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Analysis, Formation and Inhibition of Polycyclic Aromatic Hydrocarbons in Foods: An Overview

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), formed through incomplete combustion or pyrolysis of wood or gasoline, represents an important class of toxicological compounds because of its wide distribution in the environment and possible contamination of foods. This paper deals with an overview of analysis, formation and inhibition of PAHs in foods. Extraction of PAHs from food is routinely conducted by saponification of lipid with Soxhlet method, followed by purification with a Sep-Pak Florisil cartridge and partition. This method can remove more impurities than the sonication method. With HPLC, all 16 PAHs can be simultaneously separated by a gradient solvent system and detected by UV at 254 nm or fluorescence employing programmable wavelength with seven settings of excitation / emission. With GC, all 16 PAHs can also be simultaneously separated by a temperature programming method and detected by flame ionization detector (FID) or ion-trap mass detector (ITD). Although HPLC can provide adequate separation of 16 PAHs, nevertheless, the presence of impurities in food sample can interfere with the subsequent identification and separation. For GC, a number of isomeric PAHs partially overlap. Nonetheless, the application of GC-ITD can readily identify the various PAHs in foods through reconstructed ion chromatograms even in the presence of fat- or PAH-like impurities. Charcoal-grilling and smoking are the two major processing methods which can result in the formation of high amount of total carcinogenic PAHs. No carcinogenic PAHs are detected in steaming and liquid smoke flavoring. The amout of PAHs in food samples can also be greatly reduced by sorption into low-density polyethylene bags. Further research in necessary to determine how the formation of PAHs during processing can be directly retarded or eliminated.

Key words: **Polycyclic aromatic hydrocarbons, GC-MS, HPLC, processing methods.**

INTRODUCTION

In today's world the environmental pollution has become a major problem for public health. Of many pollutants distributed in nature, polycyclic aromatic hydrocarbons (PAHs) represent an important class of such compounds. PAHs, formed through incomplete combustion or pyrolysis of wood or gasoline, can be detrimental to human health if consumed in significant amounts.

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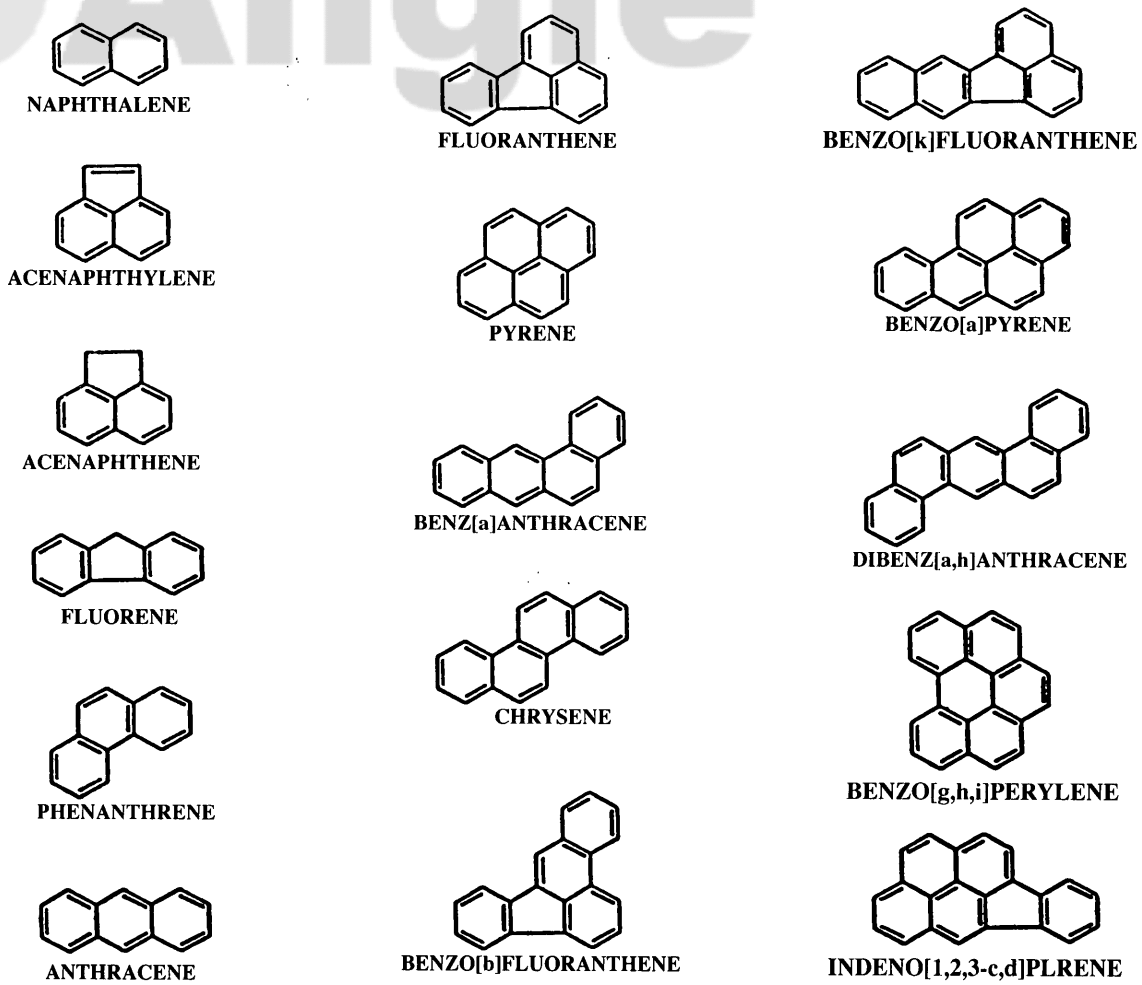

Figure 1. Structures of the 16 PAHs identified as priority pollutants by the U. S. Environmental Protection Agency.

