Analysis of Flavor Volatiles Using Headspace Solid-Phase Microextraction

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The separation of target compounds from the sample matrix is a challenge to many analytical chemists. The extraction process is generally the step at which most analyte loss occurs; therefore, efficient methods of extraction are continually being sought. Solid-phase microextraction is a relatively new technique whereby analytes of interest partition from the sample matrix into a polymeric liquid coating. The application of headspace SPME to flavor volatile compounds of orange juice was investigated. Seventeen different common flavor volatile organics from orange juice were examined. A new method was developed to successfully extract these target analytes. This technique was shown to have a linear range and detection limits well within the ranges in which the target flavor compounds naturally occur. The addition of salt to the matrix was found to significantly enhance the amount of analyte extracted into the fiber coating. The partial removal of suspended solids from the juice was found to enable a standard addition quantitation of the target analytes. The concentrations of the target analytes were determined and were calculated to be within the same range as those reported using current headspace techniques.

Keywords: *Solid-phase microextraction (SPME); flavor analysis; orange juice*

INTRODUCTION

Flavor is considered to be the resulting effect of two sensations: taste and olfaction (Fenaroli, 1971). The analysis of flavor volatiles has been a challenge to many researchers for over 40 years. The flavor industry is worldwide and incorporates a considerable amount of developmental research including the area of citrus fruit flavors (Cooper and Chapot, 1977). Of the fruit beverages, orange is by far the most popular with its delicate and complex citrus flavor (Shaw et al., 1993). The development of newer, more efficient methods for the isolation, detection and quantitation of flavor volatiles is essential. These new methods have the potential for numerous applications for the investigation of effects of factors such as storage time, shelf life, and package leaching to monitor the quality of juices. In addition, orange juice adulteration is a significant problem in the beverage industry, and monitoring such activities is vital to the industry as well as the health of the consumer. Therefore, investigation of the flavor composition can be used to determine the integrity of a product.

Much of the flavor in juices stems from the volatile components that reside in the headspace region above the juice. These components are found in very low concentrations in the presence of large amounts of water (Parliment, 1987). These conditions make extraction and isolation of the volatiles challenging for the analyst. Many different analytical methods have been developed to determine flavor concentrations in juices. Present headspace methods include steam distillation–solvent extraction, gas chromatography–olfactometry, static headspace gas chromatography, and dynamic purge and trap gas chromatography. However, the majority of these methods are very time-consuming, require exhaustive concentration steps, have memory effect problems, and require dedicated gas chromatographs equipped with headspace sampling devices. Therefore, it is evident that there is a need for a new, more rapid, and simple technique that will reduce or eliminate these problems.

Solid-phase microextraction (SPME) (Zhang et al., 1994) is a new analytical technique that can overcome these difficulties and can be applied to the detection of flavor volatiles in citrus beverages such as orange juice, grapefruit juice, and grape juice. SPME is a solvent-free method of extracting analytes from a variety of matrices by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. It uses a very simple setup and requires no additional instrumentation other than a conventional gas chromatograph (GC) with the traditional injection port. SPME eliminates preconcentration steps by directly extracting the analytes into a poly(dimethylsiloxane)-coated fiber. It has been shown to be a very sensitive method for headspace analysis and has been recommended for the quantitative analysis of flavor and fragrance compounds (Hawthorne and Miller, 1992; Zhang and Pawliszyn, 1993). As well, headspace SPME has been tested and has compared favorably to the commonly used purge and trap type analysis (MacGillivray and Pawliszyn, 1994). The use of a fiber for extraction can enhance the selectivity of the analysis because one may choose the stationary phase that best suits the analytes. By using headspace SPME, one can reduce matrix effects and interferences present in the liquid sample. The use of SPME in flavor analysis of volatile components can reduce the limitations associated with current methodologies.

This paper focuses on the development of a method to quantitatively determine the amount of certain flavor volatiles present in orange juice and to qualitatively determine certain components in other fruit juices as well.

