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The Effect of Fatty Acids and Meat Oils Combustion on PAH Formation in Smoke during Grilling Process

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HIGHLIGHTS

- Polycyclic Aromatic Hydrocarbons (PAHs) concentration formed in the smoke depended on the number of carbon atoms, the degree of double bonds, and the types of fatty acids in oil.
- Striped catfish oil containing long carbon chains and high degree of double bonds of fatty acids generated a high PAHs concentration.
- Linoleic acid and oleic acid generated higher concentrations of PAHs in the smoke, at 48.53 and 46.81 ppm, respectively
- Stearic acid and palmitic acid provided PAHs in the smoke at 6.15 and 3.87 ppm, respectively.

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Acronyms and abbreviations
PAH=Polycyclic Aromatic Hydrocarbon
GC-MS=Gas Chromatography-Mass Spectroscopy
FAME=Fatty Acid Methyl Esters
LOQ=Limit of Quantitation
LOD=Limit of Detection

ABSTRACT

Background: Oil droplets from foods can cause the formation of Polycyclic Aromatic Hydrocarbons (PAHs) in smoke and contaminate grilled food. The aim of this research was to examine the effect of the number of carbon atoms, degree of double bonds, and types of fatty acids on the formation of PAHs in smoke during grilling process.

Methods: Four fatty acids consisting of palmitic acid, stearic acid, linoleic acid, and oleic acid, and three animal oils consisting of chicken skin oil, beef oil, and striped catfish oil had been studied. The smoke obtained during the combustion of fatty acids and animal oils was collected in a PUF/XAD-2/PUF absorption tube, and the analysis of 16 major PAHs was done using Gas Chromatography-Mass Spectroscopy (GC-MS). The experiments were conducted in three replicates.

Results: Linoleic acid and oleic acid generated relatively higher concentrations of PAHs in the smoke, at 48.53 and 46.81 ppm, while stearic acid and palmitic acid provided PAHs in the smoke at 6.15 and 3.87 ppm. The rank of the highest PAH concentration levels in order of decreasing in smoke included striped catfish oil, chicken skin oil, and beef loin oil, with values of 50.22, 35.07, and 33.62 ppm, respectively. A variety of fatty acids were found in animal oils, but some fatty acids, such as arachidic acid (20:0), mead acid (20:3), behenic acid (22:0), erucic acid (22:1), cervonic acid (DHA) (22:6), lignoceric acid (24:0), and nervonic acid (24:1), were not found in chicken skin oil or beef oil. Fatty acids in the striped catfish oil had longer carbon chains (20:0, 20:3, 22:0, 22:1, 22:6, 24:0, 24:1) compared to other animal oils and a higher degree of double bonds, thus giving a higher PAHs concentration.

Conclusion: It can be concluded that PAH concentration present in the smoke of animal oils depends on the number of carbon atoms, the degree of double bonds in the molecules, and the types of fatty acids.

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Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are present in the environment, such as soil and air, and they can be formed through industrial processes as well as cooking using high temperatures; the cooking may consist of roasting, barbecuing, baking, frying, and grilling (Duedahl-Olesen and Ionas, 2021). The characteristics of PAHs are inert and hydrophobic, but when taken into the body, they undergo metabolic transformation, and active metabolites are formed. These active metabolites bind to DNA via covalent bonds, thus causing mutations and mistakes in DNA replication, which will commence the carcinogenic process (Ewa and Danuta, 2017). The United States Environment Protection Agency (EPA, 1998) has classified the PAHs with 16 substances as major pollutants. According to the Scientific Committee of European Union (EU), the most suitable indicators of carcinogenic PAHs in food are benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene (Lee et al., 2016). Furthermore, EU Regulation No. 835/2011 (EC, 2011) states that the maximum wet weight of benzo(a)pyrene and the maximum combined wet weight of benzo(a)pyrene, benzo(a)anthracene, chrysene, and benzo(b)fluoranthene allowed in smoked and heat-treated (i.e., grilling and barbecuing) meat products are 0.005 and 0.03 µg/g, respectively.

Grilling is a high-temperature process which provides food with flavor and tenderness. According to Alomirah et al. (2011), PAHs are formed when oil droplets from food are incompletely combusted and come into contact with a high-heat source. The smoke containing PAHs is then deposited on the food surface (Kao et al., 2014). Overconsumption of these grilled foods can ultimately lead to health concerns such as cancer among the public (Hur et al., 2019). Many studies addressed the factors affecting the formation of PAHs in grilled meat. The factors include the temperature used to grill the meats, grilling time, types of heat source, fat content in meats, and meat oil dripping. The grilling temperature and time displayed a significant increase in PAH concentration of poultry meat, red meat, and seafood products (Kao et al., 2014). Charcoal-grilled meat contained the highest PAHs concentration compared with gas- and oven-grilled meats (Farhadian et al., 2010). Generally, higher fat content in meat causes a higher amount of oil dripping, resulting in a higher level of PAH concentration when grilling meat. However, some study results are contrary to the findings stated above. Lee et al. (2016) compared the effect of fat content of meat on PAH concentration in the grilling process. Grilled pork belly had the highest PAH concentration, whereas beef loin had the highest fat content. In addition, Babaoglu et al. (2017) determined different fat parts of lamb in beef Kokorec (a local food of Turkey) regarding PAH concentration. Beef Kokorec with lamb tail fat (37.31%) had the highest PAH concentration, while beef Kokorec with lamb tallow fat contained the highest fat content (40.39%). The experimental results of Lee et al. (2016) and Babaoglu et al. (2017) showed that the highest concentrations of PAHs in the samples were not derived from the high fat contents existing in the samples. This may be due to other factors such as fatty acid profiles in meat and fatty acid structures.

