Solid-Phase Microextraction Method Development for Headspace Analysis of Volatile Flavor Compounds

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Solid-phase microextraction (SPME) fibers were evaluated for their ability to adsorb volatile flavor compounds under various conditions with coffee and aqueous flavored solutions. Experiments comparing different fibers showed that poly(dimethylsiloxane)/divinylbenzene had the highest overall sensitivity. Carboxen/poly(dimethylsiloxane) was the most sensitive to small molecules and acids. As the concentrations of compounds increased, the quantitative linear range was exceeded as shown by competition effects with 2-isobutyl-3-methoxypyrazine at concentrations above 1 ppm. A method based on a short-time sampling of the headspace (1 min) was shown to better represent the equilibrium headspace concentration. Analysis of coffee brew with a 1-min headspace adsorption time was verified to be within the linear range for most compounds and thus appropriate for relative headspace quantification. Absolute quantification of volatiles, using isotope dilution assays (IDA), is not subject to biases caused by excess compound concentrations or complex matrices. The degradation of coffee aroma volatiles during storage was followed by relative headspace measurements and absolute quantifications. Both methods gave similar values for 3-methylbutanal, 4-ethylguaiacol, and 2,3-pentanedione. Acetic acid, however, gave higher values during storage upon relative headspace measurements due to concurrent pH decreases that were not seen with IDA.

Keywords: *Solid-phase microextraction; isotope dilution assay; linear range; coffee; competition; headspace*

INTRODUCTION

The technique of solid-phase microextraction (SPME) offers a simple and sensitive technique for volatile compound analysis. Since the first description of the technique (Belardi et al., 1989), it has been widely adopted for use in air, water, soil, and food analysis. In fact, as of 5/99, there were about 420 publications on SPME (http://www.cm.utexas.edu/brodbelt/spme_refs.html). Several general SPME useful reviews have been published (Pawliszyn, 1997; Eisert and Pawliszyn, 1997), including one on flavor analysis (Harmon, 1997). The primary uses include quantification of compounds present in simple or complex matrices. Although different types of fibers with different affinities are available, they generally are particularly sensitive to nonpolar compounds.

There are seven commercial fiber types available: (1) poly(dimethylsiloxane) (PDMS) at 100, 30, and 7 μm sizes, (2) *polyacrylate* 85 μm, (3) PDMS/*divinylbenzene (DVB)* 65 μm, (4) PDMS/*Carboxen* 75 μm, (5) Carbowax/*DVB* 65 μm, (6) Carbowax/*template resin,* and (7) *divinylbenzene/Carboxen*/PDMS (in italics = porous adsorbents which are solid at room temperature, not in italics = liquid commercial GC phases).

Two sampling methods could be used depending on the placement of the fiber: in the sample or in the headspace of the sample. The fiber is often placed in the sample (immersion) if it is an aqueous sample free of carbohydrates, proteins, or fat, and the objective is quantification of a target analyte. In the cases where equilibrium is attained, the two sampling methods theoretically give the same results (Pawliszyn, 1997). With the headspace mode of sampling, the time to attain equilibrium is normally longer, but the fiber's lifetime is extended. Most applications use measurements under equilibrium conditions if their objectives are extraction of the matrix and quantification of compounds in the matrix.

In SPME headspace analysis, a fiber is placed in the headspace above an equilibrated sample. Given an infinite time of adsorption, two types of equilibrations take place: $K_{sample-air}$ and $K_{fiber-air}$. $K_{fiber-air}$ is highly dependent on the molecule and can be accurately estimated by its retention index on a column coated with a polymer corresponding to the fiber (Pawliszyn, 1997). Those compounds with high values of $K_{fiber-air}$ will have a large adsorption capacity on the fiber as compared to those compounds with low values.

For research on flavor-food matrix interactions, the volatile compounds in the headspace are of interest because they can travel to the nose during eating and stimulate the olfactory receptors in the nasal cavity (Linforth and Taylor, 1993). The effect of a given food component (protein, oil, carbohydrate) on the volatility of flavor compounds is usually checked by a form of headspace analysis (Roberts and Pollien, 2000). Complete extraction of the matrix would not allow the food component influences on flavor compounds volatility to be seen. An SPME method is proposed in this report that samples primarily the headspace. While the method itself is simple, the understanding of the physical chemistry of the method is more complex and is necessary for correct usage. Several factors such as the need to be in the linear range and competition effects between volatile compounds can cause biases in the quantitative determination of compounds (Coleman, 1996). Recently, it was shown that these limitations can be overcome by using isotope labeled compounds as internal standards hereby combining the advantages of SPME with those of IDA (Blank et al., 1999). IDA combined with SPME

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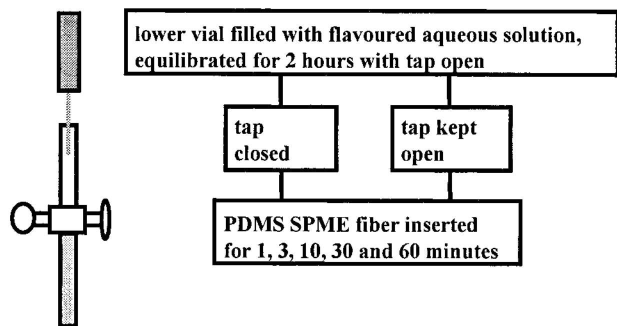

Figure 1. Method used to isolate the headspace from the sample and determine extraction location of the fiber.

was used for the first time for the solvent free quantification of caffeine in beverages (Hawthorne et al., 1992) and for hydrocarbons in wastewater (Langenfeld et al., 1996). In this paper we apply IDA, combined with SPME, for the rapid and direct quantification of selected coffee impact odorants, which were extensively studied by Grosch and co-workers (Semmelroch and Grosch, 1995, 1996). By now IDA has become an important quantification method in flavor research. Strengths and limitations of this method have recently been discussed (Milo and Blank, 1998).

