Polycyclic Aromatic Hydrocarbons in Liquid Smoke Flavorings Obtained from Different Types of Wood. Effect of Storage in Polyethylene Flasks on Their Concentrations

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Smoke flavorings are widely used as an alternative to the traditional smoking techniques. Smoke generation conditions can determine the level of polycyclic aromatic hydrocarbons (PAHs) in the smoke and, consequently, in these preparations. In this paper, the influence of the wood source on the formation of PAHs is studied. For this purpose, five liquid smoke flavorings, obtained from different types of wood, were used. Sample aliquots, including deuterated internal standards, were subjected to an alkaline treatment, extracted by liquid−liquid partition and cleaned up by means of silica tubes, followed by gas chromatography−mass spectrometry analysis. The results reveal that the flavoring obtained from poplar wood presents the highest number and concentrations of both total and carcinogenic PAHs, even though the levels of these latter are very low. It has also been observed that the storage of smoke flavorings in polyethylene flasks reduces the concentration of some PAHs.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) constitute a widespread group of contaminants in the environment and in foods (Guillén et al., 1997). There are studies which point to cereals, fats, oils, and derived products as mainly responsible for the daily intake of PAHs (Dennis et al., 1983; De Vos et al., 1990; Lodovici et al., 1995). However, smoked products have traditionally received special attention because considerable amounts of PAHs have been detected (Fretheim, 1976; Joe et al., 1984; Larsson et al., 1988; Gomaa et al., 1993; Karl and Leinemann, 1996). The presence of PAHs in these foods has been attributed to the smoking process, especially when the smoke comes into direct contact with the products.

It has been well documented that smoke generation conditions can dramatically influence the level of resulting PAHs in the smoke and, consequently, in smoked foods (Maga, 1988). Studies on the influence of smoking technology (Tóth and Potthast, 1984) have revealed that contamination with benzo[*a*]pyrene, which is often taken as a marker of the presence of PAHs, shows a close relationship to smoke generation conditions. Parameters such as wood moisture content, air supply, and combustion temperature affect the generation of PAHs. Some authors have showed that moistening the wood source during smoking gives rise to smoke with lower PAH concentrations than dry woods, because it lowers the smoke generation temperature (Maga, 1988). For the same reason, a low air supply during pyrolysis also limits the production of PAHs. On the other hand, Tóth and Blaas (1972) noted a linear increase in benzo[*a*]-pyrene concentration as the smoke generation temper-

ature was increased from 400 to 1000 °C, even though, according to other authors (Möhler, 1980), the production of PAHs decreases with temperatures higher than 800 °C. Another factor considered to be related to the production of PAHs is the wood nature. The use of hardwoods instead of softwoods has been recommended to reduce the presence of PAHs in smoke and in smoked foods (Maga, 1988). However, there are not many studies on the influence of this factor in the level of PAHs in the smoke produced, and they are not in total agreement. Results obtained by Potthast (1979) show that the PAH concentrations found in smoke coming both from softwood (pine) and from hardwood (beech) are very similar. However, another study on PAHs in fish smoked using different woods (Larsson, 1982) revealed that softwoods show a slight tendency to produce higher concentrations of heavy PAHs. For these reasons, in this paper, the influence of wood nature on the PAH content of the smoke flavorings produced is studied. Five liquid smoke flavorings were obtained in our laboratory from dry sawdust of different sources, keeping the air supply constant. The maximum temperature reached during the smoke generation was very similar in all the experiments, so that all the parameters considered as influencing PAH generation, except for the wood nature, remained the same. The effect of storing the samples in polyethylene flasks on their PAH concentrations was also studied.

EXPERIMENTAL PROCEDURES

Materials and Reagents. Solvents employed were cyclohexane and methanol, which were both HPLC grade (99.9+%).

Other reagents and materials used were potassium hydroxide, anhydrous sodium sulfate, Supelclean LC-Si solid-phase extraction (SPE) tubes, 3 mL (500 mg), and sodium chloride.

