Formation of Polycyclic Aromatic Hydrocarbons in the Smoke from Heated Model Lipids and Food Lipids

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The contents of polycyclic aromatic hydrocarbons (PAHs) in the smoke from model lipids and food lipids during heating were determined and the mechanism of PAH formation was studied. A Rancimat oil stability analyzer was used as a model system for heating model lipids and food lipids at 220 °C for 2 h and for adsorption of smoke. The various lipid degradation products and PAHs in the smoke were identified and quantified by a GC/MS technique. Results showed that model lipids were more susceptible to smoke formation than food lipids during heating, but the PAH levels were lower for the former than latter. Methyl linolenate produced the highest amount of PAHs, followed by methyl linoleate, methyl oleate, and methyl stearate. Also, soybean oil generated a larger amount of PAHs than canola oil or sunflower oil. Benzene-like compounds were found to be possible precursors for PAHs formation. Several PAH derivatives were also present in heated model lipids and food lipids.

Keywords: *Polycyclic aromatic hydrocarbon; smoke; GC-MS; lipids*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), formed through incomplete combustion of wood or gasoline, represent an important class of toxicological compounds. To date, more than 100 PAHs have been characterized (*1*, *2*). The United States Environmental Protection Agency (USEPA) has chosen 16 PAHs as priority pollutants according to their presence in the atmosphere and carcinogenicity. These PAHs include 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. Numerous studies have shown that food processing methods such as grilling and smoking may induce formation of PAHs (*3*−*7*).

Several epidemiological studies conducted in Taiwan and China revealed that the Chinese women ranked first in the world for lung cancer occurrence, probably because of absorption of smoke from heated oil in the kitchen (*8*−*10*). The high carcinogenicity and cytotoxicity of the smoke concentrate in the kitchen has been well documented (*11*−*16*). Shields et al. (*15*) reported that the smoke from heated rapeseed oil possessed a higher mutagenicity than soybean oil. However, no mutagenicity was observed for lard, sesame oil, and canola oil. Interestingly, in another study, Chiang et al. (*16*) found that smoke concentrates from soybean oil, peanut oil, and lard showed a strong cytotoxicity, and several PAHs such as benzo[*a*]anthracene, benzo[*a*]-pyrene, and dibenzo[*a*,*h*]anthracene were present. This result seems to be contradictory to a previous report by Shields et al. (*15*). Thus, it is necessary to determine the various PAHs in the smoke from cooked oil. The objectives of this study were to determine the PAHs in the smoke from heated model lipids and food lipids, and to postulate the formation mechanism of PAHs from degradation products of cooked oil.

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MATERIALS AND METHODS

Materials. Model lipids, including methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate, were purchased from Nu-chek-Prep Co. (Elysian, MN). Sixteen PAH standards, 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, and internal standard 2-methylphenanthrene, were obtained from Supelco Inc. (Bellefonte, PA). Edible oils such as soybean oil, sunflower oil, and canola oil were from DIN-HAU supermarket (Hsinchuang, Taipei). Reagents, including sodium bisulfite solution (0.1 N), soluble starch, and the Wijs reagent, were purchased from Merck Co. (Darmstadt, Germany). Chemicals such as potassium iodide, potassium hydroxide, methanol, ethanol, petroleum ether, diethyl ether, chloroform, and hydrochloric acid were also purchased from Merck Co. The reagent diazomethane used for derivatization of fatty acids was prepared from *N*-nitroso-*N*-methyl urea (Sigma Co., St. Louis, MO, USA) using a method described by Lu et al. (*17*). Solvents, including *n*-hexane and acetone, were from Mallinckrodt Co. (Kentucky, USA). Adsorbents Tenax TA 60/80 and active carbon were from Supelco Inc. The active carbon was ground into a powder and screened through mesh 60 prior to use.

Heating and Adsorption System. A Rancimat oil stability analyzer was used as a model system for heating oil. The Pyrex glass reaction tubes (17 × 2.5 cm i.d.) were made by Jeng-Mei Co. (Taipei, Taiwan). The adsorption tube (100 × 6 mm i.d.) contained 0.2 g of either adsorbent Tenax or active carbon, retained by glasswool at the top and bottom. The adsorption tube was placed on the top of the reaction tube, and both were held together with a Teflon screw cap. Adsorption tubes were conditioned at 300 °C for 2 h prior to use. Air was pumped through the reaction tube, and the flow rate was controlled by air flow adjustor.

