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Solid-phase microextraction for headspace analysis of key volatile compounds in orange beverage emulsion

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Abstract

Headspace solid-phase microextraction (HS-SPME) gas chromatography was used to analyze target flavor compounds in orange beverage emulsion. The effects of SPME fiber (PDMS 100 μm, CAR/PDMS 75 μm, PDMS/DVB 65 μm and DVB/CAR/PDMS 50/30 μm), adsorption temperature (25–45 °C), adsorption time (5–25 min), sample concentration (1–100%), sample amount (5–12.5 g), pH (2.5–9.5), salt type (K_2CO_3, Na_2CO_3, NaCl and Na_2SO_4), salt amounts (0–30%) and stirring mode were studied to develop HS-SPME condition for obtaining the highest extraction efficiency and aroma recovery. For the head space volatile extraction, the optimum conditions were: CAR/PDMS fiber, adsorption at 45 °C for 15 min, 5 g of diluted beverage emulsion (1:100), 15% (w/w) of NaCl with stirring and original pH 4. The main volatile flavor compounds were: limonene, 94.9%; myrcene, 1.2%; ethyl butyrate, 1.1%; γ-terpinene, 0.41%; linalool, 0.36%; 3-carene, 0.16%; decanal, 0.12%; ethyl acetate, 0.1%; 1-octanol, 0.06%; geranial, 0.05%; β-pinene, 0.04%; octanal, 0.03%; α-pinene, 0.03%; and neral, 0.03%. The linearity was very good in the considered concentration ranges ($R^2 \geqslant 0.97$). Average recoveries ranged from 88.3% to 121.7% and showed good accuracy for the proposed analytical method. Average relative standard deviation (RSD) for five replicate analyses was found to be less than 14%. The limit of detection (LOD) ranged from 0.06 to 2.27 mg/l for all volatile flavor compounds and confirmed the feasibility of the HS-SPME technique for headspace analysis of orange beverage emulsion. The method was successfully applied for headspace analysis of five commercial orange beverage emulsions.

Keywords: Solid-phase microextraction; Gas chromatography; Flavor compounds; Orange beverage emulsion; Extraction efficiency; Headspace analysis

1. Introduction

In soft drinks, citrus flavors are among the most popular of all flavors, and worldwide, orange flavor is the favorite of consumers (Tan, 1997). The orange flavor has been studied more than that of any other type of citrus fruit. This is partly because the orange beverage is the most popular fruit beverage worldwide, and its great demand is a result of its nutritional and sensory properties (Selli, Cabaroglu, & Canbas, 2003). Its fresh and uniquely delicate flavor is due to complex combinations of several odour components that have interdependent quantitative relationships (Maccarone, Campisi, Fallico, Rapisarda, & Sgarlata, 1998; Shaw, 1991). Important contributors to orange flavor include esters, aldehydes, ketones, terpenes, and alcohols (Nisperos-Carriedo & Shaw, 1990). These citrus flavored products are based primarily on the essential oils extracted from the peel of the fruits. Since they are not water soluble, incorporation of these flavors into soft drinks can be done by either separating out the water soluble fraction from the essential oil by extraction and distillation, or converting the oil into a water-dispersible emulsion: a beverage emulsion (Tan & Wu Holmes, 1988).

Flavor/cloud emulsions are the most important beverage emulsions which are widely used in beverages (e.g., citrus drinks) to give the products an opaque appearance and suitable aroma which is more appealing to the consumer.

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The addition of flavor/cloud emulsion changes the properties of the beverage phase, thus altering volatile compound partition. As a result, the aroma profile above the product changes and this may affect the overall perceived flavor. Flavorists use a combination of experience and science to adjust flavor formulations to compensate for the type of cloud emulsion. However, it should be possible to predict volatile behavior through scientific principles. Organoleptic emulsion attributes and emulsion flavor freshness are influenced by changes in behavior of volatile compounds in an emulsion. Flavor release followed by changing the sensory of emulsion are the most relevant defects in beverage emulsions during processing and/or storage. The behavior of emulsion flavor depends on flavor properties (e.g., type, concentration, molecular structure, boiling point, volatility and hydrophobicity of flavor compounds). One the other hand, emulsion flavor properties will be influenced by physicochemical parameters such as environment (matrix) and its physical state, chemical properties of flavor molecules and interaction of volatile flavor compounds with other molecules during processing and storage (McClements, 1999).

