Determination of Polycyclic Aromatic Hydrocarbons in Commercial Liquid Smoke Flavorings of Different Compositions by Gas Chromatography−Mass Spectrometry

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The presence of polycyclic aromatic hydrocarbons (PAHs) in five commercial liquid smoke flavorings, used in the European food industry, was studied. The samples were subjected to an alkaline treatment, extracted with cyclohexane, cleaned up by means of solid-phase extraction tubes, and analyzed by gas chromatography−mass spectrometry. Three different procedures for the cleanup were tested. The results revealed the presence of 34 PAHs, some of them with methyl substituents. In all cases, the concentrations of compounds of low molecular weight were much higher than those of high molecular weight. Relationships between smoke flavoring compositions and PAH levels were also studied. Three of the samples contained high levels of both total and carcinogenic PAHs. Benzo[*a*]pyrene was also detected in these three samples, but its concentration did not exceed the 10 μg/kg level fixed by the FAO/WHO. Finally, a relation was found, first between the concentrations of total carcinogenic PAHs and benzo[*a*]pyrene and also between the concentrations of pyrene and benzo[*a*]pyrene. The latter ratio reveals that pyrene concentration could be very useful in predicting the level of benzo[*a*]pyrene and, consequently, in estimating the carcinogenicity arising from the presence of benzo[*a*]pyrene and other carcinogenic PAHs.

Keywords: *Polycyclic aromatic hydrocarbons; smoke flavorings; gas chromatography−mass spectrometry*

INTRODUCTION

The occurrence of polycyclic aromatic hydrocarbons (PAHs) in a wide range of foodstuffs has been described (Joe et al., 1979; Dennis et al., 1983; Lawrence and Weber, 1984a,b; Takatsuki et al., 1985; De Vos et al., 1990; Chen et al., 1996; Moret et al., 1997). However, many studies have been carried out on smoked products because of the high amounts of PAHs detected in these types of foods (Thorsteinsson, 1969; Fretheim, 1976; Joe et al., 1984; Larsson et al., 1988; Gomaa et al., 1993; Karl and Leinemann, 1996). Traditional smoking techniques, in which smoke from incomplete wood burning comes into direct contact with the product, can lead to its contamination with PAHs if the process is not adequately controlled. For this reason, smoke flavorings are used as an alternative to the aforementioned traditional smoking.

The usual method for producing liquid smoke flavorings is to pyrolyze wood and collect the smoke produced in water (Hollenbeck, 1964) or by simple condensation; smoke preparations in edible oils and in hydroalcoholic or vinegar solutions are also used in the food industry (Girard, 1991). Moreover, the smoke can be adsorbed on solids such as spices, salt, sugars, starch, or proteins, resulting in dry or powdered forms (Tóth and Potthast, 1984). According to some authors, the most outstanding benefits derived from smoke flavorings are flavor reproducibility (Hollenbeck, 1964) and the possibility of controlling the PAH content of the smoked products (Gomaa et al., 1993; Yabiku et al., 1993). A lowering of the PAH content in products smoked with this type of flavoring has been reported by some authors (Gomaa et al., 1993; Chen and Lin, 1997). In addition to those advantages already pointed out, others can also be mentioned (Maga, 1988): uniform distribution of flavor throughout the product; intensifying of the flavor of traditionally smoked foods; application to a wide range of foodstuffs; possibility of use at the consumer as well as at the commercial level; savings in costs because wood and smoking equipment are not required; less environmental pollution associated with the use of smoke flavorings; and a variety of application methods such as spraying on the surface, dipping, or mixing with the food.

On the other hand, despite the benefits mentioned, it must not be forgotten that smoke flavorings come from natural wood smoke, so if PAHs are present in the smoke, they could also be present in the flavorings. Therefore, if the conditions of the smoke generation process are not controlled or the PAHs generated are not eliminated, these compounds could be present in smoke flavorings, with their consequent risk for human health. In fact, for several decades, the presence of various levels of PAHs in smoke and smoke flavorings has been reported (White et al., 1971; Potthast, 1979; Maga, 1986; Simko et al., 1992; Gomaa et al., 1993; Yabiku et al., 1993). For this reason and, taking into account the carcinogenic properties of some of these compounds, many authors have emphasized the need for a more exhaustive study of the occurrence of PAHs, both in smoke flavorings and in the foods processed with them. In relation to the elimination of PAHs from liquid

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smokes, some authors (Gorbatov, 1971; White et al., 1971) have emphasized the importance of removing the residual tars that settle out of the liquid smoke flavorings during storage. Gorbatov (1971) has also pointed out that purification steps in the production of liquid smoke flavorings do not need to be exhaustive if they are used for food surface treatments. Nevertheless, if they are going to be incorporated internally, certain components must be eliminated.