The degradation products and PAHs formed from heated model lipids and food lipids were measured using a HP 6890

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gas chromatograph coupled with a model 5973 mass selective detector (Hewlett-Packard Co., Palo Alto, CA) operating at 70 eV. Data acquisition and processing were carried out with an Hewlett-Packard HP-Chem data system.

Adsorption Efficiency of Various Adsorbents to PAHs. The PAH stock solution, containing naphthalene (353 ppm), acenaphthylene (400 ppm), acenaphthene (310 ppm), fluorene (360 ppm), phenanthrene (316 ppm), anthracene (336 ppm), fluoranthene (382 ppm), pyrene (347 ppm), benzo[*a*]anthracene (308 ppm), chrysene (352 ppm), benzo[*b*]fluoranthene (200 ppm), benzo[*k*]fluoranthene (220 ppm), benzo[*a*]pyrene (336 ppm), dibenzo[*a,h*]anthracene (157 ppm), benzo[*g,h,i*]perylene (40 ppm), and indeno[1,2,3-*c,d*]pyrene (40 ppm), was prepared by dissolving in 250 mL of acetone. Two milliliters of the stock solution was placed into the reaction tube. After evaporation to dryness with nitrogen gas, 2 g of model lipid methyl stearate was added and the reaction tube was heated at 220 °C for 2 h. Methyl stearate was selected because it is the most stable to heat. The adsorption tubes containing different types of adsorbents, 100% Tenax, Tenax/Carbon (1/1, w/w), and 100% active carbon, were used for comparison. During heating, the air flow rate was controlled at 10 L/h and the adsorption tube was replaced every 40 min, after which time the adsorption efficiency was substantially decreased. After heating, 70 mL of acetone was added to each reaction tube with an extraction rate at 3.0 mL/min by an adjustable vacuum extractor. The extract was combined, concentrated to 1.0 mL, and diluted to 2.0 mL with acetone. The solution was transferred to a brown bottle and stored at −70 °C for analysis of PAHs in the smoke by GC/MS/SIM mode.

Formation of Degradation Products and PAHs during Heating of Model Lipids and Food Lipids. Two grams of model lipids, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate as well as 2 g of food lipids, soybean oil, sunflower oil, and canola oil were each poured into separate reaction tubes for heating at 220 °C for 2 h. The air flow rate was 10 L/h, and each adsorption tube was replaced every 40 min. After heating, each adsorption tube was extracted with 70 mL of acetone with a rate of 3.0 mL/min by an adjustable vacuum extractor. The extract was combined, concentrated to 1.0 mL, and diluted to 2.0 mL with acetone. The solution was transferred to a brown bottle and stored at −70 °C for analyses of PAHs and degradation products in the smoke by GC/MS/SIM and GC/MS/scan mode, respectively. The amount of volatile compounds trapped could be calculated from the weight difference of each adsorption tube before and after heating.

Analysis of Degradation Products during Heating of Model Lipids and Food Lipids. The degradation products formed during heating of model lipids and food lipids were analyzed using GC/MS/scan mode. The scanning range was *m/z* 35−500. An HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with a He carrier gas flow rate of 1.0 mL/min and split ratio (30:1) was used. The injector temperature was 290 °C, and the column temperature was at 70 °C initially, then programmed to 290 °C at 2 °C/min and maintained at 290 °C for 20 min. The injection volume was 1.0 μL, and the GC/MS interface temperature was 290 °C. The tentative identification of degradation products was carried out by comparison of mass spectra of unknown peaks with those in the Wiley 275 mass spectra database.

Analysis of PAHs during Heating of Model Lipids and Food Lipids. The PAHs formed during heating of model lipids and food lipids were analyzed by GC/MS/SIM mode. The molecular ions (M^+) of all the PAHs were set as target ions. In addition to fluorene, the $(M\text{-}H_2)^+$ ion of each PAH was set as the qualifier ion. Because of the great ion abundance of fluorene, the $(M\text{-}H_1)^+$ was used as the qualifier ion. The GC conditions were the same as for analysis of degradation products except that the column was programmed from 70 to 250 °C at 10 °C/min, then raised to 290 °C at 5 °C/min, and maintained at 290 °C for 10 min. The injection was carried out by a manual mode and the volume was 1.0 μL. The identification of PAHs was made by comparison of unknown peaks with reference standards and co-chromatography with added standards. In addition, the various PAHs were identified by comparing Q1 ratios of unknown peaks to those of reference standards. The Q1 ratio was defined as the ratio of qualifier ion to target ion. For positive confirmation, the error should be less than 20%.