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Table 1. Equilibration Times of the Target Compounds for Orange Juice Flavor Volatiles

	equilibration time (min)	
target analyte	PDMS-coated fiber	PA-coated fiber
methanol	90	45
ethanol	120	60
ethyl acetate	5	60
2-methyl-1-propanol	5	60
methyl butyrate	12	60
ethyl butyrate	5	45
cis-3-hexen-1-ol	10	60
hexyl alcohol	5	60
α-pinene	35	60
β-myrcene	35	60
ethyl hexanoate	40	60
octanal	40	60
limonene	35	45
γ-terpinene	45	60
linalool	35	60
α-terpineol	40	45
decanal	35	60

EXPERIMENTAL PROCEDURES

Sample Preparation. Seventeen target compounds were analyzed (see Table 1). Methanol, ethanol, diethyl ether, and ethyl acetate were purchased from BDH (Toronto, ON), while the remaining compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI).

The standard mixture containing 17 of the volatile orange flavor components was made up in the laboratory. The non-water-soluble compounds were mixed together in diethyl ether, while the water-soluble compounds were mixed together in deionized water. Both standards were mixed together and diluted in water to make the stock solution used for the experiments. All water standards were made up fresh every 2 weeks and stored in a refrigerator. The diethyl ether standard was kept in a freezer and made up fresh every month. Calibration curves were made by extracting the standard from water at various concentration levels and plotting the area count versus concentration and using regression analysis to calculate the curve. Buffer solutions were made (pH 3.9) by mixing 110 mL of a 0.198 M acetic acid aqueous solution with a 15 mL of a 0.2 M sodium acetate solution and diluting to a 250 mL volume.

Standard Samples. Each sample was made up to 30 mL and put into 40 mL amber vials with a Teflon septum lid allowing for a 10 mL headspace volume. Into each vial was put a magnetic stir bar, and NaCl (BDH) was added in appropriate amounts.

Orange Juice Samples. Tropicana orange juice was purchased from the local grocery store. The juice was centrifuged and the remaining liquid portion used for analysis. The samples were prepared by pipetting 30 mL of the centrifuged orange juice into the 40 mL amber vial along with NaCl and magnetic stirrer. Various amounts of the standard solutions were spiked into the orange juice where the standard addition method was performed.

Extraction Procedure. The setup for the SPME device has been discussed previously in detail (Boyd-Boland et al., 1994). A 1 cm long 100 μm poly(dimethylsiloxane)-coated fiber or an 85-μm poly(acrylate)-coated fiber (Supelco, Canada) were used for this analysis. The fibers were conditioned in a GC injection port at 250 °C for 3 h and at 275 °C for 5 h, prior to use, respectively.

The extraction procedure is as follows: The prepared sample is put onto a magnetic stirrer, and the stirrer is set so that there is a constant vigorous stirring in the sample. The solution is maintained at ambient temperature. The needle of the SPME device is pierced through the septum of the vial, and the plunger is depressed to expose the fiber to the headspace of the solution. Once equilibrium has been reached, the fiber is withdrawn into the needle and transferred to the injection port of the GC. The needle of the SPME device penetrates the septum of the GC inlet and the fiber is exposed so that the analytes are thermally desorbed in the hot injection port and deposited onto the column where subsequent chromatographic analysis is performed.

GC-FID Analysis Conditions. Gas chromatographic analysis was performed using a Varian (Georgetown, ON) 3500 gas chromatograph equipped with a flame ionization detector (FID) and a septum programmable injector (SPI). The chromatograph was equipped with cryogenic cooling of both the injector and column oven for temperature programming. The chromatograms and quantitation information were obtained using the Varian STAR system. The target compounds were separated using a 30 m × 0.25 mm column with a 0.25 μm film of SPB-5 (Supelco).

