Solid-Phase Microextraction for Flavor Analysis

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The application of solid-phase microextraction (SPME) for flavor analysis has been studied. SPME headspace and liquid sampling were tested for 25 common flavor components in dilute aqueous solution. The addition of salt generally enhances SPME adsorption. Larger sample volume and a smaller volume of headspace over the liquid sample also increase the sensitivity of SPME-based analysis. For thermal desorption following SPME, a GC injector liner with small diameter, e.g., 1 mm i.d., improves resolution and obviates the need for cryogenic focusing. The SPME technique was applied to authentic samples: ground coffee, a fruit juice beverage, and a butter flavor in vegetable oil. The advantages and limitations of SPME are discussed.

Keywords: *Flavor analysis; SPME; headspace analysis*

INTRODUCTION

Sample preparation techniques based on adsorption have been widely used for trace analysis to preconcentrate trace compounds and/or separate analytes from sample matrix. Recently, a new variation of adsorption technique called solid-phase microextraction (SPME) has been developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Arthur et al., 1992b,c; Potter and Pawliszyn, 1992), and SPME devices are now commercially available. The key component of a SPME device is a piece of fused silica fiber (ca. 1 cm in length) coated with an adsorbent such as poly(dimethylsiloxane). When the SPME fiber is immersed in an aqueous sample, a partitioning of the compounds in the sample between the aqueous phase and the fiber surface occurs. The adsorbed compounds can then be thermally desorbed in a GC injection port. This technique has been studied mainly for analysis of pollutants in environmental water samples (Arthur et al., 1992b,c; Potter and Pawliszyn, 1992). Headspace sampling using SPME has also been reported (Zhang and Pawliszyn, 1993).

SPME is also a potentially useful technique for flavor analysis. Hawthorne et al. (1992) successfully applied SPME with an uncoated fused silica fiber for determination of caffeine in beverages. In comparison with solvent extraction, simultaneous distillation/extraction, conventional solid-phase extraction, and purge-and-trap sampling, which are the most frequently used sample preparation techniques employed in flavor analysis (Reineccius, 1993; Bartsch and Hammerschmidt, 1993), SPME is simple, rapid, solvent-free, and inexpensive. Therefore, it is of great interest to investigate further applications of SPME. In the present study, we examined liquid and headspace SPME sampling in a test solution comprising 25 common flavor components and applied this technique to the analysis of authentic food, beverage, and flavor samples.

MATERIALS AND METHODS

Flavor Test Mixture. All flavor constituents used in this study were obtained commercially; purity was established by GC. Approximately equal amounts of 25 compounds (100 μL for liquids and 100 mg for solids) were mixed together. To make a stock solution, 50 μL of the mixture was diluted to 2.0 mL with absolute ethanol. A working solution was made by taking 10 μL of the stock solution and diluting to 10.0 mL with deionized water, giving approximately 1 ppm of each flavor compound in 0.1% (v/v) aqueous ethanol.

General SPME Procedures. The SPME device was purchased from Supelco Co. (Bellefonte, PA), as was the fused silica fiber coated with poly(dimethylsiloxane) (100 μm). Unless stated otherwise, for liquid sampling, the SPME fiber was inserted into a 1.3-mL vial containing 0.5 mL of sample; the fiber remained in the liquid for 10 min under stirring. For headspace sampling, 0.5 mL of a liquid sample was placed in a 2-mL vial, where it equilibrated for 1 h prior to 2 min of SPME headspace sampling under stirring.

Analysis of Fruit Juice Beverage. In a 4-mL vial, 0.6 g of NaCl was dissolved in 3 mL of sample. After SPME liquid sampling for 10 min at ambient temperature, the SPME fiber was introduced into the GC injector in splitless mode.

For comparison, 250 mL of sample was extracted three times with 50 mL of dichloromethane (DCM). The solvent was then removed using a Kuderna-Danish evaporator and concentrated to 250 μL by a gentle nitrogen stream. Thus, a concentration factor of ca. 1000 was achieved by the solvent extraction method. One microliter was injected into the GC column using split mode (1:70).