The unsaturation present in fatty acid molecules can induce the formation of PAHs more than saturated fatty acids (Wongmaneepratip and Vangnai, 2017). The study of PAH formation by heating four fatty acids (methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate) at 220 °C for 2 h demonstrated that the PAHs content increased with the number of double bonds in the fatty acid molecules (Chen and Chen, 2001). Additionally, the number of carbon atoms also affected PAH formation. Dandajeh et al. (2018) studied the effect of pyrolysis temperature on PAH formation from hydrocarbons containing carbon atoms of C1-C7. The concentration of PAHs increased during pyrolysis at 1,050 °C and then decreased when the pyrolysis temperature was increased to 1,350 °C. However, the carbon chain length (C1-C7) used as a fuel for this experiment did not cover the number of carbon atoms of fatty acids in meats.

Animal oils have their own fatty acid profiles, i.e., each fatty acid is different both in the number of carbon atoms and the degree of double bonds. Currently, there is limited information regarding the aforementioned factors concerning PAH formation during the grilling process.

The objectives of this study are to determine the factors which affect the formation of PAHs, such as the number of carbon atoms, the degree of double bonds in a fatty acid molecule, and the types of fatty acids present in animal oils. The 16 major PAHs, classified as major pollutants, will be extracted from the smoke generated from a grilling process and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) to confirm their identity.

Materials and methods

Fatty acid samples purchased from Sigma-Aldrich (Saint Louis, USA) included palmitic acid, stearic acid, oleic acid, and linoleic acid. Chicken skin (*Gallus domesticus*), beef loin (*Bos primigenius*), and striped catfish (*Pangasianodon hypophthalmus*) were obtained from Thungkru Prachauthit 61 market (January-October, 2019). An ORBO-1500 PUF/XAD-2/PUF absorption tube was purchased from Supelco Inc. (Bellefonte, USA). The 16 mixed PAHs solution, surrogate standard D10-pyrene, and internal standard D8-naphthalene were purchased from Sigma-Aldrich (Munich, Germany). Finally, High-

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Performance Liquid Chromatography (HPLC)-and Analytical Research (AR)-grade chemical reagents were purchased from RCI LabScan (Bangkok, Thailand). The experiments were conducted in tree replicates**,** and the data included mean values with standard deviation**.**

Analysis of chemical constituents of meat oils using GC technique

-Extraction of meat oils

An oil extraction method from meat was carried out according to ISO 661, (2003). One hundred g of meat was sliced and dry-rendered to melt the meat fat at 60 °C for 40 min using an electric hotplate. The melted oil was filtered through a filter paper (Whatman No. 4) containing approximately 10 g of anhydrous sodium sulfate to remove any traces of water present in the oil. The filtered melted oil was prepared for Fatty Acid Methyl Esters (FAME) samples.

-Preparation of FAME samples

FAME samples were prepared using the method of Harrington and D'Arcy-Evens (1985). One ml of toluene was mixed with 8 mg of an oil sample, followed by the addition of 1 ml of 4% sulfuric acid in methanol. The reaction mixture was vortexed for 1 min and then placed in a water bath at 70 °C for 4 h. After cooling the reaction mixture, the toluene phase was washed three times with distilled water and dried with anhydrous sodium sulfate to remove any traces of water before being filtered through a 0.45 µm membrane filter. The chemical constituents of the filtrates were then analyzed using GC technique.

-GC conditions

GC was performed using a Shimadzu 17A with a DB-Wax fused silica capillary column (30 m×0.25 mm ID, 0.25 µm film thickness). The injector temperature and the flame ionization detector temperature were maintained at 250 °C. The column temperature program was set to start at 160 °C and held for 1 min, then increased at a rate of 2 °C/min until the temperature reached 210 °C. The column temperature was held at this temperature for 5 min (Lomsugarit et al., 2001).

Characterization of chemical constituents of smoke from the combustion of fatty acids and meat oils at 600 °C using GC-MS technique

-Preparation of smoke from the combustion of fatty acids and meat oils at 600 °C

Simulation of the grilling process and smoke trapping procedure was carried out as shown in Figure 1. The heating block was heated to 600±5 °C for 15 min. Then, 3 ml of sample in the syringe was slowly injected, one droplet at a time, into the heated tube to generate smoke. The smoke obtained from the sample combustion was trapped with a PUF/XAD-2/PUF absorption tube (Supelco) by an air pump (Resun, model AP-40, China) at 4 L/min. Then, a PUF/XAD-2/PUF absorption tube was used to extract PAHs.

-Extraction of PAHs from a PUF/XAD-2/PUF absorption

The procedure for extracting PAH was performed according to the method TO 13A (EPA, 1999). PAHs were first extracted from the PUF portion of the adsorption tube with 350 ml of 10% diethyl ether in hexane for 18 h, and from the XAD-2 resin portion with 350 ml of methylene chloride for 18 h by Soxhlet extraction method. After the extraction was complete, the extract was filtered through 10 g of anhydrous sodium sulfate to remove any trace of water. The filtrate was concentrated to 1 ml of a crude extract using a rotary evaporator; then it was transferred to a screw-cap vial to avoid contact with light and stored at -20 °C before usage. The PAH chemical constituents of the crude extract were then identified with GC-MS technique.

