Headspace Solid-Phase Microextraction as a Tool to Estimate the Contamination of Smoked Cheeses by Polycyclic Aromatic Hydrocarbons

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ABSTRACT

Headspace solid-phase microextraction (HS-SPME) was used to study polycyclic aromatic hydrocarbons (PAH) in smoked cheeses. Two types of fiber coatings and different extraction conditions were tested. The results reveal that the use of an 85- μ m polyacrylate fiber immersed in the headspace of the samples at 70°C for 60 min is suitable for the detection of PAH with no more than 4 aromatic rings. To determine if a relationship can be established between the results obtained using a solvent extraction technique and HS-SPME, 6 samples of smoked cheese previously studied by a solvent extraction method were analyzed by HS-SPME, and the results obtained by both methodologies were compared. Polycyclic aromatic hydrocarbons were identified and quantified by gas chromatography-mass spectrometry operating in selective ion monitoring mode. Among the PAH determined by the solvent extraction method, only those with 4 aromatic rings or less were detected by HS-SPME and, consequently, this technique does not allow one to determine the PAH content of smoked cheese samples under the conditions of the study. Nevertheless, the relationship between the results obtained by both techniques for some PAH revealed that HS-SPME could be useful as a screening method to distinguish among samples with different degrees of PAH contamination.

(Key words: headspace solid-phase microextraction, polycyclic aromatic hydrocarbon, smoked cheese)

Abbreviation key: GC-MS = gas chromatography-mass spectrometry, PA = polyacrylate, PAH = polycyclic aromatic hydrocarbons, PDMS = polydimethylsiloxane, SE = solvent extraction method, HS-SPME = headspace solid-phase microextraction.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) constitute a group of contaminants that are widespread in foods, because of environmental contamination or due to certain processes during their manufacture, such as smoking (Guillén et al., 1997). Taking into account that many of these compounds show carcinogenic activity as proved in experimental animals and probable in humans (IARC, 1973; 1983), the occurrence and levels of PAH in foods must be strictly controlled. However, the methods usually used for the determination of PAH in this type of matrix are generally tedious and time-consuming, and require large volumes of organic solvents. Solid-phase microextraction is a quick and simple technique, based on the use of a fused-silica fiber coated with a phase where PAH can be retained. Moreover, the sample amounts required are very small and the use of solvents is not required. Solid-phase microextraction has been used principally for the study of PAH in water samples of different origins (Langenfeld et al., 1996; Negrao and Alpendurada, 1998; Doong et al., 2000a, b), and also in soils (Liu et al., 1997; Doong et al., 2000a; Seduikiene et al., 2000), sediments (Cam et al., 2000; Pino et al., 2003) and air particulate matter (Hageman et al., 1996). The technique used in many cases is direct immersion of the fiber in the samples, but it can also be applied to the headspace (Djozan and Assadi, 1999; Doong et al., 2000a, b; Waidyanatha et al., 2003), so that liquid and solid samples can be analyzed. In headspace solid-phase microextraction (HS-SPME), the fiber is not in contact with the sample, with the advantage that the life expectancy of the fiber is longer. On the other hand, the selectivity and sensitivity of the method is strongly affected by the interactions between the analytes and the sample matrix, and the vaporization of the analytes from the sample. Hence, the nature and complexity of the sample has a strong influence on the results obtained with HS-SPME, to such an extent that Doong et al. (2000a) found that the technique was suitable to determine PAH with up to 6 aromatic rings in water samples when the extraction temperature was increased to 80°C, but that

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it was not possible to detect 6-ring PAH in soil. These same authors proposed a preheating of the samples to enhance the extraction of PAH, which produced an increase in the extracted amount of 4- and 5-ring PAH; however, no 6-ring PAH were detected.

In spite of the numerous advantages of solid-phase microextraction, the technique has not, to our knowledge, been applied to the study of PAH in foods. In this paper, HS-SPME was used to study PAH in smoked cheeses, in which the PAH concentrations were previously determined using a solvent extraction method. There were 2 objectives: first, to study the suitability of HS-SPME to determine PAH in smoked cheese, and second, to compare the results obtained by both techniques to know if a relationship between them could be established.

MATERIALS AND METHODS

Samples

The samples were 6 types of smoked cheese with percentages of dry extract varying between 45 and 65%, and with a fat content ranging from 43 to 50%, relative to the dry extract. The cheeses were designated SC1, SC2, SC3, SC4, SC5, and SC6. They were manufactured with cows', sheep's, or goats' milk, or with a mixture of them. Approximately 0.8-g ground portions were taken from the exterior zone of the cheese pieces, and were weighed in 4-mL amber vials for HS-SPME analysis. The samples used to determine the final HS-SPME testing parameters were portions of the rind of cheese SC1, because a previous study (Guillén and Sopelana, 2004) revealed that this was the most contaminated sample and that the PAH concentrations in the rind were much higher than in the exterior.