Instrumental Analysis. A Sigma 2000 gas chromatograph (Perkin-Elmer, Norwalk, CT) was equipped with both regular split/splitless and programmable temperature vaporizing (PTV) injectors. The temperature of the PTV injector (200 °C) was held constant during analysis. A 30-m, 0.25-mm i.d., 1-μm film thickness DB-Wax column (J&W Scientific, Folsom, CA) was used. The GC oven temperature was programmed as follows: 50 °C held for 2 min, increased to 220 °C at a rate of 4 °C/min. Helium was used as carrier gas. For thermal desorption, the SPME fiber remained in the injector for 3 min. Splitless injection mode was used, the split valve being opened after 2 min.

GC/MS analysis was carried out on a Varian 3400 gas chromatograph/Finnigan INCOS XL quadrupole mass spectrometer system. Compound identification was based on comparison of GC retention indices and mass spectra with those of authentic compounds.

For SPME/GC analysis, cryogenic focusing is often used to improve GC resolution. We found that the diameter of the GC injection liner can influence the peak width, especially for early eluting compounds. Therefore, instead of a conventional split/splitless injector with a 3.5-mm i.d. liner, a PTV injector with a 1-mm i.d. liner was used, and the GC resolution improved markably. Although cryogenic focusing can further sharpen the early eluting peaks, it was not necessary for the flavor compounds tested in this study under the GC conditions given above.

RESULTS AND DISCUSSION

SPME Performance. SPME headspace and liquid samplings were tested for 25 common flavor components

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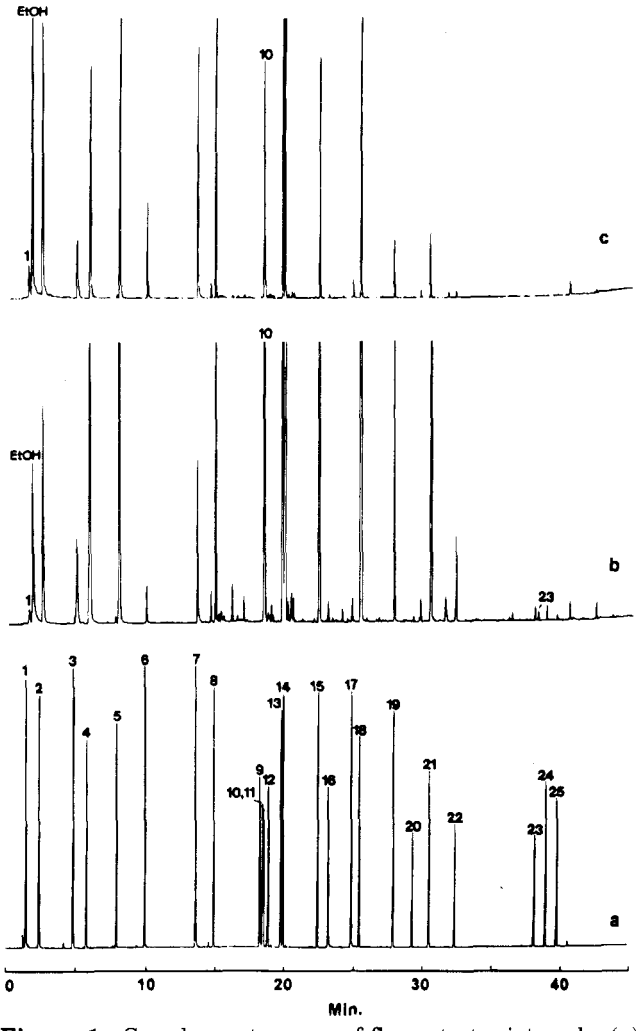

Figure 1. Gas chromatograms of flavor test mixture by (a) direct injection, (b) SPME liquid sampling, and (c) SPME headspace sampling. For component listing, see Table 1.

in dilute aqueous solution. The gas chromatograms obtained for a flavor test solution by both direct injection and SPME sampling are shown in Figure 1 (see also Table 1).

