Analysis of the Volatile Components in Vanilla Extracts and Flavorings by Solid-Phase Microextraction and Gas Chromatography

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The development and application of a solid-phase microextraction (SPME) method in the analysis of vanilla extracts and vanilla flavorings was studied. The SPME method was developed to be used in conjunction with gas chromatography mass spectrometry (GC−MS). The optimized SPME sampling parameters for the determination of the volatile components included a poly(acrylate) fiber, a 40-min sampling time at room temperature, and a 2-min desorption time. The reproducibility of the method was good, with a percent relative standard deviation between 2.5 and 6.4% for the target compounds. The data suggest that the origin of natural extracts can be readily determined from the GC profile and that differences exist between nature-identical and synthetic flavorings and the natural extracts. The method also has potential for identifying the type of vanilla extract/flavoring used to flavor food.

Keywords: *Solid-phase microextraction; vanilla extracts; flavor analysis; gas chromatography*

INTRODUCTION

Vanilla is one of the most widely used flavoring ingredients in food. Several vanilla flavoring agents are used, the most prized being natural extracts derived from the vanilla orchid. The specific taste and aroma properties of the different agents result from the blend of components present. Over 170 volatile components that contribute to flavor have been identified in natural extracts, some being present in minute amounts (Klimes and Lamparsky, 1976; Ranadive, 1992). Vanillin, *p*-hydroxybenzaldehyde, vanillic acid, *p*-methoxybenzaldehyde, and piperonal are some of the components found in the highest quantitites. High quality natural extracts are expensive and their supply is limited, therefore, nature-identical and synthetic vanilla flavorings are frequently used to flavor food. Nature-identical flavorings contain only components that are found in nature. Synthetic vanilla flavorings usually contain vanillin and/or ethylvanillin that has been synthetically produced. As natural extracts are expensive compared to nature-identical and synthetic vanilla flavorings, there have been many attempts at adulterating natural extracts or substituting less expensive vanilla flavorings for natural extracts (Riley and Kleyn, 1989; Lamprecht et al., 1994).

Many different methods have been developed to characterize vanilla extracts including high-performance liquid chromatography (HPLC), isotope ratio mass spectrometry (IRMS), gas chromatography (GC), and thin-layer chromatography (Ranadive, 1992; Belay and Poole, 1993; Lamprecht et al., 1994). HPLC allows the relative concentrations of the main components such as vanillin, *p*-hydroxybenzoic acid, and *p*-hydroxybenzaldehyde to be compared and can be used to determine the origin of the extract. Vanillin extracted from the vanilla orchid has a characteristic carbon isotope signature (−16 to − 21‰) which is very different to that of vanillin derived from lignin (−26 to −32‰) (Lamprecht et al., 1994). However, the analysis involves isolation of pure vanillin from the extract prior to isotope analysis. In addition, the IRMS equipment is expensive and not available in many laboratories. GC is potentially ideal for the analysis of complex mixtures such as natural extracts. However, traditional methods for extracting volatile components from the nonvolatile components (fats, sugars, and waxes) are both time-consuming and prone to sample loss and degradation. An alternative has been to sample the headspace only; however, conventional headspace methods such as static headspace GC or dynamic purge and trap GC methods require concentration steps and specific sampling equipment and are time-consuming (Steffan and Pawliszyn, 1996).

Solid-phase microextraction (SPME) is a relatively new solventless extraction technique that can be used in conjunction with HPLC or GC. The analytes are extracted from a variety of matrixes by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. The stationary phase, which is coated onto a fused silica fiber, is exposed to the headspace or liquid. The extracted analytes can then be thermally desorbed in the injector of the GC and subsequently swept onto the column where they are separated. This provides a simple and effective method for the selective extraction of volatile and semivolatile components from a matrix containing nonvolatile high molecular weight components. The technique has been successfully used for the analysis of volatiles from apples (Song et al., 1997), cinnamon (Miller et al., 1996), orange juice (Steffen et al., 1996), ground coffee (Yang and Peppard, 1994), and hops (Field et al., 1996).