Reagents and Materials

The solvents, reagents, and materials used for the determination of PAH by the solvent extraction method were cyclohexane and methanol, both HPLC grade (+99.9%). Other reagents and materials used were potassium hydroxide, anhydrous sodium sulfate, sodium tungstate dihydrate, sodium chloride, and Supelclean LC-Si SPE (solid-phase extraction) tubes (3 mL; 500 mg). All solvents, reagents, and materials mentioned are commercially available from Aldrich (Steinheim, Germany), Panreac (Barcelona, Spain), and Supelco (Bellefonte, PA).

The fibers used for HS-SPME analysis were polydimethylsiloxane, 100 μm , (PDMS) and polyacrylate, 85 μm , (PA) from Supelco.

Standards

The PAH standards used for the identification and quantification of the PAH were a commercial mixture of PAH standards dissolved in a mixture of dichloromethane:benzene (75:25), containing naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(c)phenanthrene, benz(a)anthracene, chrysene, 7,12-dimethylbenz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(ghi)perylene, dibenzo(a,l)pyrene, dibenzo(a,i)pyrene, and dibenzo(a,h)pyrene in concentrations of approximately 500 $\mu\text{g/mL}$; commercial individual cyclohexane solutions of 1,7-dimethylnaphthalene, 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1-methylphenanthrene, 2,3-dimethylantracene, 9,10-dimethylphenanthrene, 2-methylfluoranthene, 1-methylfluoranthene, 11H-benzo(c)fluorene, 1-methylpyrene, 3-methylchrysene, 2-methylchrysene, 5-methylchrysene, 4-methylchrysene, 6-methylchrysene, 1-methylchrysene, dibenz(a,j)anthracene, benzo(b)chrysene, picene, anthanthrene, coronene, and dibenzo(a,e)pyrene, in concentrations of 10 $\mu\text{g/mL}$; and a mixture of pure PAH dissolved in dichloromethane, containing 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, o-terphenyl, 2-methylantracene, 9-methylantracene, 3,6-dimethylphenanthrene, m-terphenyl, p-terphenyl, 11H-benzo(a)fluorene, 11H-benzo(b)fluorene, benzo(e)pyrene, and perylene, in concentrations ranging from 100 to 247.5 $\mu\text{g/mL}$. Naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, pyrene-d₁₀, p-terphenyl-d₁₄, chrysene-d₁₂, and perylene-d₁₂ were used as internal standards. The purity of these standards ranged from 97 to 99.5%.

Because some of the standards used are suspected carcinogens, precautions were taken when handling these compounds. All pure standards and solutions were obtained from Sigma Aldrich Co., Supelco, and Symta (Madrid, Spain).

Methods

Solvent extraction method. The methodology used for the determination of PAH in smoked cheese by solvent extraction (SE) has been described (Guillén and Sopelana, 2004). In brief, it included the initial extraction of fat from the sample, alkaline treatment of the extract, extraction of PAH with cyclohexane, a clean-up procedure using solid-phase extraction tubes and, finally, identification and quantification of PAH by gas chromatography-mass spectrometry (GC-MS) operating in selective ion monitoring mode.

HS-SPME method. Before use, the SPME fiber must be conditioned, that is, heated in the inlet of a gas

chromatograph at a high temperature as recommended by the manufacturer according to the type of fiber. The conditioning conditions for PDMS and PA fibers are: 250°C for 30 min and 300°C for 2 h, respectively. Once the fiber is conditioned, it is exposed to the headspace of the samples in closed vials at 70°C for 60 min. During this time, the PAH present in the headspace are adsorbed onto the fiber; then, the compounds are desorbed in the inlet of a gas chromatograph. Most researchers use desorption temperatures that range from 250 to 300°C for PDMS and from 280 to 300°C for PA. Taking into account the conditioning conditions and the recommended working temperatures for each type of fiber, together with the maximum temperature of the chromatographic column, it was decided to use 270°C for PDMS and 280°C for PA. A 5-min desorption time was selected. After desorption time in the gas chromatograph, the fiber is kept in the inlet for additional time in split mode to favor desorption of compounds that can interfere in subsequent analyses. Moreover, blanks are performed after each experiment to avoid carryover effects from one sample to another. The separation, identification, and quantification of PAH 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 $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness), coated with a nonpolar stationary phase (HP-5MS, 5% phenylmethyl siloxane). The operating conditions were as follows: oven temperature was set initially at 50°C (5 min hold), increased to 130°C at 8°C/min, and again increased to 290°C at a rate of 5°C/min (40 min hold). The temperatures of the ion source and the quadrupole mass analyzer were 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; detector temperature was held at 300°C; splitless mode was used for injection. The data acquisition mode used was selective ion monitoring mode.