Each PAH was quantified using an internal standard. The standard calibration curves were prepared for all the 16 PAHs with concentrations ranging from 0 to 2000 ppb, and the internal standard 2-methylphenanthrene (50 ppb) was added to each PAH solution. The curve of each PAH was obtained by plotting abundance ratio of each PAH to 2-methylphenanthrene against concentration ratio. The regression equation and correlation coefficient (r^2) were calculated. The content (μg/g of smoke) of each PAH in the smoke was thus obtained through the regression equation. The correlation coefficients for all the 16 PAHs ranged from 0.95 to 0.998.

Determination of Detection Limits and Quantification Limits of 16 PAHs. Both the detection limits (DL) and quantification limits (QL) were determined based on a method described by International Conference on Harmonization (*18*). Three concentrations (0.1, 0.2, and 1%) of PAH stock solutions were chosen, and triplicate analyses were performed. The linear regression equation was derived and the slope (S) was obtained. The standard deviation (σ) for the intercepts of three regression curves were calculated. Both DL and QL were measured using the following formulas:

$$DL = 3.3\sigma/S$$

$$QL = 10\sigma/S$$

Determination of Fatty Acid Composition in Food Lipids. The fatty acid compositions in food lipids were analyzed based on the method described by Lu et al. (*17*). *n*-Hexane (1.0 mL) was mixed with 0.1 g of oil sample in a vial, and the mixture (200 μL) was evaporated to dryness under nitrogen. Ethanol solution (in 0.3% KOH) (1.0 mL) was added to the vial, and the mixture was saponified at 40 °C for 60 min. After saponification, 1.0 mL of distilled water was added, and the mixture was mixed thoroughly. The nonsaponifiable fraction was extracted with petroleum ether (3 × 2 mL), and the extracts were combined and evaporated to dryness under nitrogen. Diethyl ether/methanol (9/1, v/v) (1.0 mL) was added and mixed with 2 mL of diazomethane (in diethyl ether) for esterification. After esterification, the solution was evaporated to dryness under nitrogen and mixed with 50 μL of *n*-hexane for analysis of fatty acids by GC. A Supelco SP-2330 glass column (2 m × 3 mm i.d.) with a N_2 flow rate of 40 mL/min was used. The injector and detector temperatures were 290 °C, while the column temperature was 240 °C. The injection volume was 1.0 μL and was carried out by a manual mode. The various fatty acids in the food lipids were identified by comparison of retention time of unknown peaks with reference standards. The amount of each fatty acid was expressed as the ratio of area to the total area.

Determination of Iodine Value. The iodine value of each food lipid was determined according to the AOAC method (*19*).

RESULTS AND DISCUSSION

Method Evaluation of GC/MS/SIM. Figure 1 shows the GC/MS chromatogram of 16 PAHs with detection by selected ion monitoring (SIM). By using this method, the PAH ion possessing the specific mass could be detected. As compared to GC/MS/scan mode, this method showed a higher specificity and sensitivity and thus would be more appropriate for trace component analysis such as PAH. However, the molecular ions (M^+) of most PAHs gave the most abundant peak (>80% of total ions), while the other fragment ions possessed weak intensity. This phenomenon would make it difficult for detection of low-concentration PAH by SIM mode, because of inadequate intensity of qualifier ions. In this study, when the PAH concentration in the sample was