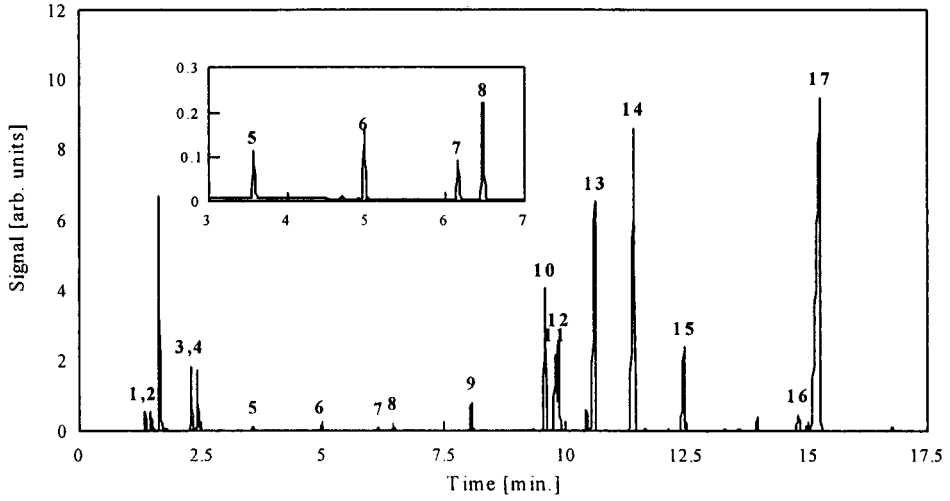
Once the analytes were extracted into the coating, they were desorbed at 250 °C for 3 min in the injection port of the GC. The column was maintained at 25 °C for 1 min after desorption, ramped at 20 °C/min to 40 °C, then further ramped at 6 °C/min to 130 °C, and finally ramped to 200 °C at 25 °C/min and held for 1 min. The FID was maintained at 300 °C. Helium (ECD grade) was used as a carrier gas; nitrogen (UHP) was used as a makeup gas; air (zero-gas) and hydrogen (UHP) made up the FID flame.

RESULTS AND DISCUSSION

Seventeen analytes commonly found in orange juice flavor volatiles were used for this study. The mixture consisted of various aldehydes, esters, alcohols, and terpenes shown in Table 1. Each orange juice component was identified in the total mixture by a comparison of the retention times of the individually extracted components. Figure 1 shows an FID chromatogram of all 17 components using the poly(acrylate) (PA) fiber coating.

Two types of fiber coatings were investigated in this study: poly(dimethylsiloxane) (PDMS) and PA. Each coating offers particular advantages; for example, the PDMS is a nonpolar coating that has been known to work very effectively on a wide range of analytes, both polar and nonpolar (Boyd-Boland et al., 1994). As well, this coating offers a greater similarity to the stationary phase of the capillary column used in all of the experiments. Conversely, the PA coating offers a more polar phase by which the more polar analytes, such as methanol and ethanol, can be more readily extracted. Figure 2 illustrates a comparison of the extraction efficiencies of the two fiber coatings for most of the analytes. The percent efficiency was calculated as the percentage of analyte from the solution extracted into the fiber coating. It can be seen that the PA coating extracts more analyte in comparison to the PDMS coating, with the exception of the terpenes. The terpenes, α-pinene, β-myrcene, γ-terpinene, and limonene, are all nonpolar and were extracted to a higher degree into the nonpolar PDMS coating. The remainder of the target analytes were extracted proportionately more into the polar PA coating. This demonstrates that the partitioning of the analytes is generally favored into the PA fiber coating relative to the PDMS fiber for most of the target compounds.

Headspace SPME is based on the equilibrium of analytes among three phases of the system. These three phases include the polymeric liquid coating, the headspace, and the aqueous solution. The theory related to this type of extraction has been discussed in detail (Zhang and Pawliszyn, 1993). SPME is an equilibrium method and, as such, once equilibrium has been reached, the concentration of the analytes can be considered constant in all three phases. The limiting step in this process is considered to be the diffusion of the analytes through the system. For this reason the equilibrium time of the system must first be determined. This can be accomplished by plotting extraction time profile



Peak Labels: 1) methanol; 2) ethanol; 3) ethyl acetate; 4) 2-methyl-1-propanol; 5) methyl butyrate; 6) ethyl butyrate; 7) cis-3-hexene-1-ol; 8) hexyl alcohol; 9) α-pinene; 10) β-myrcene; 11) ethyl hexanoate; 12) octanal; 13) limonene; 14) γ-terpinene; 15) linalool; 16) α-terpineol; 17) decanal.

Figure 1. Headspace SPME of a standard solution of target orange juice flavor volatiles using the PA fiber coating.