To date more than 100 PAHs have been characterized in nature, 16 of which were classified as "priority pollutants" according to the US Environmental Protection Agency (EPA) (Figure 1). Of these 16 PAHs, benzo[a] pyrene and dibenz[a,h]anthracene was reported to be the most carcinogenic by IARC[1,2]. Since environmental pollution is becoming a serious problem throughout the world, it is possible that PAHs may be widely distributed within the environment and thus contaminate processed foods. As many PAHs have been proven to be carcinogenic and mutagenic [1-4], the development of a fast and accurate method for determination of PAHs in foods is necessary.

The presence of PAHs in foods has been investigated by many researchers. Of the various foods, processed meat products is the major commodity found to contain high amount of PAHs. Several processing methods, including smoking [5-15], grilling [5,9,11,16-21] and roasting [22-26] have been reported to induce formation of PAHs in foods. Thus, the formation of PAHs during processing of foods poses a potential hazard to human health. To reduce the risk caused by formation of PAHs during smoking, some processors use liquid smoke flavoring instead. It has been reported that the application of liquid smoke flavoring can decrease the amount of PAHs to a considerable extent [13,14,27-29]. In addition, several authors have used specific types of packing material for adsorption of PAHs from foods [27,29,30-32].

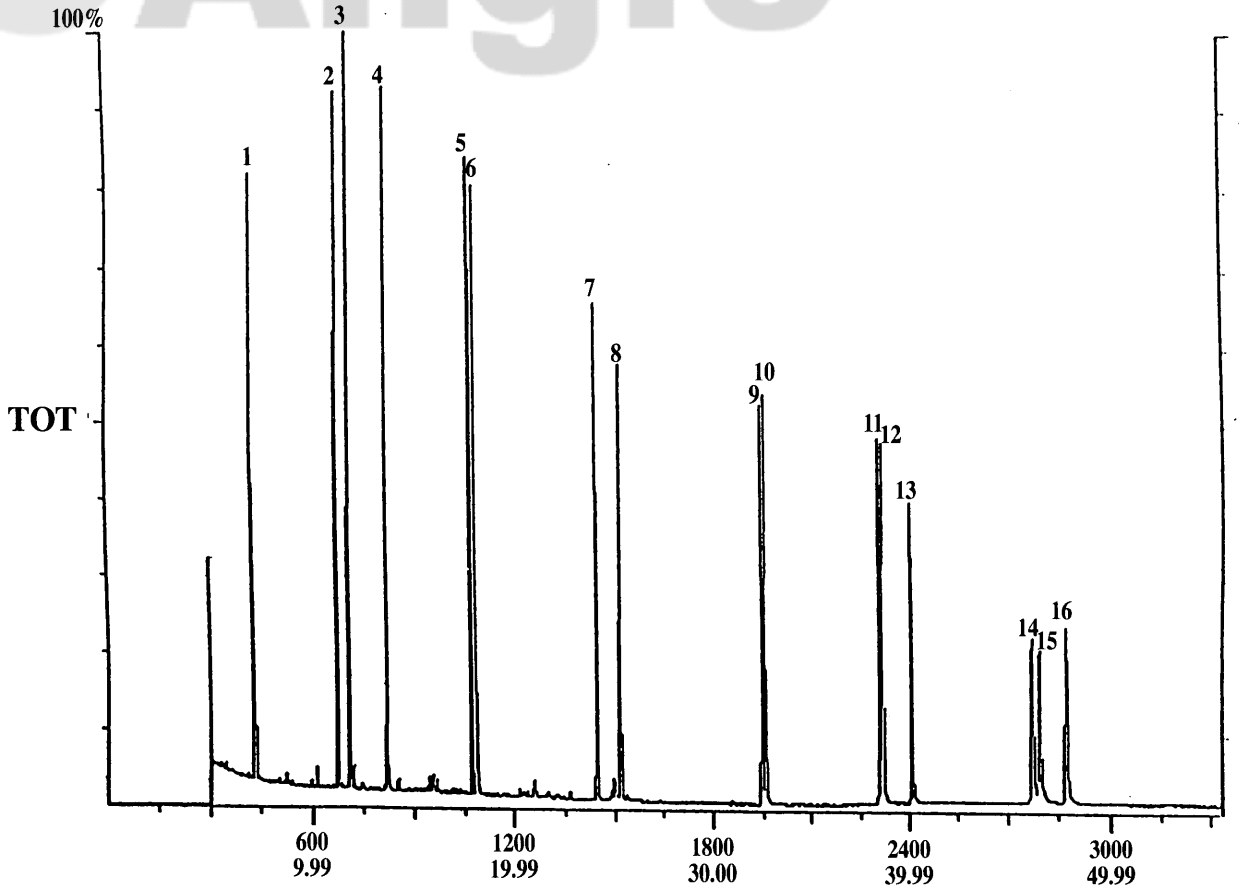

Figure 2. GC chromatogram of 16 priority PAHs with ITD detection. Chromatographic conditions described in the text. See Table 1 for peak identifications.

In view of these problems, the selection of an appropriate processing method for retarding formation of PAHs is very important. This paper deals with an overview of analysis, formation and inhibition of PAHs in foods.

TOXICITY OF PAHS

Due to the wide distribution of PAHs in the environment, the possibility of contamination of foods by PAHs has become a major concern for the public. Many reports have shown that the prolonged exposure of human body to the environment containing PAHs may induce some fatal diseases such as lung cancer and skin cancer [33,34]. According to Ames test and animal study, some PAHs such as benzo[a]pyrene and dibenz[a,h]anthracene have proved to be mutagenic and carcinogenic [35]. Many researchers believe that the formation of neoplasia may arise from the somatic mutation of body cells, which is caused by DNA damage. Haugen et al. [36] postulated that benzo[a]pyrene could be oxidized to benzo[a]pyrene-7, 8-oxide by cytochrome p-450 in the body, which in turn is hydrolyzed to benzo[a]pyrene-7, 8-diol-9, 10-epoxide by epoxidehydrolase, and then is further oxidized to benzo[a]pyrene-7,8-diol-9,10-epoxide by cytochrome p-450. The formation of benzo[a]pyrene-7, 8-diol-9, 10-epoxide, which react with DNA, could thus affect the replicating function of DNA. Of the 16 priority PAHs, benzo[a]pyrene has been reported to be the most carcinogenic [1,2]. Therefore, benzo[a]pyrene is