EXPERIMENTAL PROCEDURES

General SPME Methodology. This was the general methodology used for all analyses, except for the different vial sizes where specifically noted. Samples were placed in a 2-mL vial (normally 0.8 mL of solution) and allowed to equilibrate. Then depending on the sample, time to equilibrium was checked and took between 30 min and several hours. Crimp tops for the vials from Agilent Technologies, Palo Alto, CA (5181−1210) were found to contain few volatile impurities. The headspace of the samples at room temperature was sampled using a Varian 8200 autosampler at room temperature. An SPME fiber was inserted into the headspace for a certain amount of time depending on the study. Various fibers were used throughout these studies all from Supelco (Bellefonte, PA). The PDMS fiber used had a film thickness of 100 μm. After sampling, the fiber was placed into the injection port of the GC equipped with a 0.75 mm i.d. liner (Supelco) for 5 min at 250 °C. During the first 3 min of desorption, the purge was off, and the last 2 min with purge on further cleaned the fiber. GC separation with FID or mass spectrometric detection was used for quantification of the aroma compounds. GC conditions for FID detection: DBWAX, J&W, 30 m; 0.32 mm i.d., 0.25 μm film, 10 psi helium or 60 m 0.25 mm i.d. GC conditions for MS detection: DBWAX, J&W, 30 m; 0.25 μm i.d., 0.25 μm film, 0.8 mL/min, constant flow (5 psi at 40 °C).

Isolation of Headspace from Sample. Figure 1 shows the experimental setup and design used in the study of extraction time's effect on "true" headspace. Two 7 mL glass vials were welded to a tap. The top vial contained a cap with septum that could be pierced by the SPME fiber. The vials had been silanized with Sylon CT (5% dimethyldichlorosilane in toluene, Supelco) to avoid compound absorption. The tap that connected them had a 3 mm diameter, which could be closed off or opened. A model flavored aqueous solution was used that contained compounds of different SPME fiber affinity at levels that were in the linear range: 3-methylbutanal (10 ppm), dimethyltrisulfide (2 ppm), 2,3-diethyl-5-methylpyrazine (2 ppm), and guaiacol (10 ppm).

Measurement of Air−Water Partition Coefficients. Air−water partition coefficients at 30 °C were measured using a static method with a stainless steel cell of 312 mL. After equilibrium, the cell's headspace was pushed through a 250 mg Tenax trap at 40 mL/min. A complete method description can be found in (Chaintreau et al., 1995). The method involves a calculation based on the difference in headspace concentration (GC area) between 1 mL and 2 mL or 1 mL and 4 mL solutions. At each of two different volumes, the aroma compounds were analyzed at the following concentrations in mg/L in water: 3-methylbutanal (3.75, 7.5, and 15), dimethyltrisulfide (1, 2, and 4), guaiacol (12, 28 and 40), 2,3-diethyl-5-methylpyrazine (50, 100, and 200), and 2-isobutyl-3-methoxypyrazine (6, 12, and 24).

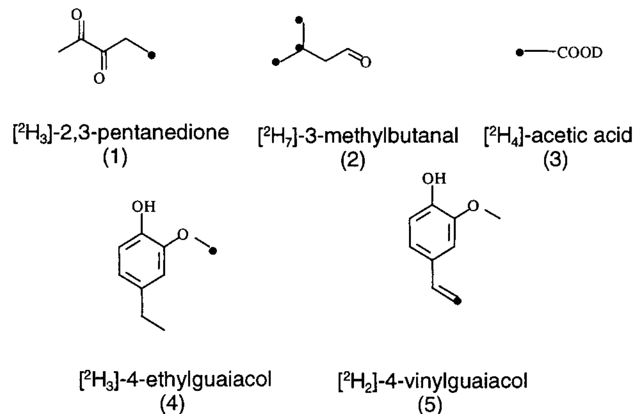

Figure 2. Chemical structures of the labeled internal standards used for quantification of the corresponding impact odorants in coffee. Symbol • indicates the labeling position for deuterium.

HPLC Lipophilicity Measurement. Compound lipophilicity was determined based on its retention time on a reversed phase HPLC column (El Tayar et al., 1985). The HPLC used was a Hewlett-Packard series 1100 with diode array and HP1097A refractive index detection (Avondale, PA). The column used (250 mm × 4 mm) was packed with Nucleosil 50-5 C18, particle size 5 μM (Macherey-Nagel, Oensinger, Switzerland). The mobile phase was made up volumetrically from various combinations (30−70%) of methanol and a solution containing 3-morpholinopropane sulfonic acid buffer (0.01 M) and *n*-decylamine (0.2% v/v). The pH of the aqueous solution was adjusted beforehand to 7.4 (4.5 for aldehydes) by addition of HCl. Retention times (t_r) were measured at room temperature with a 1.0 mL/min flow rate. The column dead time (t_o) was determined with uracil. The capacity factor was defined as $k = (t_r - t_o)/t_o$. Log k for 100% water (log k_w) was linearly extrapolated from results obtained for different mobile phase compositions.