Taking into account that smoke flavorings are widely used as an alternative to traditional smoking and that, as has been mentioned, different levels of PAHs have been found in these preparations, this paper evaluates the PAH contents of some commercial liquid smoke flavorings being used in the European food industry. The smoke flavorings selected have very different compositions. The aim of this paper is to determine if the PAH contents of these preparations are related to their composition and if their use in foods could constitute a real risk to human health. All possible PAHs, alkylated or not, present in the samples and able to be studied by gas chromatography−mass spectrometry (GC-MS), are subject of the study.

EXPERIMENTAL PROCEDURES

Samples. The samples are five commercial liquid smoke flavorings designated A, B, C, D, and E. Sample A is a smoke flavoring constituted mainly of phenol, guaiacol, and syringol derivatives, with a small proportion of lignin dimers and trimers and an insignificant proportion of carbonyl and carboxyl derivatives (Guillén and Ibargoitia, 1998, 1999). Samples B and C are smoke flavorings that contain typical smoke components in similar proportions to those found in smoke; both could be considered to be smoke condensates (Guillén and Manzanos, 1996). Smoke flavoring D also presents typical smoke components showing similar proportions to those found in smoke; however, the concentrations of its components are lower than in samples B and C. Finally, sample E also contains typical smoke components, with higher proportions of phenol, guaiacol, and syringol derivatives than of carbonyl and carboxyl derivatives; the concentrations of the components of this flavoring are also lower than those of samples B and C (Guillén et al., 1995).

Reagents and Materials. Solvents employed were dichloromethane, cyclohexane, methanol, and *n*-hexane. Dichloromethane, cyclohexane, and methanol were all of HPLC grade (99.9+%), and *n*-hexane was capillary GC grade (99+%). Other reagents and materials used were potassium hydroxide, anhydrous sodium sulfate, 6 mL Supelclean LC-Florisil SPE tubes (1 g), 3 mL Supelclean LC-Si SPE tubes (500 mg), and sodium chloride. All solvents, reagents, and materials mentioned are commercially available from Sigma, Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), Supelco (Bellefonte, PA), and Merck (Darmstadt, Germany).

Standards. A commercial mixture of deuterated standards dissolved in dichloromethane, containing 1,2-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} in concentrations of 4 mg/mL, was used. Two other PAH standard cyclohexane solutions were used: one containing anthracene, fluoranthene, pyrene, chrysene (50 μg/mL each), fluorene, benz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*ah*]anthracene, and benzo[*ghi*]perylene (10 μg/mL each), and the other containing 1-methylfluoranthene (10 μg/mL). Pure naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, 1,6-dimethylnaphthalene, phenanthrene, 9-methylanthracene, 3,6-dimethylphenanthrene, *m*-terphenyl, *p*-terphenyl, 11*H*-benzo[*b*]fluorene, triphenylene, benzo[*e*]pyrene, coronene, dibenzo[*ai*]pyrene, pyrene-d_{10}, and *p*-terphenyl-d_{14} were also used. The purity of these standards range from 97 to 99.5%. All pure standards and solutions were obtained from Sigma, Aldrich (Steinheim, Germany), Supelco (Bellefonte, PA), and Symta (Madrid, Spain).

Method. *Alkaline Treatment of the Samples.* Approximately 3.6 g of potassium hydroxide dissolved in 32 mL of methanol and the mixture of deuterated internal standards were added to 10 g aliquots of the samples of liquid smoke flavorings, and the whole mixture was heated for 3 h under reflux.

Extraction of PAHs. After the alkaline treatment, the samples were extracted by shaking in a separation funnel with 240 mL of cyclohexane plus 120 mL of methanol 50% in water, in eight steps. Afterward, the extracts were concentrated to a smaller volume and washed three times with distilled water and a small amount of sodium chloride to make phases separate more easily. Finally, the extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL.

