Optimization of Solid-Phase Microextraction Analysis for Headspace Flavor Compounds of Orange Juice

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The headspace flavor compounds of orange juice were isolated by solid-phase microextraction (SPME) fiber coated with 100 μm of poly(dimethylsiloxane) and separated by gas chromatography. The effects of the orange juice temperature from 25 to 80 °C and the adsorption time from 5 to 40 min on the equilibrium of flavor compounds between the SPME coating and the orange juice indicated that the equilibrium time decreased as the sample temperature increased. The equilibrium of the flavor compounds between the SPME coating and the orange juice required 30 min at 40 °C or 20 min at 60 °C. The amount of orange flavor compounds adsorbed by SPME coating decreased as the orange juice temperature increased from 25 to 80 °C. The resolution of the gas chromatogram increased as the inside diameter of the injection port liner decreased from 1 to 0.75 mm. The concentrations of ethyl butyrate, octanal, decanal, α-pinene, and limonene in orange juice were 0.4, 1.1, 1.0, 1.4, and 254 ppm, respectively. The coefficients of variation for the analyses of ethyl butyrate, octanal, decanal, α-pinene, and limonene ranged from 4.4% for 0.4 ppm ethyl butyrate to 1.6% for 254 ppm limonene.

Keywords: *Headspace; SPME-GC; flavor analysis; orange flavor*

INTRODUCTION

Solid-phase microextraction (SPME) is a relatively new and simple adsorption technique for the isolation of headspace flavor compounds (Arthur and Pawliszyn, 1990; Arthur et al., 1992; Zhang and Pawliszyn, 1993). SPME headspace sampling requires neither solvent extraction and purification steps nor a complicated purge-and-trap apparatus. The SPME can be inserted into a gas chromatograph (GC) injection port to separate the isolated volatile flavor compounds. An SPME unit consists of a holder and a fused silica fiber, which is coated with a layer of stationary phase such as nonpolar poly(dimethylsiloxane) or polar polyacrylate. When a SPME coating is exposed in the headspace of an airtightly sealed sample bottle, an equilibrium partition process occurs between the sample and the SPME coating (Zhang and Pawliszyn, 1993). The equilibrium partition of flavor compounds between the headspace of the sample bottle and the SPME coating mainly depends on the heating time, temperature, sample volume, and sample concentration of the bottle. Although this technique was developed mainly for the analysis of environmental samples in the beginning, the interest in using SPME for food flavor analysis has increased during the past few years. This technique has been used to analyze flavor compounds of coffee, a butter flavor in vegetable oil (Yang and Peppard, 1994), flavor compounds in a fruit beverage (Penton, 1996), essential oils in hops (Field et al., 1996), pyrazines in a food model system (Ibanez and Bernhard, 1996), and volatile compounds in vodkas (Ng et al., 1996).

The SPME-GC method is simple to use and inexpensive and does not require solvent extraction. However, SPME analysis is quite sensitive to experimental conditions such as heating temperature and time, sample volume, concentration, and sample matrix and uniformity (Yang and Peppard, 1994). The application of this technique to flavor analysis of foods and beverages still requires further modification to improve the reproducibility, sensitivity, and resolution of the chromatogram.

The flavor compounds in orange juice are 0.02% of the total weight: 75−98% of flavor compounds are hydrocarbons, 0.6−1.7% aldehydes, 1% esters, 1% ketones, and 1−5% alcohols (Sizer et al., 1988). Ahmed et al. (1978) reported that acetaldehyde, citral, ethyl butyrate, limonene, linalool, octanal, and α-pinene are the major contributors of orange juice. Octanal and decanal are important flavor compounds in orange juice (Arctander, 1969). Limonene is the major component in orange juice flavor, but it is not the most important compound in flavor quality. Moshonas and Shaw (1989) reported that up to 40% limonene is lost in aseptically packaged commercial orange juice during storage. The degradation of limonene to α-terpineol and other compounds produces off-flavor (Tatum et al., 1975). Shaw et al. (1993) classified commercial orange juice types by pattern recognition involving volatile compounds by headspace GC. Moshonas and Shaw (1997) analyzed the headspace volatile compounds of 62 different juices by dynamic headspace gas chromatography to classify these juices into three types of processing conditions. They have quantified 46 compounds in each of 62 orange juice samples, and the data were used for multivariate analysis. They have successfully classified the orange juice types according to three different processing types using principal headspace volatile compounds.

The purpose of this study was to optimize the SPME sampling and gas chromatographic conditions for the qualitative and quantitative analyses of volatile compounds in the headspace of the orange juice bottle.