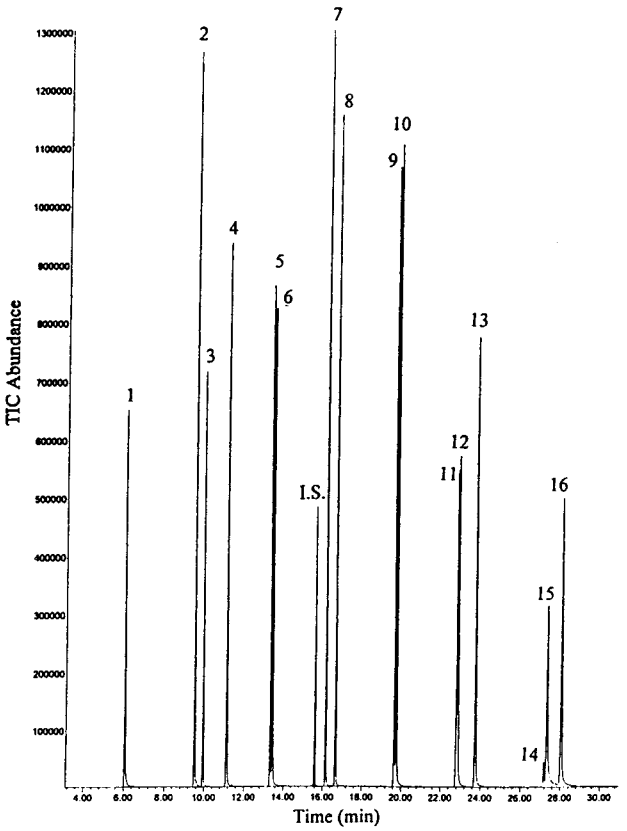

Figure 1. GC/MS/SIM chromatogram of 16 PAHs standards plus one internal standard. Peaks: 1 = naphthalene, 2 = acenaphthylene, 3 = acenaphthene, 4 = fluorene, 5 = phenanthrene, 6 = anthranthene, IS = 2-methylphenanthrene, 7 = fluoranthene, 8 = pyrene, 9 = benzo[*a*]anthrancene, 10 = chrysene, 11 = benzo[*b*]fluoranthene, 12 = benzo[*k*]fluoranthene, 13 = benzo[*a*]pyrene, 14 = indeno[1,2,3-*c,d*]pyrene, 15 = dibenzo[*a,h*]anthrance, 16 = benzo[*g,h,i*]perylene.

Table 1. Detection and Quantification Limits of 16 PAHs

PAH	DL (ppb)[a]	QL (ppb)[b]
naphthalene	0.5	1.4
acenaphthylene	0.9	2.7
acenaphthene	0.6	1.7
fluorene	0.8	2.6
phenanthrene	0.8	2.4
anthracene	1.2	3.6
fluoranthene	1.5	4.4
pyrene	1.6	4.8
benzo[*a*]anthracene	0.6	1.8
chrysene	0.4	1.3
benzo[*b*]fluoranthene	0.8	2.4
benzo[*k*]fluoranthene	0.2	0.7
benzo[*a*]pyrene	0.9	2.9
dibenzo[*a,h*]anthrancene	0.4	1.3
benzo[*g,h,i*]perylene	0.5	1.6
indeno[1,2,3-*c,d*]pyrene	0.1	0.2

[a] DL: limit of detection. [b] QL: limit of quantitation.

close to the quantification limit (2.2 ppb), several PAHs might be incorrectly identified. Under these circumstances, the PAH was confirmed by comparison of retention time and co-chromatography with added standards. This phenomenon was also observed by Mottier et al. (*20*), who determined PAHs contents in barbecued meat sausages by GC/MS. Table 1 shows the DL and QL of 16 PAHs. The average DL and QL of 16 PAHs were 0.7 and 2.2 ppb, respectively. This result is similar to that reported by the ACS Subcommittee on Environmental Analytical Chemistry (*21*). For reproducibility test, three concentrations 2, 20, and 200 ppm

Table 2. Adsorption Efficiency (%) of Various Adsorbents to 16 PAHs during Heating of Methyl Stearate at 220 °C for 2 h[a]

PAH	ring no.	100% Tenax (%)	Tenax/ carbon (%)	100% carbon (%)
naphthalene	2	105	98	40
acenaphthylene	3	104	101	29
acenaphthene	3	82	77	22
fluorene	3	78	80	11
phenanthrene	3	70	66	9
anthracene	3	73	75	5
fluoranthene	4	40	22	2
pyrene	4	41	17	ND[b]
benzo[*a*]anthracene	4	33	10	ND
chrysene	4	39	14	ND
benzo[*b*]fluoranthene	5	11	ND	ND
benzo[*k*]fluoranthene	5	11	ND	ND
benzo[*a*]pyrene	5	5	ND	ND
dibenzo[*a,h*]anthrancene	5	8	ND	ND
benzo[*g,h,i*]perylene	6	4	ND	ND
indeno[1,2,3-*c,d*]pyrene	6	8	ND	ND

[a] Average of triplicate determinations. [b] ND: not detected.