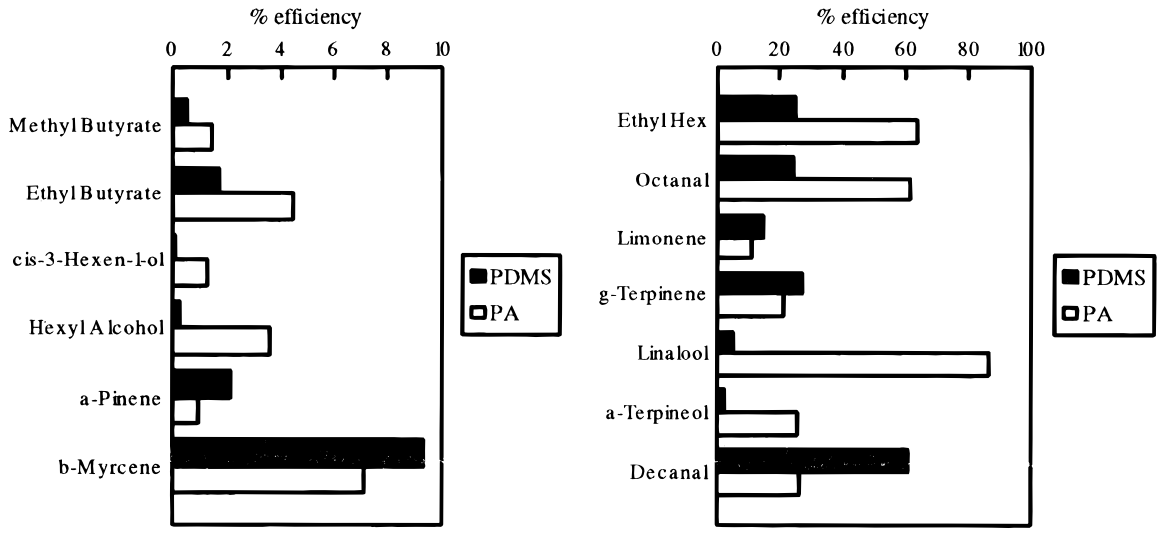


Figure 2. Comparison of the extraction efficiencies of the PDMS- and PA-coated fibers.

curves of the amount of analyte extracted versus the time of extraction. The time at which the area under the curve becomes constant is considered to be the equilibration time. Table 1 lists the equilibration times for all of the analytes extracted with both the PDMS- and PA-coated fibers. From Table 1 it can be seen that most of the analytes extracted with the PDMS-coated fiber reach equilibrium within 40 min, while the analytes extracted using the PA-coated fiber reach equilibrium within 60 min. The PA coating is a crystalline polymer so the analytes diffuse through it at a slower rate. For this reason the analytes take slightly longer to reach equilibrium with the PA coating than with the PDMS coating. Furthermore, more analytes were extracted into the PA coating than the PDMS coating; thus, it would take longer to reach equilibrium for this reason, as well. The PA fiber coating was used for the remainder of the experiments.

By decreasing the solubility of the water, the amount of analytes extracted into the headspace and finally the fiber coating is increased, which lowers the detection limits of the method. This was accomplished in this procedure by the addition of salt to the sample matrix. The "salting out" effect was studied to determine its effect on the extraction efficiency of the analytes with the two fiber coatings.

Three different salt solutions were investigated; 15% (unsaturated), 36% (saturated), and 42% (w/v) NaCl solutions were used for this study. All illustrated in Figure 3, the 36% (w/v) (supersaturated) NaCl solution enabled the highest amount of analyte to partition into the coating for most of the analytes. For α-pinene the solution with no salt was the most effective for extraction, while for ethanol and hexyl alcohol the analytes were extracted to a higher extent from a 42% (w/v) NaCl solution. The 36% (w/v) NaCl solution was used for the remainder of the experiments.

Once the preliminary investigations were completed and the instrumental and extraction parameters were optimized, the feasibility of the SPME method was investigated. The assessment of the method included investigation of precision, estimation of the linear range, and estimation of the limits of detection and quantitation.