-Determination of PAHs using GC-MS technique

Modified conditions of GC-MS analysis based on the method of Prathomtong et al. (2016) were applied. A 7,890A GC system (Agilent Technologies, Canada) with a DB-5MS column (30 m length × 0.25 mm i.d. with 0.25 µm of film thickness, Agilent Technologies, USA) was used. The injected volume of a sample was 1 µl. The oven temperature was initially set at 55 °C for 1 min, then increased to 100 °C at a rate of 2 °C/min, and subsequently increased to 320 °C at a rate of 5 °C/min; finally, it was maintained at 320 °C for 3 min. Helium was used as the carrier gas. The MS detector temperature was set at 280 °C, and MS was acquired using electron ionization at 70 eV. The mass range detected was from 45 to 450 atomic mass units and was set to selective ion monitoring mode. The PAH data were considered valid when Q value (similarity quality compared to MS data available in NIST 2017 library) was more than 80, and the number of samples with PAH levels≥Limit of Quantitation (LOQ) (Carlson et al., 2014; Chen and Chen, 2003). The PAH standards calibration curve was conducted using internal standard (D8-naphthalene) and surrogate compound (D10-pyrene). The calibration curve of PAHs was prepared by plotting eight concentrations of PAH standard solutions (0-2.5 ppm in hexane) against area. Triplicate analyses were conducted; the Limit of Detection (LOD) and LOQ were calculated using the formulas, 3.3 and 10 σ/S, respectively, where S was the slope obtained from the linear regression equation, and σ was the standard deviation (Chen and Chen 2001).

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Results

Retention time, m⁄z value, and the calibration curve of 16 PAHs standards

Classifying PAH samples was operated by comparing their retention times with those of PAHs standards. The total ion chromatogram for 16 PAH standards was demonstrated in Figure 2, and their retention times and primary ions were shown in Table 1. In a mass spectrometer, the samples' primary ions were generated, then separated based on their specific mass-to-charge ratios (m/z), and finally, the relative abundance of each ion type was recorded. Phenanthrene ($C_{14}H_{10}$) and anthracene ($C_{14}H_{10}$), fluoranthene ($C_{16}H_{10}$) and pyrene ($C_{16}H_{10}$), benzo(a)anthracene ($C_{18}H_{12}$) and chrysene ($C_{18}H_{12}$), benzo(b)fluoranthene ($C_{20}H_{12}$), benzo(k)fluoranthene ($C_{20}H_{12}$) and benzo(a)pyrene ($C_{20}H_{12}$), indeno (1,2,3-c,d)pyrene ($C_{22}H_{12}$) and benzo (g,h,i) perylene ($C_{22}H_{12}$) were identified with the same m/z of 178, 202, 228, 252, and 276, respectively. However, their respective retention times were different. This implied that those compounds were isomers. The isomeric PAHs having lower boiling points had shorter retention times than those with higher boiling points. The linear equation of the calibration curve, correlation coefficient (R^2), LOD, and LOQ for 16 PAH standards were presented in Table 2. The LOD and LOQ values ranged from 0.04 to 0.06 and 0.1 to 0.19 ppm, respectively. The correlation coefficient of the standard curves of the 16 PAHs was between 0.954-0.996 ppm.

PAH concentrations (ppm) in smoke from fatty acids combustion at 600 °C

The main saturated fatty acids in animal meat are palmitic acid (16:0) and stearic acid (18:0), which were used to examine the effect of the number of carbon atoms in the fatty acid structure on PAH formation in smoke. In addition, stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) were used to investigate the effect of double bonds on PAH formation. The results of PAH formation in smoke from fatty acids combustion are demonstrated in Table 3. Oleic acid and linoleic acid generated relatively high concentrations of PAHs, whereas stearic acid and palmitic acid provided lower PAHs.

Fatty acid compositions in animal oils and PAHs concentrations (ppm) in smoke from animal oils combustion at 600 °C

Saturated fatty acids in animal oils are mainly myristic, palmitic, and stearic acids, while monounsaturated fatty acids are palmitoleic and oleic fatty acids, and polyunsaturated fatty acids are mainly linoleic and linolenic fatty acids. In this study, animal oils, i.e. beef oil, chicken oil, and striped catfish oil, were used because they are usually one of the ingredients in local food menus. Table 4 shows the fatty acid compositions in animal oils identified using GC technique. The majority of fatty acids in beef loin oil were saturated fatty acids, whereas those of chicken skin oil and striped catfish oil were unsaturated fatty acids. Striped catfish oil contained many unsaturated fatty acids, which were not found in beef loin oil and chicken skin oil; they included arachidic acid, mead acid, behenic acid, erucic acid, cervonic acid (DHA), lignoceric acid, and nervonic acid, which contained degrees of double bonds varying from 1 to 6. Table 5 shows PAH concentrations (ppm) in smoke from animal oils combustion at 600 °C. The smoke of striped catfish oil contained the highest concentration of PAH, followed by the smoke of chicken skin oil, and finally, the smoke of beef loin oil with a value of 33.62 ppm.