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Table 1. Concentration, GC Retention, and Precision Data for Components Present in the Standard Mixture

analyte	concentration ppm	retention time, min	precision (% RSD) 25 °C	80 °C
m-methoxybenzaldehyde	0.10	9.1	3.32	8.03
ethyl benzoate	0.10	11.6	4.30	6.39
piperonal	0.10	13.3	6.40	13.50
vanillin	10.0	15.9	2.51	15.51
coumarin	10.0	17.9	5.22	10.75
ethylvanillin	10.0	18.2	5.22	14.65
p-hydroxybenzaldehyde	10.0			
vanillic acid	10.0			
protocatechuic acid	10.0			

We report here the development of a qualitative method for the analysis of flavor volatiles present in vanilla extracts/flavorings using SPME. This had been applied to the analysis of vanilla extracts (natural and synthetic) and food samples.

MATERIALS AND METHODS

Chemicals. Coumarin, ethyl vanillin, ethyl benzoate, *p*-hydroxybenzaldehyde, *m*-methoxybenzaldehyde, piperonal, protocatechuic acid, vanillin, and vanillic acid were purchased from Sigma, Australia, and used as received. Ethanol, HPLC grade, was purchased from Aldrich, Australia.

Standards and Samples. A standard mixture comprising the substances listed in Table 1 was prepared in 95:5 water:ethanol. This matrix was chosen to match the ethanol content of the diluted (1 in 10) natural extracts. Ethyl benzoate, piperonal, and *m*-methoxybenzaldehyde were present at a concentration of 0.1 ppm, while all other standards were present at concentrations of 10 ppm. The lower concentrations of ethyl benzoate, *m*-methoxybenzaldehyde, and piperonal in the standard mix were necessary as these volatile components overload the capillary GC at higher concentrations. Individual standard solutions for each of these compounds were prepared in a similar manner. Certified Bourbon, Indonesian, and Tahitian vanilla extracts (0.2% v/v in 35% ethanol), nature-identical vanilla flavoring, synthetic vanilla flavoring, and vanilla flavored food products were obtained locally. The natural extracts were diluted (1 in 10) with water prior to analysis.

General GC−MS Analysis Conditions. Gas chromatographic analysis was carried out using a Varian 3400 GC fitted with a split/splitless injector suitable for SPME analysis, a Varian 2000 mass spectrometer (MS) detector, and a Varian 9200 autosampler. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The components were separated on a 30 m × 0.2 mm column with a 0.25 μm film of DB5 stationary phase (Alltech, Australia). The injector temperature was set at 250 °C and operated in the splitless mode for 2 min unless otherwise stated. The column was maintained at 40 °C for 2 min then ramped to 200 °C at 8 °C·min^{-1} and further ramped to 250 °C at 50 °C·min^{-1}. The NIST ′92 MS Library was used to identify key components in the samples.

General Conditions for SPME Extraction. Three SPME fibers were used in this study: poly(dimethylsiloxane) (PDMS), poly(acrylate) (PA), and carbowax/poly(divinylbenzene) (CW/DVB). The thickness of the polymeric coating varied with the fiber type: the PDMS fiber coating was 100 μm thick, the PA coating was 85 μm thick, and the CW/DVB coating was 65 μm thick. All the fibers were supplied by Supelco (Australia and Canada) and were conditioned as recommended by the manufacturer. The sample or standard mixture (200 μL) was transferred to a 2.0 mL vial, which was sealed with a screw capped top containing a Teflon-lined septum. The fiber was exposed to the headspace of the sample for 40 min, unless otherwise stated. The fiber was then retracted and inserted immediately into the inlet of the GC. For nonambient temperature extractions a heating block (Thermoline, BTC 9000) was used to heat the vial and its contents. Each sample was analyzed in triplicate, using a fresh vial and aliquot for each replicate.

RESULTS AND DISCUSSION

There are several factors that influence headspace analysis by SPME. They include fiber type, extraction temperature, adsorption time, and desorption conditions. Therefore, we explored the effect of these variables on the extraction of volatiles characteristic of vanilla extracts. The sample vial volume was not varied and was fixed at 2.0 mL. This vial size is compatible with the autosampler available in our laboratory and allowed us to perform automated SPME extractions at room temperature. The components in the standard mixture (Table 1) were chosen because (a) they are known to be present in vanilla extracts, (b) they include components characteristic of natural extracts and synthetic vanilla flavorings (e.g., ethylvanillin), and (c) they have a range of volatilities and polarity.