Coffee Preparation for Fiber Comparison and Linear Range. Due to the potential problem of water vapor trapping on the SPME fiber and subsequent chromatographic resolution difficulties, analysis of hot coffee was not performed. Instead, freshly prepared R&G coffee was cooled to 4 °C and subsequently analyzed at room temperature. To check the linear range of the coffee samples, nonaromatized coffee was prepared by stripping the volatiles from the brew under vacuum (20 mmHg) at room temperature. The absence of volatiles was checked by SPME. The linear range was studied by diluting the coffee with nonaromatized coffee to achieve coffee at 50% and 25% concentration. The SPME analysis of the sample took place in duplicate at room temperature as previously described.

Quantification of Selected Volatiles in Coffee by SPME-IDA. The isotope labeled compounds **1**, **2**, **4**, and **5** (Figure 2) were custom synthesized by Aspen Research Laboratories (St. Paul, MN). [$^2H_4$]-Acetic acid was obtained by Aldrich. To quantify 4-ethylguaiacol, 4-vinylguaiacol, 2,3-pentanedione, 3-methylbutanal, and acetic acid, fresh and stored liquid coffee (cold; 50 mL) was spiked with [$^2H_3$]-4-ethylguaiacol (3.9 μg), [$^2H_2$]-4-vinylguaiacol (33.6 μg), [$^2H_3$]-2,3-pentanedione (8.6 μg), and [$^2H_7$]-3-methylbutanal (4.8 μg) in dichloromethane and [$^2H_4$]-acetic acid (2640 μg) in water. These compounds cover a wide range of physical and chemical properties and are known as important aroma compounds in coffee. Standard addition was done by preparing a standard mixture, containing the standards in a ratio of 0.2−5× over

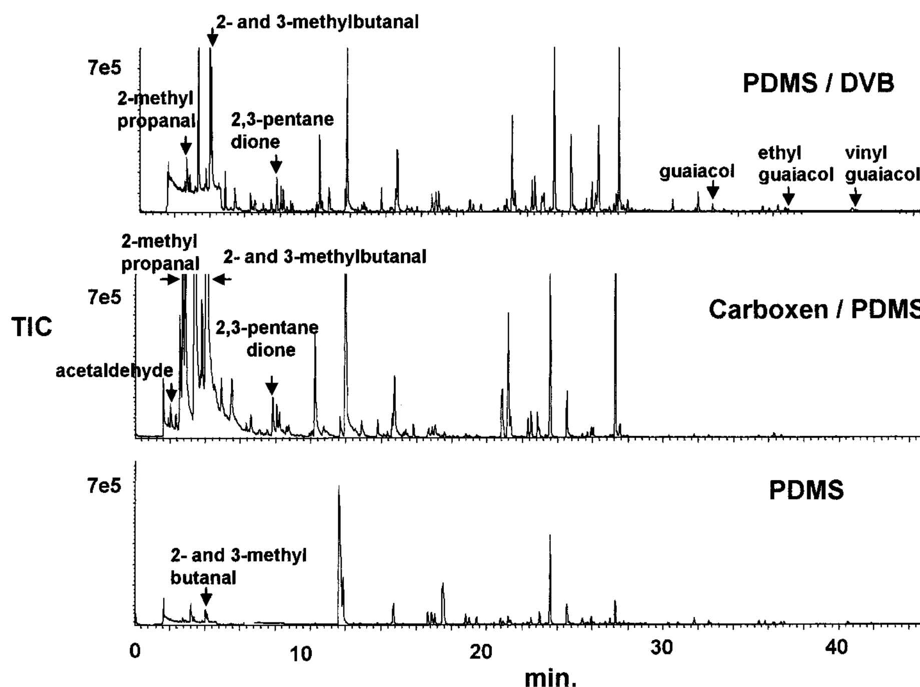

Figure 3. Comparison of different fibers for coffee brew analysis. Conditions: 1 mL in 16 mL vial, 10 min headspace adsorption, and GC-MS analysis using a DBWAX column.

their corresponding analytes and adding 0.5−1 mL of this solution to the coffee slurry. After preparation samples were kept frozen until analysis. All samples from one storage experiment were analyzed the same day with the same SPME fiber. The headspace sampling time for SPME-IDA was 60 min, while for relative measurements, i.e., without standards added, was 10 min. Thawed samples were analyzed in the order of storage time. For relative measurements, the influence of the standing time in the sequence tray was determined in a separate experiment using five identical samples which were injected after 1.5−10 h incubation at room temperature.

RESULTS AND DISCUSSION

Comparison of Different Fiber Types. The choice of fiber depends on the target compounds and therefore on the food of interest. The traditional fiber (PDMS) has very good stability and can be used for many injections (above 100). It is the first fiber tested, normally, because of this fact. However, some applications require special sensitivity to certain compounds. The PDMS fiber has very high sensitivity to nonpolar compounds but not to polar compounds. Because the target compounds often include polar compounds, other fibers were considered.