SPME exhibits some selectivity, yielding greater or lesser relative sensitivities for the various flavor compounds tested (Table 1). Under the experimental conditions employed, a detection limit of the order of 0.1−10 ppb is estimated for ethyl butyrate, ethyl hexanoate, *cis*-3-hexenyl acetate, linalool, neral, *l*-carvone, geranial, anethole, β-ionone, cinnamic aldehyde, and γ-decalactone. Ethyl acetate, limonene, *cis*-3-hexenol, and heliotropin could be detected at 0.01−1 ppm concentration. It is also not difficult to detect γ-hexalactone, hexanoic acid, phenylethyl alcohol, triacetin, and triethyl citrate at concentrations of 1 ppm. In contrast, no detectable amount of diethyl succinate, 2-methylbutyric acid, ethylvanillin, or vanillin was adsorbed by the SPME fiber from the 1 ppm test mixture. Generally, sample preparation methods based on reversed-phase adsorption or solvent extraction have low efficiency for isolation and concentration of hydrophilic compounds such as vanillin (Reineccius, 1993; Bartsch and Hammerschmidt, 1993).

When conditioned before use, the SPME fiber generated only low background in gas chromatograms. The eluted compounds due to the SPME coating bleed mostly contain silicon and are easily recognized by their distinctive mass spectra.

Table 1. Relative GC Peak Area by Different Sampling Methods and Relative Standard Deviation of SPME Liquid Sampling

		relative peak area %			
no.	compound	*a*	*b*	*c*	RSD %[b]
1	ethyl acetate	4.4	0.2	1.2	17.8
2	ethyl butyrate	5.0	2.6	11.5	6.3
3	limonene	6.4	1.2	2.6	16.5
4	ethyl hexanoate	4.3	6.9	8.4	8.6
5	*cis*-3-hexenyl acetate	4.3	7.8	12.0	10.8
6	*cis*-3-hexanol	4.9	0.3	2.1	5.9
7	benzaldehyde	5.5	1.1	6.0	5.9
8	linalool	4.5	9.0	16.0	3.2
9	diethyl succinate	3.4	<0.1	<0.1	
10	neral	2.9	7.0	5.9	2.3
11	2-methylbutyric acid	2.6	0.1	<0.1	4.4
12	γ-hexalactone	3.4	0.1	0.3	5.5
13	*l*-carvone	4.7	9.6	7.9	4.0
14	geranial	5.0	13.6	9.7	1.4
15	anethole	4.8	14.1	5.0	3.3
16	hexanoic acid	3.2	0.1	<0.1	12.2
17	phenylethyl alcohol	4.9	0.2	0.4	0.5
18	β-ionone	4.3	14.9	8.9	3.9
19	cinnamic aldehyde	4.6	2.5	0.2	3.4
20	triacetin	2.1	0.2	0.2	18.3
21	γ-decalactone	3.7	8.0	1.5	8.1
22	heliotropin	2.4	0.5	0.2	3.7
23	triethyl citrate	2.2	0.1	<0.1	2.7
24	ethylvanillin	3.3	<0.1	<0.1	
25	vanillin	3.0	<0.1	<0.1	

[a] Direct injection of flavor mixture. [b] SPME liquid sampling. [c] SPME headspace sampling.

SPME sampling is a single-batch process; i.e., the number of theoretical plates $N \leq 1$. The amount of an analyte adsorbed on the SPME fiber, and the resulting sensitivity, are determined both by adsorption kinetics and by the distribution coefficient of the compound between the fiber surface and the sample. Unlike conventional solid-phase extraction and purge-and-trap sampling techniques, in which a practically quantitative recovery is often achieved, SPME is more sensitive to experimental conditions. Any change of experimental parameters, which affect the distribution coefficient and adsorption rate, will also influence the amount adsorbed on the SPME fiber and the corresponding reproducibility.

Table 1 shows the relative standard deviations achieved using a SPME liquid sampling method followed by GC/FID analysis for 22 flavor compounds at an approximate concentration of 1 ppm. The average relative standard deviation of 7% is generally acceptable in trace organic analysis.