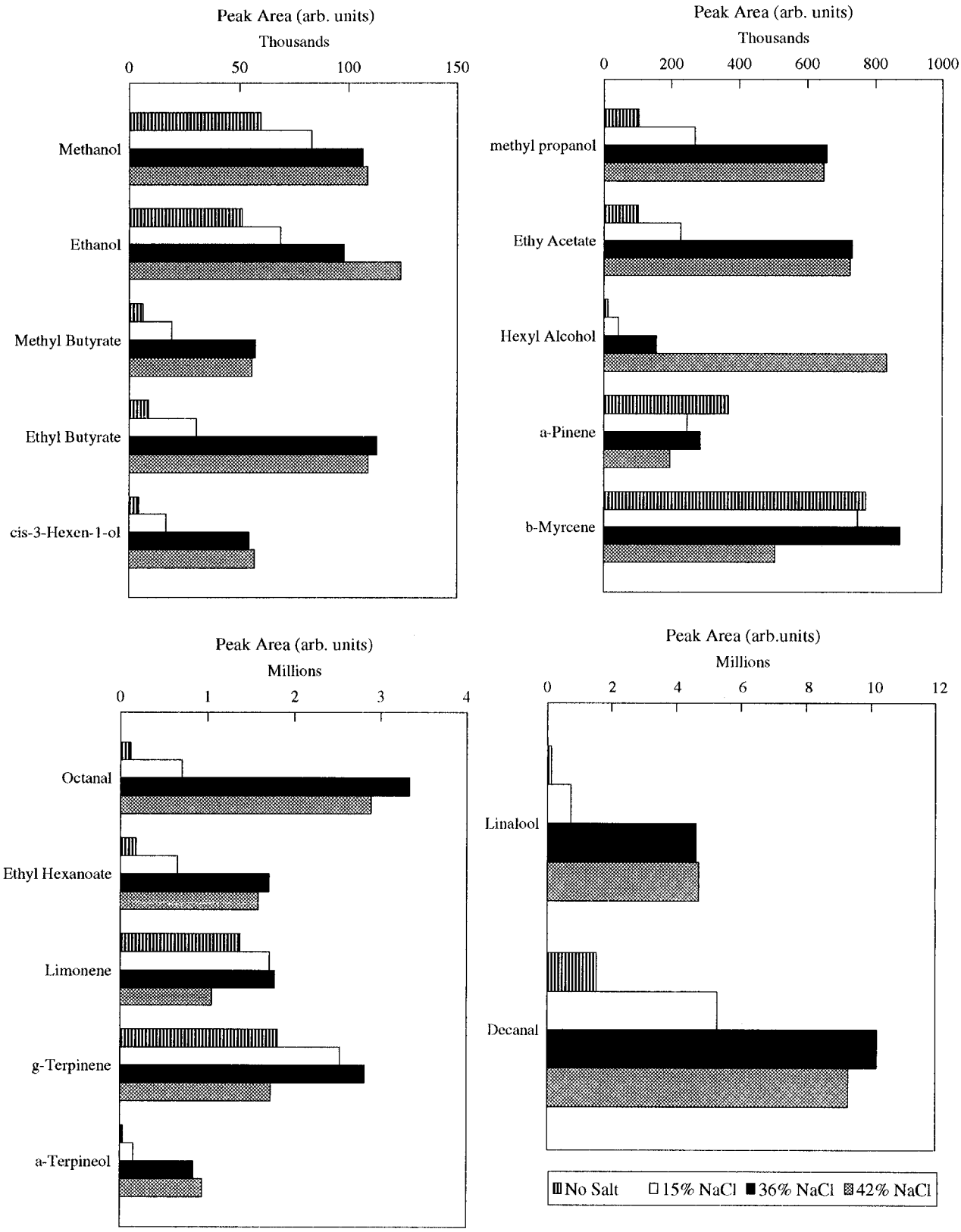


Figure 3. Relative amount extracted under various salt concentrations using a PA fiber coating for solutions containing no salt and 15, 36, and 42% (w/v) NaCl.

The precision was estimated by performing six or seven replicate extractions. The corresponding standard deviation was then calculated for these extractions and expressed as a percentage. The percent relative standard deviation (% RSD) values for the PA-coated fiber showed % RSD values ranging between 1 and 18%; most of the compounds fell below 10%.