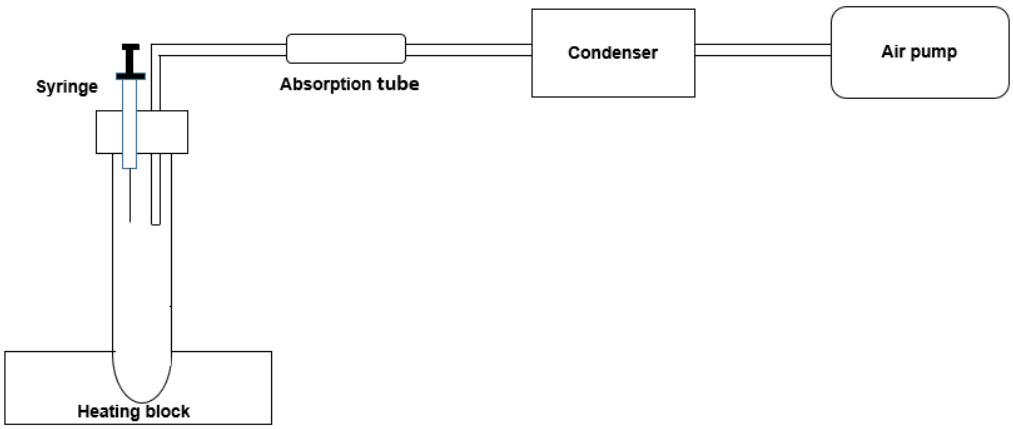


Figure 1: The experimental set-up simulating the grilling process

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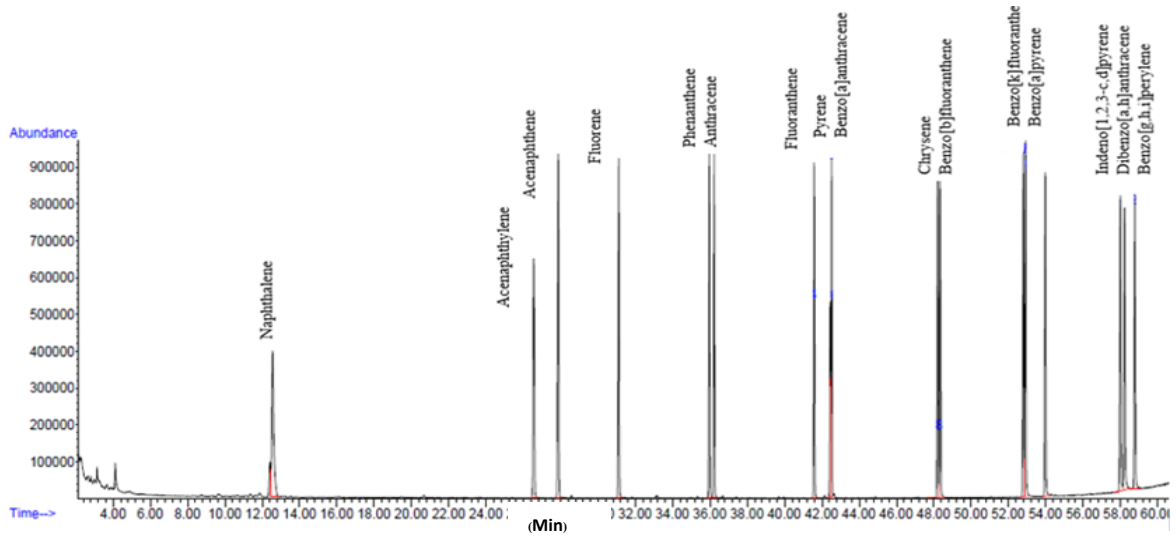


Figure 2: Total Ion Chromatogram (TIC) for 16 Polycyclic Aromatic Hydrocarbon (PAH) standards

Table 1: Retention time and, m/z value of 16 PAHs standards including their chemical structure

PAHs	**structure**	**Retention time** (min)	**m/z**
Naphthalene		554.12	128
Acenaphthylene		556.26	152
Acenaphthene		874.27	153
Fluorene		107.31	166
Phenanthrene		968.35	178
Anthracene		218.36	178
Fluoranthene		596.41	202
Pyrene		42.520	202
Benzo(a)anthracene		42.527	228
Chrysene		48.213	228
Benzo(b)fluoranthene		48.348	252
Benzo(k)fluoranthene		52.796	252
Benzo(a)pyrene		915.52	252
Indeno(1,2,3-c,d)pyrene		998.57	276
Dibenzo(a,h)anthracene		229.58	278
Benzo(g,h,i)perylene		777.58	276

PAH=Polycyclic Aromatic Hydrocarbon

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Table 2: The linear equation of the calibration curve, correlation coefficient (R^2), Limit of Detection (LOD), and Limit of Quantitation (LOQ) for 16 PAHs standards

PAHs	Linear equation	LOD (ppm)	LOQ (ppm)	R^2
Naphthalene	$Y=1.33\times10^{2}(X)-6.57\times10^{-3}$	0.06	0.19	0.995
Acenaphthylene	$Y=1.40\times10^{2}(X)-3.55\times10^{-2}$	0.04	0.11	0.996
Acenaphthene	$Y=9.38\times10^{2}(X)-1.23\times10^{-2}$	0.05	0.16	0.996
Fluorene	$Y=9.67\times10^{2}(X)-1.43\times10^{-2}$	0.03	0.10	0.994
Phenanthrene	$Y=1.27\times10^{2}(X)-6.84\times10^{-3}$	0.05	0.16	0.994
Anthracene	$Y=1.26\times10^{2}(X)-1.16\times10^{-2}$	0.04	0.12	0.993
Fluoranthene	$Y=1.39\times10^{2}(X)-1.81\times10^{-2}$	0.06	0.17	0.991
Pyrene	$Y=5.90\times10^{2}(X)-9.91\times10^{-2}$	0.06	0.18	0.954
Benzo(a)anthracene	$Y=1.22\times10^{2}(X)-1.12\times10^{-2}$	0.04	0.13	0.989
Chrysene	$Y=1.25\times10^{2}(X)-2.35\times10^{-2}$	0.04	0.12	0.987
Benzo(b)fluoranthene	$Y=1.32\times10^{2}(X)-3.38\times10^{-2}$	0.04	0.12	0.993
Benzo(k)fluoranthene	$Y=1.37\times10^{2}(X)-2.56\times10^{-2}$	0.04	0.11	0.991
Benzo(a)pyrene	$Y=1.21\times10^{2}(X)-5.61\times10^{-2}$	0.06	0.17	0.994
Indeno(1,2,3-c,d)pyrene	$Y=9.94\times10^{2}(X)-8.26\times10^{-2}$	0.05	0.15	0.996
Dibenzo(a,h)anthracene	$Y=9.47\times10^{2}(X)-1.36\times10^{-2}$	0.05	0.16	0.995
Benzo(g,h,i)perylene	$Y=9.78\times10^{2}(X)-1.22\times10^{-2}$	0.05	0.16	0.995