Cleanup Procedure. The cleanup of the extracts was carried out by means of solid-phase extraction (SPE) tubes. Two adsorbents were used, Florisil and silica, and different elution sequences were tested, to find the procedure that led to cleaner extracts and which allowed satisfactory PAH recoveries. Procedure 1 was performed as follows: The cyclohexane extract of an aliquot of smoke flavoring E was passed through a Florisil tube, filled with 1 g of the adsorbent, and PAHs were eluted with 10 mL of hexane plus 10 mL of 30% dichloromethane in hexane; this eluate was concentrated to 1 mL, passed through a second Florisil tube, and eluted with 1.5 mL of hexane first (fraction 1) and with 8.5 mL of hexane plus 10 mL of 30% dichloromethane in hexane (fraction 2). In procedure 2, the cyclohexane extract was passed through a Florisil tube and eluted as in procedure 1. The eluate obtained, after concentration to 1 mL, was passed through a silica tube, filled with 500 mg of the adsorbent, and eluted with 1 mL of hexane first (fraction 1) and 9 mL of hexane (fraction 2). Finally, in procedure 3 the extract was passed through a Florisil tube and eluted as in the other procedures. The eluate was then concentrated and passed through a silica tube, but this time the elution of PAHs was carried out with 1 mL of cyclohexane (fraction 1) and 9 mL of cyclohexane (fraction 2). Whichever procedure was used, the second fraction from the second tube was again concentrated to 1 mL and analyzed by GC-MS, whereas fraction 1 was discarded.

Identification and Quantitation by GC-MS Technique. It was carried out by means of a Hewlett-Packard gas chromatograph model HP 6890 Series, equipped with a 5973 mass selective detector and a Hewlett-Packard Vectra XM series 4 computer. The column used was a fused-silica capillary column (60 m long × 0.25 mm i.d. × 0.25 μm film thickness), coated with a nonpolar stationary phase (HP-5MS, 5% phenyl methyl siloxane). The operation conditions were the following: The oven temperature was set initially at 50 °C (0.50 min hold), increased to 130 °C at 8 °C/min, and again increased to 290 °C at a rate of 5 °C/min (50 min hold); the temperatures of the ion source and the quadrupole mass analyzer were kept at 230 and 150 °C, respectively; helium with a purity of 99.999% was used as carrier gas at a constant flow of 1.0 mL/min; injector and detector temperatures were held at 250 and 280 °C, respectively; pulsed splitless mode was used for injection with a pressure pulse of 30 psi, and 1 μL of each sample was introduced in the gas chromatograph. The data acquisition mode employed was mainly selective ion monitoring (SIM).

When scan mode is used, identification is based on both the retention times of the compounds and their mass spectra. Nevertheless, when the SIM mode is employed, complete mass spectra do not exist, so identification is based on the retention time of the main ion of the mass spectrum characteristic of each compound together with the relative abundances of the other ions selected for each PAH. When PAHs are studied, the main and most abundant ion of their mass spectra is the molecular ion, and the relative abundances of the other two or three major ions must be calculated in relation to the former. These values are compared with those obtained for standard compounds and, if they agree within a set interval (a 20% margin), the compound can be definitely identified as

Table 1. Ions (*m*/*z* Values) Selected for the Identification of Some PAHs and Relative Abundances in Relation to the Molecular Ion