Determination of the Best Fiber Coating for SPME. The fiber coatings used in this study were the PDMS, PA, and CW/DVB fibers. PDMS was trialed as it has been used successfully for the analysis of both polar and nonpolar volatile components and the phase is similar to the stationary phase coating on the GC column used for this study (Miller et al., 1996; Steffan and Pawliszyn, 1996). The PA fiber was investigated as it has been used successfully for the extraction of more polar analytes (Steffan and Pawliszyn, 1996). The mixed coating was trialed as it was considered suitable for the analysis of polar semivolatiles (Pawliszyn, 1997).

Figure 1 shows a comparison of the extraction efficiencies of the fiber coatings for the analytes extracted from the standard mixture. Each fiber was effective at extracting six of the nine target components; however, none of the fibers extracted *p*-hydroxybenzaldehyde, protocatechuic acid, or vanillic acid at concentrations of 10 ppm. All the fibers extracted the early eluting (see Table 1 for retention time data) more volatile components (e.g., ethyl benzoate, *m*-methoxybenzaldehyde and piperonal) in the greatest amounts; however, the PA fiber was superior in that it extracted more of each component. For the less volatile, later eluting components, the PA fiber was also superior. For example, the PA fiber extracted over 50% more vanillin than either the PDMS or CW/DVB fiber. The experiment was repeated using a natural Bourbon extract and the efficiencies of the different fibers for extracting the main volatiles are shown in Figure 2. The target compounds were selected because they either were in the standard mixture or were present in high concentrations in the natural extract. As observed with the standard mixture, the PA coating was the most efficient at extracting vanillin and ethyl benzoate. It was also as efficient as the other fibers at extracting three major esters identified in the extract. The PA fiber was, therefore, used for the remainder of the study.

Absorption and Desorption Conditions. A desorption temperature of 250 °C with the injector operating in the splitless mode for 2 min was sufficient to quantitatively transfer all the components from the fiber to the separation column. During desorption the column temperature was held at 40 °C for 2 min to focus the sample onto the top of the column. The fiber was thermally desorbed prior to each run by putting the fiber in the injector with the split open for approximately 5 min.

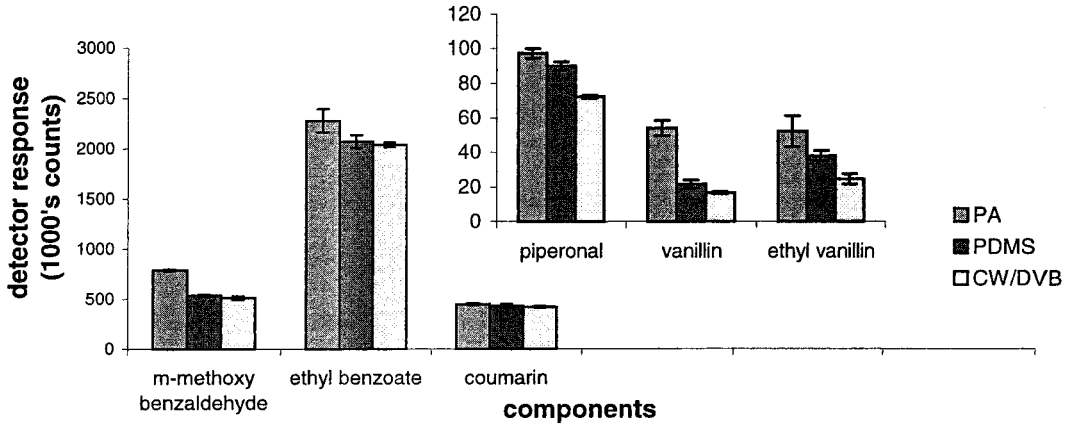

Figure 1. Comparison of the extraction efficiencies of the poly(dimethylsiloxane) (PDMS), poly(acrylate) (PA), and carbowax/poly(divinylbenzene) (CW/DVB) fibers using a prepared mixture.