All solvents, reagents, and materials above-mentioned are commercially available from Sigma, Aldrich (Steinheim, Ger-

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many), 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 and chrysene (50 µg/mL each) and fluorene, benz[a]anthracene, 7,-12-dimethylbenz[a]anthracene, benzo[b]fluoranthene, benzo-[k]fluoranthene, benzo[a]pyrene, perylene, indene[1,2,3-cd]-pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene (10 µg/mL each), and the other 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, 11H-benzo[a]fluorene, 11H-benzo-[b]fluorene, triphenylene, benzo[e]pyrene, pyrene-d_{10}, and p-terphenyl-d_{14} were also used. The purities 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).

Samples. The samples were five liquid smoke flavorings obtained in the laboratory from dry sawdust coming from different woods. Only sawdust particles smaller than 2 mm were used in the smoke generation. The process was carried out in a round-bottom flask smoke generator made of quartz. The pyrolysis was started with the use of a rheostat-controlled heating mantle, keeping the air supply constant for all the samples. The temperature was measured with a Crison thermometer 639K positioned in the center of the charge of sawdust. The smoke resulting from 100 g of sawdust was filtered by means of a glass wool filter and collected by bubbling in 150 mL of distilled water. The aqueous liquid smoke obtained was again filtered through a paper filter and, finally, stored in glass and polyethylene flasks. The woods selected for the study were oak, cherry tree, beech, poplar, and vine shoots, and the maximum temperatures reached during the process in each case were, respectively, 530, 550, 532, 536, and 559 °C. From each sample, two 10 g aliquots were taken.

It must be pointed out that the smoke flavorings obtained from beech, poplar, and vine shoots were stored in both glass and polyethylene flasks. The storage time was approximately four years for poplar and vine shoot samples, and two and a half years for beech.

Procedure. Each aliquot from the five smoke flavorings was filtered to avoid the presence of solid particles. Then, the cyclohexane internal standards solution including naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, p-terphenyl-d_{14}, chrysene-d_{12}, and perylene-d_{12}, with concentrations ranging from 0.1109 to 0.7164 µg/mL, was added, and the mixture subjected to an alkaline treatment with potassium hydroxide and methanol, by heating for 3 h under reflux. This treatment has been proved to be useful for the removal of a high proportion of interfering smoke flavoring components (Guillén et al., 2000a). Next, PAHs were extracted by liquid–liquid partition with cyclohexane, and the final extract was washed with distilled water, adding a small amount of salt to make the phases separate more easily. The extract was concentrated to a smaller volume, dried over anhydrous sodium sulfate, and finally concentrated to 1 mL. The concentrated extract was cleaned up by means of two SPE tubes filled with 500 mg of silica, activated with cyclohexane. Silica SPE tubes are preferable to Florisil tubes because the former make it possible to obtain high recoveries of PAHs by eluting them only with cyclohexane, which reduces the presence of interfering smoke flavoring compounds which elute if dichloromethane is used. The elution of PAHs from the first tube was carried out with 9 mL of cyclohexane, and the eluate was concentrated to 1 mL. This eluate was passed through a second SPE tube, but this time, two fractions were collected: the first with 1 mL of cyclohexane and the second with 9 mL of cyclohexane. The reason for the use of a second SPE silica tube is, above all, the separation of PAHs from linear hydrocarbons, which can interfere in the determination of the former, especially of

benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, and perylene. Fraction 1 was discarded, since it contained only some PAHs with low molecular weight which had been previously identified and quantified in the eluate from the first silica tube, and fraction 2 was finally analyzed by gas chromatography–mass spectrometry (GC–MS) for the identification and quantification of the PAHs present. It must be pointed out that, although the general cleanup procedure consists of two steps, in the case of the smoke flavoring obtained from vine shoots an additional cleanup step was necessary because of the presence of a higher amount of interfering compounds.

In the case of the flavorings from beech, poplar, and vine shoots, aliquots were also taken from polyethylene flasks.