Following these experiments, the linear range was estimated for the method, using each of the fiber coatings. These experiments were performed under optimum salt conditions. The measured detector response over a series of concentrations of the target analytes was plotted. These results were analyzed by linear regression which plotted the peak area versus the

Table 2. Linear Range, LOD, and LOQ for the Orange Juice Flavor Volatile Compounds Using the PA-Coated Fiber

target analyte	linear range (ppm)	LOD (ppm)	LOQ (ppm)
methanol	1.4×10^2 to 0.14	3.5×10^{-2}	1.2×10^{-1}
ethanol	9.4×10^2 to 0.94	3.9×10^{-1}	9.0×10^{-1}
ethyl acetate	2.3−0.023	3.9×10^{-3}	1.3×10^{-2}
2-methyl-1-propanol	26−0.027	4.1×10^{-3}	1.4×10^{-2}
methyl butyrate	1.2−0.0012	4.7×10^{-4}	1.0×10^{-3}
ethyl butyrate	0.55−0.00055	8.1×10^{-5}	2.7×10^{-4}
cis-3-hexen-1-ol	0.79−0.00079	1.8×10^{-4}	6.2×10^{-4}
hexyl alcohol	0.75−0.00075	9.0×10^{-5}	3.0×10^{-4}
α-pinene	0.084−0.00084	2.7×10^{-5}	9.0×10^{-5}
β-myrcene	0.013−0.00013	6.8×10^{-6}	2.7×10^{-5}
ethyl hexanoate	0.015−0.00015	3.8×10^{-6}	1.3×10^{-5}
octanal	0.014−0.00014	3.8×10^{-6}	1.3×10^{-5}
limonene	0.79−0.00079	4.7×10^{-6}	1.6×10^{-5}
γ-terpinene	0.81−0.0081	3.8×10^{-6}	1.3×10^{-5}
linalool	0.77−0.00077	4.1×10^{-6}	1.4×10^{-5}
α-terpineol	0.71−0.0071	3.6×10^{-7}	1.2×10^{-3}
decanal	0.7−0.0070	1.3×10^{-6}	4.3×10^{-6}

concentration. The range over which the r^2 value was found to be approximately 0.99 was considered to be the linear range. The linear ranges of the target analytes were found to span between 3 and 4 orders of magnitude, depending on the compound. The concentrations of the flavor volatiles found in natural orange juice fall within the linear ranges of most of the analytes. The only exception was limonene, which is generally found at very high levels in orange juice. When the limonene was extracted from the real juice and chromatographically analyzed, the response exceeded the dynamic range of the detector. Therefore, the linear range given in Table 2 for limonene does not cover the range in which it is found in orange juice.

The limit of detection (LOD) was estimated to be the concentration of analytes that produces a signal 3 times that of the noise. The detection limits were calculated by extrapolating from the lowest concentration point on the linear range calibration curve. As well, the limit of quantitation (LOQ) was estimated to be the concentration of the analytes that produces a signal 10 times that of the noise. The calculated LOQs are below the concentrations at which these analytes are found in real juice. These results are shown in Table 2. In some cases the estimated LOQ was slightly higher than the lowest concentration explored in the linear range, so the LOQ was then estimated to be the lower end of the linear range that was examined for these analytes.

Once a method has been developed, real orange juice samples were analyzed using the standard addition method. As well, other juice samples were analyzed in a purely qualitative experiment to simply determine the presence of any of the analytes investigated. These samples were not analyzed using the standard addition method.

Robards and Antolovich (1995) concluded that orange juice had the most complex matrix of all the fruit juices, so studies were done to investigate the headspace extraction from the orange juice matrix itself. One of the most prominent features of orange juice is its acidity (pH between 3.5 and 4), yet the preliminary extractions in this study were done from a water solution that had a pH of approximately 6.2. Therefore, a standard solution in water was buffered to pH 3.9, extracted using the PA-coated fiber, and compared with both a salted standard solution in water and a plain standard solution in water that was not modified to determine the potential effect of the pH of the real juice. It was found that there was a significant increase in the amount extracted from the standard in a saturated salt solution compared to the more acidic matrix conditions. In most cases more of the analytes were extracted to a higher extent (approximately a 40% increase) from the unmodified water solution (pH approximately 6.2) than from the buffered solution (pH 3.9); however, there was only a slight difference between the amount extracted from these solutions for a few of the target compounds. In general, it was concluded that a decrease in the pH of the matrix from which the analytes were extracted decreases the partitioning of the analytes from the juice into the fiber coating. Furthermore, it can be expected that the low pH of the real juice matrix could lower the extraction of some analytes into the fiber coating.