compound	ions	relative abundances
naphthalene	128, 126, 102	100, 6.63, 6.43
2-methylnaphthalene	142, 141, 115	100, 83.73, 24.39
1-methylnaphthalene	142, 141, 115	100, 86.24, 26.62
2,6-dimethylnaphthalene	156, 141, 155	100, 65.28, 33.60
fluorene	166, 165, 163	100, 91.25, 14.40
phenanthrene	178, 176, 179	100, 17.74, 14.54
anthracene	178, 176, 179	100, 17.02, 14.42
9-methylanthracene	192, 191, 189	100, 62.85, 28.96
3,6-dimethylphenanthrene	206, 191, 189, 205	100, 33.56, 28.95, 23.24
fluoranthene	202, 200, 101	100, 19.45, 17.53
pyrene	202, 200, 101	100, 20.08, 18.25
m-terphenyl	230, 231, 228	100, 20.52, 15.40
p-terphenyl	230, 231, 228	100, 19.39, 12.38
11*H*-benzo[*b*]fluorene	216, 215, 213	100, 91.19, 19.78
1-methylfluoranthene	216, 215, 213	100, 90.94, 21.23
benz[*a*]anthracene	228, 226, 229	100, 25.97, 19.02
chrysene	228, 226, 229	100, 27.42, 18.84
triphenylene	228, 226, 229	100, 30.04, 19.49
5-methylchrysene	242, 241, 239, 240	100, 50.70, 37.92, 15.15
7,12-dimethylbenz[*a*]anthracene	256, 241, 239, 240	100, 52.20, 41.11, 28.62
benzo[*b*]fluoranthene	252, 250, 253	100, 22.50, 21.96
benzo[*e*]pyrene	252, 250, 253	100, 31.36, 23.91
benzo[*a*]pyrene	252, 250, 253	100, 22.81, 21.39
perylene	252, 250, 253	100, 26.56, 21.09
indeno[123-*cd*]pyrene	276, 277, 274	100, 21.99, 18.77
dibenz[*ah*]anthracene	278, 279, 276	100, 22.08, 19.83
benzo[*ghi*]perylene	276, 277, 274	100, 23.41, 20.26
coronene	300, 150, 301	100, 22.76, 22.39
dibenzo(ai)pyrene	302, 303, 300	100, 21.66, 16.64

a certain PAH. Table 1 shows the ions (*m*/*z* values) selected for the identification of each PAH, as well as their relative abundances in relation to the molecular ion. Asterisked compounds in Table 3 were identified by taking into account their retention times (Baumard et al., 1999) as well as the relative abundances of the ions selected for their identification.

With regard to quantitation in SIM mode, this is based on the measurement of the peak area correspondent to the molecular ion of each compound, and it was carried out by means of the deuterated internal standards previously mentioned. Thus, naphthalene-d_8 was used for quantitation of naphthalene and its methyl derivatives; phenanthrene-d_{10} for phenanthrene, anthracene, and their methyl derivatives; pyrene-d_{10} for fluoranthene and pyrene; *p*-terphenyl-d_{14} for *m*-terphenyl, *p*-terphenyl, and methylfluoranthenes/pyrenes; chrysene-d_{12} for benz[*a*]anthracene and chrysene; and, last, perylene-d_{12} for PAHs with higher molecular weights. The response factors of each compound relative to the internal standard chosen for its quantitation were calculated. Some authors (Baumard et al., 1997) have mentioned the suitability of employing deuterated internal standards. These compounds are always absent from the sample, and their physical and chemical properties match with those of the target analytes, so their recoveries can be considered the same. It must also be pointed out that more than one internal standard needs to be used to guarantee the accuracy of the measurements (Baumard and Budzinski, 1997) because groups of PAHs with different sizes and shapes behave in different ways. However, despite the advantages derived from the use of this type of internal standard, there are very few papers (Nyman et al., 1993) dealing with the determination of PAHs in foods where deuterated compounds have been used.

RESULTS AND DISCUSSION

The first step of the study includes an alkaline treatment of the sample, followed by extraction with cyclohexane. These extracts contain many smoke components in addition to the PAHs and, for this reason, a cleanup step is also essential to eliminate interfering compunds. As has been previously mentioned, three

Table 2. Recovery Percentages of Deuterated Internal Standards Added to the Samples, after Different Cleanup Procedures

	procedure 1	procedure 2	procedure 3
phenanthrene-d_{10}	90.34 ± 5.56	97.10 ± 8.61	69.03 ± 11.43
pyrene-d_{10}	93.51 ± 2.18	99.29 ± 6.53	73.02 ± 13.25
p-terphenyl-d_{14}	94.89 ± 1.29	99.04 ± 5.77	88.08 ± 6.89
chrysene-d_{12}	90.05 ± 2.57	90.39 ± 5.75	88.57 ± 7.09
perylene-d_{12}	84.57 ± 1.75	53.06 ± 7.89	84.85 ± 2.51