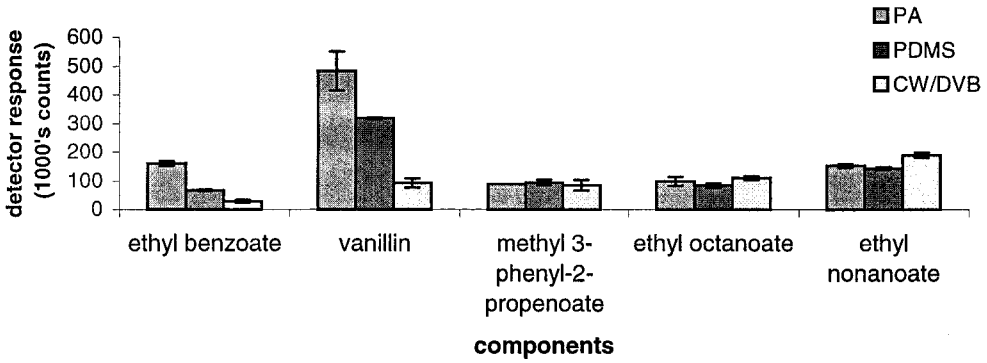

Figure 2. Comparison of the extraction efficiencies of the poly(dimethylsiloxane) (PDMS), poly(acrylate) (PA), and carbowax/poly(divinylbenzene) (CW/DVB) fibers using a natural Bourbon vanilla extract.

To extract components reproducibly from a sample it is desirable to do so when the system is at equilibrium (Pawliszyn, 1997). For SPME headspace analysis the analytes equilibrate between three phases, the liquid phase, the headspace, and the polymeric fiber coating. A plot of extraction time versus amount extracted can be used to determine the time taken for the components to reach equilibrium between the phases. The point where the curve plateaus or levels off is considered to be the equilibration time (Steffan and Pawliszyn, 1996). Therefore, to determine equilibrium or steady-state sampling conditions at room temperature, the PA fiber was exposed to the standard mixture for differing amounts of time between 5 and 100 min. Equilibrium conditions were achieved for all components in 40 min (Figure 3). The experiment was repeated using a natural vanilla Bourbon extract and similar results were obtained, with equilibrium conditions being achieved for all target components in 40 min (Figure 4).

The precision of the method was then investigated. The standard mixture was extracted several times using an absorption time of 40 min. The percent relative standard deviation (% RSD) for all the compounds was excellent and ranged between 2.5 and 6.4% for seven extractions (Table 1). When the experiment was repeated for a natural extract the % RSD for the main components gave similar values (2.6−8%).

Stirring or sonicating the sample during absorption or employing a higher extraction temperature will generally increase the rate at which steady-state conditions are achieved (Pawliszyn, 1997). We investigated the effect of using higher temperatures to reduce the equilibration time.

The standard mixture was extracted using the PA fiber at different temperatures (ambient and 40, 60, and 80 °C). As temperature increased the extraction efficiency increased for all components and was greatest at 80 °C. The largest increases in extraction were observed for the less volatile components such as vanillin, coumarin, and ethylvanillin (data not shown). Therefore, the steady-state sampling conditions were determined at 80 °C by exposing the fiber to the standard mixture for different time periods between 5 and 80 min. In general, equilibrium conditions were achieved in a shorter time. For example, it was achieved within 20 min for the more volatile components (*m*-methoxybenzaldehyde and piperonal) and achieved after 30 min for the less volatile components (ethylvanillin and coumarin) (Figure 5). While this method results in shorter absorption times, the error (expressed as standard deviation) incurred between replicates was greater than for the same experiment conducted at room temperature. The precision of the method was also investigated. The standard mixture was extracted several times using a 30-min absorption time. The percent relative standard deviation for all the compounds ranged between 6.4 and 15.5% for seven extractions (Table 1), indicating that the reproducibility of the method was poorer than for the same experiment conducted at room temperature. The poorer reproducibility between replicates for the high-temperature experiment is not surprising since the extraction process was done manually and involved quickly removing the fiber from the vial (held at 80 °C) and inserting it into the GC inlet to minimize temperature changes.