Coffee. Three fibers were compared for the analysis of coffee volatiles: PDMS, Carboxen/PDMS, and PDMS/DVB. Figure 3 shows the chromatographic profiles of the coffee volatiles. The PDMS fiber showed the lowest overall sensitivity. Carboxen/PDMS was the most sensitive for small molecules such as 2-methylpropanal and acetaldehyde. However, our experience is that there is a peak-tailing effect of highly volatile compounds when analyzing liquid samples with this fiber. PDMS/DVB gave the overall best sensitivity, especially for molecules such as guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol. Likewise, this fiber was chosen for juice analysis because it had the best overall sensitivity for various juice aroma compounds (Miller and Stuart, 1999) and for polar analytes in water (Gorecki et al., 1998).

Organic Acids. Three fibers thought to be more sensitive to polar compounds were compared. Carboxen/

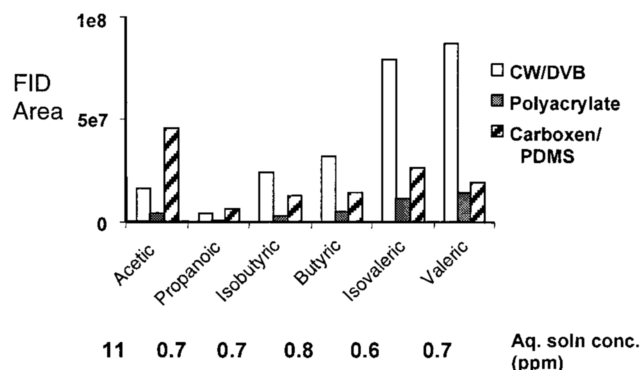

Figure 4. Comparison of different fibers for organic acid analysis. Conditions: aqueous solution (0.2 mL) in 2 mL vial, 30 °C, pH 2, saturated in NaCl, and 60 min headspace adsorption.

PDMS is especially sensitive for small molecules. Figure 4 shows that it had the best sensitivity toward acetic and propanoic acid but not for the others. For best overall sensitivity to the range of acids, Carbowax/PDMS was chosen. This fiber was also chosen as the best for analysis of polar pyrazines and furfural (Coleman, 1996).

Polar Compounds of Low Volatility. Compounds such as furaneol, sotolon, and vanillin are important aroma compounds in many foods including fruits, coffee, and meats. However, their concentrations are usually at the ppb or low ppm levels. SPME has difficulty in detecting polar compounds of low volatility at the levels they are found in foods. In our model system studies, the concentration was chosen to be very high (2000 ppm) in order to have sufficient quantity in the headspace for detection. Five fibers were compared, and PDMS/DVB was found to give the best sensitivity (Figure 5). There is a need for development of fibers that are sensitive to polar molecules such as Nafion-coated fibers (Goreckiet al., 1998).

Sensitivity and Reproducibility. Many other publications report excellent sensitivity and reproducibility

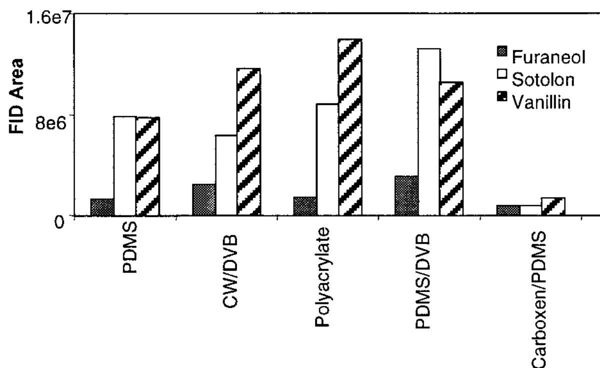

Figure 5. Comparison of different fibers for analysis of polar compounds of low volatility. Conditions: 0.8 mL of the flavored aqueous solution of 2000 ppm for each compound in 2 mL vial, 30 °C, and 10 min headspace adsorption.

of this method when using long extraction times (Roberts et al., 1996; Jelen et al., 1998; Miller and Stuart, 1999). Generally, sensitivity depends on the compound analyzed as nonpolar compounds are easily detected at the ppb levels, while it is sometimes difficult to detect polar compounds at ppm levels. In analyzing R&G coffee at room temperature in SIM GC-MS mode with a PDMS/DVB fiber, the following compounds could be detected: vanillin (7.7 ppm), furaneol (116 ppm), ethylguaiacol (2.3 ppm), guaiacol (5.7 ppm), 4-vinylguaiacol (40 ppm), β-damascenone (0.2 ppm), 2,3-diethyl-5-methylpyrazine (0.4 ppm), and 2-ethyl-3,5-dimethylpyrazine (1 ppm). This sensitivity was obtained when using a 5 min fiber adsorption time, except for furaneol where 30 min was required. The reproducibility of the method also often depends on the compound, but on average, we find a CV of about 7% with a range from 2 to 15, similar to other publications (Yang and Peppard, 1994).