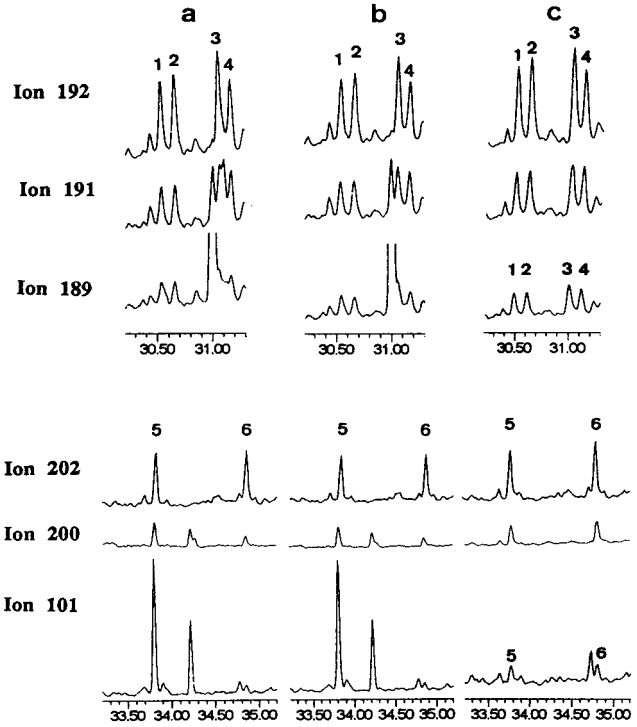

Figure 1. Extracted chromatograms of ions 192, 191, 189, 202, 200, and 101 in (a) the eluate coming from the first Florisil tube used for the cleanup (procedures 1−3), (b) the second fraction from the second Florisil tube (procedure 1), and (c) the second fraction from the silica tube (procedures 2 and 3). The peaks designated 1, 2, 3, 4, 5, and 6 correspond to 3-, 2-, 9-, and 1-methylphenanthrenes, fluoranthene, and pyrene, respectively.

cleanup procedures were tested. As indicated under Experimental Procedures, procedure 1 involved the use of two Florisil tubes and PAHs were collected in the second fraction obtained from the second tube. To determine the effectiveness of this procedure, the recoveries of the deuterated PAHs, added at the beginning of the process as internal standards, were determined from the second fraction collected from the second Florisil tube and are shown in Table 2. It must be pointed out that recoveries of naphthalene-d_8 and acenaphthene-d_{10} are not included because a high proportion of these compounds elutes in the first fraction. It can be observed that the recovery percentages for the rest of the deuterated PAHs are very similar for all of the compounds (90.05−94.89%) except for that of perylene-d_{12}, which is slightly lower (84.57%).

However, despite the good recoveries obtained with procedure 1, the eluate coming from the second Florisil tube still contained several interfering ions, as can be observed in Figure 1. This shows the extracted ion chromatograms corresponding to the ions selected for the identification of 3-methylphenanthrene (1), 2-methylphenanthrene (2), 9-methylphenanthrene (3), and 1-methylphenanthrene (4) (*m*/*z* 192, 191, and 189) and

Table 3. PAHs Detected in Smoke Flavorings A−E and Their Concentrations, Expressed in Micrograms per Kilogram