To better understand these changes, it is necessary to have quantitative information about the most characteristic aromatic compounds in orange beverage emulsion. This information allows us for the modification of some processing conditions. On the other hand, the analysis of key volatile flavor compounds in beverage emulsion plays a significant role in evaluating emulsion flavor freshness. There are a few techniques to monitor changes of volatile flavor compounds. Headspace solid-phase microextraction (HS-SPME) is an optional technique that may be more suitable than the conventional methods for evaluating the release of volatile flavor compounds from orange beverage emulsion. Solid-phase microextraction (SPME) is a simple adsorption technique for the isolation of headspace flavor compounds (Arthur & Pawliszyn, 1990; Zhang & Pawliszyn, 1993). It is a sample preparation technique based on sorption procedure (absorption and/or adsorption, depending on the fiber coating), which is useful for extraction and concentration analyses either by submersion in a liquid phase or by exposure to a gaseous phase (Arthur, Killam, Buchholz, & Pawliszyn, 1992). SPME is a fast, convenient, solventless extraction technique that can be used to extract analytes from both liquid and solid matrices. However, SPME analysis is quite sensitive to experimental conditions such as heating temperature, extraction time, sample volume, concentration, and sample matrix and uniformity (Yang & Peppard, 1994). For HS-SPME, two processes need to occur to successfully extract flavor compounds: release of analytes from the matrix followed by partitioning of analytes into the extracting phase (Pawliszyn, 1995). As such, SPME requires careful development of these experimental parameters, which strictly depend on the type of food sample and matrix characteristics (Roberts, Pollien, & Milo, 2000). SPME has been used to analyze volatile flavor compounds in fruit juice beverages and nectars (Arthur & Pawliszyn, 1990; Lambropoulou & Albanis, 2002; Liu & Yang, 2002; Riu-Aumatell, Castellari, Lopez-Tamames, Galassi, & Buxaderas, 2004; Yang & Peppard, 1994), ground coffee, butter flavored vegetable oil (Yang & Peppard, 1994), baby food (Bianchi, Careri, Mangia, & Musci, 2006), wine (Carrillo, Lopez, & Tena, 2006; Mejıas, Marın, Moreno, & Barroso, 2003) and to determine the organic contaminants in water samples (Li, Zhong, Xu, & Sun, 2006; Wen, Zhou, Xu, Jin, & Feng, 2006; Yang, Zeng, Maruya, Mai, & Ran, 2007), environmental pollutants in soil (Prosen, Fingler, Kralj, & Drevenkar, 2007), organophosphorus insecticides in fruits (Fytianos, Raikos, Theodoridis, Velinova, & Tsoukali, 2006), cocaine and cocaethylene in plasma (Álvarez, Bermejo, Tabernero, Fernández, & López, 2007). Hence, partitioning, release or binding behavior of volatile compounds above a beverage emulsion can be measured under equilibrium headspace conditions using SPME technique. HS-SPME provides information on the composition of volatile fractions that contribute to perceived aroma. For reproducible SPME results, some variables must be controlled during the extraction process. These include sample agitation, sampling method (headspace vs. immersion), sample pH, ionic strength, volume, time and temperature (Pawliszyn, 1997).

The aim of this study was to develop the SPME condition for quantitative and qualitative analyses of headspace volatile compounds released from an orange beverage emulsion. This method development has not been carried out on date for beverage emulsion. Validation of the method based on HS-SPME-GC was then carried out by plotting calibration curve, evaluating linearity, reproducibility, recovery, limit of detection (LOD) and limit of quantification (LOQ).