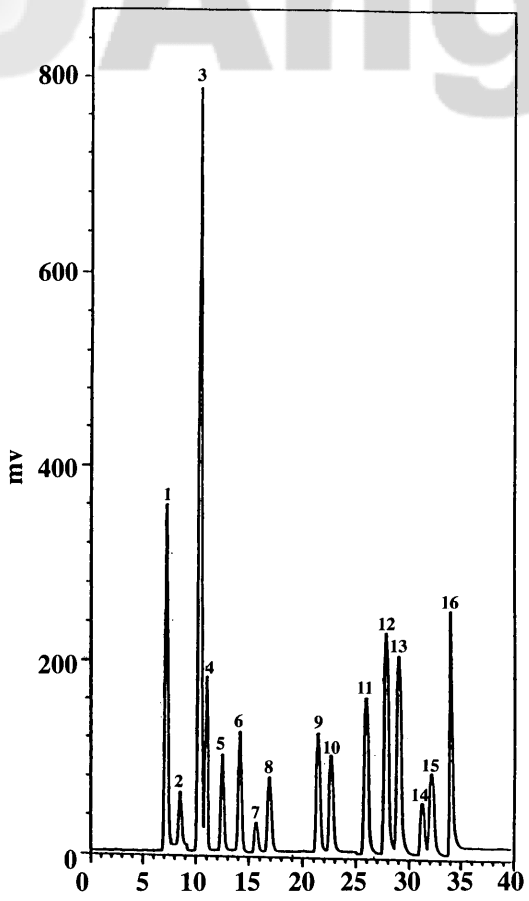

Figure 3. HPLC chromatogram of 16 priority PAHs produced by programmable fluorescence detection. The following settings were used (excitation wavelength/ emission wavelength): λ_1 = 270 nm/340 nm (Peaks 1,3,4), λ_2 = 320 nm/533 nm (Peak 2), λ_3 = 254 nm/375 nm (Peak 5), λ_4 = 260 nm/420 nm (Peaks 6-7), λ_5 = 254 nm/390 nm (Peaks 8-10), λ_6 = 260 nm/420 nm (Peaks 11-15), λ_7 = 293 nm/498 nm (Peak 16). Chromatographic conditions described in the text. See Table 1 for peak identifications.

usually used as a reference indicator of air of food pollution. In 1987 the joint FAO/WHO Expert Committee on Food Additives announced that the amount of benzo[a]pyrene in foods should not exceed 10 ppb. Grimmer [37] reported that PAHs containing four or more rings are more susceptible to inducing malignant tumors in mice than those containing two or three rings. Similar results were observed by Davis et al., [34] and Tuominen et al. [38]. These authors demonstrated that PAHs with four or more rings, or with nitro groups are more carcinogenic than with other PAHs. Apparently the toxicity or carcinogenicity of PAHs is well correlated with the structure and position of the benzene ring.

ANALYSIS OF PAHS IN FOODS

I. *Extraction*

The major problems associated with analysis of PAHs in foods are as follows: (1) Most PAHs are present in trace amounts (ppb or ppt) in foods, which can make extraction difficult; (2) Many PAH-like impurities can be coextracted with PAHs from foods, which can interfere with the subsequent separation and identification of PAHs; (3) Most PAHs are structurally similar and present in isomeric forms, which make their separation and identification difficult.

The most common method for extraction of PAHs from foods usually involves saponification of lipids by methanolic KOH, followed by liquid-liquid partition and liquid-solid chromatography. Joe et al. [39] extracted PAHs from wheat germ by sonication with cyclohexane as the extracting solvent, followed by purification with partition and column chromatography. The recovery obtained ranged between 78 and 97% Kolarovic and Traitler [40] extracted PAHs from vegetable oil by cyclohexane and a caffeine-formic acid solution, followed by purification with a silica gel column and high-performance thin-layer chromatography (HPTLC). In another study, Joe et al. [10] used 1,1,2-trichloro-1,2,2-trifluoroethane to extract PAHs from alcoholic KOH digests of smoked meat, followed by purification with a deactivated silica gel-alumina column and partition between dimethyl sulfoxide and cyclohexane. Lawrence and Weber [23] extracted PAHs from meat and fish products by saponification of lipids with methanolic KOH under reflux, followed by purification with a Florisil column and partition with dimethyl sulfoxide and hexane. Takatsuki et al. [41] extracted PAHs from fish by alkaline digestion, extraction with n-hexane, and purifica-