Identification and Quantification of PAHs. This was carried out with a Hewlett-Packard gas chromatograph model HP 6890 Series, equipped with a mass selective detector 5973 and a Hewlett-Packard Vectra XM Series 4 computer. The column used was a fused-silica capillary column (60 m long × 0.25 mm inner diameter × 0.25 µm film thickness), coated with a nonpolar stationary phase (HP-5MS, 5% phenyl methyl siloxane). The operation conditions of the GC–MS were the following: the temperature of the injection port was held at 250 °C; the oven temperature was set initially at 50 °C (0.50 min hold), increased to 130 °C at 8 °C/min and 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 gas was used as the carrier gas at a constant flow of 1 mL/min; the 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 selective ion monitoring (SIM). Identification of PAHs was based on the retention time of standard compounds and on the main ion of the mass spectrum characteristic of each compound together with the relative abundances of two more major ions; for the identification of asterisked PAHs in Table 1, the retention times given by Baumard et al. (1999) were used, together with the relative proportions of the ions selected for these compounds found in a reference coal tar pitch sample. On the other hand, quantification was based on the area of the peak corresponding to the main ion selected for each compound. Deuterated PAHs were used as internal standards, and they were added at the beginning of the process.

RESULTS AND DISCUSSION

As has been described previously, the samples were subjected to an alkaline treatment, extracted with cyclohexane, dried over anhydrous sodium sulfate, and cleaned up by means of silica SPE tubes, before the determination of PAHs by GC–MS (SIM). A typical SIM chromatogram of the second fraction obtained from the second silica tube, corresponding to the beech flavoring sample stored in a glass flask, is given in Figure 1. Table 1 shows the PAHs identified in the five samples of liquid smoke flavorings stored in glass flasks and their concentrations, µg/kg. Each value comes from duplicate analyses of two different aliquots from each smoke flavoring sample, and only peaks with a signal-to-noise ratio equal to or higher than 3 have been considered. The concentrations of total and carcinogenic PAHs are also given in this table.

First, it must be noticed that Table 1 shows a wide range of PAHs, which include compounds with two, three, four, and five aromatic rings, such as naphthalene, phenanthrene, pyrene, and benzo[a]pyrene, even though compounds with low molecular weights are the most abundant. It must also be pointed out that PAH concentrations follow two similar patterns in all the samples: First, the concentrations of PAHs generally decrease as the molecular weight of the compounds

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Table 1. Concentrations of the PAHs Identified in Some Smoke Flavorings from Different Woods, Stored in Glass and in Polyethylene (PE) Flasks, μg/kg

