Solid Phase Microextraction Application in Gas Chromatography/ Olfactometry Dilution Analysis

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Gas chromatography/olfactometry (GC/O) based on dilution analysis (e.g., CharmAnalysis or aroma extraction dilution analysis) gives an indication of what compounds are most important (most potent) to the aroma of foods. The application of solid phase microextraction to the preparation of samples for GC/O dilution analysis was shown to be feasible by varying the fiber thickness and length to achieve various absorbant volumes.

Keywords: *Flavor release; gas chromatography olfactometry; Charm; solid phase microextraction (SPME)*

INTRODUCTION

Solid phase microextraction (SPME) is a sample preparation technique based on absorption, which is useful for extraction and concentration of analyses either by submersion in a liquid phase or by exposure to a gaseous phase (Aurthur, 1992). Following exposure of the fiber to the sample, absorbed analytes can be thermally desorbed in a conventional gas chromatography (GC) injection port. SPME has been commercially available since 1993 and now is available with various adsorbent materials and various coating thicknesses. The fiber behaves as a liquid solvent selectively extracting analytes with more polar fibers having a greater affinity for polar analytes. Extraction is based on the diffusion and partitioning of the analytes (Zhang and Pawliszyn, 1993, 1995).

SPME first found application in the evaluation of pollutants in water (Belardi and Pawliszyn, 1989). Since then, SPME has been used in an array of fields including the compositional analysis of water, air, essential oils, caffeine, ground coffee, apple volatiles, pharmaceutical products, insect pheromones, and botanicals (Hawthorne et al., 1992; Yang and Peppard, 1995; Field et al., 1996; Malosse et al., 1995; Matich et al., 1996).

Compounds have odor activity when they volatilize from a substance into the headspace at concentrations above their detection threshold. Because aroma compounds are usually present in a headspace at levels too low to be detected by GC, headspace extraction also requires concentration. Some methods used for extraction and concentration of headspace include purge and trap, static headspace, liquid−liquid, and solid phase extraction.

The significance of each aroma compound's contribution to a perceived aroma can be estimated by its potency using a method based on dilution to threshold such as CharmAnalysis or aroma extraction dilution analysis (AEDA) (Acree, 1997; Grosch, 1993). These methods utilize GC/olfactometry (GC/O), which provides measurement selectivity of components that have odor activity and is as much as 100 times more sensitive than flame ionization detection. This paper describes a method to use SPME sampling to conduct a dilution analysis of a static headspace. Dilution analysis provides the essential differentiation between odor active and non-odor active volatiles for aroma analysis. The quantitative aspect of using flavor dilution analysis makes this a unique and vitally important application of SPME.

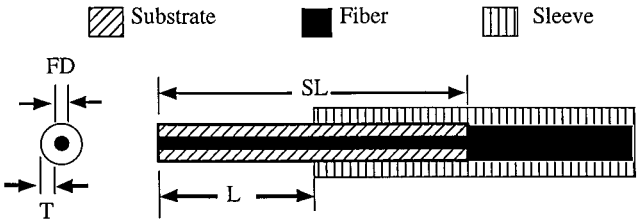

Figure 1. Diagram of SPME fibers used in GC/O−SPME; T is the substrate thickness, L is the substrate exposed to the vapor phase outside the sleeve, FD is the fiber diameter, and SL is the total fiber length.

EXPERIMENTAL PROCEDURES

Sample Preparation. A solution of six standard chemicals (benzaldehyde, octanol, 1,8-cineole, citral, decanal, 2-acetyl-3-methylpyrazine) was prepared in ethanol at 1% (w/w). The six standards were chosen to represent common structures of odor active compounds. The relatively high concentration of the standard solution was necessary to achieve measurable volatile concentration from ethanol. Solute concentration should be a factor only when approaching saturation levels. In food samples, solvent would vary as would concentration of odorants. The solution was stirred with a magnetic stirring bar and allowed to equilibrate for 1 h prior to extraction. Solution (50 mL) was held in a 100 mL glass bottle with a Teflon-faced butyl septum lid.

SPME Extraction. Figure 1 shows a diagram depicting the SPME apparatus. SPME fibers with 95, 30, and 7 μm thicknesses of poly(dimethylsiloxane) (PDMS) coating and the manual holder were obtained from Supelco Co. (Bellefonte, PA). PDMS was selected to demonstrate this method because it is the most commonly used and most versatile SPME fiber and it was commercially available in three thicknesses. Before

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initial use, the fibers were conditioned for 1 h at 250 °C for the 95 and 30 μm fibers and for 2 h at 320 °C for the 7 μm fiber. Before each extraction, the fiber was held at 225 °C for 5 min and allowed to come to room temperature for 10 min. The plunger depth was set at 3 cm to allow for maximum desorption into the GC by injecting into the hottest part of the injection port. It was found to be important that the holder be tightly assembled to obtain consistent results. When sampling, the fiber was injected 1 cm above the solution surface. The fiber was in the injection port to desorb volatiles for 2 min.