Linear Range. The high sensitivity of SPME fibers to most compounds leads to a linear range that is usually under 1 ppm. This may mean that for analysis of natural products, where compounds have different affinities for the fiber and are present at various concentrations, that all compounds may not be in the linear range. Above this concentration, there is less and less increase in the amount bound for equal increases in concentration. Solutions to this problem include dilution of the matrix, reducing the adsorption time so the linear range needs are met, or quantification by using isotope labeled standards, which behave identically to the target compound.

To stay in the linear range for coffee analysis, a short adsorption time was chosen, and the linear range was checked in the following way. Coffee was analyzed at 100, 50, and 25% strength, using nonaromatized coffee for dilution. If the percent decrease in a compound's adsorption corresponded to the dilution, the linear range needs were met. Table 1 shows that for most compounds, this was the case. Certain rather polar compounds (2,3-pentanedione, pyrazine, furfural, and 2-acetylfuran) showed slight nonlinear behavior. Compounds showing slight nonlinear behavior are not necessarily those in the highest concentration as those four compounds had 2.7, 0.7, 17, and 5% peak areas, respectively. What they do have in common is a relatively low affinity to a PDMS fiber as seen by their calculated values based on their linear retention index (Pawliszyn, 1997): $K_{\mathrm{PDMS\ fiber-air}} = 390$, 570, 1350, and 3020, respectively. This method of dilution in the matrix

Table 1. Linearity Determination for 1 Min SPME Headspace Analysis of Coffee Using a PDMS/DVB Fiber[a]

	% area of full strength	
	50% strength brew	25% strength brew
3-hexanone	47	nd[b]
2,3-pentanedione	58	34
pyrazine	60	nd[b]
2-methyltetrahydrofuran-3-one	56	23
2-methylpyrazine	53	24
2,5-dimethylpyrazine	51	nd[b]
2,6-dimethylpyrazine	48	22
2-ethylpyrazine	52	25
2-ethyl-6-methylpyrazine	46	nd[b]
2-ethyl-3-methylpyrazine	54	nd[b]
furfural	57	27
2-acetylfuran	57	27
furfuryl acetate	57	25
5-methylfurfural	52	24
furfurylpropanoate	50	nd[b]
2-furfurylfuran	50	27
N-methyl-2-carboxaldehyde-pyrrole	50	23
median	52	25

[a] Dilutions of full strength brew were made with nonaromatized brew. [b] Not detected: nd.

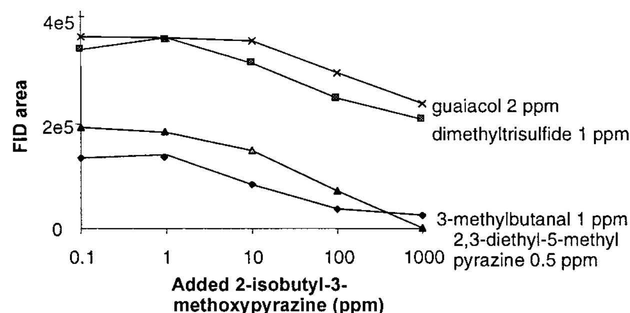

Figure 6. Illustration of competition on SPME fiber showing how an added nonpolar compound can greatly influence other compounds' adsorption. Conditions: PDMS fiber, aqueous solution (0.8 mL) in 2 mL vial, 30 °C, and 30 min headspace adsorption.

can give direction about the linear range of the method for the target compounds in a complex system.

Competition. Competition for adsorption by fibers are important to realize and control for. Researchers have demonstrated many instances of competition. Ethanol replaced acetone and isoprene on PDMS/DVB (Grote and Pawliszyn, 1997). Methyl isobutyl ketone replaced 2-propanol, 2-methyl-2-propanol, and tetrahydrofuran with Nafion coated fibers (Goreckiet al., 1998). Alkylpyridines replaced pyridine (50 ppm) with CW/DVB and PDMS (Coleman, 1996). Farmesene replaced butyl acetate upon high adsorption times (Song et al., 1997). In addition, Maillard reaction compounds at 50 ppm were adsorbed to a greater degree when in a mix of 5 as compared to 20 (Coleman, 1996). However, competition effects were not found between pesticides in drinking water (Dugay et al., 1998).

Figure 6 shows how added 2-isobutyl-3-methoxypyrazine influenced the fiber's adsorption of four other compounds. Up to 1 ppm of added pyrazine caused little effect. However, higher levels resulted in marked decreases of the other compounds. Compounds with high $K_{\mathrm{fiber-air}}$, such as 2-isobutyl-3-methoxypyrazine, are highly adsorbed by the fiber and are those which can reduce the adsorption of other compounds. Competition phenomena occur once the concentration exceeds the upper limit of the linear range. Short sampling times

can be used to reduce the possibility for fiber overloading and resulting biases, especially when both compounds of low and high affinity for the fiber are analyzed (Goreckiet al., 1998).

Competition effects can affect the results when two samples are being compared for amounts of target volatiles. If, for example, one of the samples has a large concentration of a compound with a high $K_{fiber-air}$ that the other does not, adsorption amounts of the target volatiles would be affected. In this case, the two samples could not be adequately compared unless isotopic standards were used for quantitation.