RESULTS AND DISCUSSION

HS-SPME Procedure

Before choosing the final HS-SPME conditions, tests with different fibers and different extraction temperatures and times were made. Taking into account the findings of several authors (Cam et al., 2000; Doong et al., 2000a, b; Pino et al., 2003), it seems clear that the most appropriate fiber coatings for the study of PAH in matrices such as water, sediments, or soils are polyacrylate (85 μ m) and polydimethylsiloxane (100 μ m), even though newer types of fiber coatings are continuously being developed (Djozan and Assadi, 1999; Yu et

al., 2002). Therefore, the behavior of these 2 fibers was tested with rind samples of cheese SC1. The ratio between the PAH areas obtained with PA and PDMS after extractions of the headspace at 70°C over 60 min is shown in Table 1. It can be concluded from this table that, from naphthalene to anthracene, PA results in higher PAH areas than PDMS, whereas the areas of fluoranthene and pyrene are lower when a PA fiber is used. This matches well with the results of Doong et al. (2000a, b) and Pino et al. (2003) because, according to these authors, PA enhances the extraction efficiency of PAH with 2 and 3 rings. Given that PAH with more than 4 rings have not been detected with either of the fibers, PA coating was selected.

In regards to extraction temperature, there are very few studies on PAH where the effect of different extraction temperatures on HS-SPME has been studied. Doong et al. (2000a, b) studied HS-SPME in water samples at 25, 60, and 80°C with a 100- μ m PDMS fiber, and the results varied depending on the PAH. The highest areas for PAH with 2 and 3 aromatic rings were obtained with an extraction temperature of 60°C, whereas 80°C was better for PAH with higher molecular weights. Moreover, 5- and 6-ring PAH could only be detected when an extraction temperature of 80°C was used. Other authors (Liu et al., 1997; Seduikienė et al., 2000) have used an extraction temperature of 60°C to study PAH in soils by HS-SPME, whereas Djozan and Assadi (1999) chose 80°C for the extraction of PAH from water samples. In our study, experiments were made at 50, 60, 70, 80, and 90°C with rind samples of cheese SC1, and the results obtained revealed that all PAH areas increase with the extraction temperature. These findings differ from those of Doong et al. (2000b), who observed a decrease in sensitivity for the PAH with a low number of aromatic rings when the extraction temperature was increased to 80°C, using a 100- μ m PDMS fiber during an extraction time of 90 min. The ratios between the PAH areas obtained at 60 and 50°C, at 70 and 60°C, at 80 and 70°C, and at 90 and 80°C with a PA fiber after 60 min extractions are shown in Table 1. These ratios reveal that the increase of extraction temperature from 50 to 60°C produces the highest increase in the areas of PAH with 2 and 3 aromatic rings, whereas the highest increase in fluoranthene and pyrene areas is obtained by raising the extraction temperature from 60 to 70°C. In all cases, the area increase is generally more pronounced for PAH of higher molecular weight. It can also be observed that temperatures above 70°C produce increases in the areas of low molecular weight PAH very similar to those achieved at 70°C; however, from phenanthrene onwards, the area growth goes down as the temperature increases. On the other hand, the use of extraction tem-

Table 1. Ratios between the areas (A) of the peaks corresponding to the polycyclic aromatic hydrocarbons (PAH) identified in the rind of smoked cheese sample SC1 by headspace solid-phase microextraction in different conditions of fiber coating, extraction temperature, and extraction time.

Polycyclic aromatic hydrocarbon	Fiber coating ¹ A _{PA} /A _{PDMS}	Extraction temperature ²				Extraction time ³ A _{120min} /A _{60min}
		A _{60°C} /A _{50°C}	A _{70°C} /A _{60°C}	A _{80°C} /A _{70°C}	A _{90°C} /A _{80°C}	
Naphthalene	3.77	1.14	1.03	1.08	1.01	0.96
2-Methylnaphthalene	3.31	1.25	1.05	1.10	1.09	1.07
1-Methylnaphthalene	3.41	1.27	1.08	1.09	1.09	1.07
2,6-Dimethylnaphthalene	2.95	1.46	1.11	1.12	1.17	1.11
1,7-Dimethylnaphthalene	2.82	1.48	1.19	1.08	1.19	1.11
1,6-Dimethylnaphthalene	2.91	1.50	1.18	1.09	1.19	1.08
1,4-+2,3-Dimethylnaphthalenes	†	1.56	*	**	**	1.14
1,5-Dimethylnaphthalene	3.13	1.55	1.18	1.12	1.20	1.11
Dimethyl-/ethyl-naphthalene	3.05	1.56	1.20	1.12	1.19	\$
Acenaphthylene	4.45	1.68	1.27	1.11	1.23	1.12
Acenaphthene	†	1.63	1.23	1.13	1.22	1.06
Fluorene	3.15	1.96	1.53	1.20	1.36	1.19
Phenanthrene	1.69	2.40	2.10	1.58	1.49	1.57
Anthracene	1.98	2.34	2.14	1.66	1.51	1.49
3-Methylphenanthrene	†	2.40	2.34	1.87	1.61	1.65
2-Methylphenanthrene	†	2.28	2.24	1.83	1.55	1.64
9-Methylphenanthrene	†	**	*	1.89	1.57	1.76
1-Methylphenanthrene	†	2.25	2.27	1.95	1.55	1.53
Dimethylphenanthrene or isomer (2)	†	**	**	**	*	#
Dimethylphenanthrene or isomer (5)	†	**	**	*	1.66	#
Dimethylphenanthrene or isomer (7)	†	**	**	*	1.64	#
Fluoranthene	0.89	2.00	2.23	1.95	1.59	1.77
Pyrene	0.80	2.08	2.35	1.99	1.65	1.64

¹Extractions of 60 min at 70°C.²Extractions of 60 min with a polyacrylate (PA) fiber.³Extractions at 70°C with a polyacrylate (PA) fiber.