PAH=Polycyclic Aromatic Hydrocarbon

Table 3: PAHs concentrations (ppm) in smoke from fatty acids combustion at 600 °C

PAHs	Ring no.	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)
Naphthalene	2	2.71±0.01	3.08±0.21	25.47±0.51	17.82±0.40
Acenaphthylene	3	0.41±0.36	0.45±0.23	ND	4.57±0.01
Acenaphthene	3	0.42±0.11	0.08±0.02	ND	4.87±0.01
Fluorene	3	ND	ND	1.39±0.33	2.56±0.62
Phenanthrene	3	0.09±0.02	0.75±0.46	ND	2.93±0.22
Anthracene	3	0.09±0.00	0.76±0.28	15.82±0.01	4.26±0.70
Fluoranthene	4	ND	ND	0.48±0.32	ND
Pyrene	4	ND	0.41±0.02	ND	ND
Benzo(a)anthracene	4	ND	ND	ND	ND
Chrysene	4	ND	0.17±0.02	ND	ND
Benzo(b)fluoranthene	5	0.10±0.11	0.16±0.35	1.01±0.01	1.34±0.07
Benzo(k)fluoranthene	5	0.05±0.01	0.15±0.23	0.98±0.01	0.94±0.16
Benzo(a)pyrene	5	ND	ND	0.50±0.22	1.74±0.33
Indeno(1,2,3-c,d)pyrene	6	ND	0.14±0.03	0.98±0.02	2.10±0.21
Dibenzo(a,h)anthracene	5	ND	ND	ND	0.88±0.50
Benzo(g,h,i)perylene	6	ND	ND	0.18±0.04	4.52±0.01
Total 16 PAHs		3.87	6.15	46.81	48.53

ND=Not Detected
Mean±standard deviation of triplicate analyses
PAH=Polycyclic Aromatic Hydrocarbon

Table 4: Fatty acid compositions (% total fatty acids by peak area) identified using gas chromatography technique in animal oils

Fatty acids		% Fatty acids composition		
		Beef loin oil	Chicken skin oil	Striped catfish oil
Myristic acid	14:0	5.74	0.7	4.15
Myristoleic acid	14:1		0.18	0.26
Palmitic acid	16:0	28.31	24.5	30.16
Palmitoleic acid	16:1	3.85	0.35	0.35
Stearic acid	18:0	17.59	5.57	8.27
Oleic acid	18:1	43.44	39.15	33.23
Linoleic acid	18:2	0.78	26.48	14.22
Linolenic acid	18:3		2.2	3.22
Arachidic acid	20:0			0.36
Paullinic acid	20:1	0.29	0.33	1.01
Eicosadienoic acid	20:2		0.22	0.86
Mead acid	20:3			0.76
Arachidonic acid	20:4		0.32	0.64
Behenic acid	22:0			0.49
Erucic acid	22:1			0.56
Cervonic acid (DHA)	22:6			0.65
Lignoceric acid	24:0			0.34
Nervonic acid	24:1			0.47
Total saturated fatty acid		51.64	30.77	43.77
Total monounsaturated fatty acid		47.58	40.01	34.85
Total polyunsaturated fatty acid		0.78	29.22	21.38

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Table 5: PAHs concentrations (ppm) in smoke from animal oils combustion at 600 °C

PAHs	Ring no.	Beef loin oil	Chicken skin oil	Striped catfish oil
Naphthalene	2	5.50±0.61	4.70±0.22	4.98±0.85
Acenaphthylene	3	2.45±0.30	3.13±0.70	2.91±0.01
Acenaphthene	3	3.61±0.28	4.28±0.60	4.21±0.48
Fluorene	3	ND	4.30±0.26	4.48±0.40
Phenanthrene	3	3.58±0.48	3.89±0.01	4.50±0.80
Anthracene	3	2.99±0.62	ND	3.82±0.01
Fluoranthene	4	0.36±0.06	ND	0.31±0.26
Pyrene	4	2.70±0.28	0.15±0.01	3.93±0.23
Benzo(a)anthracene	4	2.62±0.22	2.26±0.66	3.30±0.70
Chrysene	4	2.56±0.17	2.23±0.06	3.23±0.01
Benzo(b)fluoranthene	5	1.99±0.66	2.22±0.14	2.62±0.02
Benzo(k)fluoranthene	5	1.92±0.01	2.14±0.62	2.53±0.20
Benzo(a)pyrene	5	0.68±0.18	1.53±0.02	2.00±0.52
Indeno(1,2,3-c,d)pyrene	6	0.98±0.45	1.33±0.01	1.70±0.13
Dibenzo(a,h)anthracene	5	0.96±0.01	1.42±0.22	1.13±0.56
Benzo(g,h,i)perylene	6	0.73±0.15	1.52±0.08	1.95±0.42
Total 16 PAHs		33.62	35.07	50.22

ND=Not Detected
Mean±standard deviation of triplicate analyses
PAH=Polycyclic Aromatic Hydrocarbon

Table 6: Benzo (a) pyrene and 4 PAHs concentration (ppm) in smoke from fatty acids and animal oils combustion at 600 °C and maximum level defined in EU regulation No 835/2011 in smoked meats (EC, 2011).