Effect of Extraction Time on "True" Headspace. Various analytical techniques exist that give us information about the volatile compounds in the food matrix or in the headspace above the food matrix. If the goal is complete extraction of the food matrix, techniques such as simultaneous distillation extraction (SDE), solvent extraction, or exhaustive SPME are used. Exhaustive SPME occurs when the fiber is left in the sample headspace or sample matrix until equilibrium is reached (the maximum possible amount is adsorbed on the fiber). None of these techniques gives absolutely complete and accurate quantitations of the compound amounts because of biases induced by the method: e.g. choice of solvent, losses of highly volatile compounds for SDE, and higher adsorption of nonpolar compounds for SPME.

Static headspace analysis samples the volatiles in the air above an equilibrated sample. In dynamic headspace analysis, an inert gas is blown above the sample and collected or analyzed directly. Purge-and-trap in this context refers to purging of the sample by this inert gas, that is bubbled through the sample, and then trapped by an absorbent trap before analysis. These dynamic methods are more sensitive than static headspace analysis because they induce additional volatilization of compounds that were not in the headspace at equilibrium. In studies that compared gas- versus SPME-sampled static headspace, SPME had 2−5 and 1800-fold the sensitivity for the more nonpolar alcohol and ester compounds in beer (Jelenet al., 1998) and for nonpolar compounds in juice (Miller and Stuart, 1999), respectively. Both of these studies used a 60 min exposure time of the SPME fiber to the headspace during which time the matrix was extracted (exhaustive SPME). When using a shorter sampling time, the profiles of conventional and SPME headspace analysis are more similar, although the less volatile compounds are detected better with SPME and the highly volatile compounds with static headspace (Yang and Peppard, 1994).

Although short sampling times with SPME have reduced sensitivity as compared to exhaustive SPME, it better approaches static headspace. The rate constants for $K_{fiber-air}$ are much faster than for $K_{sample-air}$. SPME sampling of volatiles in the headspace occurs in the order of minutes, while sampling of the liquid often needs hours. This can be seen by the different equilibration times between molecules: those mainly sampled from the headspace take minutes, while those which also include liquid extraction take hours to reach equilibrium. Ai (1997) found that the time to reach adsorption equilibrium with the fiber is an order of magnitude shorter in the gas phase than in the aqueous phase. This is explained by convection in the gas phase which maintains a steady-state diffusion at the SPME polymer surface. Short sampling times can be used for quantitation as there is a linear relationship between the adsorbed analyte amount and the initial sample concentration (Ai, 1997).

Figure 1 shows the method used to determine the effect of fiber extraction time on the sampling of the "true" headspace. In the tap closed situation, the fiber samples the equilibrium ("true") headspace. With the tap open, the fiber, air, and sample start to come to a three-phase equilibrium (sample, fiber, headspace). The fiber can induce additional volatilization of compounds from the sample into the air which it can adsorb. The effect of different lengths of time where the fiber was inserted into the headspace was determined.

The results are dependent on the compound analyzed (Figure 7). 3-Methylbutanal and dimethyltrisulfide are compounds with a high $K_{air-water}$ and low to moderate $K_{fiber-air}$ (Table 2). These compounds are present at high concentrations in the headspace. In fact, the amount in the headspace was enough to saturate the fiber. Thus, little difference was found between the tap open and tap closed situation.

2,3-Diethyl-5-methylpyrazine and guaiacol are compounds with low $K_{air-water}$ and moderate to high $K_{fiber-air}$. They showed marked differences between the tap open and tap closed situations. These two compounds are in low concentrations in the headspace, yet the fiber has a high affinity for them. Therefore, with the tap-open, the fiber induces a volatilization of these compounds from the matrix. For compounds such as these, short extraction times should be used to minimize extraction of the matrix if headspace sampling is the goal. This experiment used two vials connected by a tap, where a small opening limited diffusion. Fiber extraction of the matrix may have been even higher in the tap-open situations if diffusion was not limiting.

Confirmation of these differences between compounds were demonstrated using the normal 2 mL sampling vial (Figure 8). As adsorption time was increased, 3-methylbutanal and dimethyltrisulfide showed little increases after the initial 1 min adsorption time. These compounds with low $K_{fiber-air}$ have concentrations in the headspace which probably overload the fiber's capacity. After a 1 min adsorption, these compounds have already reached about 80% of the fiber's 60 min adsorption capacity. However, guaiacol and 2,3-diethyl-5-methylpyrazine showed large increases as time of adsorption was increased. Comparing their 1−60 min adsorption areas, 3-methylbutanal and dimethyltrisulfide less than doubled, but guaiacol and 2,3-diethyl-5-methylpyrazine rose more than five times their level. This shows that not only the headspace but also the liquid was being extracted for guaiacol and 2,3-diethyl-5-methylpyrazine, which are two compounds with high $K_{fiber-air}$ values.