† = Compound not identified with polydimethylsiloxane (PDMS) or polyacrylate (PA) fiber.

* = Compound not identified at one of the temperatures tested.

** = Compound not identified at any of the temperatures tested.

\$ = Compound not identified after 60 min of extraction.

= Compound not identified after either of the extraction times tested.

peratures of 80 and 90°C does not permit the detection of PAH with higher molecular weight than fluoranthene and pyrene in smoked cheeses, even though these temperatures allow the detection of some dimethylphenanthrenes. Taking into account all these findings, it was decided to keep the extraction temperature at 70°C, because at 80 and 90°C, the working conditions are more extreme, and maintaining a constant temperature in the thermostatic bath over the extraction time is difficult.

With regard to the extraction time, the selection of this parameter has a great influence on the extraction efficiency of the different fibers, due to differences in the equilibration time of PAH when different coatings are used. Thus, the extraction time selected can determine which fiber is more suitable for a specific study or if a certain PAH is detected or not, depending on the nature of the coating and on its thickness. Doong et al. (2000a) observed that PAH with up to 6 aromatic rings could be detected with an 85- μ m PA fiber from 75 min onwards at 60°C in water samples. The most frequently

used extraction times vary from 30 to 120 min for PA fibers. Although it is not necessary to reach extraction equilibrium for all PAH, the extraction time should be sufficient to permit the detection of the PAH with higher molecular weights. In our study, tests were made with extraction times of 60 and 120 min with a PA fiber at 70°C, and the ratios between PAH areas are shown in Table 1. It is observed that, from naphthalene to fluorene, there are hardly any differences between the 2 times, but from phenanthrene onwards, the differences between the areas are higher, reaching a relationship between HS-SPME areas of 1.77 for fluoranthene. Nevertheless, the time increase from 60 to 120 min does not allow the detection of additional PAH, so the extraction time was set to 60 min.

Although all the extraction parameters mentioned above have an individual effect on the results obtained by HS-SPME, there are also influences of some parameters on others. Thus, the extraction temperature has an influence on the equilibrium time of PAH and, consequently, on the extraction efficiency of the fibers for a

selected extraction time. In turn, the extraction time can determine which fiber has higher extraction efficiency, depending on the extraction temperature. There is another parameter, the type of sample matrix, which has a great influence on the results obtained. Doong et al. (2000a) studied PAH in soils by HS-SPME and found that 6-ring PAH could not be detected by HS-SPME, with an extraction temperature of 80°C or with an extraction time of 120 min.

Results Obtained by HS-SPME

Table 2 shows the PAH identified in the external region of the smoked cheese samples analyzed and their concentrations, expressed as area counts divided by 10^3 , of the peak corresponding to the ion selected for the quantification of each PAH which, except for acenaphthene (153), coincides with the molecular ion. It can be seen that most of the PAH identified by HS-SPME are compounds with 2 and 3 aromatic rings, even though 2 PAH with 4 rings were also identified: fluoranthene and pyrene. Naphthalene and phenanthrene were identified in all samples, and acenaphthylene, fluorene, and anthracene were identified in most of them. It can also be observed from Table 2 that monomethyl- and dimethyl-naphthalenes are the only alkylated PAH identified by HS-SPME and were present in most of the samples studied.

In regards to the PAH area counts, it can be observed that the highest values correspond to naphthalene and its alkyl derivatives, followed by phenanthrene, acenaphthylene, and fluorene, even though the order of these latter compounds varies depending on the sample evaluated. With regard to fluoranthene and pyrene, it is noteworthy that their area values are very similar in all samples where they have been identified, ranging from 4.26 to 6.72 for fluoranthene, and from 2.77 to 5.47 for pyrene. Lastly, the areas of the methyl derivatives of naphthalene are lower than those of their parent PAH and, among the methyl derivatives, the monomethyl are in higher concentrations than the dimethyl derivatives, except for 1,6-dimethylnaphthalene in sample SC4.

