthracene, chrysene and phenanthene.

Table 4.5 Comparison between PAHs concentration (µg/ g of chicken*g of oil) which deposited on the steamed chicken surface and PAHs concentration in commercial grilled chicken

PAHs	PAHs concentration µg/g of chicken*g of oil (ppm)				
	Smoked chicken in the experiment				Commercial grilled chicken
	250 °C		400 °C		
	Rep.1	Rep.2	Rep.1	Rep.2	
Naphthalene	N/A	N/A	0.265	0.105	0.690
Acenaphthylene	N/A	N/A	N/A	N/A	N/A
Acenaphthene	N/A	0.018	N/A	N/A	N/A
Fluorene	N/A	N/A	N/A	N/A	N/A
Phenanthene	N/A	N/A	N/A	N/A	1.127
Anthracene	N/A	N/A	N/A	N/A	N/A
Fluoranthene	N/A	N/A	N/A	N/A	N/A
Pyrene	N/A	N/A	0.084	N/A	0.459
*Benzo[a]anthracene	N/A	N/A	0.011	N/A	0.147
*Chrysene	N/A	N/A	0.012	N/A	0.086
*Benzo[b]fluoranthene	N/A	N/A	N/A	N/A	N/A
Benzo[k]fluoranthene	N/A	N/A	N/A	N/A	N/A
*Benzo[a]pyrene	N/A	N/A	N/A	N/A	N/A
Benzo[g,h,i]perylene	N/A	N/A	N/A	N/A	N/A
Dibenzo[a,h]anthracene	N/A	N/A	N/A	N/A	N/A
Indeno[1,2,3-c,d]pyrene (6)	N/A	N/A	N/A	N/A	N/A

Table 4.6 showed summary comparison between PAHs concentration in smoke which deposited on the steamed chicken surface and PAHs concentration in commercial grilled chicken. Commercial grilled chicken was found to generate the largest amount of total PAHs (2.509 µg/ g chicken) than the amount of total PAHs from heating temperature at 400 °C (0.238 µg/ g chicken) and 250 °C (0.018 µg/ g chicken), respectively.

Table 4.6 Summary comparison between PAHs concentration (µg/ g of chicken*g of oil) which deposited on the steamed chicken surface and PAHs concentration in commercial grilled chicken

PAHs	EU Regulation (No 835/2011) Max PAHs in smoked meats	PAHs concentration µg/ g of chicken*g of oil (ppm)		
		250 °C	400 °C	Commercial grilled chicken
Total PAHs	-	0.018	0.238 ± 0.188	2.509
Benzo[a]pyrene	0.005	N/A	N/A	N/A
Sum of Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[a]pyrene	0.030	N/A	0.023	0.233

According to commission regulation (EU) No 835/2011, benzo[a]pyrene and sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene of smoked meat and smoked meat products should not be exceed 0.005 µg/g of chicken and 0.03 µg/g of chicken respectively. Benzo[a]pyrene and sum of benzo[a]pyrene, benzo[a]anthraxcene, benzo[b]fluoranthene and chrysene concentration in smoked chicken from dropping chicken oil at 250 °C and 400 °C not apparently exceeded the safety standard of benzo[a]pyrene and sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene set by (EU) No 835/2011. However, this study was focused only one variable which was grilling temperature. Nevertheless, there are many factors that may affect the amount of PAHs in grilled food.

Furthermore, sum of benzo[a]pyrene, benzo[a]anthraxcene, benzo[b]fluoranthene and chrysene concentration in commercial grilled chicken was 0.233 µg/ g of chicken which was exceeded the safety standard.

Steamed chicken in this study was hung and smoked by dropping chicken oil but commercial grilled chicken made from fresh chicken skewer on stick in order to grill above charcoal. Therefore, chicken and processing in this study were different in commercial. There are some factors which affect PAHs concentration in commercial grilled chicken as followings.