Relative and Absolute Quantification of Volatiles Using SPME. Absolute and accurate quantification of volatiles using SPME is difficult considering the possible biases caused by the linear range, competition phenomena, different affinities to the fiber, and the effect of sampling time. Using an external standard curve for absolute quantification with SPME can only be done if the matrix can be completely mimicked: for example, for quantification in water (Langenfeldet al., 1996). An external standard can be used, however, to obtain relative values (Roberts and Pollien, 2000). Standard addition has also been reported to function for quantification in water (Langenfeldet al., 1996),

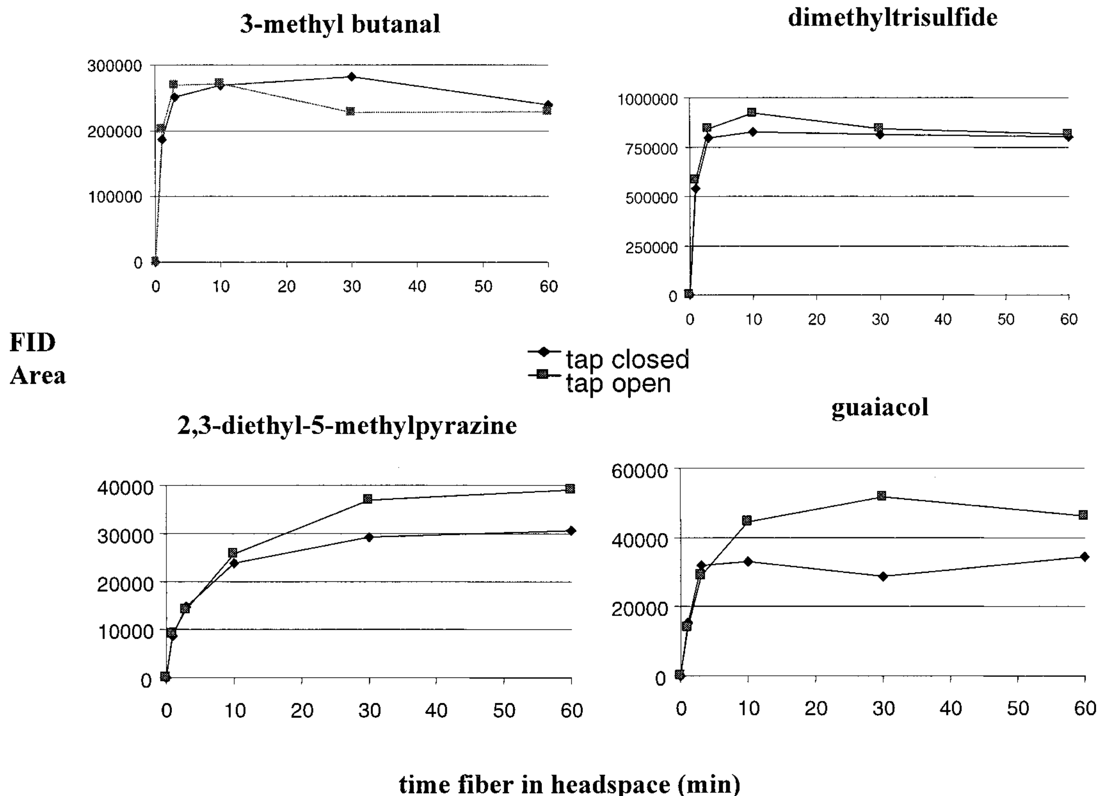

Figure 7. Effect of extraction time on headspace amounts, where the tap closed situation corresponds to true equilibrium headspace and the tap open situation shows induced volatilization by the SPME fiber.

Table 2. Partition Coefficients for Flavor Compounds

	$K_{air-water}$ (30 °C)	$K_{PDMS\ fiber-air}$[a]	lipophilicity log k_w
3-methylbutanal	1.4×10^{-2}	270	1.24
dimethyltrisulfide	2.2×10^{-2}	5700	2.16
guaiacol	2.6×10^{-4}	18 000	0.97
2,3-diethyl-5-methyl-pyrazine	5×10^{-4}	35 000	1.73
2-isobutyl-3-methoxy-pyrazine	3.2×10^{-3}	45 000	2.43

[a] Calculated from linear retention index (RI) (Pawliszyn, 1997): $\log K_{fiber-air} = 0.00415^{*}\ RI - 0.188$.

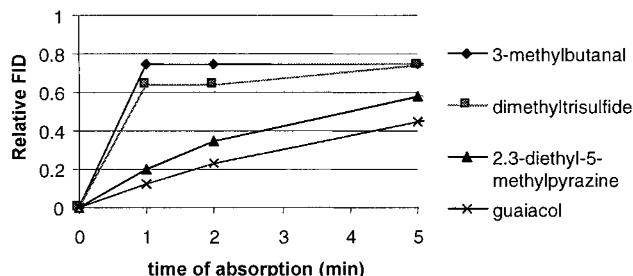

Figure 8. Effect of time of adsorption on FID area (relative to value at equilibrium) for compounds of different properties. Conditions: PDMS fiber with 2 mL sampling vial containing 0.8 mL of 1 ppm flavored aqueous solution.

although linear range needs would still have to be met. Quantification of all compounds using one internal standard does not give absolute quantification due to different partition coefficient values among compounds (Harmon, 1997). Absolute quantification can be obtained by using isotope dilution assays (IDA), i.e., compounds labeled with stable isotopes as internal standards (Blank et al., 1999). In some cases, however, e.g. studies on the kinetics of aroma losses or aroma degradation, relative measurements compared to a reference would be sufficient if the method accurately determines changes in the samples. This is demonstrated for selected impact odorants of liquid coffee as affected by room-temperature storage.