GC Parameters. An HP5890 GC with a fused silica capillary column DB-1 liquid phase (0.33 μm) with column dimensions of 25 m × 0.20 mm was used. Carrier gas was helium at 25 cm/s linear velocity. The temperature program was an initial 3 min at 40 °C and then a ramp rate of 4 °C/min to 200 °C followed by 25 °C/min to 250 °C and held at 250 °C for 15 min. The injection purge on the GC was off for the initial 1 min.

Optimization of Exposure Time. The 95 μm fiber was exposed to the standard solution for 1, 5, 7, 10, 15, 20, 25, 60, and 90 min. The shortest time at which 95% of maximum product was extracted for all compounds was determined to be the optimum exposure time for the PDMS fiber.

Dilution Analysis. Each fiber was exposed to the headspace of the standard solution for the determined optimum time. Each fiber was fully exposed (1.0 cm), approximately two-thirds exposed (0.58 cm), and approximately one-third exposed (0.28 cm) to the headspace standard solution. Exposure length (L in Figure 1) was controlled by creating two additional notches in the SPME holder. After the injector septum had been pierced with the manual SPME instrument, the appropriate amount of fiber corresponding to the amount exposed during sample adsorption was desorbed onto the column for 1.5 min followed with a full exposure desorption in another injection port for 5 min. Relative concentration was calculated on the basis of the area of the corresponding GC peak. Each measurement was conducted in triplicate.

CharmAnalysis of Example. CharmAnalysis was conducted on an HP5890 GC modified by DATU, Inc. (Geneva, NY) (Acree et al., 1984; Acree, 1997). GC/O conditions were the same as the GC parameters described above. Commercial brewed coffee (50 mL) was held in a 100 mL glass bottle with a Teflon-faced butyl septum lid at 28 °C. The coffee was stirred with a magnetic stirring bar and allowed to equilibrate for 1 h prior to extraction (15 min). To calculate relative retention indices, the SPME was exposed to the headspace of an ethanol solution of ethyl esters from C_4 to C_{18} using identical sampling and measurement procedures except detection was by FID.

RESULTS AND DISCUSSION

1,8-Cineole was the slowest to achieve 95% maximum absorption and thus the limiting compound, requiring 10 min to achieve absorption. The optimum exposure time was thus concluded to be 10 min.

Actual fiber dimensions were obtained from Supelco. All fibers were 10 mm long. The thicknesses were 95, 30, and 7 μm with nominal coating volumes of 0.612, 0.132, and 0.0121 mm^3, respectively. However, measurement of the 7 μm fiber with an Olympus microscope at 100× magnification showed an actual thickness of 13 μm, giving a fiber volume of 0.054 mm^3. When fractions of the fiber were exposed (100, 58, or 28%), the resulting exposed three volumes of the 95, 30, and 7 μm fibers were 0.612, 0.349, 0.171; 0.132, 0.075, 0.037; and 0.054, 0.036, 0.018 mm^3, respectively. For each fiber thickness the GC peak areas were reproducible at <5% standard deviation. As shown in Figure 2 each compound produced linear plots of peak area versus exposed fiber volume (R^2 > 0.90). Slopes of each line are indicative of the amount of analyte in the gas phase and its affinity for the fiber. Weight values were not used in Figure 2 because absolute weight quantification was not evaluated in this experiment. Because there were no deviations from the linear trend in any of the compounds tested, it was assumed that this method can be applied to volatiles. An exposure of the fiber fully sheathed (zero exposure) to the experimental conditions resulted in <5% of the 1,8-cineole and ethanol, and no other compounds could be detected; thus, analyte movement along the protected fiber did not significantly affect the results. There was not a noticeable difference in peak shapes with the different size fibers.

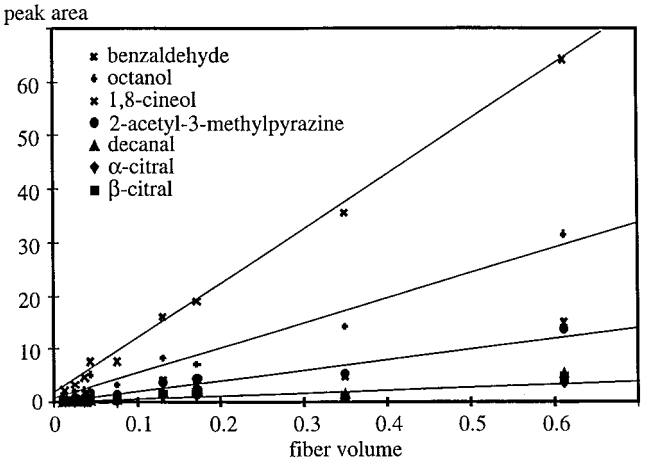

Figure 2. Average GC response (×10^{-4}) for each chemical with different SPME thickness and/or exposure length. The standard deviations were <5%, and the R^2 values ranged between 0.90 and 0.99.