	oak	cherry tree	beech		poplar		vine shoots	
	glass	glass	glass	PE	glass	PE	glass	PE
naphthalene	23.56 ± 4.14	23.79 ± 1.46	27.00 ± 0.55	12.63 ± 0.04	172.77 ± 1.17	5.21 ± 2.57	70.51 ± 11.41	7.79 ± 0.10
2-methylnaphthalene	11.70 ± 0.40	9.27 ± 1.56	8.58 ± 0.54	2.89 ± 0.02	109.02 ± 1.00	2.64 ± 0.1	28.43 ± 0.59	1.71 ± 0.01
1-methylnaphthalene	11.00 ± 0.74	9.28 ± 0.32	6.78 ± 0.70	2.84 ± 0.10	81.05 ± 0.10	28.43 ± 1.50	21.71 ± 1.40	2.98 ± 0.01
2,6-dimethylnaphthalene	2.83 ± 0.21	1.64 ± 0.52	2.24 ± 0.20	1.28 ± 0.06	17.90 ± 0.33	1.38 ± 0.06	5.72 ± 2.04	1.10 ± 0.01
dimethylnaphthalene	2.69 ± 0.08	1.74 ± 0.44	2.14 ± 0.19	1.03 ± 0.03	24.36 ± 0.07	0.94 ± 0.04	5.16 ± 0.86	1.02 ± 0.05
1,6-dimethylnaphthalene	3.38 ± 0.08	2.28 ± 0.24	1.84 ± 0.08	1.60 ± 0.03	15.03 ± 0.42	1.09 ± 0.05	4.22 ± 0.92	1.36 ± 0.00
dimethylnaphthalene	1.19 ± 0.03	[a]	0.91 ± 0.11		9.92 ± 1.07			
dimethylnaphthalene					4.20 ± 0.28			
fluorene	2.61 ± 0.04	1.65 ± 0.01	1.58 ± 0.62	1.06 ± 0.01	15.07 ± 0.08	1.15 ± 0.12	2.84 ± 0.13	0.72 ± 0.01
phenanthrene	1.51 ± 0.29	1.17 ± 0.11	2.45 ± 0.01	1.42 ± 0.01	6.07 ± 0.03	1.64 ± 0.21	1.20 ± 0.06	0.58 ± 0.08
anthracene		0.41 ± 0.01			1.75 ± 0.04	0.46 ± 0.04		
3-methylphenanthrene*	0.29 ± 0.07	0.23 ± 0.00	0.37 ± 0.03	0.36 ± 0.01	1.26 ± 0.01	0.32 ± 0.04	0.28 ± 0.01	0.13 ± 0.02
2-methylphenanthrene*	0.40 ± 0.13	0.32 ± 0.01	0.52 ± 0.03	0.52 ± 0.01	1.60 ± 0.01	0.45 ± 0.04	0.41 ± 0.01	0.17 ± 0.03
2-methylanthracene*					0.56 ± 0.03			
9-methylphenanthrene*	0.29 ± 0.07	0.25 ± 0.01	0.41 ± 0.04	0.38 ± 0.02	1.11 ± 0.01	0.35 ± 0.00	0.30 ± 0.00	0.13 ± 0.01
1-methylphenanthrene*	0.26 ± 0.06	0.20 ± 0.02	0.32 ± 0.03	0.31 ± 0.01	0.81 ± 0.01	0.27 ± 0.01		
dimethylphenanthrene/ anthracene			0.18 ± 0.05		0.33 ± 0.03			
dimethylphenanthrene/ anthracene	0.28 ± 0.08		0.46 ± 0.10		0.62 ± 0.06	0.33 ± 0.02		
dimethylphenanthrene/ anthracene						0.54 ± 0.05		
fluoranthene	0.25 ± 0.03	0.24 ± 0.08	0.36 ± 0.04	0.37 ± 0.00	0.53 ± 0.03	0.60 ± 0.01	0.25 ± 0.06	0.15 ± 0.03
pyrene	0.23 ± 0.03	0.24 ± 0.04	0.43 ± 0.11	0.21 ± 0.00	0.62 ± 0.03	0.59 ± 0.06	0.23 ± 0.00	0.17 ± 0.06
m-terphenyl	0.19 ± 0.07	0.28 ± 0.06	0.10 ± 0.04	0.19 ± 0.01	0.10 ± 0.00	0.33 ± 0.01	0.10 ± 0.00	0.13 ± 0.01
p-terphenyl	0.08 ± 0.03	0.28 ± 0.05	0.14 ± 0.08	0.07 ± 0.00	0.07 ± 0.00	0.35 ± 0.08	0.08 ± 0.00	0.08 ± 0.00
11H−benzo[*a*]fluorene					0.49 ± 0.02			
methyl-fluoranthene/pyrene	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00		0.29 ± 0.01	0.04 ± 0.01		
methyl-fluoranthene/pyrene	0.03 ± 0.01	0.04 ± 0.00	0.04 ± 0.01		0.33 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.01 ± 0.00
methyl-fluoranthene/pyrene		0.03 ± 0.01			0.23 ± 0.01	0.04 ± 0.02		
benz[*a*]anthracene	0.12 ± 0.04	0.17 ± 0.04	0.16 ± 0.03	0.07 ± 0.08	0.24 ± 0.01	0.20 ± 0.00	0.05 ± 0.00	0.08 ± 0.02
chrysene+triphenylene	0.09 ± 0.02	0.27 ± 0.16	0.12 ± 0.01	0.11 ± 0.00	0.34 ± 0.00	0.39 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
benzo[*b*]fluoranthene					0.07 ± 0.00	0.07 ± 0.01		
benzo[*k*]fluoranthene					0.07 ± 0.00	0.05 ± 0.01		
benzo[*e*]pyrene					0.03 ± 0.00			
benzo[*a*]pyrene			0.04 ± 0.01		0.06 ± 0.02	0.06 ± 0.00		
perylene						0.07 ± 0.00		
benzo[*ghi*]perylene	0.02 ± 0.00	0.08 ± 0.02	0.05 ± 0.04	0.03 ± 0.00	0.04 ± 0.01	0.06 ± 0.00		
total PAHs	63.03	53.89	57.24	27.37	467.4	47.57	141.62	18.38
carcinogenic PAHs	0.21	0.44	0.32	0.18	0.78	0.77	0.14	0.15