2. Experimental

2.1. Chemicals and materials

The standard solution of orange volatile compounds including α-pinene (99.5%), ethyl butyrate (99.7%), β-pinene (98.5), 3-carene (98%), myrcene (95%), limonene (99%), γ-terpinene (98.5%), octanal (98%), decanal (95%), linalool (95%), and citral (95%) (neral and geranial) were supplied by Fluka (Buch, Switzerland). Internal standard, butyl acetate (98%) was obtained from Aldrich (Steinheim, Germany). The SPME device, SPME fiber assortment kit no. 4, 20 ml glass vial, teflon coated rubber septa and aluminum caps were supplied by Supelco Inc. (Bellefonte, USA). Gum Arabic food grade was provided by Colloides Naturels International Co. (Rouen, France). Xanthan gum was donated by CP Kelco (Chicago, USA). Citric acid, sodium benzoate and potassium sorbate (p.a. $\geqslant$ 95%) were purchased from Fisher Scientific (Pittsburgh, PA). Potassium carbonate, sodium carbonate, sodium chloride, sodium hydrogen carbonate and sodium sulfate (p.a. $\geqslant$ 99%) were supplied by Merck (Darmstadt, Germany). Valencia cold pressed orange oil was provided by

Danisco (Cultor, Aarhus, Denmark). Deionized water was used to prepare standard solutions. Five commercial orange beverage emulsions were purchased from Danisco (Cultor, Aarhus, Denmark), Givaudan (Dubendorf, Switzerland) and Symrise (Nördlingen, Germany).

2.2. Standard preparation

Stock standard solutions of 20 mg/l (% w/v) of flavor compounds were individually prepared in deionized water. These flavor compounds were detected by comparing retention time with those of known standard compounds by standard addition technique and by comparison of mass spectra using the NIST library (version 2.0). For quantitative analysis and method validation, different stock standard solutions containing 500 mg/l (% w/v) of each flavor compound were used to prepare working standard solution that contained different concentration of target flavor compounds. Stock standard solution of 1000 mg/l (% w/v) of butyl acetate was prepared as internal standard. Five levels of concentration were prepared to cover the appropriate range for each compound and to plot the calibration curve. The range of concentrations for the working standard solution of volatile flavor compounds was estimated on the basis of concentration as reported by Shaw (1991) and preliminary work. Concentration of working standard solution that ranged from 1.5 to 20 mg/l (% w/v) was prepared for most flavor compounds except limonene and myrcene. For quantitative analysis, the stock solution of myrcene and limonene were diluted to yield suitable concentration ranges of 4–40 mg/l and 30–300 mg/l (% w/v), respectively. The standard solutions were stored at 4 °C. Butyl acetate was used as an internal standard.

2.3. Orange beverage emulsion preparation

A representative orange beverage emulsion composed of gum Arabic (13.5% w/w), xanthan gum (0.3% w/w), orange oil (10% w/w), sodium benzoate (0.1% w/w), potassium sorbate (0.1% w/w), citric acid (0.5% w/w) and deionized water was prepared for the SPME development procedure. A beverage emulsion is usually composed of two phases: water phase and oil phase. To prepare the water phase, sodium benzoate, potassium sorbate and citric acid were dispersed in 60 °C deionized water using a high shear blender (Waring blender 32BL80, New Hartford, CO, USA). While mixing the mixture, gum Arabic was gradually added to the 60 °C deionized water and mixed for 3 min to facilitate hydration. The gum solution was kept overnight at room temperature to fully hydrate and then mixed until complete dissolution (Buffo, Reineccius, & Oehlert, 2001). To prepare the water phase, xanthan gum solution was prepared separately by dissolving xanthan gum in deionized water and then mixed with Arabic gum solution by using a high speed blender (Silverson L4R, Buckinghamshire, UK). Using a 50% (w/w) solution of citric acid, pH of water phase was adjusted as required. While mixing the water phase, cold pressed orange oil was gradually dispersed in the water phase to provide an initial coarse emulsion. The coarse emulsion was prehomogenized using a Silverson high shear blender for 1 min and then passed through a high pressure homogenizer (APV, Crawley, UK), for three passes (30, 28 and 25 MPa).

2.4. Model beverage emulsion

For quantitative analysis and method validation, a model cloud emulsion was prepared by using the same materials as the real beverage emulsion excluding orange oil. The model beverage emulsion was prepared by incorporating known amounts of standard solutions as oil phase in the water phase and diluted to yield suitable concentration.