Table 3. Contents of Smoke (g) Formed during Heating of Model Lipids and Food Lipids at 220 °C for 2 h

sample	weight of smoke
methyl stearate	0.166 ± 0.011[a]
methyl oleate	0.241 ± 0.011
methyl linoleate	0.426 ± 0.001
methyl linolenate	0.512 ± 0.003
soybean oil	0.021 ± 0.001
sunflower oil	0.013 ± 0.001
canola oil	0.016 ± 0.002

[a] Mean of triplicate analyses ± standard deviation.

of PAHs solutions were each analyzed six times separately, and the coefficient of variation (CV%) for all the PAHs ranged from 0.24 to 6.57%, from 0.11 to 6.98%, and from 1.12 to 8.79%, respectively.

Adsorption Efficiency of Various Adsorbents to PAHs during Heating of Model Lipids. Table 2 shows the adsorption efficiency (expressed as percentage) of three adsorbents to PAHs during heating of model lipids. All of the PAHs could be adsorbed onto 100% Tenax; however, the adsorption efficiency decreased significantly with PAHs having more than three rings. With four rings, the efficiency drops below 50%. This is probably because of the boiling point difference, i.e., the PAHs with high molecular weight would be difficult to vaporize and thus the adsorption efficiency decreased. The adsorption capacity of adsorbent Tenax/carbon to PAHs with three rings or less was similar to that of 100% Tenax; however, no PAHs with five rings and above were adsorbed by the former. Also, the adsorption capacity of active carbon was inferior to that of 100% Tenax or Tenax/carbon. From the above results, the following conclusions could be drawn: (i) Active carbon might possess high adsorption capacity for compounds with low molecular weight. In contrast, Tenax could adsorb compounds with high molecular weight such as PAHs and (ii) Tenax could adsorb more smoke than active carbon.

Smoke Components in Heated Model Lipids and Food Lipids. Table 3 shows the amount of smoke adsorbed onto the Tenax-containing reaction tube during heating of model lipids and food lipids at 220 °C for 2 h. The amount of smoke in food lipids was lower than that in model lipids, probably because the former was found to contain the antioxidant tocopherol (in soybean oil) and BHT (in sunflower oil and canola oil). It is also

Table 4. Contents of PAHs (μg/g of Smoke) in the Smoke during Heating of Model Lipids and Food Lipids at 220 °C for 2 h[a]