Coffee Aroma Degradation: Comparison of Absolute and Relative SPME Measurements. SPME was chosen as the method to monitor changes in volatile concentrations due to the high affinity of the PDMS/DVB fiber to coffee volatiles and the ability for high sample throughput. After storage of a liquid coffee for up to 4 weeks at room temperature, compounds such as 4-vinylguaiacol, 2,3-pentanedione, and 3-methylbutanal were drastically reduced, whereas acetic acid seemed to be present in higher amounts as determined by SPME in the headspace above coffee. However these changes may be partly caused by the possible biases mentioned above. Therefore, we determined the changes of potent odorants upon storage of coffee expressed as relative changes in peak area (total ion counts; TIC) and compared them to those which were obtained by using stable isotopes as internal standards. As illustrated in Figure 9 a,b) the relative losses of 4-vinylguaiacol, 2,3-pentanedione, and 3-methylbutanal during storage were the same for the two methods. Therefore, relative TIC measurements reflect the true concentration changes in these coffee impact odorants under the analytical conditions chosen.

The increase in acetic acid observed by relative TIC measurements (Figure 9b) could be explained by the change in pH of the liquid coffee from 5.25 to 4.85 during the storage period. At a lower pH a higher proportion of undissociated acid is available, which will partition into the headspace and be adsorbed on the SPME fiber. A similar increase was also found for 4-ethylguaiacol

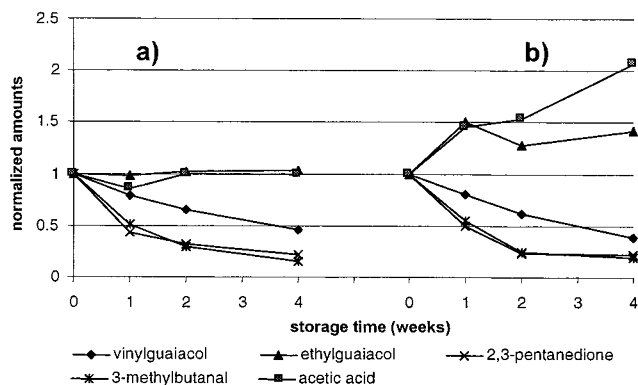

Figure 9. Comparison of the degradation of aroma impact compounds during storage (25 °C) of liquid coffee measured by (a) HS-SPME-IDA and (b) HS-SPME via peak areas of total ion current. Normalization was done based on the absolute concentration of each individual component at time zero for (a) and the peak area at time zero for (b).

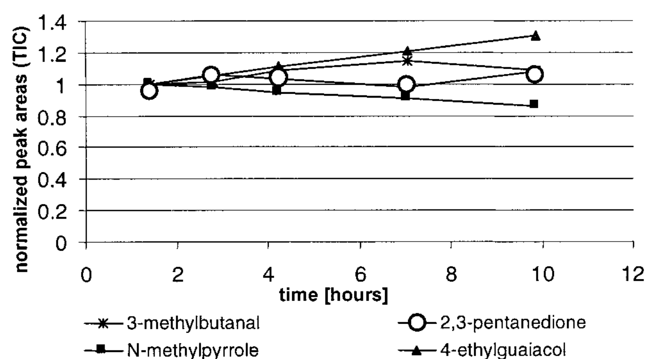

Figure 10. Changes of coffee aroma volatiles as affected by standing time in the sequence tray.

although pH changes will not account for this. We assume that the system was not fully equilibrated for this compound after 1.5 h due to a very low volatility. Therefore, samples that were analyzed after a longer standing time in the sequence may result in an apparent increase of compounds with low volatility (this was also the case for alkylpyrazines). Since the stored samples were analyzed in the order of the storage time (after a blank vial, 0d, 1w, 2w, and 4w), we determined the influence of "standing time" in the sequence tray on the TIC area. Figure 10 shows that indeed there is a slight apparent increase for 4-ethylguaiacol (and alkylpyrazines) and a decrease for the very labile *N*-methylpyrrole depending on the time in the sequence tray. The influences due to pH changes and/or equilibration time are, however, fully compensated by HS-SPME-IDA. The latter results (Figure 9a) clearly demonstrate that there is no increase in absolute concentration neither for acetic acid nor for 4-ethylguaiacol during storage of liquid coffee at room temperature. Keeping in mind the effect of long equilibration times for compounds with low volatility we can summarize that SPME reflected well the overall changes in the volatile fraction of stored coffee and seemed not to be biased by competition and or linear range phenomena.

CONCLUSIONS

SPME offers certain advantages over other sampling techniques. It is high through-put and does not require extended sample preparations resulting in time-savings. Likewise, it is inexpensive and avoids the use of solvents for extraction. Although highly sensitive to nonpolar compounds, more developments in fibers with high sensitivity to polar compounds would be useful. While the usage is simple, several factors need to be taken into consideration for reliable comparisons. Competition effects may skew results if a compound with a high $K_{fiber-air}$ is present at high concentrations. If relative values are measured, the compound concentrations should be in the linear range. Compounds at high concentrations or with high affinity to the fiber can be brought into the linear range by short-time sampling. Short-time sampling (1 min) also better estimates "true" headspace, which is the goal in some studies, as opposed to sample extraction. Examples with coffee show that under the analysis conditions chosen, biases due to competition or linear range excesses were controlled, as checked by isotope dilution assays.

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