Salt Effect. Generally, the presence of electrolyte in an adsorption system can influence the adsorption in two ways: changing the properties of the phase boundary and decreasing the solubility of hydrophobic compounds in the aqueous phase. The latter is more often observed in analytical chemistry, being referred to as "salting out". The salting out effect is widely used to increase the sensitivity of an analytical method. Figure 2 shows the salt effect on SPME adsorption for some of the flavor compounds investigated in this study. In most cases SPME sensitivity changed significantly with increasing salt concentration. The influence of salt on SPME adsorption gave rise to four types of behavior among the flavor components studied: (a) For most compounds tested here, adsorption increases with increasing salt concentration (ethyl butyrate, *cis*-3-hexenol, benzaldehyde, linalool, neral, γ-hexalactone, *l*-carvone, phenylethyl alcohol, cinnamic aldehyde, γ-decalactone, heliotropin, and triethyl citrate). (b) Adsorp-

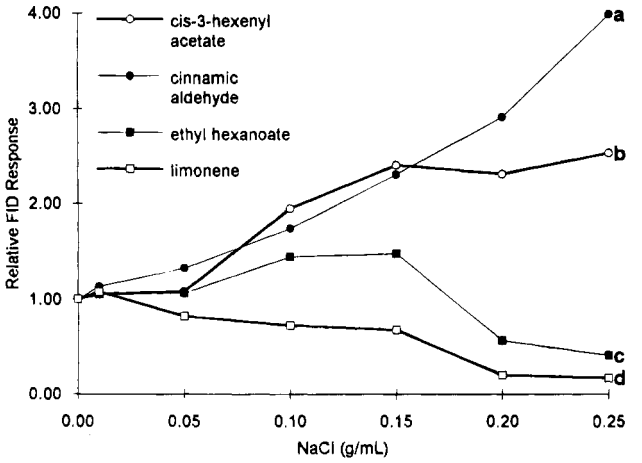

Figure 2. Four types of salt effects on SPME liquid sampling of a flavor mixture at a concentration of 1 ppm per component. Similar effects were observed for (a) ethyl butyrate, *cis*-3-hexenol, benzaldehyde, linalool, neral, γ-hexalactone, *l*-carvone, phenylethyl alcohol, cinnamic aldehyde, γ-decalactone, heliotropin, and triethyl citrate; (b) *cis*-3-hexenyl acetate, ethyl acetate, and geranial; (c) ethyl hexanoate, hexanoic acid, and triacetin; and (d) limonene, anethole, and β-ionone.

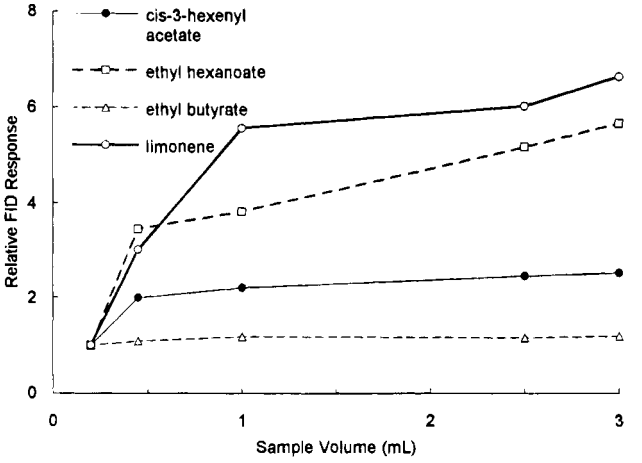

Figure 3. Influence of sample volume on SPME liquid sampling with a gas/liquid phase ratio of 1:1.

tion increases initially and then levels off at higher salt concentration (*cis*-3-hexenyl acetate, ethyl acetate, and geranial). (c) Adsorption increases initially and then decreases with increasing salt concentration (ethyl hexanoate, hexanoic acid, and triacetin). (d) Adsorption decreases with higher salt concentration (limonene, anethole, and β-ionone). Salt concentration was also found to have a similar effect on SPME headspace sampling.

Sample Volume. Although SPME adsorption is generally not quantitative, the concentration change of the sample after SPME adsorption cannot be ignored, especially if a small sample volume is used. The amount adsorbed on the SPME fiber is dependent not only on the initial sample concentration but also on the sample volume. This is illustrated in the case of liquid sampling in Figure 3. While the ratio of liquid phase and its headspace was kept constant at 1:1, the sample volume (liquid phase) was increased from 200 μL to 3 mL. With increasing sample volume, the extent of SPME adsorption increased rapidly initially and then remained relatively constant at larger volume. A similar relationship between SPME adsorption and sample volume was also observed for SPME headspace sampling. The flat part of the curve does not necessarily mean saturated adsorption, especially if the analyte concentration is low. When the concentration change after adsorption is no longer significant, SPME adsorption is practically independent of sample volume. SPME sensitivity can be increased by taking a larger sample volume, if the initial sample volume is small and the trace analyte is strongly adsorbed by the SPME fiber.