PAH	methyl stearate	methyl oleate	methyl linoleate	methyl linolenate	soybean oil	sunflower oil	canola oil
naphthalene	ND[b]	ND	ND	ND	ND	ND	ND
acenaphthylene	4.1 ± 0.9	17.0 ± 0.4	45.0 ± 1.8	87.2 ± 2.2	ND	21.1 ± 1.4	12.9 ± 1.2
acenaphthene	ND	ND	8.4 ± 1.0	49.7 ± 1.1	72.5 ± 2.2	14.5 ± 0.2	62.2 ± 3.1
fluorene	ND	37.7 ± 1.8	ND	ND	84.9 ± 0.8	21.7 ± 1.7	59.5 ± 2.5
phenanthrene	ND	26.2 ± 1.2	54.6 ± 5.8	24.6 ± 3.1	83.2 ± 4.5	33.0 ± 6.6	60.4 ± 4.3
anthracene	2.1 ± 0.4	ND	17.8 ± 2.4	60.0 ± 1.2	31.2 ± 2.6	10.3 ± 1.8	61.1 ± 2.6
fluoranthene	ND	ND	89.6 ± 3.0	109.0 ± 5.5	98.2 ± 6.3	44.0 ± 2.4	78.1 ± 5.4
pyrene	ND	ND	81.6 ± 4.1	50.0 ± 3.1	87.5 ± 3.4	40.1 ± 4.6	68.6 ± 3.6
benzo[*a*]anthracene	3.8 ± 1.1	27.2 ± 0.3	44.9 ± 0.5	52.7 ± 2.6	46.8 ± 1.2	21.5 ± 1.0	39.0 ± 1.0
chrysene	7.6 ± 1.1	29.9 ± 1.3	ND	ND	58.0 ± 6.6	24.6 ± 3.1	44.5 ± 5.2
benzo[*b*]fluoranthene[c]	ND	27.5 ± 0.2	24.0 ± 1.2	8.4 ± 0.1	29.8 ± 3.1	13.3 ± 1.8	ND
benzo[*k*]fluoranthene[c]	ND	26.5 ± 3.8	ND	11.3 ± 0.1	30.6 ± 1.1	15.5 ± 1.5	ND
benzo[*a*]pyrene[c]	ND	8.4 ± 2.2	9.3 ± 1.7	18.6 ± 2.8	16.1 ± 1.1	5.9 ± 0.9	10.1 ± 0.3
dibenzo[*a,h*]anthracene[c]	ND	21.2 ± 1.1	49.0 ± 2.1	25.6 ± 2.4	15.4 ± 1.2	5.8 ± 0.5	8.8 ± 1.3
benzo[*g,h,i*]perylene[c]	ND	ND	ND	ND	ND	ND	ND
indeno[1,2,3-*c,d*]pyrene[c]	ND	4.3 ± 1.3	2.7 ± 0.1	5.9 ± 0.2	8.2 ± 2.4	1.6 ± 0.2	9.9 ± 0.8
total	17.6	225.9	426.9	503.0	662.4	272.9	515.1

[a] Mean of triplicate analyses ± standard deviation. [b] ND: not detected. [c] The precision of the quantitative results of these PAHs need further evaluation because of low adsorption efficiency.

possible that the free fatty acid esters in model lipids were more susceptible to oxidation than triglyceride which was the major form in food lipids. The smoke extract in model lipids was found to contain a higher amount of fatty acid degradation products than those in food lipids. For model lipids, methyl linolenate produced the highest amount of smoke, followed by methyl linoleate, methyl oleate, and methyl stearate. Apparently, this result could be attributed to the degree of unsaturation. Methyl linolenate was the most susceptible to oxidation, and the smoke should be more readily formed than the other model lipids during heating. However, the iodine values for the three food lipids were slightly different. The iodine values were 132.86, 132.82, and 100.84 for soybean oil, sunflower oil, and canola oil, respectively. 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%). 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. In another study, Chiang et al. (*16*) demonstrated that during heating soybean oil could form a greater amount of smoke than peanut oil or lard. A similar result was reported by Shields et al. (*15*), who studied the mutagenicity of heated cooking oil.

Table 4 shows the amount of PAHs in the smoke during heating of model lipids and food lipids. Methyl linolenate was found to generate the largest amount of PAHs (503.0 ppm), followed by methyl linoleate (426.9 ppm), methyl oleate (225.9 ppm), and methyl stearate (17.6 ppm). All the smoke from four model lipids contained benzo[*a*]anthracene. With the exception of methyl stearate, both benzo[*a*]pyrene and dibenzo[*a,h*]-anthracene were present in the smoke of the other three model lipids. This result clearly indicated that the degree of unsaturation of fatty acids could affect the variety and amount of PAHs formed in the smoke. The variety of PAHs formed in the smoke from methyl linolenate and methyl linoleate were similar, and a slight difference was observed for methyl oleate. For instance, acenaphthylene, anthracene, fluoranthene, and pyrene were present in the smoke of methyl linolenate and methyl linoleate. In contrast, both fluorene and chrysene were found in the smoke of methyl oleate.