[a] Not identified.
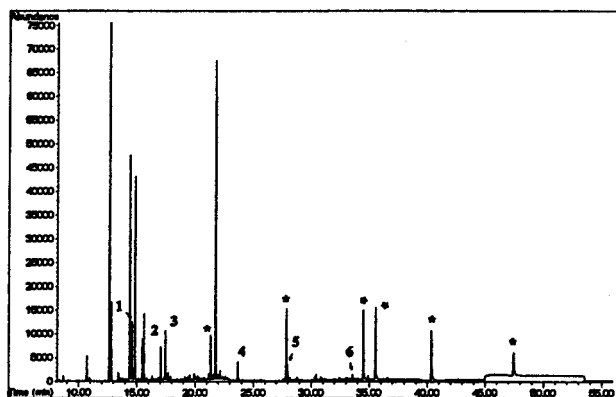
Figure 1. SIM chromatogram of the second fraction obtained from the second silica tube during the cleanup of the beech smoke flavoring sample: (1) Naphthalene; (2) 2-methylnaphthalene; (3) 1-methylnaphthalene; (4) fluorene; (5) phenanthrene; (6) fluoranthene. Asterisked peaks correspond to deuterated internal standards.

increases; second, isomers in each sample present very similar PAH levels, except for anthracene or naphthacene, which are either in lower concentrations than their isomers, or absent.

If we compare the concentrations both of individual and of total PAHs, it is observed that they are very similar in the oak, cherry tree, and beech samples, whereas poplar has not only the highest values for individual and total PAH concentrations, but also the greatest number of identified compounds. The high concentration of PAHs in the vine shoot sample, compared to oak, cherry tree, and beech, is due to higher concentrations of naphthalene and its methyl derivatives in the former. However, the levels of the rest of the PAHs in the vine shoot sample are very similar to those observed in the others; PAHs with molecular weight above 228 have not been identified in this sample.

In relation to all PAHs with high molecular weight, which include most of the carcinogenic compounds, poplar has the highest number, even though in very low concentrations. With regard to the levels of specifically carcinogenic PAHs, the highest value also corresponds to poplar (0.78 μg/kg) and the lowest to vine shoots (0.14 μg/kg). As for benzo[*a*]pyrene, which is the only compound for which a limit has been established, it has been detected in poplar and in beech, but its levels are much lower than the maximum of 10 μg/kg fixed by the FAO/WHO (1987).

Oak, cherry tree, and beech, considered as hardwoods (Pallu, 1971; Maga, 1988), produce less light PAHs than vine shoots (softwood, Pallu, 1971), and these four woods produce less of both light and heavy PAHs than poplar, which is considered as hardwood (Pallu, 1971; Maga, 1988) or softwood (Pallu, 1971) by different authors. Thus, the wood nature influences the amount of PAHs

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produced during its pyrolysis, but individual studies of each wood are necessary to establish the importance of this influence.

Benzo[*a*]pyrene concentration has been considered as an indicator of the carcinogenic PAH concentration in smoke and smoke flavoring samples (Potthast, 1979). Furthermore, in a previous paper (Guillén et al., 2000b), a fairly constant ratio was observed between the concentrations of pyrene and benzo[*a*]pyrene in smoke flavorings, for which reason the possibility of using pyrene concentration as an indicator of benzo[*a*]pyrene and carcinogenic PAH concentrations arose. The explanation for the relation between pyrene and benzo[*a*]-pyrene concentrations could be found in the formation mechanism of these compounds in the wood pyrolysis process; this could occur through pathways in which the condensation of butadiene with PAHs forms new PAHs with an additional aromatic ring (McNeil, 1963; Cypres, 1987). The results obtained here confirm previous results in beech and poplar flavorings, with ratios between concentrations of pyrene and benzo[*a*]pyrene near 10; oak, cherry tree, and vine shoot samples have so low pyrene concentrations that benzo[*a*]pyrene would be under the detection limit of the method, as could be expected from the above.