2.5. HS-SPME procedure

For SPME analysis, 5 g of the diluted orange beverage emulsion (1:100) was transferred into a 20 ml serum vial containing a microstirring bar. Subsequently, 1.5 g NaCl and 1 μl mixed butyl acetate as internal standard was added into the vial. The vial was sealed with a Teflon-lined septa and screw cap, and then immersed in a water bath at 45 °C. The SPME fiber coated with CAR/PDMS (carboxen/polydimethylsiloxane) was manually exposed to the sample headspace for 15 min at 45 °C to reach equilibrium. The sample was continuously agitated with a magnetic stirring bar during the extraction process to allow faster equilibrium condition. Finally, the fiber was withdrawn into the needle holder and immediately introduced into the GC injection port and held for 8 min to completely desorb the volatile compounds. In this work, four different fibers, five extraction times, three adsorption temperatures, stirring, four sample amounts, four salt amounts, four salt types, four pH values and four sample concentrations were analyzed to develop the SPME procedure for headspace analysis of orange beverage emulsion.

2.6. GC–FID condition

The volatile compounds were analyzed using a Hewlett–Packard 6890 GC equipped with a flame ionization detector (FID) and a DB-Wax column (J&W Science, i.d. = 0.25 mm, length = 30 m, film thickness = 0.25 μm, Supelco, MA). The GC injection port was equipped with a 0.75 mm i.d. liner (Supelco) to minimize peak broadening. For the headspace analysis of orange beverage emulsion, the injection was performed in the split mode (1:40) for 8 min at 250 °C. Oven temperature was programmed at 45 °C isothermally for 5 min, then ramped to 51 °C at 1 °C/min and held for 5 min at 51 °C then increased to 160 °C at 5 °C/min and finally raised to 250 °C at 12 °C/min and held for 15 min at the final temperature. Helium was used as the carrier gas with a flow-rate of 1.1 ml/min. Injector and detector temperatures were 250 and 270 °C, respectively.

2.7. GC–TOFMS condition

The volatile compounds were initially detected and confirmed using a Hewlett-Packard 6890N GC system (Wilmington, DE) equipped with Electron Ionization-Time-of-Flight Mass Spectrometer (TOFMS, Pegasus III, Leco Corp., St. Joseph, MI, USA). The same GC column and operating conditions were later used to analyze the flavor compounds in orange oil and emulsion. Helium was used as carrier gas with flow-rates 1.4 ml/min. Mass spectra in the electron impact (EI) mode were generated at 70 eV (Hognadottir & Rouseff, 2003). For orange flavor analysis, the injections were done in the split mode (1:100 and 1:200) with the injector temperature held at 250 °C.

2.8. Data analysis

The data obtained from GC–TOFMS was processed using the ChromaTOF software version 2.4 (LECO Corporation). The volatile flavor compounds of orange oil and beverage emulsion were detected by matching mass spectra fragment with the NIST library version 2.0 and confirmed by comparing retention times and mass spectra of unknowns with those of known standards. Subsequently, the peaks were verified by running the known standard solutions and samples, respectively. Among the 84 volatile flavor compounds detected by GC–MS, 12 orange oil compounds were selected on the basis of their abundance and their impact on fresh orange aroma.

2.9. Statistical analysis

Statistical analyses were performed using Minitab v. 13.2 and Statsoft Statistica v. 6.1. A one-way ANOVA was carried out on the peak areas. Significant differences were evaluated by the Fisher test at 95% confidence level. Two-way ANOVA was also used to study the effects between two factors.

3. Results and discussion

3.1. Identification

In qualitative analysis, 84 volatile flavor compounds were detected by the HS-SPME-GC–TOFMS in Valencia cold pressed orange oil. Among the volatile compounds, only 12 flavor compounds were chosen as representative of the terpenes, alcohols, ketones, and aldehydes present in Valencia cold pressed orange oils: α-pinene, ethyl butyrate, β-pinene, 3-carene, myrcene, limonene, γ-terpinene, octanal, decanal, linalool, neral and geranial were detected. In previous study (Hognadottir & Rouseff, 2003), these flavor compounds have been reported as important volatile compounds in orange oil (Hognadottir & Rouseff, 2003). The results showed that monoterpene hydrocarbons constituted the main volatile compound in orange oil. α-pinene, β-pinene, 3-carene, myrcene, limonene and γ-terpinene were the most important terpene hydrocarbons in orange oil. Limonene was reported to be by far the most abundant monoterpene in cold pressed orange oil followed by myrcene (Table 1).