1. During commercial grilling, the chicken oil drippings fall on the charcoal, and hence, the PAHs formed come up with smoke, and thus the adherence of PAHs to the meat surface might be possible. However, the experiments were controlled weight of chicken oil which was dropped on to the heat sauce while commercial grilling was not controlled weight of chicken oil. Oil dripping in commercial grilled chicken may be more likely to occur. That may results to increase PAHs formation.
2. Charcoal was used as a heat sauce for commercial grilling. The formation of PAHs during charcoal grilling at high temperature may be due to the incomplete combustion of charcoal. That possibly results to increase PAHs concentration. This result seems to be similarly to the report by Dyremark et al. (1994). Dyremark et al. (1994) studied emission of PAHs in smoke from grilling of meat using hardwood charcoal as fuel (with and without meat). The levels from the grilled meat samples are slightly higher than those from the pure charcoal samples. The major source of PAHs emitted to the local air environment from charcoal grilling of lean meat is the combustion of the charcoal itself.

The results of this study indicated that dropping chicken oil onto heat source could result to PAHs formation. Moreover, formed PAHs could deposit on chicken meat which was hung above the heat source. In addition, higher temperature of heat source results to higher PAHs concentration in both of smoke and chicken meat.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In grilling process, oil dropping from food on a hot charcoal may cause the PAHs contamination in Yakitori product. This work investigated the influence of different temperatures of heat source on PAHs concentrations in smoke and chicken meat.

For PAHs formed in whole smoke during oil dropping on heat source, the high heat source temperature generated more amounts and types of PAHs than low temperature. Total 16 PAHs concentrations in smoke generated by dropping oil at 250 and 400 °C were 15.02 and 91.09 µg/ g oil, respectively. In addition, smoke formed by dropping oil at 250 °C contained 3 PAHs, while smoke formed at 250 °C contained 3 PAHs.

For PAHs in chicken meat, the steamed chicken sample smoked with the dropping oil at 400 °C contained the larger amount of total PAHs than sample smoked at 250 °C. Moreover, sample smoked at 400 ^{o}C contained benzo[a]anthraxcene, benzo[b]fluoranthene and chrysene which are the carcinogen indicators in food according to Commission Regulation (EU) No 835/2011.

In conclusion, this result clearly indicated that temperature of heat source of oil dropping affected PAHs formation in smoke. Moreover, PAHs generated in smoke can deposit on steamed chicken meat since PAHs are lipophilic and have very low aqueous solubility. However, this study was focused only one factor which was grilling temperature.

5.2 Recommendations

Since oil pyrolysis causes by PAHs formation the results possibly vary. The experiment should be performed more than two replications to confirm the results.

According to food and food processing affected the PAHs formation but this study was focused only on one factor which was grilling temperature. The effect of oil dropping on charcoal should be further studied.