The low PAH concentrations found in these samples, independently of the wood type, compared with those found in liquid smoke flavorings by other authors (White et al., 1971; Gomaa et al., 1993; Yabiku et al., 1993), reveal that the temperature of the process (530−559 °C) is adequate to obtain smoke flavorings with very low carcinogenic PAH contents; this agrees with the results obtained by some authors, who concluded that the optimum temperature for wood pyrolysis should be between 400 and 600 °C (Girard, 1991).

In a previous paper on changes in smoke flavoring composition during storage in polyethylene receptacles (Guillén and Manzanos, 1996), a migration of some compounds from the flavoring toward the wall of the flasks was observed; compounds such as alkylbenzene derivatives and some alkylated polycyclic aromatic compounds showed this tendency. Other authors (Simko and Bruncková, 1993) have also found a lowering of PAH concentrations in a liquid smoke flavoring stored in polyethylene packaging material. For these reasons, beech, poplar, and vine shoot flavorings were stored in polyethylene flasks, and their PAH content was determined, to study if this type of material could have an influence on their PAH concentrations.

Table 1 gives the PAHs identified in the samples stored in polyethylene flasks and their concentrations, μg/kg. This table also shows the total and carcinogenic PAH concentrations found in these samples. It can be noticed that, in most of the cases, the concentrations of PAHs are lower in the aliquots from polyethylene flasks, and in general, the higher the initial PAH concentration, the greater the decrease observed; thus, the main reduction is found in the poplar sample. This reduction is higher for the lightest PAHs, and as the molecular weight of the compounds increases, the degree of reduction decreases. The variations in PAH concentrations of benz[*a*]anthracene to benzo[*ghi*]perylene are very slight. With regard to methyl derivatives, in general, the dimethyl derivatives undergo less reduction than the monomethyl derivatives; however, a fixed tendency cannot be established for the latter compounds. The lowerings in the concentrations of phenanthrene, an-

thracene, and their monomethyl derivatives, given as the ratio between the concentrations of each PAH in the glass and in the polyethylene flask, are very similar, ranging from 1.0 to 1.7 in beech, from 3.0 to 3.9 in poplar, and from 2.1 to 2.4 in vine shoots. Methylfluoranthenes/pyrenes show a decrease which is higher than that of their parent PAHs and even higher than that of other compounds with lower molecular weights (see the poplar sample in Table 1). The decrease in beech PAH concentrations is smaller than in poplar and vine shoots. This could be explained by the low concentration of PAHs in this sample, especially for those of higher molecular weight, or by its storage time, being approximately half that of poplar and vine shoots.

Therefore, to sum up, it could be stated that when smoke flavorings are kept in polyethylene flasks for a certain period of time, a decrease in the concentration of PAHs is observed, as the concentration in the sample is higher and as the molecular weight of the PAHs is lower.

It only remains to add that the determination of the decrease undergone by those compounds whose concentrations are very close to the detection limits is difficult, because their quantification is subject to greater errors.

LITERATURE CITED

Baumard, P.; Budzinski, H.; Garrigues, P.; Dizer, H.; Hansen, P. D. Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. *Mar. Environ. Res.* **1999**, *47*, 17−47.

Cypres, R. Aromatic hydrocarbons formation during coal pyrolysis. *Fuel Process. Technol.* **1987**, *15*, 1−15.

Dennis, M. J.; Massey, R. C.; McWeeny, D. J.; Knowles, M. E. Analysis of polycyclic aromatic hydrocarbons in UK total diets. *Food Chem. Toxicol.* **1983**, *21*, 569−574.

De Vos, R. H.; Van Dokkum, W.; Schouten, A.; De Jong-Berkhout, P. Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984−1986). *Food Chem. Toxicol.* **1990**, *28*, 263−268.

FAO/WHO. *31st Report of the Joint Committee on Food Additives*; WHO: Geneva, Switzerland, 1987; p 759.

Fretheim, K. Carcinogenic polycyclic aromatic hydrocarbons in Norwegian smoked meat sausages. *J. Agric. Food Chem.* **1976**, *24*, 976−979.