	smoke flavoring				
	A	B	C	D	E
naphthalene	677.55 ± 47.16	508.99 ± 43.57	62.47 ± 9.29	9.74 ± 0.04	18.92 ± 1.97
2-methylnaphthalene	351.97 ± 47.09	98.94 ± 16.09	52.01 ± 10.46	1.72 ± 0.05	7.69 ± 0.36
1-methylnaphthalene	363.90 ± 28.35	81.24 ± 13.17	94.98 ± 71.12	2.42 ± 1.05	6.16 ± 1.39
2,6-dimethylnaphthalene	127.21 ± 10.29	13.21 ± 3.00	25.72 ± 3.05	0.77 ± 0.01	3.28 ± 0.36
dimethylnaphthalene	151.97 ± 2.40	13.41 ± 5.78	33.47 ± 3.24	0.52 ± 0.02	2.80 ± 0.35
1,6-dimethylnaphthalene	118.66 ± 10.71	10.96 ± 0.36	23.86 ± 4.54	0.80 ± 0.13	3.13 ± 0.06
fluorene	380.45 ± 13.15	33.79 ± 0.37	119.96 ± 2.67	0.62 ± 0.04	1.22 ± 0.04
phenanthrene	664.92 ± 39.33	38.53 ± 0.89	56.93 ± 5.42	0.94 ± 0.18	2.37 ± 0.16
anthracene	118.36 ± 0.06	8.81 ± 0.45	11.79 ± 0.01		
3-methylphenanthrene*	35.27 ± 1.61	3.51 ± 0.06	12.52 ± 0.43	0.24 ± 0.03	0.61 ± 0.08
2-methylphenanthrene*	39.17 ± 1.23	3.79 ± 0.15	14.06 ± 0.54	0.35 ± 0.03	0.81 ± 0.02
2-methylanthracene*	17.18 ± 0.39	1.60 ± 0.04	5.64 ± 0.15		0.79 ± 0.18
9-methylphenanthrene*	31.80 ± 2.23		13.98 ± 0.05	0.27 ± 0.02	0.63 ± 0.16
1-methylphenanthrene*	21.23 ± 0.73	2.58 ± 0.06	9.25 ± 0.44		
9-methylanthracene	1.75 ± 0.16				
dimethylphenanthrene/anthracene	3.39 ± 0.37	0.65 ± 0.11	3.23 ± 0.41		
fluoranthene	43.26 ± 0.61	12.11 ± 2.43	19.28 ± 0.14	0.20 ± 0.01	0.66 ± 0.01
pyrene	33.50 ± 0.77	12.71 ± 1.18	22.62 ± 0.03	0.16 ± 0.01	0.64 ± 0.04
m-terphenyl	2.27 ± 1.17	1.13 ± 0.11	0.86 ± 0.01	0.14 ± 0.06	0.33 ± 0.10
p-terphenyl	1.01 ± 0.26	0.54 ± 0.35	0.66 ± 0.26	0.12 ± 0.06	0.30 ± 0.01
methylfluoranthene/pyrene	1.95 ± 0.28	1.71 ± 0.03	3.49 ± 0.16		
methylfluoranthene/pyrene	3.03 ± 0.82	2.72 ± 0.36	5.44 ± 0.10	0.02[a]	
methylfluoranthene/pyrene	2.40 ± 0.52	2.14 ± 0.18	4.64 ± 0.13		
benz[*a*]anthracene	4.41 ± 0.07	1.53 ± 0.01	3.50 ± 0.24	0.07 ± 0.03	0.11 ± 0.02
chrysene + triphenylene	3.34 ± 0.11	1.42 ± 0.00	3.03 ± 0.00		0.25 ± 0.01
benzo[*b*]fluoranthene	1.48 ± 0.08	0.60 ± 0.19	1.31 ± 0.18		
benzo[*k*]fluoranthene	1.72 ± 0.18	0.78 ± 0.17	1.45 ± 0.15		
benzo[*a*]fluoranthene*	0.62 ± 0.01	0.27 ± 0.08	0.39 ± 0.01		
benzo[*e*]pyrene	1.74 ± 0.20	0.72 ± 0.06	1.37 ± 0.01		0.08 ± 0.01
benzo[*a*]pyrene	2.86 ± 0.39	1.11 ± 0.12	2.18 ± 0.08		
perylene	0.59 ± 0.30	0.29 ± 0.09	0.37 ± 0.00		
indeno[123-*cd*]pyrene	1.57 ± 0.20	0.38 ± 0.04	1.10 ± 0.00		
dibenzanthracene	0.41 ± 0.03	0.22 ± 0.19	0.51 ± 0.37		
benzo[*ghi*]perylene	1.94 ± 0.09	0.63 ± 0.02	1.70 ± 0.53		
total PAHs	3195.35	854.26	599.32	19.31	50.78
carcinogenic PAHs	17.53	6.76	14.45	0.07	0.44
carcinogenic PAHs/benzo[*a*]pyrene	6.13	6.09	6.63		
pyrene/benzo[*a*]pyrene	11.71	11.45	10.38		

[a] Identified in only one of the aliquots.