Comparison of Results Obtained by HS-SPME and SE

The PAH identified by both techniques are shown in Table 2. The cheese samples selected for this study had already been studied by an SE method, so the values corresponding to the latter, which express concentrations of PAH in micrograms per kilogram of sample exterior, were taken from a previous paper (Guillén and Sopelana, 2004). In all cases, values come from duplicate analyses of each sample. If the results ob-

tained by both techniques are compared, it can be seen that HS-SPME does not allow the detection of as many PAH as SE. Although the temperature used for the generation of the headspace of the samples (70°C) would favor the vaporization of the heaviest compounds, all the compounds identified have 4 aromatic rings or less, whereas SE permits the detection of PAH with up to 6 rings. This matches with the results obtained by Doong et al. (2000a). According to them, HS-SPME is not useful for detecting PAH with more than 5 aromatic rings in a complex matrix such as soil. However, it is observed in this study that even though PAH with 5 rings were determined by SE in some of the samples, they were not detected by HS-SPME. This could be due to the low volatility of PAH with 5 or more rings and the strong attractive interactions between PAH and cheese lipids, which could hinder the escape of high molecular weight PAH to the vapor phase. However, when aqueous samples are studied, the escaping tendency of PAH is favored by the repulsive interactions existing between PAH and water, given that most of these compounds are highly hydrophobic. When using HS-SPME, there is competition among all compounds present in the headspace, according to their affinity for the fiber coating, which determines the compounds that are finally adsorbed. Moreover, when an HS-SPME analysis is performed, it is not possible to isolate PAH from other compounds, which can interfere in their identification, so there is a chance that PAH retained in the fiber cannot be correctly identified due to interference. Therefore, the amount and nature of the compounds present in the headspace can impede the adsorption and identification of PAH. Thus, it can be observed that some PAH, such as 2,6- and 1,5-dimethylnaphthalenes, identified by SE in all samples studied, were not identified by HS-SPME in sample SC2, even though their concentrations are of the same order as in sample SC5.

In relation to the PAH identified by both techniques, in general, the value of the area obtained by HS-SPME is related to the concentration determined by SE. Thus, the samples with higher PAH concentrations also have higher HS-SPME areas, and the same is true for lower concentrations. However, when the molecular weight of the PAH increases, as well as the number of alkylated groups, the response given by HS-SPME is lower, and it is not possible to observe differences among samples with this technique. This can be observed in the case of fluoranthene and pyrene from the data in Table 2. Table 3 shows the ratios between the values obtained by HS-SPME and SE for the PAH identified by both techniques. In the case of some compounds, such as naphthalene, fluorene, and phenanthrene, this relationship could be considered constant in all the samples studied.

Table 2. Polycyclic aromatic hydrocarbons (PAH) identified in the external region of the smoked cheeses studied (SC1 to SC6) and their concentrations, obtained by a solvent extraction method (SE) and by headspace solid-phase microextraction (SPME).

