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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-μm 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 μg/mL; commercial individual cyclohexane solutions of 1,7-dimethylnaphthalene, 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1-methylphenanthrene, 2,3-dimethylanthracene, 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 μg/mL; and a mixture of pure PAH dissolved in dichloromethane, containing 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, *o*-terphenyl, 2-methylanthracene, 9-methylanthracene, 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 μg/mL. Naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, *p*-terphenyl-d_{14}, chrysene-d_{12}, and perylene-d_{12} 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