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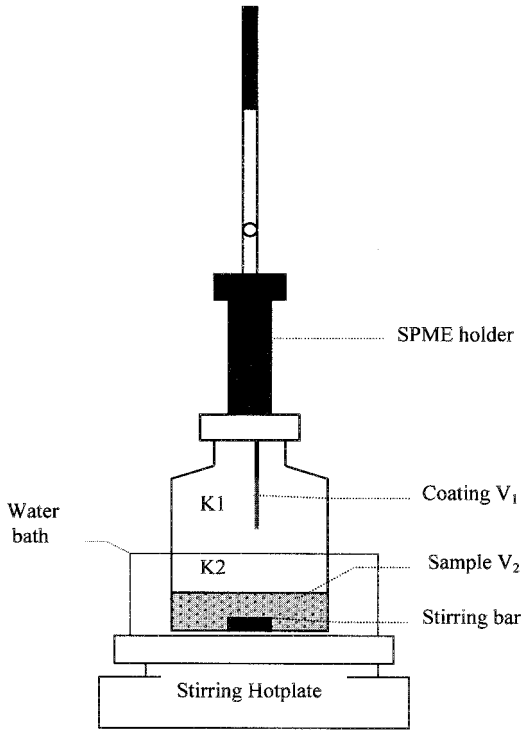

Figure 1. Diagram for the isolation of headspace flavor compounds of orange juice by SPME.

MATERIALS AND METHODS

Materials. A premium single strength orange juice was obtained from a grocery store (Kroger, Columbus, OH). Ethanol, ethyl butyrate, α-pinene, myrcene, octanal, limonene, linalool, decanal, and valencene of reagent grade were purchased from Aldrich Chemical Co. (Milwaukee, WI). A SPME fiber coated with 100 μm poly(dimethylsiloxane), micro stirring bars (10 × 3 mm), 6 mL serum bottles, Teflon coated rubber septa, and aluminum caps were purchased from Supelco Inc. (Bellefonte, PA).

Headspace Volatile Compound Analysis of Orange Juice by SPME-GC. The diagram for the isolation of headspace orange juice flavor compounds by SPME is shown in Figure 1. A 1 mL aliquot of orange juice was transferred into a 6 mL serum bottle containing a micro stirring bar. The sample bottle was airtightly sealed by a Teflon septum and an aluminum cap. The SPME fiber coated with 100 μm of poly(dimethylsiloxane) was manually inserted into the headspace of the sample bottle. The SPME coating which isolated headspace flavor compounds by adsorption was injected into the GC injection port at 220 °C and kept for 2 min for the desorption of flavor compounds. The injection port was lined with a 0.75 mm i.d. splitless glass liner. The desorbed flavor compounds were separated by a Hewlett-Packard 5890 GC (Avondale, PA) with capillary column (30 m × 0.53 mm i.d.) coated with a 2.65 μm film of 5% phenyl substituted methylpolysiloxane from Supelco and a flame ionization detector. The temperature of GC was programmed from 60 to 120 °C at 10 °C/min, then increased to 200 °C at 4 °C /min, and held for 10 min at the final temperature.

Effects of Temperature and Time on the Equilibrium of Flavor Compounds. To determine the effects of heating temperature and time on the equilibrium of flavor compounds between the SPME coating and headspace of sample bottle, the sample bottles were maintained at 25, 40, 50, 60, or 80 °C for 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min.

Identification of Flavor Compounds. The flavor compounds of orange juice were identified by comparing the retention times of GC peaks with those of authentic compounds under the identical experimental conditions.

Reproducibility of Flavor Compound Analyses by SPME-GC. The reproducibility of flavor compounds analyses by SPME-GC was determined by analyzing the quantities of ethyl butyrate, α-pinene, octanal, limonene, and decanal in orange juice in six replicates.

Preparation of Deodorized Orange Juice by Vacuum Evaporation and Solvent Extraction. The deodorized orange juice from single strength orange juice was prepared by the following steps.

The flavor compounds of single strength orange juice with 11.8 °Brix were removed by a combination of vacuum evaporation and solvent extraction. The orange juice was concentrated from 11.8 to 45 °Brix in a vacuum rotary evaporator (Brinkmann Instrumental, Inc., Westbury, NY). The residual flavor compounds in the concentrated juice, mainly oil soluble and low-volatile terpenes such as limonene and valencene, were extracted twice by hexane (1:1) in a separatory funnel. The juice layer was separated and drained from the hexane layer in a separatory funnel.