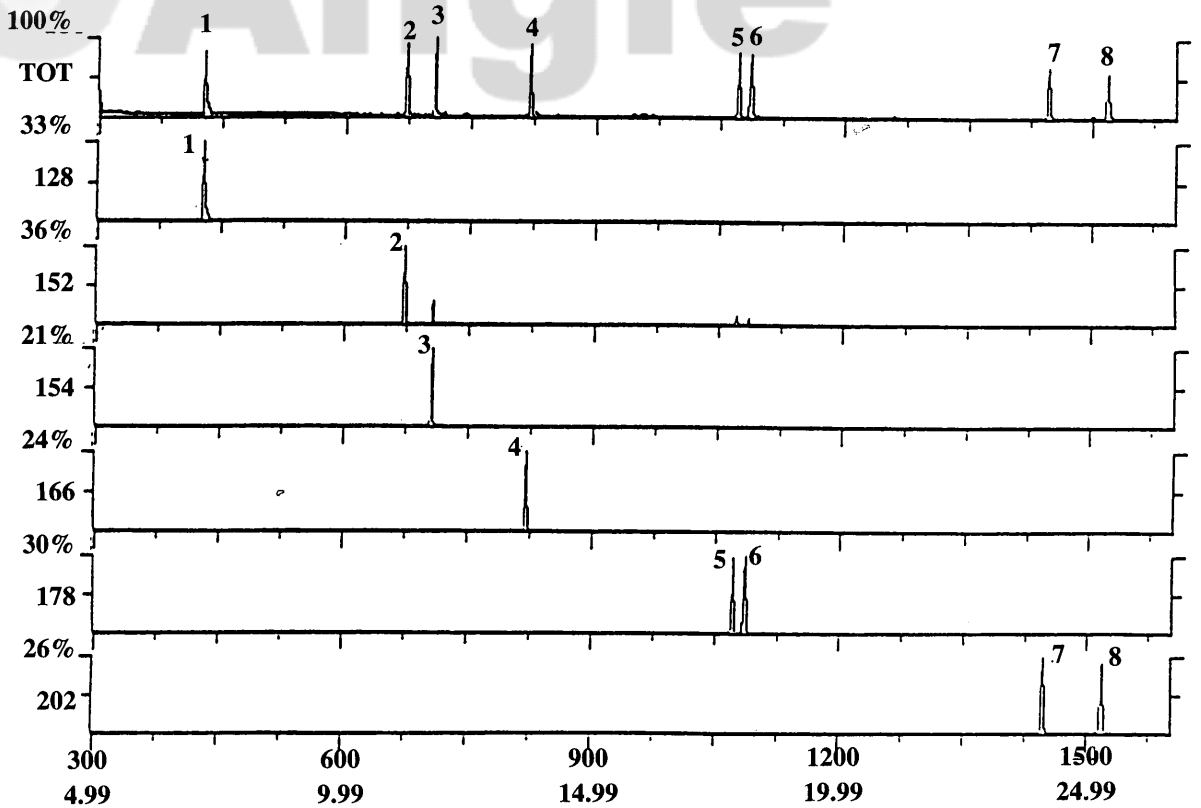

Figure 4. Total and reconstructed ion chromatograms of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene standards detected by ITD. See Table 1 for peak identifications.

tion with a silica gel column. A similar study was conducted by Hopia et al. [42], who used cyclohexane and dimethylformamide to extract PAHs from vegetable oil, followed by purification with a SiO_2 column. Coates and Elzerman [43] pointed out that the extraction of PAHs from plant tissue by sonication is a convenient and reliable method. Karlesky et al. [44] compared two extraction procedures, i.e., dimethyl sulfoxidepentane partition and solid phase (amino-bonded packing material) extraction, and found that the former resulted in a lower recovery than the latter. Dong et al. [45] developed a fast method to extract PAHs from soil, water and waste oil. This method is easy since only sonication is used for extraction; however, the HPLC system may be contaminated. In a recent study Chen et al. [15] evaluated several extraction methods of PAHs from meat products and concluded that the soxhlet extraction of PAHs followed by purification with a Sep-Pak Florisil cartridge removed more impurities than the sonication method. The authors also reported that saponification and partition with dimethyl sufoxide and cyclohexane are necessary for extraction of PAHs from meat products by UV detection, however, with fluorescence detection, partition can be omitted. Nevertheless, many PAH-like impurities are still present on the HPLC chromatogram, indicating that the development of a more sophisticated method is necessary for removal of these impurities from samples.

II.*Analysis of PAHs by GC-MS*

The separation of PAHs has been previously achieved by thin-layer chromatography (TLC). Doremire et al. [20] used TLC to determine 3,4-benzopyrene in roasted meat. However, the method proved to be lengthy and failed to resolve various PAHs in foods. Gas chromatography in combination with mass spectrometry (GC-MS)