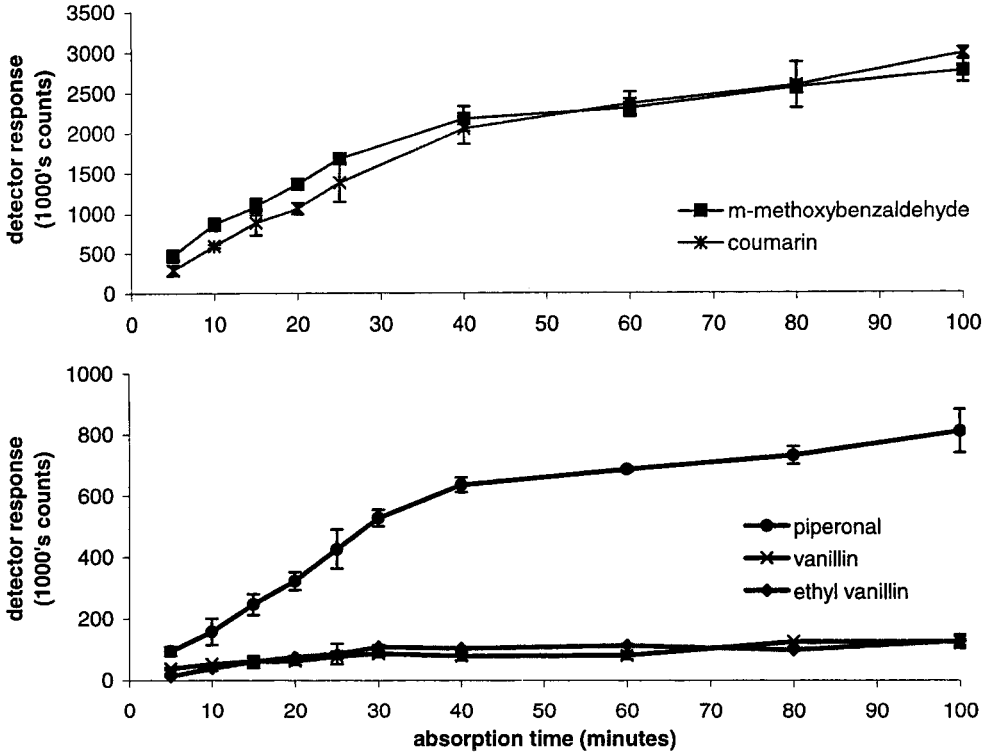

Figure 3. Effect of absorption time at room temperature on the extraction efficiency of the poly(acrylate) fiber using a standard mixture.

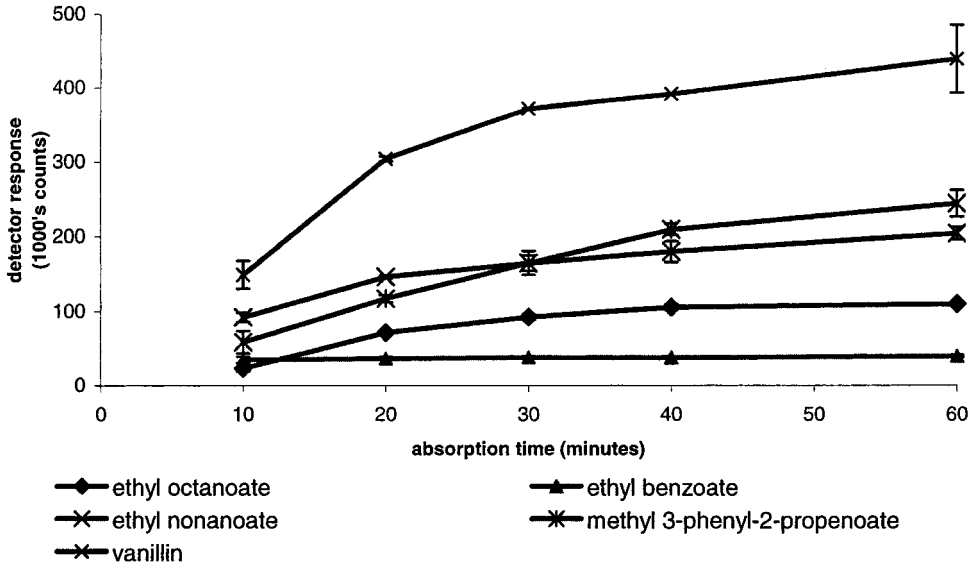

Figure 4. Effect of absorption time at room temperature on the extraction efficiency of the poly(acrylate) fiber using a natural Bourbon vanilla extract.

Having completed the temperature work, we felt that the extra sampling time required to achieve steady-state conditions at room temperature was preferable to the extra labor required to process the samples manually at higher temperature. In addition, the automated process gave better precision over the manual process. Therefore, a PA fiber, using a desorption time of 2 min, an extraction temperature of 25 °C, and an absorption time of 40 min was used to extract volatile components from vanilla extracts and flavorings.