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APPENDIX A

Calibration curves of PAHs standard

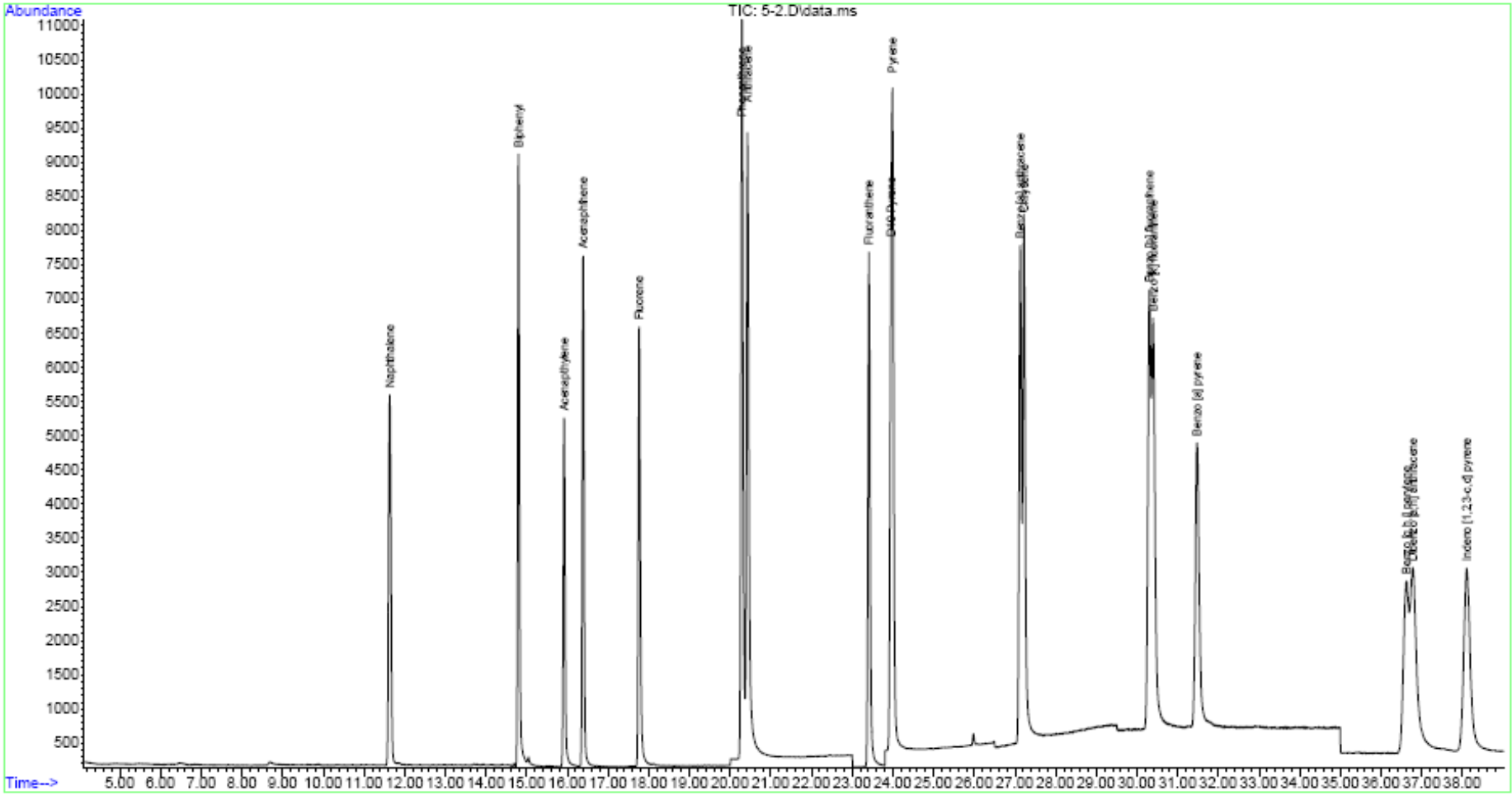

Figure A.1 The chromatogram of 16PAHs in mixed standard solution

Table A.1 Linear equations of 16 carcinogenic PAHs

Type of PAH	Linear eq.
Naphthalene	$Y = 1.18x10^{6} x (X)$
Acenaphthylene	$Y = 8.70x10^{5} x (X)$
Acenaphthene	$Y = 6.16x10^{5} x (X)$
Fluorene	$Y = 7.68x10^{5} x (X)$
Phenanthrene	$Y = 2.22x10^{6} x (X)$
Anthracene	$Y = 2.20x10^{6} x (X)$
Fluoranthene	$Y = 1.08x10^{6} x (X)$
Pyrene	$Y = 1.08x10^{6} x (X)$
Benzo(a)anthracene	Y = 9.46x105x (X)
Chrysene	Y = 9.88x105x (X)
Benzo(b)fluoranthene	Y = 1.08x106x (X)
Benzo(k)fluoranthene	Y = 1.13x106x (X)
Benzo(a)pyrene	Y = 9.50x105x (X)
Dibenzo(a,h)anthracene	Y = 1.05x106x (X)
Benzo(g,h,i)perylene	Y = 8.55x105x (X)
Indeno(1,2,3-c,d)pyrene	Y = 9.71x105x (X)

Y is abundance.