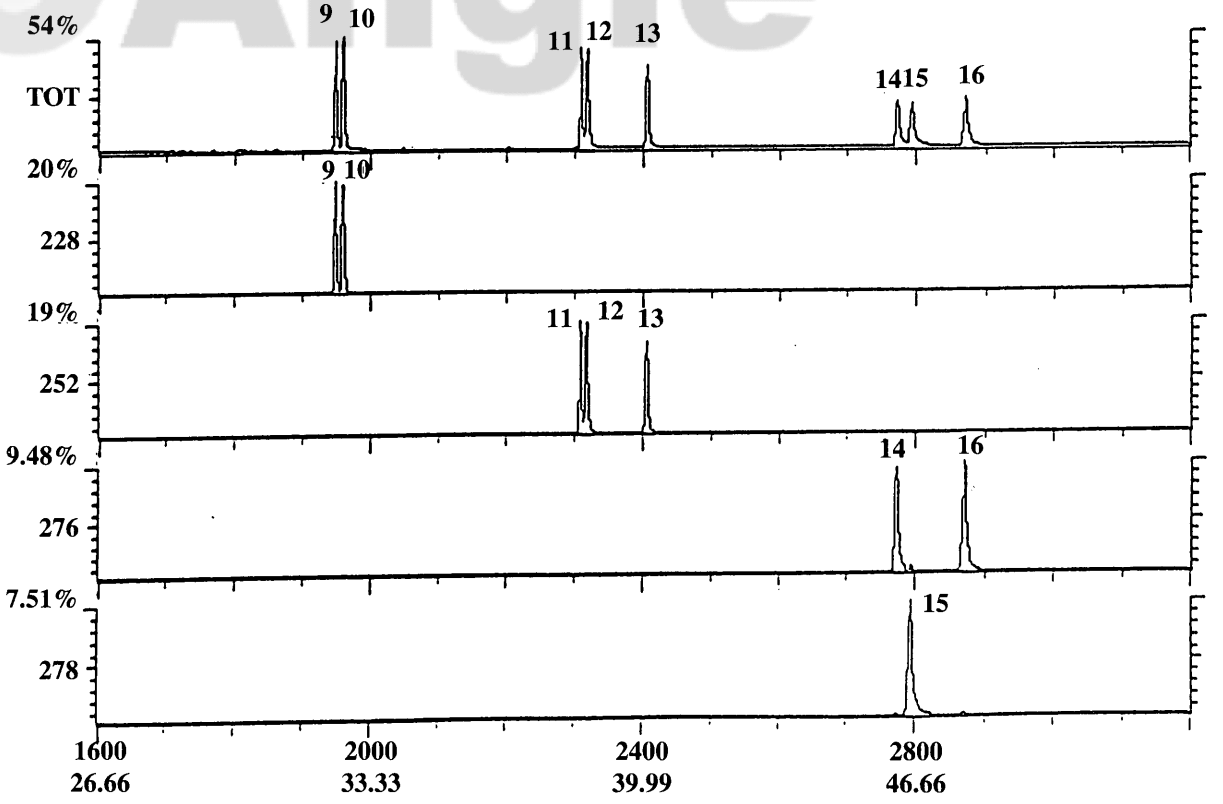

Figure 5. Total and reconstructed ion chromatograms of benzo[a]anthracene, chrysene, benzo[a]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-c,d]pyrene standards detected by ITD. See Table 1 for peak identifications.

has been widely used to determine PAHs in foods. Kolarovic and Traitler [40] determined PAHs in vegetable oil by GC with a glass capillary column (30 m×0.3 mm I.D.) coated with OV-17-SE-30 (1:1), and a temperature programming method with flame ionization detection (FID) and mass spectrometry (MS). Nineteen PAH standards were resolved, however, some peaks, phenanthrene and anthracene, and benzo[b]fluoranthene and benzo[k]fluoranthene, were partially overlapped. The amounts of total PAHs ranged from 22.93 ppb in grapeseed oil to 750.25 ppb in peanut oil. Afolabi et al. [46] analyzed PAHs in some Nigerian preserved freshwater fish species by GC with a methyl silicone quartz capillary column (25 m×0.2 mm i.d.) coated with SE-54, and a temperature programming method with a FID detector and a MS. A total of 37 PAH standards were resolved. The authors also reported that the carcinogenic and mutagenic PAHs in the smoked products were 2-10 times higher than products from the other preservation methods. In a similar study, Lawrence and Weber [23] used a DB-5 30-m fused silica column to separate 18 PAH standards by GC with FID and MS detection, and the total carcinogenic PAH levels ranged from non-detectable up to several hundred ppb in some fish products. Karlesky et al. [44] resolved 16 PAHs standards by using a 30-m, DB-5 fused silica capillary column coated with SE-54 of 0.25 mm i.d. and a temperature programming method with FID detection. However, phenanthrene and anthracene (peaks 5 and 6), benzo[b]fluoranthene and benzo[k]fluoranthene (peaks 11 and 12), dibenzo[a,h] anthracene and indeno[1,2,3-c,d]pyrene (peaks 14 and 15) were partially overlapped. Hopia et al. [42] determined PAHs in vegetable oil by GC with a

Table 1. Retention times of 16 priority PAHs using UV, fluorescence (FL) and ion-trap detection (ITD)[b].

Compound	Peak number	Retention time (min)[a]		
		UV	FL	ITD
Naphthalene	1	6.9	7.0	7.1
Acenaphthylene	2	8.2	8.3	11.2
Acenaphthene	3	10.1	10.1	11.5
Fluorene	4	10.7	10.8	13.4
Phenanthrene	5	12.2	12.3	17.5
Anthracene	6	13.9	14.0	18.0
Pyrene	7	15.5	15.5	24.1
Fluoranthene	8	16.7	16.8	25.2
Benzo[a]anthracene	9	21.3	21.4	32.2
Chrysene	10	22.5	22.6	32.4
Benzo[b]fluoranthene	11	25.8	25.9	38.3
Benzo[k]fluoranthene	12	27.7	27.8	38.4
Benzo[a]pyrene	13	28.8	28.9	40.0
Dibenzo[a,h]anthracene	14	31.1	31.2	46.3
Benzo[g,h,i]perylene	15	32.0	32.1	47.5
Indeno[1,2,3-c,d]pyrene	16	33.3	34.0	40.1

[a] Average of five injections.
[b] Data are from a report by Chiu et al. (1996)

capillary column (25 m × 0.20 mm I.D. SE-54 fused silica) and a temperature programming method with MS (selected ion monitoring, SIM) detection. A total of 33 PAHs were resolved, and the amounts of total PAHs ranged from 0.71 to 4600 ppb in vegetable oil. Tuominen et al. [38] analyzed PAHs in cereal products by employing a cross-linked methyl silicone capillary column (12 m × 0.20 mm i.d.) and a temperature programming method with MS. A total of 25 PAHs were resolved and the amounts of total PAHs ranged from 25 to 4500 ppb in cercal products. Castello and Gerbino [47] developed a GC-MS technique to resolve 16 PAH standards by using a DB-5 capillary column, 5% phenyl-95% methyl polysiloxane-bonded phase, 30 m × 0.32 mm I.D., film thickness 0.25 μm, and a temperature programming method with FID and ion-trap mass detector (ITD). Johnston et al. [48] further used GC-ITD to analyze PAHs in smoke-exposed food and packing materials from three separate fires. A DB624 capillary column (75 m × 0.53 mm i.d.) with 3-μm film thickness and a temperature programming method with ITD were used to determined 4 PAHs, and the amounts of naphthalene ranged from 1 to 4200 ppb. In a recent study Chiu et al. [49] developed a temperature programming method and used a GC-ITD technique to resolve 16 PAH standards (Figure 2). A DB-5 capillary column (30 m × 0.32 mm I.D.) with 0.25 μm film thickness and a Varian 1077 splitless injector with flow rate at 1.0 ml/min was used. Although capillary GLC has higher resolution power, PAHs may be degraded if exposed to high temperatures during separation. Also, a number of isomeric PAHs such as benzo[b]fluoranthene and benzo[k]fluoranthene are difficult to separate. In view of these problems, many high-performance liquid chromatographic (HPLC) methods have been developed to separate PAHs from foods.