Trace residual hexane in the juice was removed by vacuum rotary evaporator. The concentrated, hexane treated, and vacuum deodorized orange juice was diluted back to 11.8 °Brix with distilled water. This orange juice without volatile flavor compounds was designated as deodorized orange juice and served as a solvent for ethyl butyrate, α-pinene, octanal, limonene, and decanal for the preparation of standard calibration lines.

Calibration Lines of Flavor Compounds in Orange Juice. To determine the concentrations of ethyl butyrate, α-pinene, octanal, limonene, and decanal in orange juice, the calibration lines of the standard compounds in deodorized orange juice were prepared. It should be pointed out that the volatility of flavor compounds changes according to the sample matrices.

The concentrations of ethyl butyrate, α-pinene, octanal, limonene, and decanal in a single strength orange juice were estimated from the reports of Shaw (1991) and Chen et al. (1993) and our preliminary experiment. Ethyl butyrate was added to deodorized orange juice to obtain 0, 025, 0.50, and 1.00 ppm. Similarly, 0, 0.25, 0.50, and 1.00 ppm octanal, 0, 0.25, 0.50, and 1.00 ppm decanal, 0, 0.5, 1.0, and 2.0 ppm α-pinene, and 0, 50, 100, and 200 ppm limonene orange juices were prepared. The deodorized orange juice containing a standard compound was mixed well and stored at 4 °C overnight for flavor equilibrium. The calibration line of ethyl butyrate, α-pinene, octanal, limonene, or decanal was obtained by plotting GC peak area vs different concentrations of each standard compound in the deodorized orange juice.

RESULTS AND DISCUSSION

Effects of Heating Temperature and Time on the Equilibrium of Flavor Compounds. The reproducibility and sensitivity of headspace volatile compounds analyses by SPME are greatly influenced by the vapor pressure of flavor compounds in the bottle. To obtain the large partition coefficient of flavor compounds between SPME coating and orange juice, more compounds have to pass through the orange juice, which has a low diffusion coefficient compared to the headspace gas phase, into the headspace to reach the SPME coating. To increase the diffusion of flavor compounds through orange juice into the headspace, the orange juice was agitated using a magnetic stirring bar as shown in Figure 1.

The effects of heating temperature and time of the sample bottle on the analysis of headspace orange flavor compounds with SPME-GC are shown in Figure 2. The GC peak area of total flavor compounds decreased as the heating temperature increased from 25 to 80 °C. The equilibrium of orange flavor compounds between SPME coating and headspace was indicated by the point where the slope of the curve leveled off. The minimum time required to reach the equilibrium was 15 min at 80 °C, 20 min at 60 °C, 25 min at 50 °C, 30 min at 40 °C, and 50 min at 25 °C. The equilibrium time decreased as the sample temperature increased as shown in Figure 2.

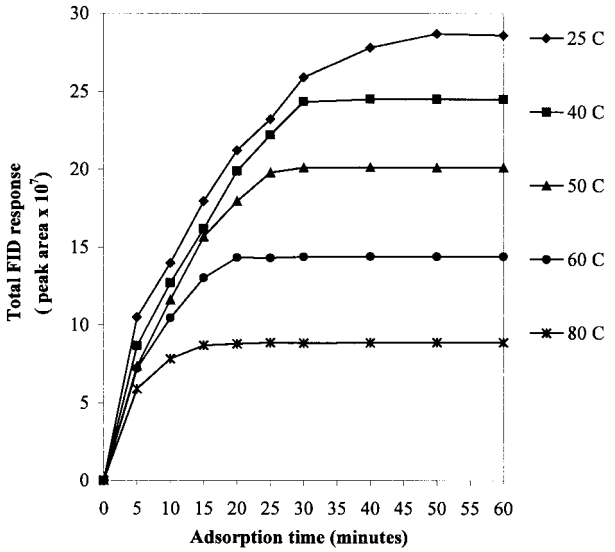

Figure 2. Effects of temperature and time on the equilibrium of flavor compounds between the SPME coating and the headspace of orange juice.