X is concentration (ppm).

APPENDIX B

Experimental data

Table B.1 Gram of dropping chicken oil in determination of PAHs in whole smoke

Sample		Chicken Oil (g.)
250 °C	Rep1	0.99
	Rep2	1.05
400 °C	Rep1	2.04
	Rep2	2.08

Table B.2 PAHs concentration and Q-value in whole smoke from heating chicken oil at 250 °C

Target	Rep1		Rep2	
	Concentration (ppb)	Q value	Concentration (ppb)	Q value
Naphthalene	4059.46	99	10695.9	91
Acenapthylene	152.97	1	217.16	1
Acenaphthene	5650.91	33	95.22	1
Fluorene	556.11	72	562.09	38
Phenanthrene	229.84	97	289.46	93
Anthracene	Below Cal	1	N.D	
Fluoranthene	119.58	1	133.32	21
Pyrene	134.54	67	367.5	17
Benzo [a] anthracene	0.00	90	236.65	94
Chrysene	7.43	1	72.67	7
Benzo [b] fluoranthene	Below Cal	1	9.24	1
Benzo [k] fluoranthene	N.D.		64.01	25
Benzo [a] pyrene	50.67	57	10.9	1
Benzo [g,h,i] perylene	Below Cal	52	Below Cal	1
Dibenzo [a,h]	Below Cal	67	5.87	1
Indeno [1,2,3-c,d] pyrene	9.68	1	20.7	1

Note: ND = Not Detected, Below Cal = Below Calibration

Table B.3 PAHs concentration and Q-value in whole smoke from heating chicken oil at 400 °C

Target	Rep1		Rep2	
	Concentration (ppb)	Q-value	Concentration (ppb)	Q-value
Naphthalene	91497.71	95	40749.3	71
Acenapthylene	2628.81	1	4788.03	1
Acenaphthene	22970.96	41	2734.31	59
Fluorene	12256.83	65	10132.49	74
Phenanthrene	21764.9	99	7139.15	100
Anthracene	22137.08	99	194.83	56
Fluoranthene	377.34	1	925.21	9
Pyrene	2486.76	86	740.23	93
Benzo [a] anthracene	194.35	44	39.91	36
Chrysene	304.14	75	112.51	38
Benzo [b] fluoranthene	105.26	83	62.09	76
Benzo [k] fluoranthene	164.98	66	Below Cal	1
Benzo [a] pyrene	181.53	59	9.22	77
Benzo [g,h,i] perylene	2.37	77	Below Cal	60
Dibenzo [a,h]	67.15	91	Below Cal	94
Indeno [1,2,3-c,d] pyrene	23.68	82	26.04	55

B.1 Calculation of PAHs in µg/g of oil unit

Example: Calculation of naphthalene in µg/g of oil

From Table B.2, naphthalene concentration in Rep 1 = 4059.46 ppb = 4.059 ppm

= 4.059 µg/ ml

Final volume of solution was 2 ml

So total naphthalene in final volume solution = 4.059 µg/ ml × 2 ml = 8.118 µg

From Table B.1, total dropping oil at 250 °C in Rep 1 = 0.99 g.