Table 2. Total PAHs and carcinogenic PAHs contents (ppb) in duck breast steak by various processing methods[c]

	Time(hr)	Total PAHs	Carcinogenic PAHs[a]
steaming	0.5	4.3	ND[b]
	1.0	8.51	ND
	1.5	8.71	ND
roasting	0.5	89.59	14.44
	0.6	115.53	15.37
	0.8	127.72	15.02
smoking	0.5	154.23	18.68
	1.0	174.33	20.31
	1.5	206.35	35.14
	2.0	317.65	41.48
	3.0	526.76	52.57
charcoal-grilling of skined duck	0.5	151.42	10.22
	1.0	200.21	11.61
	1.5	299.72	16.00
charcoal-grilling of duck without skin	0.5	182.01	24.36
	1.0	275.91	23.97
	1.5	319.28	24.75
liquid smoke flavoring		87.14	1.37
liquid-smoked duck breast steak		0.32	ND

a. Carcinogenic PAHs include benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, and dibenzo[a,h]anthracene.
b. ND: not detectable.
c. Data are from a report by Chen and Lin (1996)

III. *Analysis of PAHs by HPLC*

Schmidt et al. [50] was the first to use a chemically-bonded C_{18} column to separate PAHs. Since then, reversed-phase HPLC based on a C_{18} column has become the most popular mode for separation of PAHs. Lawrence and Weber [24] developed an isocratic solvent system of water-acetonitrile (30:70, v/v) to separate 15 PAHs with a C_{18} column and flow rate at 2.0 or 3.0 ml/min. Detection was made by UV at 254 nm and fluorescence at 250 nm/>370 nm (excitation/emission). However, the separation time of this method is too long (60 min) and the capacity facor (k') is between 0.45 and 35.64 as reported by Chen et al. [15]. In addition, some peaks (acenaphthene and fluorene)(were) overlapped. Takatsuki et al. [41] employed an isocratic solvent system of acetonitrile-H_2O (80:20, v/v) and a C_{18}

column with flow rate at 1.0 ml/min and detection by fluorescence (370 nm/410 nm) to resolve 10 PAHs. However, some peaks, anthracene, fluoranthene, and pyrene were not adequately resolved. To improve the separation some authors used gradient elution instead. Joe et al. [39] developed a gradient solvent system, which consisted of water (A) and methanol-acetonitrile (50:50, v/v) (B). In the beginning 80% B was increased to 100% B in 20 min, then held at 100% B for 20 min. After equilibration of the column, 100% B was changed to 80% B in 5 min, and held at 80% B for 20 min. A C_{18} column with flow rate at 1.0 ml/min and detection by UV or fluorescence was used to resolve 13 PAHs. The drawback of this method resides with the solvent system employed which is too complicated, and some peaks, benzo[b]fluoranthene and benzo[a]pyrene, were partially overlapped. Yabiku et al. [14] used the same method to resolve seven PAHs with fluorescence detection at 365 nm/418 nm. Gomma et al. [28] developed a binary gradient system, in which the initial mobile phase was 60% acetonitrile in water for 5 min, then changed to 100% acetonitrile in 15 min, held at 100% for 15 min, then decreased to 60% acetonitrile over 10 min, and held at 60% for 15 min. All 16 PAHs were detected by UV at 254 nm while 12 PAHs were detected by fluorescence at 254 nm/375 nm. In a recent study Chen et al. [15] developed a gradient solvent system of acetonitrile-water (55:45, v/v), which was initially maintained for 2 min, then linearly programmed to 100% acetonitrile over a 23-min period, and maintained for 15 min. All 16 PAHs were adequately separated by a C_{18} column with detection by UV at 254 nm. However, acenaphthene and fluorene were sometimes partially overlapped depending on environmental temperature. In a later study Chiu et al. [49] further reported that with environmental temperature at 20°C both acenaphthene and fluorene were adequately resolved. Furthermore, with fluorescence detection and six settings of programmable wavelength only 15 PAHs were detected because acenaphthylene has too low of a fluorescence quantum yield to be detected as reported by Chen et al.,[15] To remedy this problem an increased concentration of acenaphthylene and the use of seven settings of programmable wavelength yielded results in which all 16 PAHs could be detected simultaneously [49](Figure 3).

IV. *Comparison of Extraction of PAHs by Sonication and Saponification-partition*

An ideal extraction technique theoretically should allow complete removal of impurities from sample so that the subsequent separation and identification of PAHs by GC-MS or HPLC would not be disturbed. Saponification is often employed to remove unwanted lipid, free fatty acid and water-soluble impurities. However, it has been reported that benzo[a]pyrene is decomposed under alkaline conditions [41]. The authors also suggested the addition of Na_2S to the alkaline digestion mixture as an antioxidant can prevent decomposition of benzo[a]pyrene. Purification is often used following saponification to remove some disturbed substances, such as aliphatic hydrocarbons, fatty acids, phenol, and PAH-like substances [49,51,52]. However, many PAH-like impurities are still present in the sample as shown by many fat-like components on both GC and HPLC chromatograms [49]. Thus, a further purification step is necessary. Joe et al. [10] developed a partition procedure by using dimethyl sulfoxide and cyclohexane as partition solvents to remove disturbed substances. Nevertheless, some PAH-like impurities are still present in the sample [15]. In view of this problem a more sophisticated extraction method is required for meat products. Sonication methods have also been used by many authors to extract PAHs from foods [10,23,24,52]. One of the advantages of using sonication is that extraction time and solvent consumption can be greatly reduced. However, in a recent study Chen et al. [15] reported that with soxhlet extraction grilled duck was found to contain four PAHs, while with sonication grilled duck was found to contain only two PAHs. The authors also postulated that some PAHs in duck breast can form complexes with lipid or protein, and solvents such as hexane or

methylene chloride may fail to extract PAHs from the matrix by sonication. Nevertheless, sonication may be applicable to some other types of samples such as plant tissue, soil and water because it is very simple and convenient to operate [45,53].