Polycyclic aromatic hydrocarbon	SC1		SC2		SC3		SC4		SC5		SC6	
	SE ¹	SPME ²	SE	SPME	SE	SPME	SE	SPME	SE	SPME	SE	SPME
Naphthalene	175.74	1661.59	40.14	352.49	27.97	342.23	31.41	326.82	18.80	196.71	9.32	235.70
2-Methylnaphthalene	129.97	437.02	23.70	86.09	24.33	108.99	26.79	75.15	25.73	72.28	5.32	24.36
1-Methylnaphthalene	82.30	275.72	16.33	56.38	13.56	58.93	24.16	69.61	13.20	36.51	3.10	14.70
2,6-Dimethylnaphthalene	115.10	162.49	13.43	— ³	21.22	48.73	37.54	44.27	13.94	16.55	2.45	6.62
1,7-Dimethylnaphthalene	91.63	150.51	10.73	17.79	17.72	43.88	31.41	43.88	12.07	15.27	2.30	5.27
1,6-Dimethylnaphthalene	93.01	146.14	12.20	17.74	14.43	33.49	82.29	105.43	10.05	11.59	1.98	4.22
1,4+2,3-Dimethylnaphthalenes	39.09	71.46	7.30	—	9.17 ⁴	19.44	19.96 ⁴	21.74	—	—	—	—
1,5-Dimethylnaphthalene	21.01	35.96	2.40	—	3.87	11.45	14.91	22.33	2.50	10.27	0.66	—
Dimethylnaphthalene or isomer	79.32	152.71	3.15	—	4.28	42.41	11.37	37.26	—	—	—	—
Acenaphthylene	44.48	453.66	11.03	107.73	4.28	40.04	1.79	10.44	1.31	—	0.36	4.99
Acenaphthene	10.37	—	2.31	8.66	3.30	—	1.46	—	0.85	—	0.28	—
Fluorene	37.86	223.03	8.92	40.44	8.05	42.51	8.42	34.89	2.03	8.54	0.75	—
Phenanthrene	42.90	179.40	18.09	57.89	6.65	27.83	13.85	47.76	3.76	14.35	1.93	8.53
Anthracene	7.04	41.17	4.16	27.79	1.23	15.87	1.42	3.48 ⁴	0.44 ⁴	6.70	0.12	—
<i>o</i> -Terphenyl	2.24	—	0.36	12.48	1.45	—	—	—	3.61	—	0.76	—
3-Methylphenanthrene	7.20	—	1.91	—	1.76	—	5.54	—	0.92 ⁴	—	0.66	—
2-Methylphenanthrene	10.25	—	3.45	—	2.44	—	8.24	—	1.20	—	0.88	—
2-Methylanthracene	5.85	—	1.76	—	—	—	—	—	—	—	—	—
9-Methylphenanthrene	6.15	—	2.12	—	1.74	—	5.99	—	0.95	—	0.72	—
1-Methylphenanthrene	6.85	—	2.32	—	1.52	—	7.08	—	0.75	—	0.53	—
Dimethylphenanthrene or isomer (1)	1.16	—	—	—	—	—	—	—	—	—	—	—
Dimethylphenanthrene or isomer (2)	1.40	—	—	—	0.55	—	1.96	—	0.40	—	0.27	—
Dimethylphenanthrene or isomer (3)	0.79	—	—	—	0.52	—	2.04	—	0.38	—	0.19	—
Dimethylphenanthrene or isomer (4)	1.07	—	—	—	—	—	—	—	—	—	—	—
Dimethylphenanthrene or isomer (5)	2.53	—	—	—	1.97	—	7.44	—	1.28	—	0.81	—
Dimethylphenanthrene or isomer (6)	2.71	—	—	—	1.12	—	4.55	—	0.82	—	0.53	—
Dimethylphenanthrene or isomer (7)	3.08	—	1.87	—	0.88	—	14.17	—	0.70	—	0.36	—
Dimethylphenanthrene or isomer (10)	1.01	—	—	—	—	—	—	—	—	—	—	—
Fluoranthene	5.22	5.75	2.50	4.26	0.58	6.72	1.09	—	0.48	5.34	0.44	—
Pyrene	4.83	4.99	1.81	2.77	0.54	5.47	1.52	—	0.62	4.73	0.79	—
<i>m</i> -Terphenyl	0.21	—	0.14	—	0.12	—	—	—	0.32	—	0.33	—
<i>p</i> -Terphenyl	0.09	—	0.08	—	0.07	—	—	—	0.10	—	0.11	—
2-Methylfluoranthene	0.22	—	0.12	—	—	—	—	—	—	—	—	—
Methylfluoranthene or isomer	0.18	—	0.15	—	—	—	—	—	—	—	—	—
Methylfluoranthene or isomer	0.16	—	0.09	—	—	—	—	—	—	—	—	—
1-MFt.+11H-B(a)fl. ⁵	0.94	—	0.68	—	—	—	—	—	—	—	—	—
11H-Benzo(b)fluorene	0.38	—	0.16	—	—	—	—	—	—	—	—	—
11H-Benzo(c)fluorene	0.18	—	0.12	—	—	—	—	—	—	—	—	—
Methylfluoranthene or isomer	0.21	—	0.23	—	—	—	0.26	—	—	—	0.04	—
Methylfluoranthene or isomer	0.23	—	0.24	—	—	—	0.57	—	0.05 ⁴	—	0.08	—
1-Methylpyrene	0.24	—	0.24	—	—	—	0.30	—	—	—	0.04	—
Benz(a)anthracene	0.39	—	0.28	—	—	—	—	—	0.04	—	0.05	—
Chrysene+Triphenylene	0.64	—	0.46	—	0.12	—	—	—	0.11	—	0.11	—
Benzo(b)fluoranthene	0.19	—	0.09	—	—	—	—	—	—	—	—	—
Benzo(j+k)fluoranthenes	0.26	—	0.14	—	—	—	—	—	—	—	—	—
Benzo(e)pyrene	0.13	—	0.13	—	—	—	—	—	0.05 ⁴	—	—	—
Benzo(a)pyrene	0.20	—	0.08	—	—	—	—	—	—	—	—	—
Indeno(1,2,3-cd)pyrene	0.11	—	—	—	—	—	—	—	—	—	—	—
Benzo(ghi)perylene	0.11	—	0.05	—	—	—	—	—	0.04	—	0.04	—

¹PAH concentrations, expressed in $\mu\text{g/kg}$ of exterior. These values have been taken from Guillén and Sopelana (2004).²Arbitrary area counts of the quantification ion, divided by 10^3 .³Not identified.⁴Identified only in one of the samples.⁵Possibly 1-Methylfluoranthene+11H-Benzo(a)fluorene.

Previous studies have shown that a relationship can be established between concentrations of pyrene and benzo(a)pyrene in some matrices, such as smoke flavorings (Guillén et al., 2000a, b) or charbroiled hamburgers (Greenberg et al., 1993), and this could be very useful

to predict the level of benzo(a)pyrene from the concentration of pyrene. The existence of this relationship can be explained considering that each PAH is formed from another with a smaller number of aromatic rings by addition of small units such as acetylene to aryl radicals

Table 3. Ratios between the concentrations obtained by headspace solid-phase microextraction (HS-SPME) and solvent extraction (SE) for the polycyclic aromatic hydrocarbons identified by both techniques in the external region of the samples studied (smoked cheeses SC1 to SC6).