3.2. SPME procedure

3.2.1. SPME fiber screening

In this research, four SPME fibers (PDMS 100 μm, CAR/PDMS 75 μm, PDMS/DVB 65, DVB/CAR/PDMS 50/30 μm) were evaluated to determine their effectiveness in extraction of volatile compounds from orange beverage emulsion. As shown in Fig. 1a–c, the fibers with a medium-polar coating appeared to be more efficient for the extraction of orange flavor compounds; while the non-polar fiber coating PDMS that was the most inefficient. The results illustrated that the highest extraction efficiency for the target volatile flavor compounds except for decanal, linalool, neral and geranial were associated with the CAR/PDMS fiber followed by DVB/CAR/PDMS and PDMS/DVB fibers. Therefore, the CAR/PDMS fiber was chosen as the optimum fiber coating in this study with respect to the number of detected substances as well as the signal intensity. The bipolar fiber coating CAR/PDMS was also selected as the optimum fiber coating for orange juice flavor (Rouseff, Bazemore, Goodner, & Naim, 2001).

3.2.2. Effect of extraction time

In this study, five extraction times 5, 10, 15, 20 and 25 min were analyzed to investigate the effect of extraction time on the equilibrium of volatiles between the SPME coating and headspace of sample. Fig. 2 shows the extraction time profiles for the target volatile compounds. The shortest acceptable time, from the point of view of analyte detection limit, was chosen. The amount of flavor compounds adsorbed by SPME was found to not always increase at the same rate with increasing extraction time. The results showed that increasing extraction time increased overall extraction yield of most flavor com-

Table 1
Volatile flavor compounds of Valencia cold pressed orange oil detected by HS-SPME-GC using TOFMS and quantified by FID

No.	Compound	Similarity	LRI[a]	FID area %
1	Ethyl acetate	793	840	0.1
2	α-Pinene	951	1176	0.03
3	Ethyl butyrate	949	1264	1.1
4	β-Pinene	929	1590	0.04
5	3-Carene	931	1751	0.16
6	Myrcene	885	1917	1.2
7	Limonene	911	2100	94.9
8	γ-Terpinene	917	2272	0.41
9	Octanal	842	2654	0.03
10	Decanal	952	3521	0.12
11	Linalool	940	3616	0.36
12	1-Octanol	895	3631	0.06
13	Neral	944	3699	0.03
14	Geranial	874	3744	0.05

[a] Linear retention indices for a DB-Wax column.