Girard, J. P. The Smoking. *Meat and Meat Products Technology*; Acribia: Zaragoza, Spain, 1991; pp 183−229.

Gomaa, E. A.; Gray, J. I.; Rabie, S.; Lopez-Bote, C.; Booren, A. M. Polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavorings. *Food Addit. Contam.* **1993**, *10*, 503−521.

Guillén, M. D.; Manzanos, M. J. Some changes in an aqueous liquid smoke flavoring during storage in polyethylene receptacles. *Z. Lebensm.-Unters.-Forsch.* **1996**, *202*, 24−29.

Guillén, M. D.; Sopelana, P.; Partearroyo, M. A. Food as a Source of Polycyclic Aromatic Carcinogens. *Rev. Environ. Health* **1997**, *12*, 133−146.

Guillén, M. D.; Sopelana, P.; Partearroyo, M. A. Study of several aspects of a general method for the determination of polycyclic aromatic hydrocarbons in liquid smoke flavorings by gas chromatography−mass spectrometry. *Food Addit. Contam.* **2000a**, *17*, 27−44.

Guillén, M. D.; Sopelana, P.; Partearroyo, M. A. Determination of Polycyclic Aromatic Hydrocarbons in Commercial Liquid Smoke Flavorings of Different Compositions by Gas Chromatography−Mass Spectrometry. *J. Agric. Food Chem.* **2000b**, *48*, 126−131.

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Joe, F. L., Jr.; Salemme, J.; Fazio, T. Liquid chromatographic determination of trace residues of polynuclear aromatic hydrocarbons in smoked foods. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 1076−1082.

Karl, H.; Leinemann, M. Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. *Z. Lebensm.-Unters.-Forsch.* **1996**, *202*, 458−464.

Larsson, B. K. Polycyclic Aromatic Hydrocarbons in Smoked Fish. *Z. Lebensm.-Unters.-Forsch.* **1982**, *174*, 101−107.

Larsson, B. K.; Pyysalo, H.; Sauri, M. Class separation of mutagenic polycyclic organic material in grilled and smoked foods. *Z. Lebensm.-Unters.-Forsch.* **1988**, *18*, 546−551.

Lodovici, M.; Dolara, P.; Casalini, C.; Ciappellano, S.; Testolin, G. Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Addit. Contam.* **1995**, *12*, 703−713.

Maga, J. A. *Smoke in Food Processing*; CRC Press: Boca Raton, FL, 1988.

McNeil, D. The nature and origin of coal tars. *Gas and Coke* **1963**, November, 717−720.

Möhler, K. The Smoking; Acribia: Zaragoza, Spain, 1980.

Pallu, R. Drying and Smoking. Effect of the temperature on meat and meat products. *Meat Products in France*; Pallu, R., Ed.; Paris, France, 1971; Vol. 1, pp 107−139.

Potthast, K. The influence of smoking technology on the composition of polycyclic hydrocarbons in smoked meat products, smoke condensates and in waste gases from smoking plant. *Fleischwirtschaft* **1979**, *10*, 1515−1523.

Simko, P.; Bruncková, B. Lowering of polycyclic aromatic hydrocarbons in a liquid smoke flavor by sorption into polyethylene packaging. *Food Addit. Contam.* **1993**, *10*, 257−263.

Tóth, L.; Blaas, W. The effect of smoking technology on the content of carcinogenic hydrocarbons in smoked meat products. II. Effect of smoldering temperature of wood and of cooling, washing and filtering of smoke. *Fleischwirtschaft* **1972**, *52*, 1419−1422.

Tóth, L.; Potthast, K. Chemical aspects of the smoking of meat and meat products. *Adv. Food Res.* **1984**, *29*, 87−158.

White, R. H.; Howard, J. W.; Barnes, C. J. Determination of polycyclic aromatic hydrocarbons in liquid smoke flavors. *J. Agric. Food Chem.* **1971**, *19*, 143−146.

Yabiku, H. Y.; Martins, M. S.; Takahashi, M. Y. Levels of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons in liquid smoke flavor and some smoked foods. *Food Addit. Contam.* **1993**, *10*, 399−405.