Comparison of Liquid and Headspace Sampling. For liquid and solid samples, three phases are generally involved during the SPME process, i.e., a gas phase, a condensed phase (liquid or solid), and an adsorption phase. A SPME fiber can be placed either in the headspace or in the liquid phase of a liquid sample. Because different phase boundaries are involved, adsorption from the headspace and liquid phases can be different. However, if the property change of the adsorption surface in gas and liquid phases can be ignored, the adsorbed amount of an analyte on the SPME fiber is thermodynamically independent of the sampling method, because at equilibrium the chemical potential of a compound in the three phases is the same. This can be understood by the following equilibrium considerations.

Suppose the SPME fiber is held in the gas phase of a liquid sample. For the equilibria

$$[A]_l \rightleftarrows [A]_g \rightleftarrows [A]_a$$

there are $K_1 = [A]_g/[A]_l$ and $K_2 = [A]_a/[A]_g$, where [A] is the concentration of analyte A in liquid phase (l), in gas phase (g), and in SPME adsorption phase (a); K_1 is the distribution constant for A between gas phase and liquid phase; and K_2 that between coating phase and gas phase.

Thus, if the SPME fiber is held in the liquid phase, for the equilibrium

$$[A]_l \rightleftarrows [A]_a$$

it is obvious $K = K_1K_2 = [A]_a/[A]_l$.

Although the presence of a gas phase does not change the distribution of an analyte between the liquid phase and the adsorption phase at equilibrium, the volume of the gas phase does influence the actual amount adsorbed on the fiber. For a given amount of analyte with larger headspace volume, more analyte goes into the gas phase and less remains in the liquid phase and on the fiber. Apparently, gas volume effects are stronger for analytes with higher vapor pressure. The same is true for SPME liquid sampling.

Figure 4 shows the influence of headspace volume on SPME headspace sampling. As expected, the extent of SPME adsorption decreased with increasing headspace volume, the amount of liquid phase being kept constant. Similar trends were also observed for SPME liquid sampling. To obtain higher sensitivity in SPME-based methods, the sample headspace should be kept as small as possible.

Zhang and Pawliszyn (1993) differentiated liquid sampling and headspace sampling using the equations

$$n = C_0V_aV_lK/(KV_a + V_l)$$

for liquid sampling and

$$n = C_0V_aV_lK/(KV_a + K_lV_g + V_l)$$

for headspace sampling, where n is the amount of the analyte adsorbed on the fiber; C_0 is the initial concentration of the analyte; and V_a, V_l, and V_g are the

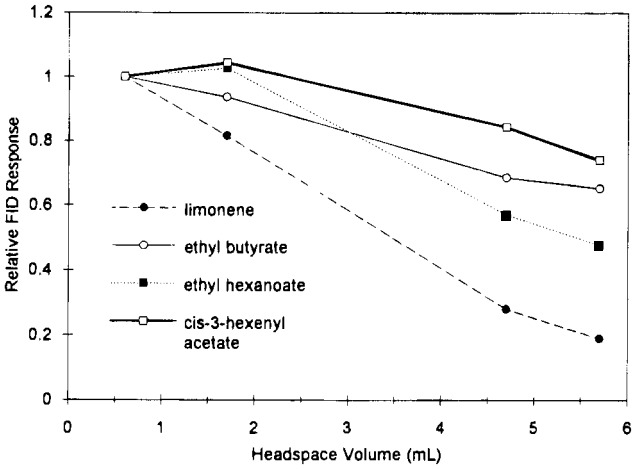

Figure 4. Influence of gas phase volume on SPME headspace sampling with 0.5 mL of solution.

volumes of adsorption, liquid, and gas phases, respectively. According to the two equations, the sensitivity of headspace sampling methods can never be higher than that of the corresponding liquid sampling method, because generally $K_i V_g > 0$. In fact, however, both equations can be obtained for liquid and headspace sampling, depending on whether the amount of analyte present in the gas phase is ignored (the first equation) or considered (the second equation).