In comparison to model lipids, the smoke from food lipids were found to contain a larger variety of PAHs (Table 4). For instance, sunflower oil contained 14 PAHs, while soybean oil had 13 PAHs, and canola oil had 12 PAHs. Interestingly, for the amount of PAHs formed, soybean oil produced the highest amount (662.4 μg/g), followed by canola oil (515.1 μg/g), and sunflower oil (272.9 μg/g). As explained before, the high degree of unsaturation in soybean oil could account for this result. Likewise, canola oil contained a high amount of linolenic acid (10.69%) and thus should be more susceptible to PAH formation than sunflower oil. This result also revealed that linolenic acid played a more important role for PAH formation than linoleic acid. The three most carcinogenic PAHs, benzo[*a*]anthracene, benzo[*a*]pyrene, and dibenzo[*a,h*]anthracene, were also present in food lipids. The level of benzo[*a*]anthracene in soybean oil was 3−4 times higher than benzo[*a*]pyrene or dibenzo[*a,h*]anthracene. This finding seems to be different from a report by Li (*14*), who found that dibenzo[*a,h*]-anthracene was present at a concentration 12 times higher than benzo[*a*]pyrene, during heating of soybean oil at 265 °C. However, in another study, Chiang et al. (*16*) reported that the content of benzo[*a*]pyrene was 10 times larger than benzo[*a*]anthracene or dibenzo[*a,h*]-anthracene during heating of soybean oil at 250 °C. This difference may be attributed to both the qualitative and quantitative techniques employed. Both Li (*14*) and Chiang et al. (*16*) used thin-layer chromatography coupling with fluorescence scanning densitometer and high performance liquid chromatography with fluorescence detector to analyze PAHs, respectively, which may affect the accuracy of quantification. Also, no GC-MS technique was used for identification, and the results may be misinterpreted. According to a report by Li (*14*), the amount of PAH formed in the smoke could be converted on a volume basis. Thus, the concentration of benzo[*a*]pyrene in soybean oil, sunflower oil, and canola oil could be estimated as 16.9, 3.8, and 8.1 μg/m^3, respectively. In a similar study, Chiang et al. (*16*) estimated that a concentration (19.6 μg/m^3) of benzo[*a*]-pyrene was produced in the smoke during heating of

Table 5. Formation of PAH Derivatives from Model Lipids and Food Lipids during Heating at 220 °C for 2 h

model lipids	PAH derivative	*m/z*	relative content
methyl oleate	1,2-dihydro-12-methyl-benzo[*a*]anthracene	209, (244)[a]	≤0.1%
	2-hydroxy-2-pentyl-1-acenaphthenone[b]	231, 252, (260)	≤0.1%
methyl linoleate	1,6-dimethyl-decahydro-naphthalene	81, (95), 166	0.1−1.0%
	2,3-dimethyl-decahydro-naphthalene	(95), 151, 166	≤0.1%
	4a,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1*H*)-naphthalenone	95, (136), 178	≤0.1%
	1,2-dihydro-5-pentadecyl-acenaphthylene[b]	43, 152, (167)	≤0.1%
	4,5,7,8,9,10,11,12-octahydrobenzo[*a*]pyrene	202, 217, (260)	≤0.1%
	1,2,3,6,7,8,9,10,11,12-decahydrobenzo[*a*]pyrene	205, 219, (262)	≤0.1%
methyl linolenate	1-naphthalenecarboxyaldehyde	127, 128, (156)	≤0.1%
	1-butyl-4-pentyl-1,2,3,4-tetrahydro-naphthalene	131, 187, (258)	≤0.1%
	1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalenone[b]	(55), 109, 194	≤0.1%
	4a-methyl-7-(1-methylethyl)-octahydro-2(1*H*)-naphthalenone	67, (81), 208	0.1−1.0%

[a] Values in parentheses represent the molecular ion, while values with underline represents the base ion. [b] Means that these PAH derivatives were also present in the smoke of soybean oil.

soybean oil at 250 °C for 30 min. In this study, the total concentrations of PAHs in the smoke during heating were 0.7, 0.2, and 0.4 μg/L for soybean oil, sunflower oil, and canola oil, respectively. However, there is no maximum allowable concentration for PAHs in the atmosphere in Taiwan. On the basis of the average respiration rate (6 L/min) of humans, the average absorption of PAHs per min may be postulated as 4.2, 1.2, and 2.4 μg for cooked soybean oil, sunflower oil and canola oil, respectively. It has been estimated that one cigarette could produce 20−40 μg of PAHs per min (*22*). In other words, with soybean oil as cooking oil the amount of PAH absorbed into the body every 5−10 min would be equivalent to that produced by one cigarette per minute.