V. *Comparison of Analysis of PAHs by GC-MS and HPLC*

Theoretically, an ideal separation technique should allow complete resolution of all 16 PAHs with low detection limit, high reproducibility, high sensitivity and short retention time. However, it is often impossible to achieve these goals simultaneously. Therefore, one must choose a particular technique for a specific type of sample so that the best results can be obtained. HPLC is often used in combination with UV, fluorescence or diode-array detection. Fluorescence detection has been found to have 20-320 times higher sensitivity than UV detection [15,45,54,55]. Diode-array detection permits the rapid scanning of PAHs spectra in the sample on the line during HPLC analysis so that a positive identification can be confirmed. Also, the purity of each peak on the chromatogram can be assessed by diode-array detection. GC is often used in combination with flame ionization detection (FID), photoionization detection (PID), multimode ionization detection (MMID), quadrupole analyzer mass spectrometry (QUAD), and ion-trap mass detection (ITD). The highest sensitivity was found for ITD, HPLC with fluorescence detection and QUAD operated in single-ion monitoring (SIM) mode [47]. The detection limits for ITD have been reported to be 2-80 pg, for fluorescence 10-20 pg and for QUAD 100-500 pg [47]. One of the drawbacks in using HPLC with fluorescence detection is that the proper excitation and emission wavelength has to be selected for a particular PAH, and thus, the maximum sensitivities for all 16 PAHs are difficult to obtain simultaneously even if various settings of programmable wavelength are employed at the same time. For QUAD, the characteristic ion mass has to be free of interferences. Thus, for complicated food samples such as meat products, the application of fluorescence or QUAD would be difficult for identification and quantitation. The newly developed ion-trap mass detector permits the entire mass spectra to be obtained with a sensitivity greater than that of QUAD (SIM) and the identification of compounds by means of automatic library search, and the simultaneous use of ITD and FID allows quantitation to be carried out without the need for standard samples of all of the detected PAHs [47]. Johnston et al. [48] further demonstrated that the various PAHs in food samples could be identified even in the presence of fat-or PAH-like impurities. In addition to GC-MS, the combination of HPLC with mass spectrometry (HPLC-MS) is also a powerful tool for identification and quantitation of PAHs. Electron ionization MS with the moving belt interface has been reported to provide high sensitivity and selectivity, as well as structural information such as molecular weight, functional qroups, and elemental composition [56].

The resolution power of a chromatographic system depends primarily upon column efficiency and separation selectivity. GC is often used for separating complex PAHs because of its superior column effciency, however, when a MS detector is used, the separation selectivity for PAHs isomer becomes more important than column effciency. Thus, HPLC can often provide more selective separation of PAHs isomers as reported by Quilliam and Sim [56]. The authors also reported that more peaks are observed in LC/MS mass chromatograms than in those from GC-MS. Interestingly, in a recent study Chiu et al. [49] reported that with GC-ITD more PAH compounds were identified in smoked chicken than those with HPLC and diode-array detection. Apparently the difference may be attributed to the presence of many PAH-like impurities in the sample, which can interfere with the subsequent identification of PAHs by HPLC. In addition, the presence of isomeric PAHs can still be easily distinguished through reconstructed ion chromatogram by GC-ITD [48,49]. Many minor peaks present on the total ion chromatogram of smoked chicken were reported by Chiu et al. [49]. However, with reconstructed ion chromatogram

the various PAHs in smoked chicken can still be clearly distinguished by comparison with that of PAH standards, which are shown in Figures 4 and 5. Table 1 shows the retention times of 16 PAHs using fluorescence and ITD detection. The retention time of GC is higher than that of HPLC, and a number of isomeric PAHs are partially overlapped (Figure 2 and Table 2).

FORMATION OF PAHS IN FOODS

I. *Grilling*

It has been well documented that the various processing methods such as smoking and grilling can induce formation of PAHs in foods. Lijinsky and his coworkers [15,17] were the first to observe the formation of carcinogenic PAHs in charcoal-grilled and smoked foods. The benzo[a]anthracene concentrations ranged from non-detectable to 50.4 ppb and 31.0 ppb were detected, respectively. The authors also postulated that during grilling the melted fat which drips on the hot charcoals is pyrolyzed under high temperature, and the PAHs formed in the smoke are then deposited on the meat surface as the smoke rises. In addition, the authors also found that fatty meats yield grilled products with higher levels of PAHs than lean meat. These findings were further supported by Fritz [18], Toth and Blaas [19], Doremire et al. [20], Larsson et al., [9], and Maga [11]. In general, the higher grilling temperature, the greater the formation of PAHs. Masuda et al. [16] reported that only minor amounts of PAHs were formed by grilling fish in an electric broiler. However, no carcinogenic PAHs were observed in the grilled products heated by electric source [17]. This result was further confirmed by Grimmer and Hidebrandt[57] ,Toth and Blaas[19], Lintas and De Matthaeis[8], and Larsson et al.[9]. The variation of PAH levels in grilled foods is directly dependent upon the method of cooking and type of heat source used. Grilling over charcoal, the most frequently used fuel, results in low PAH levels, depending on the fat content of the food and which form the fat is present. Also, the use of pine or spruce cones gives rist to high PAH levels in the grilled product [9,18,19,58,59]. In a review dealing with the formation of PAHs in meat products during grilling, Fretheim [21] concluded that the best ways to prevent formation of PAHs were to (1) avoid excessive heating, (2) keep melted fat away from heat source, and (3) use lean meat for grilling.