Application of SPME to Real Samples. A preliminary investigation was carried out to determine if SPME-GC−MS could potentially be used to discriminate between different types of extracts and flavorings. A Bourbon, Tahitian, and Indonesian extract (from a common supplier) were each analyzed in triplicate. They were distinguishable from each other by the presence of key components or fingerprint regions unique to the extract (Figure 6). For example, the Tahitian extract was distinguishable because of the presence of large amounts of *p*-methoxybenzaldehyde and an unidentified aromatic component having a retention time of 12.8 min, which were absent from the other extracts. The large amount of *p*-methoxybenzoic acid methyl ester present compared to the trace amount found in the Indonesian and Bourbon extracts was also a distinguishing feature. The Bourbon and Indonesian extracts were distinguishable from each other by the different relative amounts of key components such as hexanoic acid, 5-propenyl-1,3-benzodioxole, and ethyl nonanoate.

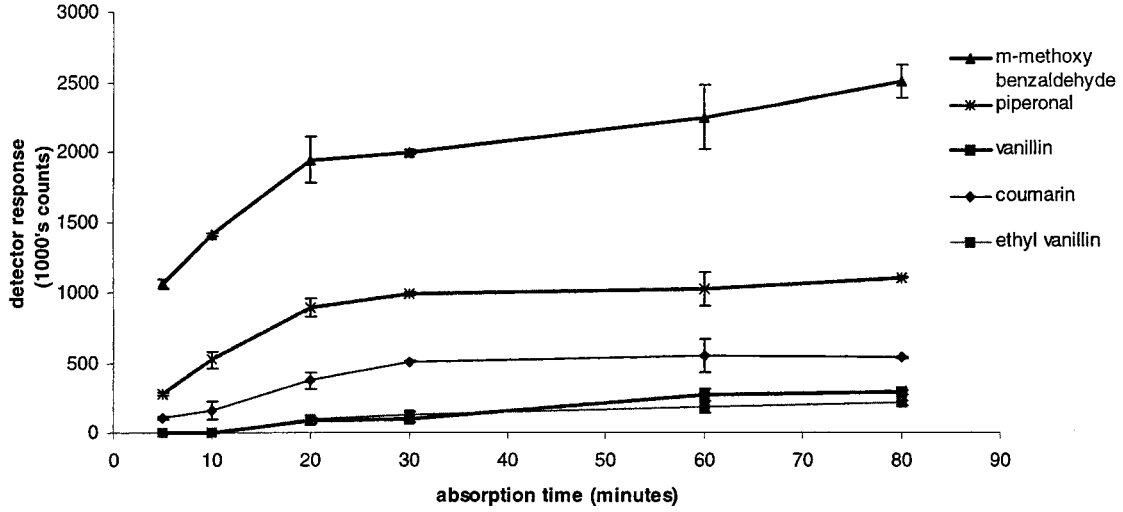

Figure 5. Effect of absorption time at 80 °C on the extraction efficiency of the poly(acrylate) fiber using a standard mixture.