PAHs	EU Regulation (No 835/2011) Max PAHs in smoked meats	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Beef loin oil	Chicken skin oil	Striped catfish oil
Benzo(a)pyrene	0.005	ND	ND	0.50±0.22	1.74±0.33	0.68±0.18	1.53±0.02	2.00±0.52
Sum of Benzo(a)anthracene Chrysene Benzo(b)fluoranthene Benzo(a)pyrene	0.030	0.10±0.11	0.33±0.58	1.51±0.23	3.08±0.02	7.85±0.58	8.24±0.88	11.15±1.25

ND=Not Detected
Mean±standard deviation of triplicate analyses
PAH=Polycyclic Aromatic Hydrocarbon

Discussion

The structure of a typical fatty acid molecule contains an aliphatic chain with a carboxylic acid group, and can be classified as either saturated or unsaturated (McClement and Decker, 2017). Stearic acid and palmitic acid are saturated, while linoleic acid and oleic acid are unsaturated. Previous studies have reported that the formation of PAHs can be related to the number of carbon atoms in a molecule (Dandajeh et al., 2018). At 1,050 °C, concentrations of PAH increased with an increase in the carbon number of the hydrocarbon chains from C1–C7; however, at 1,350 °C, concentrations of PAH tended to decrease (Dandajeh et al., 2018). The results confirmed that the number of carbon atoms affects PAHs formation; the higher the number of carbon atoms, the higher the formation of PAHs (both types and concentrations). Generally, fatty acids in foods contain a high number of carbon atoms, for example, palmitic acid (16:0) in butter, cheese, milk, and meat, and stearic acid (18:0) in butter, meat, poultry, fish, and grain products (Wood et al., 2008). The majority of PAHs in this study were 2-3 and 5-6 fused benzene rings, whereas four fused benzene rings , such as fluoranthene, pyrene, benzo(a)anthracene, and chrysene, were hardly detected. These might be forming the larger PAHs, for example, fluoranthene might convert to benzo(b)fluoranthene or benzo(k)fluoranthene.

The formation of PAHs in smoke from the combustion of palmitic acid consisted of 2-4 fused benzene rings of PAHs (i.e. naphthalene, acenaphthylene, acenaphthene, phenanthrene, and anthracene) and five fused benzene rings (i.e. benzo(b)fluoranthene and benzo(k)fluoranthene); this was while the formation of PAHs in smoke from the combustion of stearic acid consisted of 2-4 fused benzene rings of PAHs (i.e. naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, pyrene, and chrysene) and 5-6 fused benzene rings (i.e. benzo(b)fluoranthene, benzo(k)fluoranthene, and indeno(1,2,3-c,d)pyrene). Stearic acid generated more amounts and types of PAHs than palmitic acid. The sum of concentrations of 2-4 fused benzene rings of PAHs from stearic acid and palmitic acid was 5.69 and 3.72 ppm respectively; however, the sum of concentrations of five fused benzene rings of PAHs from stearic acid and palmitic acid was 0.46 and 0.15 ppm respectively. Thermal decomposition of fatty acids with a high number of carbon atoms can form small substrates as starting materials for PAHs formation; which include ethylene or 1,3-butadiene (Llamas et al., 2017). According to GC-MS analysis,

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methyl-, 1-butyl-2-ethyl-, and 1-methyl-2-pentyl- were found when palmitic acid and stearic acid were subject to a heating source. These compounds can form PAHs through methyl addition and cyclisation (Dandajeh et al., 2018). Decomposition of palmitic acid and stearic acid can result in the generation of carbon-centered radicals which can propagate PAH formation by ß-elimination via the small precursors formed, such as methane, ethyne, 1,3-butadiene, and ethylene (Llamas et al., 2017). Palmitic acid generated fewer types and lower concentrations of PAHs than stearic acid. During the grilling process, fatty acids are broken into small molecules, which are then recombined to form PAHs. Consequently, the number of carbon atoms in fatty acid affects the number of these small molecules. The number of small molecules provided by palmitic acid combustion was lower than those of stearic acid combustion. PAHs which found were pyrene, chrysene, and indeno(1,2,3-c,d) pyrene. The results were similar to those of Dandajeh et al. (2018), which indicated an increase in PAH concentration with the number of carbon atoms.

Another factor regarding the fatty acid structure which influences PAH formation was the double bond present in the molecule. Stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) were used to investigate the effect of double bonds on PAH formation. The combustion of linoleic acid generated the highest concentration of PAHs in the smoke, with a value of 48.53 ppm, whereas oleic acid and stearic acid had concentrations of 46.81 and 6.15 ppm, respectively (Table 3). The sum of concentrations of 2-4 fused benzene rings of PAHs or light PAHs (i.e. naphthalene, acenaphthylene, fluorene, phenanthrene, acenaphthene, anthracene) and the 5-6 fused benzene rings of PAHs or heavy PAHs (i.e. benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene) from linoleic acid were 37.01 and 11.52 ppm, respectively. The results also revealed that the linoleic acid combustion provided six heavy PAHs (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo (g,h,i) perylene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene). According to the results of GC-MS analysis, the oleic acid and linoleic acid combustions generated 1-methyl, 2-methyl, and benzene, which were precursors of PAH formation (Chen and Chen 2001). PAH formation from saturated fatty acids could be done through degradation to form low molecular weight compounds, followed by Diels-Alder reactions. In contrast, PAH formation from unsaturated fatty acids could occur through intramolecular cyclization, which occurs more readily, leading to the highest amount of PAH formation (Chen and Chen 2001). In addition, light PAHs could be converted to heavy PAHs through hydrocarbon radicals addition (Llamas et al., 2017).