of fluoranthene (5) and pyrene (6) (*m*/*z* 202, 200, and 101), both in the eluate coming from the first Florisil tube (Figure 1a) and in the second fraction collected from the second Florisil tube (Figure 1b). It can be noticed that, even though some interfering ions are removed with the second Florisil tube, the eluate obtained is not clear enough to allow a correct identification of the PAHs. For this reason, the second cleanup procedure described under Experimental Procedures was tested, in which the mixture of hexane/dichloromethane used in procedure 1 to elute PAHs from the second tube was substituted by hexane. Some authors (Moret et al., 1996) have pointed out that the PAH retention ability of silica is lower than that of Florisil, so possibly hexane could be able to elute all PAHs, avoiding the use of dichloromethane, which shows high affinity for some interfering smoke flavoring components. Thus, the eluate obtained from the silica tube is cleaner than that of Florisil, as can be observed in Figure 1c, which corresponds to the second fraction from the silica tube. The recovery percentages obtained with procedure 2 are also given in Table 2. It can be observed that recoveries of phenanthrene-d_{10}, pyrene-d_{10}, *p*-terphenyl-d_{14}, and chrysene-d_{12} are very similar or even slightly higher than those obtained with procedure 1; however, the recovery of perylene-d_{12} is considerably lower.

A third cleanup procedure was tested to increase the recovery of perylene-d_{12}. This involved the use of a Florisil tube followed by a silica tube, as in procedure 2, but using cyclohexane to obtain the eluate from this second tube. The recovery percentages are given in Table 2 (procedure 3). It can be noticed that recoveries of phenanthrene-d_{10}, pyrene-d_{10}, and *p*-terphenyl-d_{14} are lower than those obtained with procedures 1 and 2. This is probably due to the fact that these compounds elute in the first fraction in a higher proportion than in procedure 2, so the amount recovered in the second fraction is lower. With regard to chrysene-d_{12} and perylene-d_{12}, it is observed that their recoveries are of the same order as those achieved in procedure 1. Because the latter procedure provided the best results concerning a correct identification of PAHs and the recoveries obtained were also good, it was decided to apply it to the rest of the smoke flavoring samples.

As previously mentioned, five commercial liquid smoke flavorings were studied. Table 3 gives the results obtained, expressed in micrograms per kilogram. These values come from duplicate analyses of two different aliquots of each liquid smoke flavoring sample. It is noteworthy that a great number of PAHs have been detected of both low and high molecular weights. In addition, the presence of methyl and dimethyl PAH derivatives should be noted. To the best of our knowledge, the occurrence in commercial smoke flavorings of such a great number of PAHs has not been reported before. Some authors (Potthast, 1979; Maga, 1986) have also found a wide number and range of PAHs in smoke

coming from various smoke generators or from different wood sources, including methyl and dimethyl derivatives.

In all of the samples, the concentration of the low molecular weight PAHs is higher than that of high molecular weight compounds and, in general, as the molecular weight of the PAHs increases, their concentrations decrease. Moreover, the samples in which the levels of PAHs with low molecular weights are low are almost free of PAHs of high molecular weight. These results are in agreement with those obtained by Potthast (1979) and Maga (1986) for smoke; however, these findings are not supported by other authors (Gomaa et al., 1993; Yabiku et al., 1993). Besides, in agreement with Potthast (1979) and Maga (1986), the concentrations of methyl and dimethyl PAH derivatives in all of the samples are lower than those of the corresponding parent PAHs; for example, the levels of methylphenanthrenes are always lower than that of phenanthrene.

Among the samples studied, there are three, A, B, and C (see Table 3), that have higher concentrations of PAHs than the other two, samples D and E. Sample A has the highest concentrations of these compounds. The composition of this flavoring differs considerably from that of smoke, because it is constituted almost exclusively of lignin monomers and dimers (phenol, guaiacol, and syringol derivatives). The high concentration of PAHs in this sample could be due to the use of very high temperatures in its manufacturing process (Tóth and Potthast, 1984) or to the concentration of PAHs during the elimination of the carbonyl and carboxyl derivatives present in smoke. Samples B and C are smoke condensates in which smoke components and also PAHs are in significant concentrations; however, samples D and E are smoke flavorings in which both the smoke components and the PAHs are in low concentrations. Therefore, the concentration of PAHs in these four latter samples are in agreement with the levels of the other smoke components.

Results in Table 3 show that, in general, the smoke flavorings here studied have much lower PAH levels than the smoke samples studied by Potthast (1979) and Maga (1986). Thus, the levels of PAHs in the smokes reported by the first author range between 70 and 170 μg/kg for benzo[*a*]pyrene and between 1460 and 3830 μg/kg for anthracene; these compounds were found by Maga to vary between 41 and 74 μg/kg and between 31 and 47 μg/kg, respectively. On the other hand, Gomaa et al. (1993) found levels of PAHs in commercial smoke flavorings ranging between 0.1 and 3.4 μg/kg for benzo[*a*]pyrene and between 0.1 and 6.8 μg/kg for anthracene, whereas Yabiku et al. (1993) reported levels ranging between 0.1 and 336.6 μg/kg for benzo[*a*]pyrene and between 1.3 and 2240.3 μg/kg for anthracene.