Zhang and Pawliszyn (1993) reported that the principle of SPME is the equilibrium partition process of flavor compounds and the orange juice in the bottle. The amount of flavor compounds absorbed on the SPME coating can be determined from the equation $n = C_0V_1V_2K_1K_2/(K_1K_2V_1 + K_2V_3 + V_2)$ according to Zhang and Pawliszyn (1993). n is the mass of the flavor compound absorbed by the SPME coating, C_0 is the initial concentration of the flavor compound in the orange juice, and V_1, V_2, and V_3 are the volumes of SPME coating, the orange juice volume, and the headspace volume, respectively. K_1 is the partition coefficient of the flavor compound between the SPME coating and headspace; K_2 is the headspace and the orange juice partition coefficient. The n value and the equilibrium only depend on the partition coefficient (K) of volatile compounds between SPME coating and orange juice. Since $K = K_1K_2$ (Zhang and Pawliszyn, 1993), the whole partition coefficient is controlled by both the partition coefficient K_1 between SPME coating and headspace gas phase and the partition coefficient K_2 between the headspace gas phase and orange juice.

The equilibrium curves of Figure 2 indicate that the higher the temperature of the sample, the shorter the time required to reach equilibrium. As the temperature of the sample bottle increased, more molecules of flavor compounds obtained energy from the heating and moved to the headspace so that K_2 increased. However, the adsorption is an exothermic process; more molecules adsorbed on the stationary phase of SPME fiber diffused to gas phase with increasing temperature, which inversely decreased the partition coefficient K_1. Since $K_1 \gg K_2$ for most organic compounds (Zhang and Pawliszyn, 1993), the net effect of the increased temperature decreases the K. This may explain why the amount of orange flavor compounds adsorbed at a higher temperature was less than that adsorbed at a lower temperature under the same adsorption period.

The SPME coating adsorbed more flavor compounds, and a longer time was needed to reach the adsorption equilibrium at a lower temperature of the sample bottle. To have good reproducibility for the quantitative analysis of headspace flavor compounds, the partition coefficient should reach an equilibrium state. Since 50 min at 25 °C to reach the equilibrium seems to be too long and the 80 °C may decompose flavor compounds, the combination of 40 °C for 30 min or 60 °C for 20 min was suitable for the headspace sampling for orange flavor compounds. The SPME headspace flavor compounds sampling at 60 °C for 20 min was chosen for this study.

Table 1. Reproducibility for the Determination of Major Flavor Compounds in a Single Strength Orange Juice

replicates	ethyl butyrate (ppm)	α-pinene (ppm)	octanal (ppm)	limonene (ppm)	decanal (ppm)
1	0.432	1.378	1.089	251.05	1.005
2	0.400	1.391	1.050	254.28	0.925
3	0.391	1.343	1.054	248.26	0.987
4	0.380	1.389	1.059	256.25	0.995
5	0.403	1.402	1.020	255.71	1.015
6	0.397	1.470	1.010	260.01	1.007
SD	0.017	0.042	0.029	4.130	0.033
ave	0.400	1.395	1.047	254.26	0.989
CV (%)	4.36	3.00	2.71	1.63	3.32

Reproducibility of Flavor Compounds Analyses by SPME and GC. The concentrations and coefficients of variation of ethyl butyrate, α-pinene, octanal, limonene, and decanal in single strength orange juice are shown in Table 1. The coefficients of variation for the compounds range from 4.36% for 0.4 ppm ethyl butyrate and 1.63% for 254 ppm limonene. The low coefficients of variation for important orange flavor compounds indicated that the SPME-GC under the analytical conditions used was very good for the analysis of flavor compounds of orange juice.

Gas Chromatogram and Identification of Orange Juice Flavor Compounds. A typical gas chromatogram of orange flavor compounds which were isolated by SPME at 60 °C for 20 min and separated by GC is shown in Figure 3. The chromatographic peaks are well-separated, symmetrical, and sharp. The small size 0.75 mm i.d. splitless liner in the GC injection port greatly improved the GC resolution compared to the 1 mm i.d. liner (data not shown). Ethanol (retention time of 2.647 min of Figure 3), ethyl butyrate (6.300 min), α-pinene (9.261 min), mycerene (10.215 min), limonene (11.585 min), linalool (13.004 min), decanal (15.975 min), and valencene (25.305 min) have been identified. These compounds have been reported as important in orange juice (Arctander, 1969; Ahmed et al., 1978; Moshonas and Shaw, 1989). The chromatograms of flavor compounds in foods will be influenced by the conditions used for the isolation and separation of flavor compounds. The chromatogram of flavor compounds in an orange juice determined by nonpolar 5% phenyl substituted methylpolysiloxane SPME coating material shown in Figure 3 is most likely different from that analyzed by polar SPME coating.