Nevertheless, liquid and headspace samplings do differ in kinetics. Besides possible differences in rates of evaporation, dissolution, and diffusion in gas and liquid phases, the concentration difference between liquid phase and its headspace can make SPME liquid and headspace samplings very different. In general, the adsorption rate is higher when the concentration of analyte is higher. If an analyte exists predominantly in liquid phase, a SPME liquid sampling method is more sensitive than a headspace sampling method for a given sampling time and vice versa. This fact can be used to separate the volatile from the less volatile compounds by sampling the headspace.

APPLICATIONS FOR FLAVOR ANALYSIS

Espresso-Roast Ground Coffee. SPME is useful for headspace sampling of solid samples. Figure 5 shows a comparison of gas chromatograms obtained from espresso-roast ground coffee using conventional and SPME headspace sampling methods. As indicated by the gas chromatograms, the conventional headspace sampling method generally is more sensitive for highly volatile compounds, while the SPME headspace method picks up more of the less volatile compounds. An additional advantage of SPME sampling is that it prevents water from entering the GC and so avoids possible damage to the GC column.

Fruit Juice Beverage. A fruit juice beverage was analyzed by GC/MS following both SPME liquid sampling and solvent extraction with DCM. The gas chromatograms obtained are shown in Figure 6. Most of the flavor components extracted by DCM were also concentrated on the SPME fiber, albeit with somewhat different relative recoveries. The sensitivity of SPME achieved in this example is comparable to or higher than that of the conventional solvent extraction method for most esters, such as ethyl isovalerate, ethyl hexanoate, isoamyl butyrate, hexanyl acetate, *cis*-3-hexenyl acetate, *cis*-3-hexenyl butyrate, *cis*-methyl cinnamate, and *trans*-methyl cinnamate, for terpenoids, such as linalool,

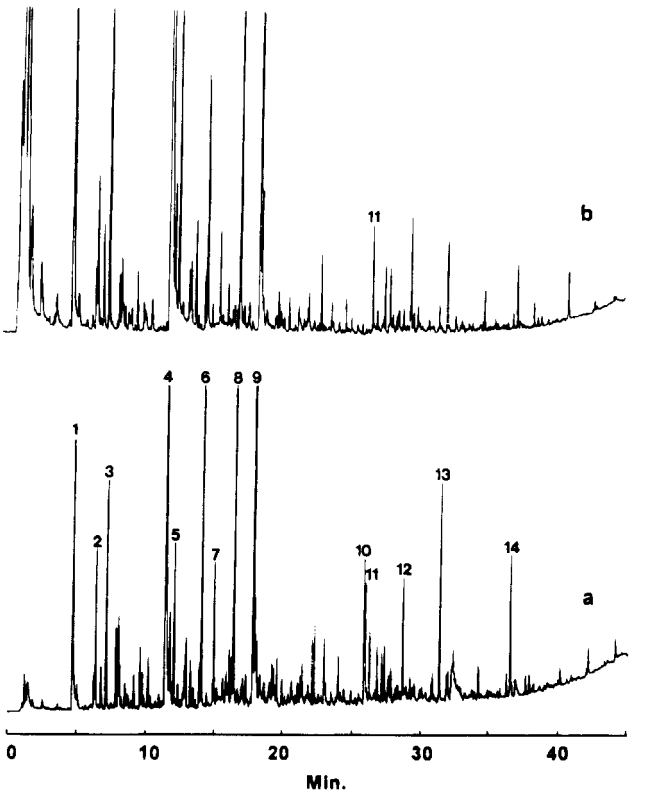

Figure 5. Gas chromatograms of espresso-roast ground coffee: (a) SPME headspace sampling at 120 °C; (b) 1-mL conventional headspace injection at 120 °C. Peaks: (1) pyridine; (2) 2-methylpyrazine; (3) acetol; (4) acetic acid; (5) hydroxyacetone acetate; (6) furfuryl acetate; (7) 5-methylfurfural; (8) γ-butyrolactone; (9) furfuryl alcohol; (10) maltol; (11) 2-acetylpyrrole; (12) pentanal; (13) 4-vinylguaiacol; (14) 3-hydroxypyridine.

β-terpineol, and α-terpineol, and for γ-decalactone. Obviously, the adsorption of fatty acids by SPME is very poor. However, the low affinity of SPME for fatty acids can be advantageous, e.g., by reducing possible interference from these compounds in an instrumental analysis of other trace flavor constituents.