Polycyclic aromatic hydrocarbon	SC1	SC2	SC3	SC4	SC5	SC6
Naphthalene	9.45	8.78	12.24	10.40	10.46	25.29
2-Methylnaphthalene	3.36	3.63	4.48	2.81	2.81	4.58
1-Methylnaphthalene	3.35	3.45	4.35	2.88	2.77	4.74
2,6-Dimethylnaphthalene	1.41	—	2.30	1.18	1.19	2.70
1,7-Dimethylnaphthalene	1.64	1.66	2.48	1.40	1.27	2.29
1,6-Dimethylnaphthalene	1.57	1.45	2.32	1.28	1.15	2.13
1,4+2,3-Dimethylnaphthalenes	1.83	—	2.12	1.09	*	—
1,5-Dimethylnaphthalene	1.71	—	2.96	1.50	4.11	—
Dimethylnaphthalene or isomer	1.93	—	9.91	3.28	*	*
Acenaphthylene	10.20	9.77	9.36	5.83	—	13.86
Acenaphthene	—	3.75	—	—	—	—
Fluorene	5.89	4.53	5.28	4.14	4.21	—
Phenanthrene	4.18	3.20	4.18	3.45	3.82	4.42
Anthracene	5.85	6.68	12.90	2.45	15.23	—
<i>o</i> -Terphenyl	—	34.67	—	*	—	—
3-Methylphenanthrene	—	1.28	—	—	—	—
2-Methylphenanthrene	—	1.19	—	—	—	—
9-Methylphenanthrene	—	1.76	—	—	—	—
Fluoranthene	1.10	1.70	11.59	4.50	11.13	9.93
Pyrene	1.03	1.53	10.13	3.26	7.63	4.96

* = Compound not identified by SE or by HS-SPME; — = compound not identified by HS-SPME.

during pyrolysis (Frenklach and Warnatz, 1987). Therefore, the existence of some relationships among certain PAH could be expected. It can be observed from the ratios in Table 3 that there is no proportionality between SE and HS-SPME results for pyrene; for this reason, it was not possible to use pyrene to predict the approximate level of benzo(a)pyrene when using HS-SPME. However, Table 3 shows that both techniques provide proportional results for phenanthrene in different samples. Therefore, this PAH, easily detectable by both methodologies, could be used as an indicator of the presence of benzo(a)pyrene, because it can act as precursor of pyrene and consequently of benzo(a)pyrene by the route above mentioned. Nevertheless, it must be demonstrated that there is a relationship among the concentrations of phenanthrene, pyrene, and benzo(a)pyrene obtained by the SE method in smoked cheeses. Table 4 shows the ratios between the concentrations of phenanthrene and pyrene, pyrene and benzo(a)pyrene, and phenanthrene and benzo(a)pyrene, for each sample. It can be observed that, except for sample SC6, the ratio phenanthrene/pyrene can be considered of a

similar order in the samples of this study. The other 2 ratios, despite the fact that they can only be calculated for 2 samples, are of the same order. If the phenanthrene areas obtained by HS-SPME in samples SC2 and SC4 are considered (see Table 2), it can be seen that in sample SC2, where the level of benzo(a)pyrene is 0.08 $\mu\text{g/kg}$ of exterior, the phenanthrene area is 57,890 (57.89×10^3), whereas in sample SC4, with an HS-SPME value of 47,760, benzo(a)pyrene has not been identified by SE. Therefore, it could be thought that phenanthrene area counts around 50,000 in selective ion mode could be related to a possible presence of benzo(a)pyrene, which may be associated with other PAH of different degrees of carcinogenicity (Potthast, 1979); it must be pointed out that the value of phenanthrene area related to the possible presence of benzo(a)pyrene will probably vary among equipments and laboratories.

It can be concluded that HS-SPME, under the conditions used in this study, does not permit identification of PAH with more than 4 aromatic rings in samples of smoked cheeses. A relationship between the results

Table 4. Ratios between the concentrations of phenanthrene and pyrene, pyrene and benzo(a)pyrene, and phenanthrene and benzo(a)pyrene, obtained by solvent extraction in smoked cheese samples (SC1 to SC6).

Ratio	SC1	SC2	SC3	SC4	SC5	SC6
Phenanthrene/Pyrene	8.88	9.99	12.31	9.11	6.06	2.44
Pyrene/Benzo(a)pyrene	24.15	22.63	— ¹	—	—	—
Phenanthrene/Benzo(a)pyrene	214.50	226.13	—	—	—	—

¹Benzo(a)pyrene was not identified.

obtained by the SE and HS-SPME methods has been observed, but it is not constant for all samples tested, nor for all PAH identified. Consequently, although HS-SPME as used here does not allow one to determine the total PAH content of smoked cheese samples, it can be used as a screening tool to differentiate those samples that present a certain degree of PAH contamination and to identify those samples which need an exhaustive study of their PAH content. Furthermore, phenanthrene area could be considered a possible indicator of the presence of benzo(a)pyrene and other carcinogenic PAH, but this possibility should be confirmed by more extensive studies with a greater number of samples containing benzo(a)pyrene. Analysis by HS-SPME takes very little time to perform compared with classical methodologies, and it does not require the use of reagents or solvents. Finally, samples are studied almost directly, without the need of previous manipulation.