So total naphthalene from dropping of chicken oil 1 g. = 8.118 µg/ 0.99 g of oil

= 8.2 µg/ g of oil

Table B.4 Gram of dropping chicken oil, gram of chicken meat and smoking time in determination of PAHs in chicken meat

Sample		Chicken Oil (g.)	Time (h:min)	Chicken weight (g.)		
				Before Smoking	After Smoking	After Freeze-drying
250°C	Rep1	1.12	2:49	18.14	14.55	5.93
	Rep2	1.03	2:25	20.59	17.17	6.04
400°C	Rep1	2.07	3:17	20.24	14.30	6.29
	Rep2	2.24	2:33	16.01	10.96	5.52

Table B.5 PAHs concentration deposited on the steamed chicken surface at 250 °C of heat source temperature and Q-value

PAHs (ppb)	Rep1				Rep2	
	Chicken	Q-value	Smoke	Q-value	Chicken	Q-value
Naphthalene	586.24	44	19312.58	87	466.34	24
Acenaphthylene	18.08	1	226.3	1	29.81	1
Acenaphthene	10.28	1	11527.13	29	55.85	90
Fluorene	273.9	30	3835.06	37	202.83	53
Phenanthene	N.D.		N.D.		N.D.	
Anthracene	N.D.		N.D.		N.D.	
Fluoranthene	298.3	25	186.41	1	1860.7	57
Pyrene	547.94	60	314.7	1	178.74	1
Benzo[a]anthracene	24.29	54	Below Cal	1	87.05	58
Chrysene	41.18	57	6.4	1	89.01	55
Benzo[b]fluoranthene	Below Cal	54	Below Cal	54	Below Cal	1
Benzo[k]fluoranthene	N.D.		Below Cal	77	Below Cal	1
Benzo[a]pyrene	602.27	54	153.72	75	Below Cal	1
Benzo[g,h,i]perylene	Below Cal	52	Below Cal	52	N.D.	
Dibenzo[a,h]anthracene	Below Cal	1	41.64	1	Below Cal	1
Indeno[1,2,3-c,d]pyrene	9.24	1	10.32	1	30.19	48

Table B.6 PAHs concentration deposited on the steamed chicken surface at 400 °C of heat source temperature and Q-value

PAHs (ppb)	Rep1				Rep2	
	Chicken	Q-value	Smoke	Q-value	Chicken	Q-value
Naphthalene	1723.25	85	44000.57	89	648.84	91
Acenaphthylene	227.66	1	4014.68	1	95	1
Acenaphthene	261.93	68	2286.48	18	397.49	89
Fluorene	280.02	1	6423.91	81	75.78	1
Phenanthene	N.D.		6408.38	98	N.D.	
Anthracene	N.D.		568.47	91	N.D.	
Fluoranthene	507.14	61	1652.66	1	539.69	57
Pyrene	544.6	95	1067.28	1	92.41	1
Benzo[a]anthracene	70.38	90	66.42	1	136.89	49
Chrysene	76	97	103.57	58	38.66	14
Benzo[b]fluoranthene	0.68	69	Below Cal	1	N.D.	
Benzo[k]fluoranthene	N.D.		Below Cal	1	N.D.	
Benzo[a]pyrene	68.67	1	8.44	6	274.61	59
Benzo[g,h,i]perylene	N.D.		Below Cal	1	Below Cal	52
Dibenzo[a,h]anthracene	7.15	58	Below Cal	1	Below Cal	1
Indeno[1,2,3-c,d]pyrene	10.71	1	31.06	72	N.D.	

B.2 Calculation of PAHs in µg/ g of chicken*g of oil unit

Example: Calculation of naphthalene in chicken meat

From Table B.6, naphthalene concentration in Rep 1 = 1723.25 ppb = 1.723 ppm

= 1.723 µg/ ml

Final volume of solution was 2 ml

So total naphthalene in final volume solution = 1.723 µg/ ml × 2 ml = 3.446 µg

From Table B.4, total dropping oil at 400 °C in Rep 1 = 2.07 g.

So total naphthalene in chicken meat from dropping of chicken oil 1 g.

= 3.446 µg/ 2.07 g of oil

= 1.665 µg/ g of oil

From Table B.4, chicken weight at 400 °C in Rep 1 = 6.29 g

So total naphthalene in chicken meat 1 g from dropping of chicken oil 1 g.

= (1.665 µg/ g of oil)/ 6.29 g of oil

= 0.265 µg/ g of chicken*g of oil

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