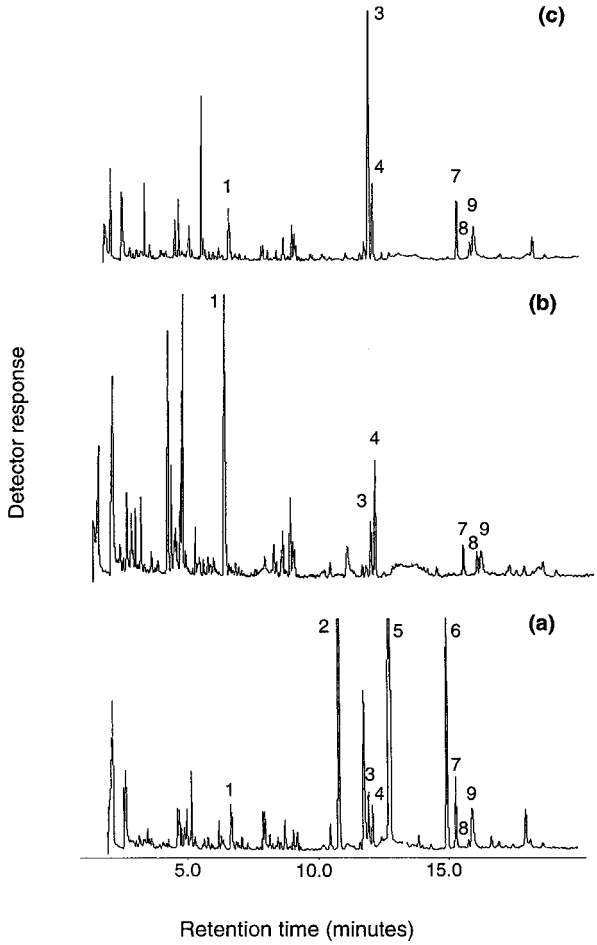

Figure 6. Gas chromatographic profile of headspace volatile components sampled by solid-phase microextraction at room temperature from (a) Tahetian natural vanilla extract; (b) Indonesian natural vanilla extract, and (c) Bourbon natural vanilla extract. The optimized conditions are given in the Experimental Section. Peak identification: 1 = ethyl hexanoate; 2 = *p*-methoxybenzaldehyde; 3 = 5-propenyl-1,3-benzodioxole; 4 = ethyl nonanoate; 5 = unidentified component; 6 = *p*-methoxybenzoic acid methyl ester; 7 = 3-phenyl-2-propenoic acid methyl ester; 8 = ethyl decanoate; 9 = vanillin.

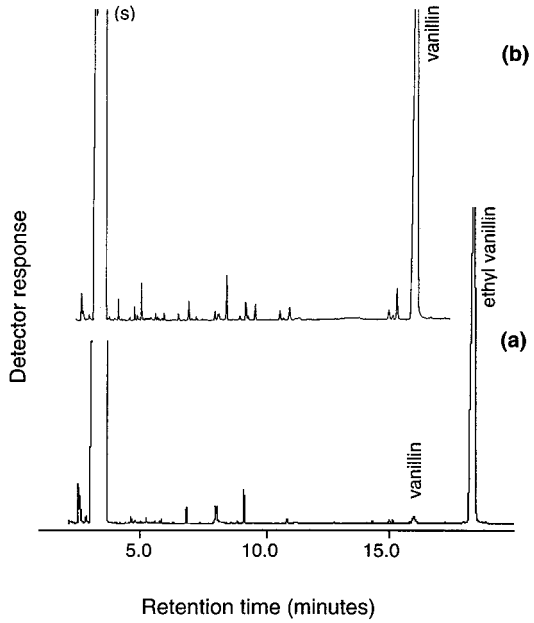

Figure 7. Gas chromatographic profile of headspace volatile components sampled by solid-phase microextraction at room temperature from (a) synthetic vanilla flavoring and (b) nature-identical Bourbon flavoring. The optimized conditions are given under Materials and Methods. (s) = solvent.

However, as these are natural extracts, natural variation between extracts is to be expected. Whether the differences observed here, particularly for Bourbon and Indonesian, are sufficient to distinguish between these extracts from a range of suppliers is currently under study. The relative concentrations of key components and the differences in the profiles of the natural extracts from a number of sources are being measured by using this SPME-GC−MS method.

Samples of nature-identical Bourbon flavoring and synthetic vanilla flavoring were analyzed next and compared against each other and the natural extracts. The synthetic vanilla flavoring was easy to identify, in that it had a relatively simple chromatogram with only a few major components present (Figure 7a). Another key difference was the presence of ethylvanillin, which does not occur naturally in vanilla extracts. The nature-identical Bourbon flavoring was clearly different than the natural Bourbon extract. The vanillin content was extremely high, and the straight chain esters such as ethyl nonanoate and ethyl decanoate, characteristic of natural vanilla extracts including Bourbon, were absent (Figure 7b).