Preparation of Deodorized Orange Juice. The principle behind the headspace analysis of flavor compounds by SPME is the equilibrium partition process of flavor compounds between SPME coating and the headspace of orange juice bottle. The vapor pressure of volatile compounds is greatly influenced by sample matrices. To determine the effect of orange juice matrix on the SPME analysis as a preliminary study, limonene was spiked into 20 mL of deodorized orange juice and 20 mL of distilled water to obtain 50 ppm. The 50 ppm limonene in deodorized orange juice and water were analyzed by SPME-GC as a preliminary study. The amounts of limonene isolated from deodorized orange juice and water by SPME were quite different. This preliminary study shows that the sample matrix affects

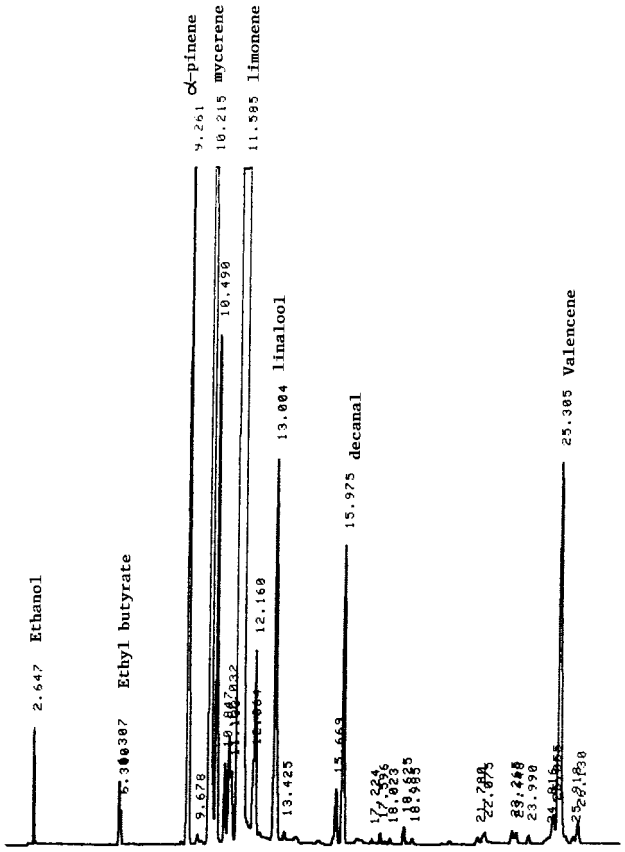

Figure 3. Gas chromatogram of orange juice flavor by the SPME headspace sampling.

Table 2. Regression Equations between Flavor Compounds (ppm) and GC Peak Areas (Electronic Counts)[a]

compd	regression eq	R^2	concn range (ppm)
ethyl butyrate	$Y = 0.2891X_1 + 0.015$	0.99	0.1−1.2
n-octanal	$Y = 0.4913X_2 + 0.003$	1.00	0.1−1.3
decanal	$Y = 0.2010X_2 + 0.066$	0.99	0.1−1.1
α-pinene	$Y = 0.3428X_2 + 0.092$	0.99	0.2−2.0
limonene	$Y = 17.922X_3 + 9.462$	0.99	20−250

[a] Y = compound in parts per million. X_1 = electronic counts of GC peak area ($\times 10^{-4}$). X_2 = electronic counts of GC peak area ($\times 10^{-5}$). X_3 = electronic counts of GC peak area ($\times 10^{-7}$).

the vapor pressure of volatile compounds, and the sensitivity of headspace analysis by SPME greatly depends on the sample matrix. For the quantitative analyses of ethyl butyrate, octanal, decanal, α-pinene, and limonene in orange juice by SPME-GC, the calibration lines of these compounds were obtained by adding different contents of standard compounds to deodorized orange juice instead of water. The deodorized orange juice prepared by a combination of vacuum rotary evaporation and solvent extraction was practically volatile-free according to the SPME-GC.

Calibration Lines of Standard Compounds. The calibration linear regression lines of ethyl butyrate, octanal, decanal, α-pinene, and limonene are shown in Table 2. Linear relationships between GC peak areas and the concentrations of standard compounds were better than $R^2 = 0.99$ for five standard compounds. These high correlations between the concentrations and GC peak areas also indicated that the deodorized orange juice was capable of forming a stable matrix with standard compounds to produce a reproducible equilibrium between SPME coating and the headspace of orange juice. The concentrations of ethyl butyrate, α-pinene, octanal, limonene, and decanal in a single strength orange juice were 0.400, 1.395, 1.049, 254.26, and 0.989 ppm, respectively (Table 1). The concentration of limonene is the highest in orange juice and is approximately 200 times higher than other compounds in orange juice.

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