The formation of PAHs in smoke from the combustion of oleic acid consisted of 2-4 fused benzene rings of PAHs, which were composed of 25.47 ppm of naphthalene, 1.39 ppm of fluorene, 15.82 ppm of anthracene, 0.48 ppm of fluoranthene; and 5-6 fused benzene rings, composed of 1.01 ppm of benzo(b)fluoranthene, 0.98 ppm of benzo(k)fluoranthene, 0.50 ppm of benzo(a)pyrene, 0.98 ppm of indeno(1,2,3-c,d)pyrene, and 0.18 ppm of benzo(g,h,i)perylene. The formation of PAHs in smoke from the combustion of linoleic acid consisted of 2-4 fused benzene rings of PAHs, and were composed of 17.82 ppm of naphthalene, 4.57 ppm of acenaphthylene, 4.87 ppm of acenaphthene, 2.56 ppm of fluorene, 2.93 ppm of phenanthrene, and 4.26 ppm of anthracene. Additionally, 5-6 fused benzene rings were composed of 1.34 ppm of benzo(b)fluoranthene, 0.94 ppm of benzo(k)fluoranthene, 1.74 ppm of benzo(a)pyrene, 2.10 ppm of indeno(1,2,3-c,d)pyrene, 0.88 ppm of dibenzo(a,h) anthracene, and 4.52 ppm of benzo(g,h,i)perylene, respectively.

Although the total concentrations of PAHs from the oleic acid and linoleic acid combustions were similar (46.81 ppm of PAHs from the oleic acid combustion, 48.53 ppm of PAHs from the linoleic acid combustion), more than half of the total concentration of PAHs from the oleic acid combustion was naphthalene at 25.47 ppm. However, linoleic acid combustion provided more types of PAHs than the oleic acid combustion. Previous studies have also shown that the degree of double bond affects PAH formation, and a higher degree of double bonds induces higher PAH content and heavier PAHs (Min et al., 2018). As mentioned above, higher degrees of double bonds leads to PAH formation from unsaturated fatty acids through intramolecular cyclization; this occurs more readily, leading to higher content of PAHs and heavier PAHs (Chen and Chen, 2001; Llamas et al., 2017).

According to this study, a saturated fatty acid (stearic acid, 18:0) provided fewer numbers and compositions of PAHs compared to oleic acid (18:1) and linoleic acid (18:2). This was because unsaturated fatty acids consisted of alkenes in the molecule (Wood et al., 2008). The decomposition of these alkenes resulted in the production of methane, 1,3-butadiene, ethylene, and allyl radical. The allyl radical was further decomposed to give 1,3-butadiene (Llamas et al., 2017). Furthermore, unsaturated fatty acids were considered to produce more PAH than saturated fatty acids due to their structure, making it easier to form free radicals and/or small molecules (Wongmaneepratip and Vangnai, 2017). These explanations supported the experimental results of this study. The decomposition of linoleic acid (18:2) resulted in the highest contents of 2-4 fused benzene rings and 5-6 fused benzene rings.

To understand the effects of the number of carbon atoms and double bonds on PAH formation in smoke,

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PAHs from palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) were prepared and compared. The degree of double bonds had a stronger impact on PAH formation than the number of carbon atoms. The total number of PAHs increased approximately 0.63 times when the length of the carbon chain increased from C16 to C18; however, PAH formation increased approximately eight times when the degree of double bonds increased from 0 to 2. Six out of 5 PAHs-or 6-fused benzene rings were found for the linoleic acid combustion (11.52 ppm), but only two PAHs were found for the palmitic acid combustion (0.15 ppm).

Among the concentrations of animal oils tested, the smoke of striped catfish oil contained the highest concentration of PAHs, with a value of 50.22 ppm, followed by the smoke of chicken skin oil with a value of 35.07 ppm, and finally, the smoke of beef loin oil with a value of 33.62 ppm (Table 5). The sum of concentrations of 2-4 fused benzene rings of PAHs from striped catfish oil, chicken skin oil, and beef loin oil was 38.29, 24.92, and 26.37 ppm, respectively; this amount for the 5-6 fused benzene rings of striped catfish oil, chicken skin oil, and beef loin oil was 11.93, 10.15, and 7.25 ppm. Striped catfish oil contained a variety of fatty acids, some of which were not found in chicken skin oil and beef loin oil. The fatty acids found only in striped catfish oil included arachidic acid (20:0), mead acid (20:3), behenic acid (22:0), erucic acid (22:1), cervonic acid (DHA) (22:6), lignoceric acid (24:0), and nervonic acid (24:1) (Wood et al., 2008). Fatty acids of striped catfish oil had a higher number of carbon atoms and a higher degree of double bonds, resulting in the highest PAH concentration (Table 5).