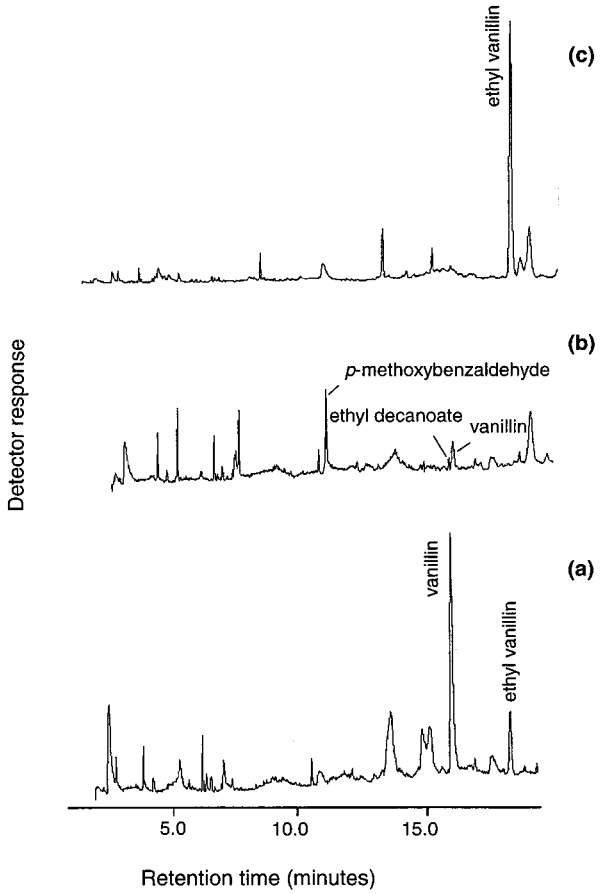

Figure 8. Gas chromatographic profile of headspace volatile components sampled by solid-phase microextraction at room temperature from (a) yogurt and (b) ice cream and at 80 °C from (c) custard powder. The optimized conditions are given under Materials and Methods.

The SPME-GC−MS method was also used to tentatively identify the source of vanilla flavoring used in some common food products. Locally produced yogurt, ice-cream, and custard powder were sampled. The chromatogram of the volatiles emitted by yogurt showed the presence of ethylvanillin, indicating that synthetic flavoring was used (Figure 8a). Ice cream, labeled as containing natural vanilla extract, was similarly analyzed. The presence of *p*-methoxybenzaldehyde and the straight chain ethyl decanoate and the absence of ethylvanillin indicated that a natural extract was used to flavor the ice cream (Figure 8b). Custard powder was also analyzed to see if the method was applicable to solid samples. At room temperature the concentrations of volatiles given off was minimal so the high-temperature method (absorption time 30 min at 80 °C) was employed. The presence of ethylvanillin indicated that a synthetic vanilla flavoring was used (Figure 8c).

In summary, using SPME as the extraction tool, GC can be used to analyze the complex mixture of components present in natural extracts. The key components (e.g., vanillin, ethyl benzoate, piperonal, *m*-methoxybenzaldehyde) routinely identified by HPLC are extracted, as well as a range of esters.

Of the fibers tested the PA fiber was superior for extracting the flavor volatiles. The volatile components were sufficiently concentrated on the coated fiber to be analyzed directly; therefore, no sample preparation is required. At 25 °C the automated SPME method was suitable for the analysis of extracts and most food samples. The high-temperature SPME was suitable for analyzing samples with low concentrations of volatiles.

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LITERATURE CITED

Belay, M.; Poole, C. Determination of vanillin and related flavor compounds in natural vanilla extracts and vanilla flavored foods by thin-layer chromatography and automated multiple development. *Chromatographia* **1993**, *37*, 365−373.

Field, J. A.; Nickerson, G.; Derryl, D. J.; Heider, C. Determination of essential oils in hops by headspace solid-phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 1768−1772.

Klimes, I.; Lamparsky, D. Vanilla volatiles−A comprehensive analysis. *Int. Flavors Food Addit.* **1976**, *Nov/Dec*, 272−276.

Lamprecht, G.; Pichlmayer, F.; Schmid, E. Determination of the authenticity of vanilla extracts by stable isotope ratio analysis and component analysis by HPLC. *J. Agric. Food Chem.* **1994**, *42*, 1722−1727.

Miller, K.; Poole, C.; Pawlowski, T. Classification of the botanical origin of cinnamon by solid-phase microextraction and gas chromatography. *Chromatographia* **1996**, *42*, 639−646.

Pawliszyn, J. *Solid-phase microextraction-theory and practice*; Wiley-VCH: New York, 1997.

Song, J.; Gardner, B.; Holland, J.; Beaudry, R. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of-flight mass spectrometry. *J. Agric. Food Chem.* **1997**, *45*, 1801−1807.

Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 2187−2193.

Ranadive, A. Vanillin and related flavor compounds in vanilla extracts made from beans of various global origins. *J. Agric. Food Chem.* **1992**, *40*, 1922−1924.

Riley, K.; Kleyn, D. Fundamental principles of vanilla/vanilla extract processing and methods of detecting adulteration in vanilla extracts. *Food Technol.* **1989**, *Oct*, 64−77.

Yang, X.; Peppard, T. Solid-phase microextraction for flavour analysis. *J. Agric. Food Chem.* **1994**, *42*, 1925−1930.

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