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REFERENCES

- Cam, D., S. Gagni, L. Meldolesi, and G. Galletti. 2000. Determination of polycyclic aromatic hydrocarbons in sediment using solid-phase microextraction with gas chromatography-mass spectrometry. *J. Chromatogr. Sci.* 38:55–60.
- Djozan, D., and Y. Assadi. 1999. Monitoring of polycyclic aromatic hydrocarbons in water using headspace solid-phase microextraction and capillary gas chromatography. *Microchem. J.* 63:276–284.
- Doong, R.-A., S.-M. Chang, and Y.-C. Sun. 2000a. Solid-phase microextraction and headspace solid-phase microextraction for the determination of high molecular-weight polycyclic aromatic hydrocarbons in water and soil samples. *J. Chromatogr. Sci.* 38:528–534.
- Doong, R.-A., S.-M. Chang, and Y.-C. Sun. 2000b. Solid-phase microextraction for determining the distribution of sixteen US environmental protection agency polycyclic aromatic hydrocarbons in water samples. *J. Chromatogr. A.* 879:177–188.
- Frenklach, M., and J. Warnatz. 1987. Detailed modeling of PAH profiles in a sooting low-pressure acetylene flame. *Combust. Sci. Technol.* 51:265–283.
- Greenberg, A., C.-H. Hsu, N. Rothman, and P. T. Strickland. 1993. PAH profiles of charbroiled hamburgers: Pyrene/b[a]p ratios and presence of reactive PAH. *Polycycl. Aromat. Comp.* 3:101–110.
- Guillén, M. D., and P. Sopelana. 2004. Occurrence of polycyclic aromatic hydrocarbons in smoked cheeses. *J. Dairy Sci.* 87:556–564.
- Guillén, M. D., P. Sopelana, and M. A. Partearroyo. 1997. Food as a source of polycyclic aromatic carcinogens. *Rev. Environ. Health* 12:133–146.
- Guillén, M. D., P. Sopelana, and M. A. Partearroyo. 2000a. Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavourings of different composition by gas chromatography-mass spectrometry. *J. Agric. Food Chem.* 48:126–131.
- Guillén, M. D., P. Sopelana, and M. A. Partearroyo. 2000b. Polycyclic aromatic hydrocarbons in liquid smoke flavourings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. *J. Agric. Food Chem.* 48:5083–5087.
- Hageman, K. J., L. Mazeas, C. B. Grabanski, D. J. Miller, and S. B. Hawthorne. 1996. Coupled subcritical water extraction with solid-phase microextraction for determining semivolatile organics in environmental solids. *Anal. Chem.* 68:3892–3898.
- IARC. 1973. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 3. IARC, Lyon, France.
- IARC. 1983. Polynuclear aromatic compounds, part 1: Chemical, environmental, and experimental data. Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 32. IARC, Lyon, France.
- Langenfeld, J. J., S. B. Hawthorne, and D. J. Miller. 1996. Quantitative analysis of fuel-related hydrocarbons in surface water and wastewater samples by solid-phase microextraction. *Anal. Chem.* 68:144–155.
- Liu, Y., M. L. Lee, K. J. Hageman, Y. Yang, and S. B. Hawthorne. 1997. Solid-phase microextraction of PAHs from aqueous samples using fibers coated with HPLC chemically bonded silica stationary phases. *Anal. Chem.* 69:5001–5005.
- Negrao, M. R., and M. F. Alpendurada. 1998. Solvent-free method for the determination of polynuclear aromatic hydrocarbons in wastewater by solid-phase microextraction-high-performance liquid chromatography with photodiode array detection. *J. Chromatogr. A.* 823:211–218.
- Pino, V., J. H. Ayala, A. M. Alfonso, and V. Gonzalez. 2003. Micellar microwave-assisted extraction combined with solid-phase microextraction for the determination of polycyclic aromatic hydrocarbons in a certified marine sediment. *Anal. Chim. Acta* 477:81–91.
- Potthast, K. 1979. The influence of smoking technology on the composition of polycyclic hydrocarbons in smoked meat products, smoke condensates and in waste gases from smoking plant. *Die Fleischwirtschaft* 59:1515–1523.
- Seduikienė, I., V. Vickackaitė, and R. Kazlauskas. 2000. Solid phase microextraction of polycyclic aromatic hydrocarbons from soil. *Chem. Anal. (Warsaw)* 45:835–842.
- Waidyanatha, S., Y. Zheng, and S. M. Rappaport. 2003. Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography-mass spectrometry. *Chem. Biol. Interact.* 145:165–174.
- Yu, J., L. Dong, C. Wu, L. Wu, and J. Xing. 2002. Hydroxyfullerene as a novel coating for solid-phase microextraction fiber with sol-gel technology. *J. Chromatogr. A.* 978:37–48.