Butter Flavor in Vegetable Oil. For vegetable oil-based samples, only headspace sampling can be applied using the SPME technique. The sensitivity of SPME-based methods is strongly dependent on sample matrix. For example, the presence of organic solvents decreases the adsorption of analytes on the SPME fiber (Arthur et al., 1992a). The same is true for oil-based samples; the sensitivity is much less than for comparable samples in aqueous solution, because the solubility of flavor compounds is generally much higher in vegetable oil than in water. Therefore, SPME of flavors in vegetable oil often requires sampling at higher temperature. The gas chromatogram of a butter flavor in vegetable oil using SPME headspace sampling is shown in Figure 7. SPME sampling is effective for detecting the characteristic components of butter flavor such as diacetyl, δ-decalactone, and δ-dodecalactone. Alternative methods for the analysis, e.g., involving static headspace sampling, simultaneous distillation/extraction, or purge-and-trap sampling, would be insufficiently sensitive and/or considerably more time-consuming.

SUMMARY

SPME is a useful tool for flavor analysis and can be considered as complementary to commonly used meth-

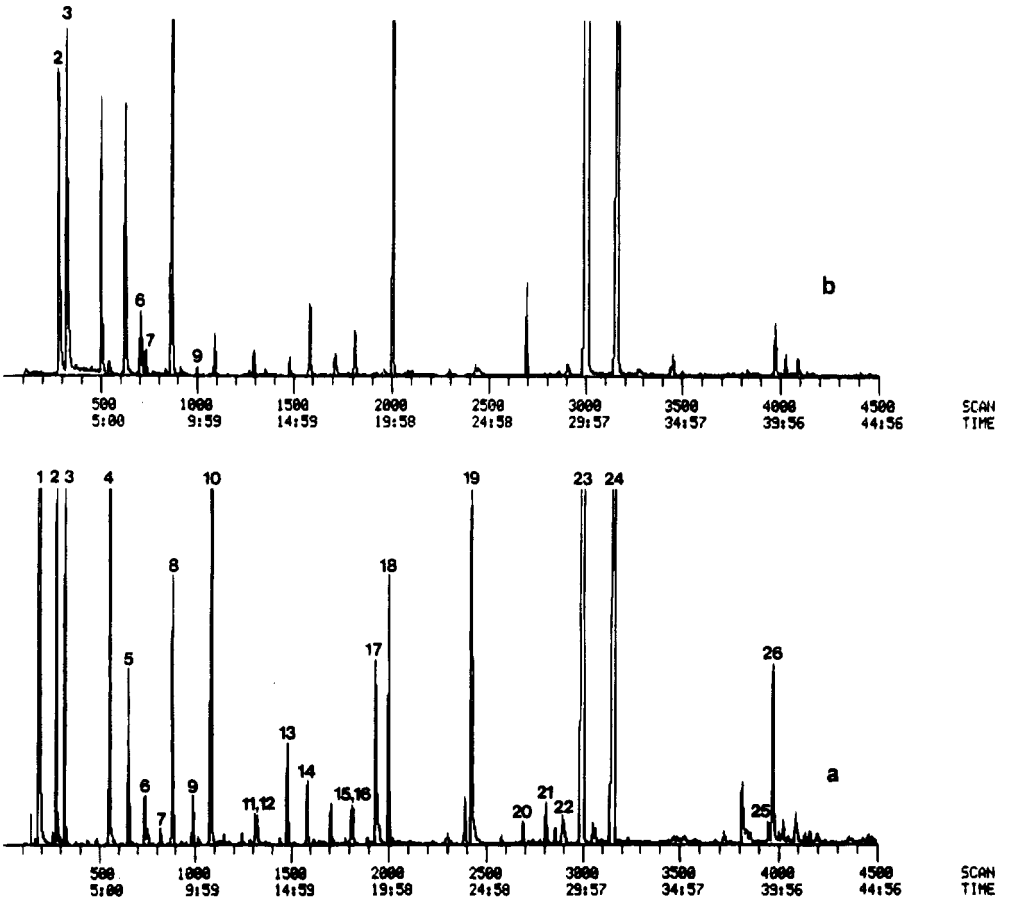

Figure 6. GC/MS chromatograms of fruit juice beverage by (a) DCM extraction and (b) SPME liquid sampling. Peaks: (1) dichloromethane; (2) ethyl butyrate; (3) ethyl isovalerate; (4) limonene; (5) ethyl hexanoate; (6) isoamyl butyrate; (7) hexanyl acetate; (8) *cis*-3-hexenyl acetate; (9) hexanol; (10) *cis*-3-hexenol; (11) *cis*-3-hexenyl butyrate; (12) furfural; (13) benzaldehyde; (14) linalool; (15) β-terpineol; (16) butyric acid; (17) 2-methylbutyric acid; (18) α-terpineol; (19) hexanoic acid; (20) *cis*-methyl cinnamate; (21) 1-(2-furyl)-2-hydroxyethanone; (22) furaneol; (23) *trans*-methyl cinnamate; (24) γ-decalactone; (25) dodecanoic acid; (26) (hydroxymethyl)furfural.