Table 1. CharmAnalysis Results of SPME Coffee Headspace Compared to CharmAnalysis Results Using Serial Solvent Extraction with Freon 113 and Ethyl Acetate (Deibler et al., 1998) and Stable Isotope Dilution Assay Producing Odor Active Values (OAV) (Semmelroch and Grosch, 1996)[a]

compound	OSV SPME	OSV Charm	OSV OAV	ref	CAS Registry No.[b]
2,4,5-trimethylthiazole	100	33	0	2	13623-11-5
3-methoxy-2-isobutyl-pyrazine	54	42	32	6	24683-00-9
sotolon	34	100	37	5	28664-35-9
abhexon	31	25	0.2	7	698-10-2
vanillin	29	64	0.7	10	121-33-5
furaneol	26	57	49	3	3658-77-3
4-vinylguaiacol	24	70	20	9	7786-61-0
4-ethylguaiacol	15	19	0.2	8	2785-89-9
2-isopropyl-3-methoxy-pyrazine	15	10	0	4	25773-40-4
2-furfurylthiol	14	90	100	1	98-02-2

[a] For comparison all data were converted to OSV [OSV = (potency/potency max)$^{0.5}$ × 100] (Acree, 1997). Reference number refers to peaks in Figure 3. [b] Supplied by the authors.

Table 1 lists the most odor potent compounds detected in brewed coffee headspace using GC/O−SPME, their odor spectrum values (OSVs) and the OSVs from previously published experiments (Deibler et al., 1998; Semmelroch and Grosch, 1996). An OSV is an expression of odor potency normalized to the most potent odorant detected and transformed as the square root, allowing for comparison of results between separate studies (Acree, 1997). All of the compounds detected from the SPME extraction have been previously found in brewed coffee (Figure 3). Although the same compounds were among the most potent odorants in all three experiments, the data from OAV experiments yielded them

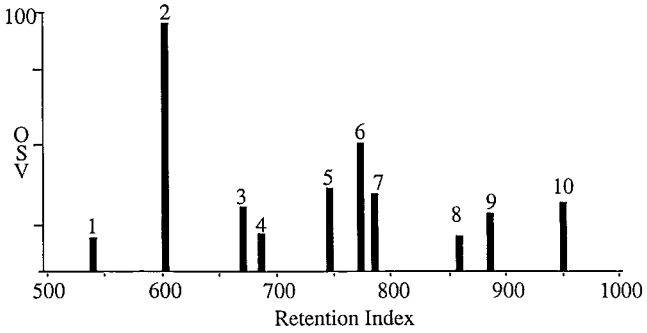

Figure 3. Odor spectrum of CharmAnalysis of brewed coffee achieved by varying PDMS SPME coating thickness and length. Numbers refer to compounds in Table 1.

in a different order. The GC/O−SPME data were very similar to the data produced by solvent extraction. The order difference between SPME and solvent extraction is reasonable for the two different coffee samples, but the difference between OAV and SPME was as extreme as the difference between coffee and chocolate. Because OAV is based on threshold, modified compositional measurements will differ from compositional measurements on solvent extracts. The results presented here indicate that the SPME−GC/O method is at least as good and gives results similar to those by solvent extraction. The ability to conduct a full GC/O dilution analysis to determine the odor potency of compounds of coffee verifies the usefulness of this method for complex food systems, especially those that are difficult to extract. To the extent that GC/O−SPME allows for the detection of compounds that would coelute with solvents, this method is an improvement over solvent extraction for GC/O.

CONCLUSIONS

A linear dilution analysis can be accomplished using SPME fibers of different thicknesses and exposure lengths. When applied to aroma analysis by GC/O (e.g., CharmAnalysis and aroma extraction dilution analysis), headspace samples can be diluted almost 2 orders of magnitude (1 to 1/50). This method could be invaluable for the analysis of fatty foods, solid foods, and aqueous foods, for which nonvolatiles create artifacts during solvent extraction. However, the data obtained from GC/O−SPME are modulated by the relative solubility of the volatiles in the fiber coating in exactly the same way solvent extraction modulates composition. Applicable to the analysis of complex systems (botanicals, foods, and fragrances), in retronasal aroma simulations of physiological headspaces, or in any system in which it is desirable to extract and concentrate the odorants in the headspace, the method is limited only by the solubility of the volatiles in the fiber coating. It is recommended that equilibration times be determined using the thickest coating and that the quantity of fiber exposed be carefully measured to get consistent and meaningful results.

Although extending this method to direct immersion into liquid samples may show similar results, the extraction of nonvolatiles would be expected to create artifacts in the same way solvent extraction does. The greatest benefit of this technique to gas phase dilution analysis is the elimination of solvent extraction and concentration. Additionally, SPME allows for headspace measurements with a greater sensitivity than other conventional methods of analysis such as purge and trap (Chaintreau et al., 1995). However, essential to the adoption of the technique for routine GC/O dilution analysis will be the commercial availability of fibers with nominal thicknesses that match closely their actual dimensions. At present the range of dilutions limited by the available fibers is a little more than 5 decades. However, combining the technique with different injector split ratios could extend this range.

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