II. *Smoking*

The presence of PAHs in smoked foods has been investigated by Lijinsky and Shubik[5], Potthast and Eigner[6], Panalaks[7], Lintas and De Matthaeis[8], Larsson[9], Joe et al.[10], Maga[11], Simko et al.[13], Gomaa et al.[28], Yabiku et al.[14], and Chen et al.[15], The variation of PAHs in smoked foods can be attributed to the type of generator, temperature of combustion, degree of smoking, time of preparation, wood composition, and fat content of the products [5,11,60,61]. Maga[12] reported that the smoke source can dramatically influence both the PAHs level and the type of compounds present in the smoke and subsequently their deposition on the surface of smoked foods. In a study dealing with the effect of mesquite wood on PAHs formation in smoked beef, Maga [11] reported that a total of 31 individual PAHs having a composite concentration of 1250 ppb of wood was measured in condensed mesquite wood smoke, while a total of 22 PAHs were found in hickory smoke. The major PAHs in mesquite and hickory smoke included phenanthrene, fluoranthene, and pyrene, with concentrations at 204, 162, and 155 ppb, respectively, for the former and 114, 94 and 104 ppb, for the latter. It was also found that the total PAH concentration for hickory smoke condensate was approximately half that of mesquite. This result demonstrates that the formation of numerous types and amounts of PAHs can be dependent upon the composition of mesquite or hickory wood.

III. *Roasting*

It has been well established that roasting can accelerate PAHs formation, and the amount formed depends upon time and temperature. Lawrence and Weber [24] determinated PAHs in Canadian samples of milk powder and found that

direct heating can induce higher amount of formation of carcinogenic PAHs. Noco [25] also found that the benzo[a]pyrene level can be increased from 0.5 to 2.0 ppb in coffee prepared from oven-toasted coffee bean. In a recent study Chen and Lin [26] reported that eleven PAHs were detected after roasting of duck breast at 200℃ for 40 min, and in most cases the amounts of PAHs increased along with increasing roasting time. The formation of PAHs during roasting may be due to some food components, such as fatty acid, triglyceride and cholesterol, which under high temperature heating may degrade to form PAHs [22].

INHIBITION OF PAHS IN FOODS

Due to the formation of PAHs during smoking of foods, the potential hazard of smoked foods to human health has become a major concern for the public. To remedy this problem some processors used liquid smoke flavoring (LSF) in the smoking process to lower PAHs content in smoked foods [13,14,27,28,29]. It was found that the use of UTP-1 LSF in the smoking process lowered the benzo[a]pyrene concentration by two orders compared to the PAHs content in meat products smoked traditionally [13]. Similar result was reported by Gomaa et al. [28], who found that sausage processed with LSF contained smaller benzo[a]pyrene concentrations than those in sausage and breakfast beef processed with woodsmoke. Apparently the reduction of PAHs content by LSF can be attributed to the sorption of flavor compounds from foods by packing materials such as polyethylene [27,29-32]. To demonstrate this effect Simko and Brunckova [29] investigated the changes in PAH concentrations spiked to LSF which was filled into low-density polyethylene packaging. Results showed that the lowering of PAHs concentration is due to diffusion processes of PAHs into packaging material. The authors postulated that the PAHs migrated from the strongly polar medium into the non-polar medium, where van der Waals dispersive forces had the decisive influence in the sorption of non-polar PAHs into packaging material. In a later study dealing with kinetics of PAHs sorption from LSF into low density polyethylene packaging, Simko et al. [27] concluded that the elimination of PAHs is a complex process which involves the diffusion of PAHs into a solution followed by the sorption on the wall and eventually subsequent diffusion inside the packaging material. Although the PAHs level can be lowered in LSF, the recycling of packaging material may become an important problem. For instance, PAHs concentrated in packaging material from contaminated foods could migrate into non-polar foods such as vegetable oil, sausage, etc. Thus, the exclusion of their use for food packaging should be the best way to prevent recontamination of foods from PAHs.

From the preceding discussion it can be concluded that charcoal-grilling and smoking are the two major processing methods which can accelerate high amount of PAHs formation. However, in addition to charcoal-grilling and smoking, some other processing methods such as steaming can also cause PAHs formation. In a study dealing with the effects of various processing methods on PAHs formation, Chen and Lin [26] concluded that with processing time from 0.5 to 1.5 h, charcoal-grilling of duck meat with skin contained the highest amount of total PAHs, followed by charcoal-grilling of duck meat without skin, smoking, roasting, steaming and LSF. For carcinogenic PAHs, smoking contained the highest amount, followed by charcoalgrilling and roasting, while no carcinogenic PAHs were detected for steaming and LSF (Table 2). Further research is necessary to determine how the PAHs formation during food processing can be retarded through application of LSF or sorption of a specific type of packaging material.

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食品中多環芳香族碳氫化合物的分析、形成與抑制：綜論

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摘　要

多環芳香族碳氫化合物（PAHs）由木材或石油燃燒不完全所形成。PAHs廣泛的分布於自然界的環境且有可能污染到食品，因此PAHs亦為相當重要的毒性化合物。本文主要對於食品中PAHs的分析、形成與抑制作一綜合回顧與討論。一般萃取食品中PAHs的方法都是以Soxhlet法萃取油脂並進行皂化，再以Sep-Pak Florisil cartridge進行純化後再進行分配，此法較超音波震盪法可除去更多雜質。以HPLC進行分析，使用梯度溶劑系統配合紫外光波長（254 nm）或七段式的螢光激發／放射波長可同步分離偵測16種PAHs；以GC進行分析，使用梯度溫度系統和火焰解離偵檢器（FID）或離子捕捉質譜儀（ITD）亦可同步分離偵測16種PAHs。HPLC可以完全分離16種PAHs，但食品中所含的雜質會干擾到PAHs的分離鑑定；GC無法完全分離某些PAHs的異構物，但使用GC-ITD卻可輕易鑑定出食品中的PAHs，且不會受到不純物質的干擾。碳烤與煙燻為兩種會導致致癌性PAHs大量形成的主要加工方法，使用蒸氣和液燻則未發現有致癌性PAHs的形成。食品中的PAHs亦可使用聚乙烯塑膠袋吸附的方法去除。更多的研究必須進行以防止食品於加工過程中PAHs的形成。

關鍵詞：多環芳香族碳氫化合物，氣相層析法－質譜儀，高效率液相層析法，加工方法。