In relation to the content of benzo[*a*]pyrene, which is the most commonly determined PAH, it must be noticed that it has been detected only in samples A−C, which are those with higher total PAH contents, and their levels are of the same order as those found by Gomaa et al. (1993). However, these concentrations do not exceed the limit of 10 μg/kg fixed by the FAO/WHO for liquid smoke flavorings (FAO/WHO, 1987) in any of the cases. It must be pointed out that there are other PAHs for which no legal limits exist but having carcinogenic properties that can be considered, in some cases, of the same order as those of benzo[*a*]pyrene, as can be seen in Table 4. This table shows the degree of carcinogenicity of a group of PAHs, expressed by means of the Iball index *I* (Szentpály, 1984; Barone et al., 1996), which is proportional to the fraction of subject animals that show a carcinogenic response divided by the mean latent period. Therefore, there are PAHs apart from benzo[*a*]pyrene for which levels cannot be ignored when the risk derived from the use of these preparations as flavoring agents is evaluated. The concentrations of total and carcinogenic PAHs in all of the samples, expressed in micrograms per kilogram, are given in Table 3. It must be noticed that PAHs with uncertain carcinogenicity have also been included in the group of carcinogenic PAHs. Table 3 also shows the ratios between the total concentration of carcinogenic PAHs and that of benzo[*a*]pyrene and between the concentrations of pyrene and benzo[*a*]pyrene. It can be observed that, in general, the flavorings with higher total PAH concentrations (A−C) also have higher levels of carcinogenic PAHs, whereas flavorings D and E, which have low PAH concentrations, do not contain either benzo[*a*]pyrene or other carcinogenic PAHs except for benz[*a*]anthracene and chrysene. A close relationship has been found between the level of carcinogenic PAHs and the level of benzo[*a*]pyrene, as can be observed in Table 3; thus, the ratio between both groups is near 6 in the samples in which these compounds have been detected. These findings agree with those of Potthast (1979), who stated that the ratio of benzo[*a*]pyrene to high molecular weight PAHs hardly varies. On the other hand, another relation has been observed between the concentrations of pyrene and benzo[*a*]pyrene, which ranges from 10.38 in sample C to 11.71 in A. Because this value also seems to be quite constant, pyrene concentration could be considered as a guide to benzo[*a*]pyrene concentration. This has the advantage of predicting the presence of the latter compound and of estimating its level using pyrene concentration. This determination is easier than that of benzo[*a*]pyrene because of the higher concentrations of pyrene and its good chromatographic resolution, free of interferences. Nevertheless, despite the usefulness of this latter ratio, the levels of other carcinogenic PAHs cannot be ignored and all of them must be considered when the risk derived from the consumption of foods treated with smoke flavorings is evaluated.

Table 4. Carcinogenicity of Some PAHs According to the Iball Index *I*

compound	*I*	compound	*I*
dibenzo[*ai*]pyrene	74	benzo[*c*]phenanthrene	04
benzo[*a*]pyrene	72	dibenz[*aj*]anthracene	04
dibenzo[*ah*]pyrene	68	dibenz[*ac*]anthracene	03
dibenzo[*ae*]pyrene	50	benzo[*e*]pyrene	02
dibenz[*ah*]anthracene	26	phenanthrene	00
benz[*a*]anthracene	07	fluoranthene	00
chrysene	05	triphenylene	00

From a practical point of view, given that the recommended dose of smoke flavorings in foods ranges from 1 to 3 g/kg, with a maximum value of 6 g/kg for some flavorings, it can be concluded that none of the preparations studied would lead to a benzo[*a*]pyrene level of 0.03 μg/kg in the final products, which is the maximum allowed as a result of treatment with smoke flavorings. However, because benzo[*a*]pyrene represents only a small percentage of the total carcinogenic PAHs detected (15−16%), further studies should be carried out on the global effect of all carcinogenic PAHs and legal measures should be updated.

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