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, acids, and fatty acid esters. Also, most of these degradation products were present in heated food lipids. In addition, several hydroperoxides could be broken down to form cyclic compounds from model lipids methyl oleate, methyl linoleate, and methyl linolenate. For instance, the smoke of methyl oleate was found to contain 1-methyldocecyl-benzene, while that of methyl linoleate contained 2,5-dihydroxybenzoic acid methyl ester and 2,3-dimethyl-4-pentylbenzoic acid. Likewise, methyl linolenate was found to produce benzaldehyde and the other benzene ring-containing compounds. These benzene ring-containing compounds may be postulated to form through the following routes: (i) The degradation product cyclohexene may be further oxidized to form benzene. (ii) The degradation products containing conjugated carbon−carbon double bonds may react with dienophilic compounds to form benzene ring-containing compounds through a Diels−Alder reaction. For example, *trans-trans*-2,4-decadienal may react with 2-butene to form 4-pentyl-2,3-dimethyl-benzoic acid. A similar theory was proposed by Henderson et al. (*23*), who reported that butenediol may react with alkoxy radical to form alkoxybutadiene, which in turn results in the formation of a benzene ring-containing compound in the presence of dienophile like propene. (iii) Fatty acid could be oxidized to form hydroperoxide and then undergo a subsequent intramolecular reaction for cyclic compound formation such as cyclohexene. For example, 9-hydroperoxide, formed from linolenic acid, may be degraded to form 2,4,7-decatrienal and then undergo further oxidation to form 8-hydroperoxide. This oxidation product could be broken down and result in formation of benzaldehyde and propanal (*24*). (iv) Polyunsaturated fatty acids such as linoleic acid and linolenic acid could undergo polymerization to form cyclic monomers or dimers (*25*).

Several reports have indicated that benzene is a probable precursor for PAH formation. According to a study by Mestres and Sola (*26*), the benzene-containing compound may react with conjugated-diene-containing degradation products such as 1,3-butadiene to form PAH through Diels−Alder cycloaddition. In addition, the degradation products such as cyclohexene may be oxidized to form benzene, which in turn reacts with C_4 compound for formation of naphthalene and other PAHs (*27*). Although no naphthalene was present in the smoke, several benzene-like compounds such as 1,2-hexenediol (from methyl linoleate) and 2-cyclohexene-1-one as well as 4,4-dimethyl-2-cyclohexene-1-one (both from methyl linolenate) were found to occur. This result demonstrated that these benzene-like compounds may undergo further reaction to form naphthalene derivatives, namely, 1,2-dimethyl-naphthalene, 1,3-dimethyl-naphthalene, and decahydro-1,6-dimethyl-naphthalene. Also, the formation of monomers or dimers through polymerization and intramolecular cyclization should be more susceptible to PAH formation than the other routes.

From the experimental results, it may be postulated that the variety of PAH formed in the smoke correlated well with benzene formation from fatty acids. Of the various fatty acids, stearic acid is the most difficult for intramolecular cyclization to occur. Thus, PAH formation from stearic acid is probably through degradation to form low-molecular-weight compounds, followed by Diels−Alder reactions. In contrast, the PAH formation from oleic acid, linoleic acid, and linolenic acid could proceed through intramolecular cyclization which occurs more readily for unsaturated fatty acids. Therefore, linolenic acid is the most susceptible to forming cyclic compounds and should result in the highest amount of PAH formation. As the fatty acid composition of food lipids is more complicated than model lipids, the mechanism for PAH formation may be different from the latter. Nevertheless, the content of PAH formed in the smoke of food lipids could be estimated by degree of unsaturation of fatty acids.

Table 5 shows the relative contents of PAH derivatives from heated model lipids and food lipids. Six PAH

derivatives were found in heated methyl linoleate, while four and two were present in heated methyl linolenate and methyl oleate, respectively. Interestingly, only 2-hydroxy-2-pentyl-1-acenaphthenone, 1,2-dihydro-5-pentadecyl-acenaphthylene, and 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalenone were detected in cooked soybean oil, and the levels of these were lower than in model lipids. No PAH derivatives were present in both the sunflower oil and canola oil, probably because the amounts were too small to detect. As no standards are commercially available, these PAH derivatives could only be identified by means of a mass spectral library search. They are present in trace amounts and are probably precursors or end products of formation of various PAHs. Further research is necessary to study the formation of PAHs in real food systems during heating.

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