Table 3. Concentrations of Orange Juice Flavor Volatiles from a Centrifuged Sample Using Headspace SPME with a PA-Coated Fiber

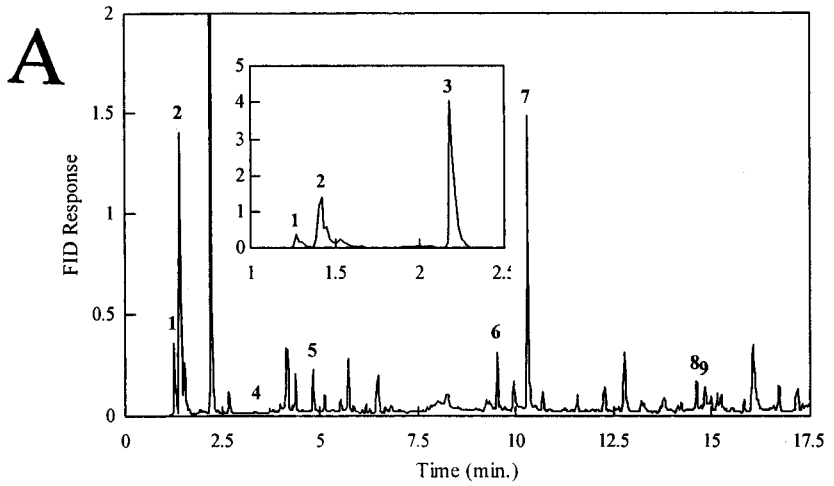
target analyte	concn (ppm)	target analyte	concn (ppm)
methanol	72	α-pinene	0.038
ethanol	4.7×10^2	β-myrcene	ND[a]
ethyl acetate	0.29	ethyl hexanoate/octanal	0.089
2-methyl-1-propanol	0.18	limonene	43
methyl butyrate	0.01	γ-terpinene	0.26
ethyl butyrate	0.34	linalool	0.22
cis-3-hexen-1-ol	0.025	α-terpineol	0.26
hexyl alcohol	0.06	decanal	0.013

[a] ND, not determined.

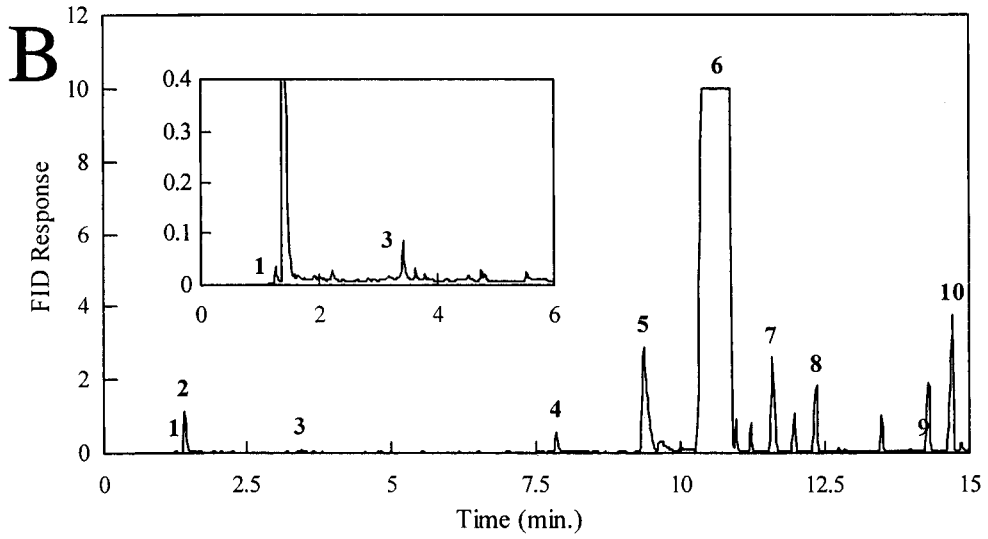
The standard addition method was applied to the real juice to quantify the target compounds. A sample of Tropicana juice was used. Because of the large amount of suspended solids in the juice, the samples were centrifuged to reduce the partitioning of the target compounds into these suspended solids. The solids were disposed of and headspace SPME was performed on the remaining liquid portion of the sample. β-Myrcene was found to coelute with a nontarget compound in the real juice so it was not accurately integrated. It was also found that when the real sample was centrifuged, the amount of limonene present in the juice was reduced so significantly that the amount extracted was within the dynamic range of the detector. Table 3 shows the amounts extracted from the centrifuged juice using the standard addition method. The determined concentrations of the target analytes in the juice generally agree with the literature values as cited by Nisperos and Shaw (1990).