All the 16 PAHs were detected in the smoke generated from the striped catfish oil combustion. The smoke generated from the chicken skin oil combustion did not contain anthracene and fluoranthene, and the smoke from the beef loin oil combustion did not have fluorene. The main fatty acid in chicken skin oil was oleic acid of 39.15% (Table 4). According to Table 3, the combustion of oleic acid provides anthracene of 15.82 ppm and fluoranthene of 0.48 ppm. But, there was no anthracene and fluoranthene found from the chicken skin oil combustion; benzo(a)anthracene of 2.26 ppm, however, was observed. Similarly, most of the composition of fatty acids regarding beef loin oil was oleic acid of 43.44% (Table 4). The combustion of oleic acid (Table 3) generated fluorene of 1.39 ppm, but no benzo(a)anthracene was found. However, oil combustion results indicated that there was no fluorene found in the beef loin oil combustion, but 2.62 ppm benzo(a)anthracene was found. This could be explained by the fact that low molecular weight PAHs may be converted to high molecular weight PAHs (Llamas et al., 2017). When temperatures rise above 500 °C, carbon-carbon bonds are broken down to form free radicals. These radicals recombine to form ethylene, acetylene, and 1,3-butadiene, and finally, form aromatic ring structures by condensation reactions (Llamas et al., 2017). The aromatic ring structures formed become resistant to thermal degradation. Moreover, those hydrocarbon radicals can combine with low molecular weight PAHs to form higher molecular weight PAHs (Llamas et al., 2017). These results were similar to the study reported by Nie et al. (2019), which indicated that PAH content generated from heating fatty acid increases with increasing the number of double-bonds.

Benzene was probably the precursor of PAH formation (Chen and Chen 2001). Animal oils contain both saturated and unsaturated fatty acids (Wood et al., 2008), and the possible pathways of PAH formation are through methyl addition, cyclization, and vinyl addition (Dandajeh et al., 2017). The concentration of PAHs in the smoke of animal oils depends on the number of carbon atoms, the degree of double bonds, and the types of fatty acids present (Nie et al., 2019). Previous studies have shown the effects of fat content and the grilling procedure of different meats and how they play a pivotal role in the formation of PAHs (Lee et al., 2016; Nie et al., 2019). Grilled pork belly had the highest PAH concentration when compared with grilled beef loin, grilled beef rib, and grilled pork neck lean (Lee et al., 2016). To overcome PAHs produced in the smoke, an alternative grilling process must be designed. This process includes an apparatus to remove meat-oil dripping and smoke during the grilling process. The content of four PAHs decreased from a range of 48 to 89% when the meat-oil dripping from the grilling of pork and beef meats was removed, and from a range of 41 to 74% when the smoke was removed (Lee et al., 2016). Lee et al. (2016) found that the reduction of the number of PAHs in meat was different due to different fat contents. However, not only fat content but also the types of fatty acids in meat before grilling influence PAH formation.

Table 6 presents the concentration (ppm) of benzo(a)pyrene and four PAHs in smoke from the combustion of fatty acids and animal oils at 600 °C. According to EU Regulation No. 835/2011 (EC, 2011) for smoked meat products, benzo(a)pyrene is defined as a marker for PAH content, and the maximum level allowed is 0.005 µg/g of wet weight. In addition, the maximum combined level of the four PAHs (benzo(a)pyrene, chrysene, benzo(a)anthracene, and benzo(b)fluoranthene) is 0.03 µg/g of wet weight. These four PAHs are the most suitable indicators of carcinogenic PAHs in food (Lee et al., 2016) due to their occurrence and toxicity (EFSA, 2008). It was demonstrated that the different oils used exceeded the maximum levels of PAHs permitted by the

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EU Regulation (No 835/2011), which only permits 0.03 ppm or below in the four PAHs. The highest PAH concentration, in terms of the four main PAHs, was from the combustion of striped catfish oil, followed by the combustion of chicken skin oil, and finally, the beef loin oil, with PAH concentrations of 11.15, 8.24, and 7.85 ppm, respectively. The highest concentration of benzo(a)pyrene was present in the smoke generated from striped catfish oil, followed by the chicken skin oil, and the beef loin oil, with values of 2, 1.53, and 0.68 ppm, respectively. If all the PAHs generated in the smoke of oil combustion contaminate the grilled food, the maximum level of benzo(a)pyrene and four PAHs in grilled food defined in EU Regulation No 835/2011 will be exceeded (EC, 2011). To reduce PAH formation in smoke from oil droplets during grilling, preheating the meat and meat wrapping before charcoal grilling were proposed (Farhadian et al., 2011).

Conclusion

Grilling provides unique flavors to food. However, oil droplets from foods can form PAHs in smoke and contaminate grilled food. The effects of the number of carbon atoms, degree of double bonds, and types of fatty acids regarding the 16 major PAH formation in smoke were determined in this study. Palmitic acid (16:0) generated fewer types and lower concentrations of PAH compared with stearic acid (18:0), indicating that the number of carbon atoms in fatty acids affects PAH formation in smoke. Moreover, linoleic acid and oleic acid generated relatively high concentrations of PAHs and heavy PAHs, while stearic acid generated the lowest PAH concentration and heavy PAHs. The degree of double bonds generated higher PAH content and heavy PAH and had a stronger impact on PAH formation compared with carbon atoms. The highest concentration of the 16 major PAHs was found in the smoke of striped catfish oil, followed by the smoke of chicken skin oil, and the smoke of beef loin oil. It was concluded that the PAH concentration in the smoke of animal oils was not only affected by fat content, but also by the types of fatty acids in the meat before grilling. Future studies should simulate a grilling process using charcoal instead of a heating block to combine the effects of charcoal and oil droplets on the formation of PAHs in smoke in a real-life charcoal grilling process.

Authors' contributions

M.N. collected data, performed the analysis, and wrote the manuscript. Under the supervision of T.K. and P.V., W.M. contributed to critical revision of manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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