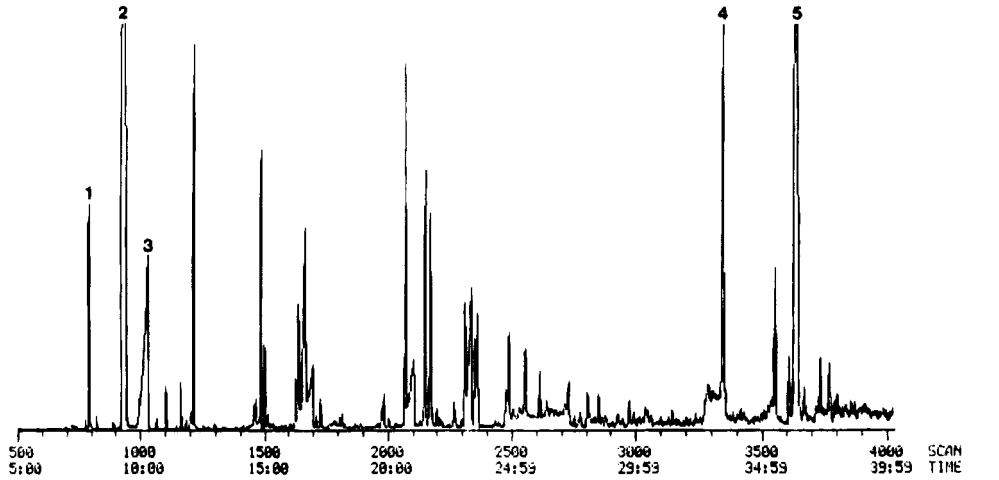

Figure 7. GC/MS chromatogram of butter flavor in vegetable oil by SPME headspace sampling at 160 °C. Peaks: (1) ethyl acetate; (2) diacetyl; (3) acetic acid; (4) δ-decalactone; (5) δ-dodecalactone.

ods such as solvent extraction, simultaneous distillation/extraction, conventional solid-phase extraction, and purge-and-trap sampling. However, unlike these techniques, in which the quantitative recovery of analytes is necessary for quantitation, SPME sampling is a single-batch process, so that quantitative adsorption is often very difficult, if not impossible. Results obtained using SPME strongly depend on experimental conditions and sample matrix. Any changes in experimental conditions that affect the adsorption distribution will be reflected in the sensitivity and reproducibility of the analytical method. An external calibration method for SPME generally is not suitable for quantitation, because a synthetic matrix can hardly match that of an authentic sample. Although the matrix effect could be significantly reduced by diluting samples and saturating with salt, it is not always applicable. Standard addition and isotopic dilution could also be used for quantitative analysis by SPME (Hawthorne et al., 1992).

Nevertheless, SPME provides many advantages over

conventional sample preparation techniques. SPME is very simple; it takes only a few minutes to complete and uses no solvent. It can be applied to flavor analysis of solid, liquid, and gaseous samples, especially for quickly screening the volatile composition. Recent enhancements to the technique of SPME have been provided by the introduction of a SPME autosampler device (Berg, 1993), allowing for more reproducible operation. SPME fibers with various coating materials and different film thicknesses are now available, which will broaden the scope of applications for SPME.

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