In headspace SPME the rate-limiting step is considered to be the diffusion of the analytes from the aqeuous phase to the headspace. However, in this complex juice, the matrix itself might cause the rate-limiting step to be the transport of the analytes through the juice. One might consider the transport of a target analyte through this system as follows: because of the presence of suspended solids, and potential emulsions, a portion of the target analytes might be "trapped" in the pore or cell of these solids (such as pulp), and for it to be extracted, it must diffuse through the static water within the cell, penetrate the cell wall, diffuse through the liquid juice, and partition into the headspace and then into the fiber.

As well, both grape juice and grapefruit juice were also analyzed in a qualitative manner to determine if headspace SPME would extract the targt compounds from other juices that were investigated in orange juice.



Peak labels: 1. methanol, 2. ethanol, 3. ethyl acetate, 4. methyl butyrate, 5. ethyl butyrate, 6. β-myrcene, 7. limonene, 8. α-terpineol, 9. decanal.



Peak labels: 1. methanol, 2. ethanol, 3. methyl butyrate, 4. α-pinene, 5. β-myrcene, 6. limonene, 7. γ-terpinene, 8. linalool, 9. α-terpineol, 10. decanal.

Figure 4. GC/FID chromatogram of a headspace SPME of (A) Welch's grape juice and (B) concentrated grapefruit juice sample.

Chromatographic analyses were performed using GC/FID for these samples. It was found with both of the juices that the addition of salt to the juice increased the amount of analyte extracted into the fiber. Figure 4A shows a chromatogram of the headspace SPME of a Welch's grape juice from concentrate. The juice was extracted both with and without the addition of a standard solution. This was done so that the target analytes in the real (unspiked) juice could be identified by comparing the retention times of the target analytes that were spiked into the juice to those in the unspiked juice. As illustrated, the following compounds matched retention times with the peaks eluted from the extraction of the grape juice: methanol, ethanol, ethyl acetate, methyl butyrate, ethyl butyrate, β-myrcene, limonene, linalool, α-terpineol, and decanal. A Mr. Pure grapefruit juice from concentrate was also analyzed by headspace SPME. The chromatogram of the extraction is shown in Figure 4B. As indicated, the following compounds were peaks that matched with peaks from the juice extraction: methanol, ethanol, methyl butyrate, ethyl butyrate, α-pinene, β-myrcene, limonene, γ-terpinene, linalool, α-terpineol, and decanal.

CONCLUSIONS

A method for the extraction and analysis of flavor volatiles from orange juice was developed. The analysis was performed with a PA-coated fiber which was found to extract more of the target flavor volatile analytes than the commonly used PDMS-coated fiber. Using a centrifuged orange juice sample, the developed method was shown to have linear range and detection limits that were well within the ranges in which the target flavor compounds naturally occur. The reproducibility was found to range between 1 and 20% RSD, but for most analytes it was found to be below 10% RSD. Addition of salt to the matrix enhanced the amount of analyte extracted into both fiber coatings. The concentrations of the target analytes were quantified using the method of standard additions, and the values were found to be within the same range as the amounts reported using current headspace techniques. It has been also shown that SPME can also readily extract flavor volatiles from other fruit juices and that some of the same analytes found in orange juice were found in grape and grapefruit juices. Therefore, headspace SPME was found to be an effective technique when applied to the analysis of flavor volatiles.

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