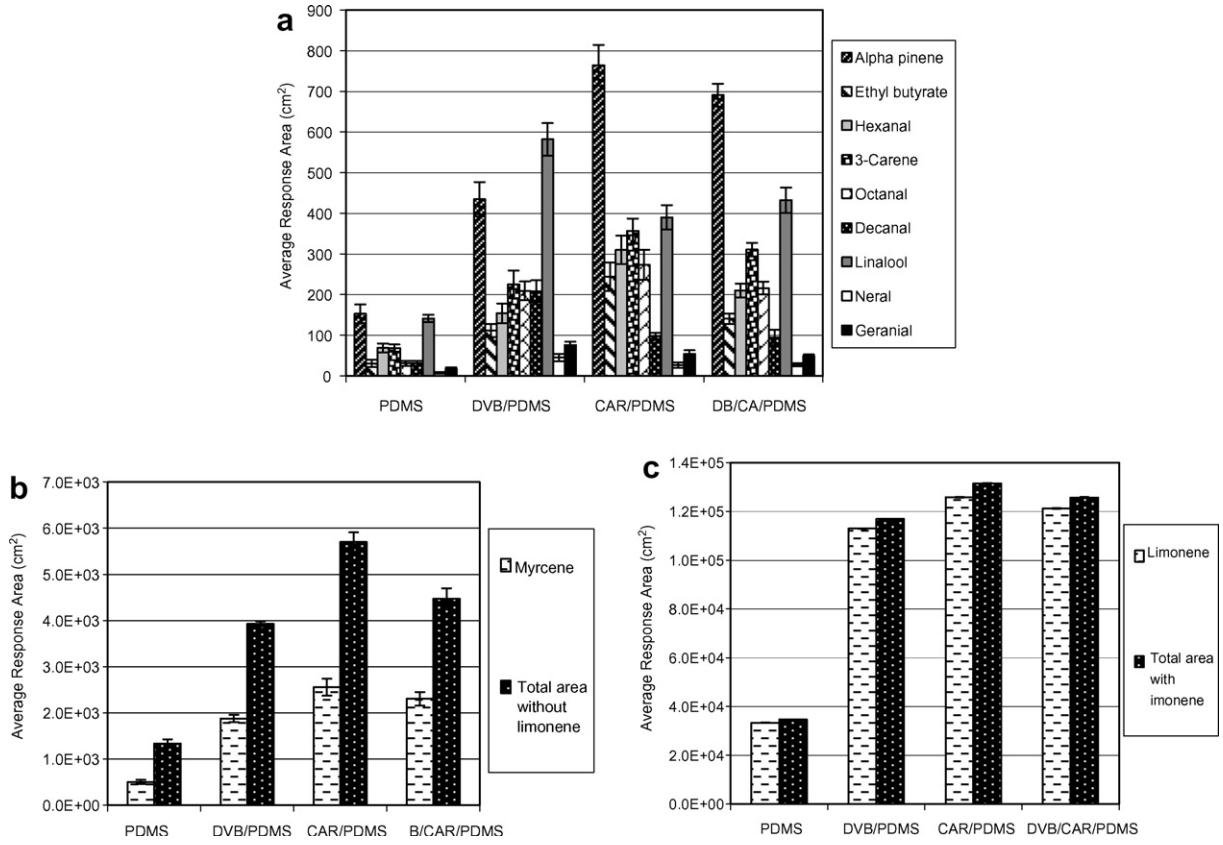

Fig. 1. Influence of type of fiber on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene.

pounds except γ-terpinene and octanal. The increase in analyte with increasing of extraction time was also reported in wine (Mejıas et al., 2003), beverage (Dong & Wang, 2006), and fruits and fruit juice (Simplıcio & Boas, 1999). No significant differences ($p > 0.05$) were found between the amount of myrcene and limonene extracted for 15 min, 20 min and 25 min. Hence, the extraction time for subsequent analyses was then fixed at 15 min as 85% of the total flavor compounds was adsorbed at 15 min.

3.2.3. Effect of adsorption temperature and sample agitation

Firstly, the effect of adsorption temperatures 25, 35 and 45 °C was studied at static sampling condition without agitation. The results showed that there were no significant differences ($p > 0.05$) between different extraction temperatures under static sampling condition without stirring (data not shown).

Interactive effects between adsorption temperature and stirring were also studied at the same levels of adsorption temperature under dynamic sampling condition. It is well known that extraction rate was strongly influenced by stirring and temperature due to faster equilibrium reached. Fig. 3a–c shows that combination of stirring and adsorption temperature had a significant ($p < 0.05$) positive affect on the overall extraction efficiency. This was because stirring caused turbulence in the liquid and gaseous phases (Zhang, Yang, & Pawliszyn, 1994) and increased the partition coefficient and diffusion rate of the analyte into the fiber. This observation was also in agreement with the previous study on orange juice (Jia, Zhang, & Min, 1998), and wine (Carrillo et al., 2006). In this study, stirring was found to be a more significant ($p < 0.05$) factor to extract the target orange volatile compounds than adsorption temperature alone because the average total peak areas under stirring mode improved 2-, 4- and 7.5-folds at 25, 35 and 45 °C, respectively. Adsorption temperature was also an effective factor to increase extraction efficiency under dynamic sampling condition in this study.

3.2.4. Effect of adsorption temperature and salt addition

The effect of salt addition was also determined in combination with adsorption temperature. Results obtained for extraction yield of the target volatile compounds at 25, 35 and 45 °C with and without NaCl are shown in Fig. 4. In general, the addition of salts led to an increase of the extraction yield because of the salting-out effect. Salting out increased the ionic strength of the aqueous solution and, in this way, could decrease the solubility of organic analytes; thus, partitioning the volatile flavor compounds from the aqueous solution to the headspace and the fiber coating was improved. This observation was also reported in previous study on wine (Mestres, Busto, & Guasch, 2002; Mestres, Sala, Marti, Busto, & Guasch, 1999), where extraction efficiency increased with the addition of salt.

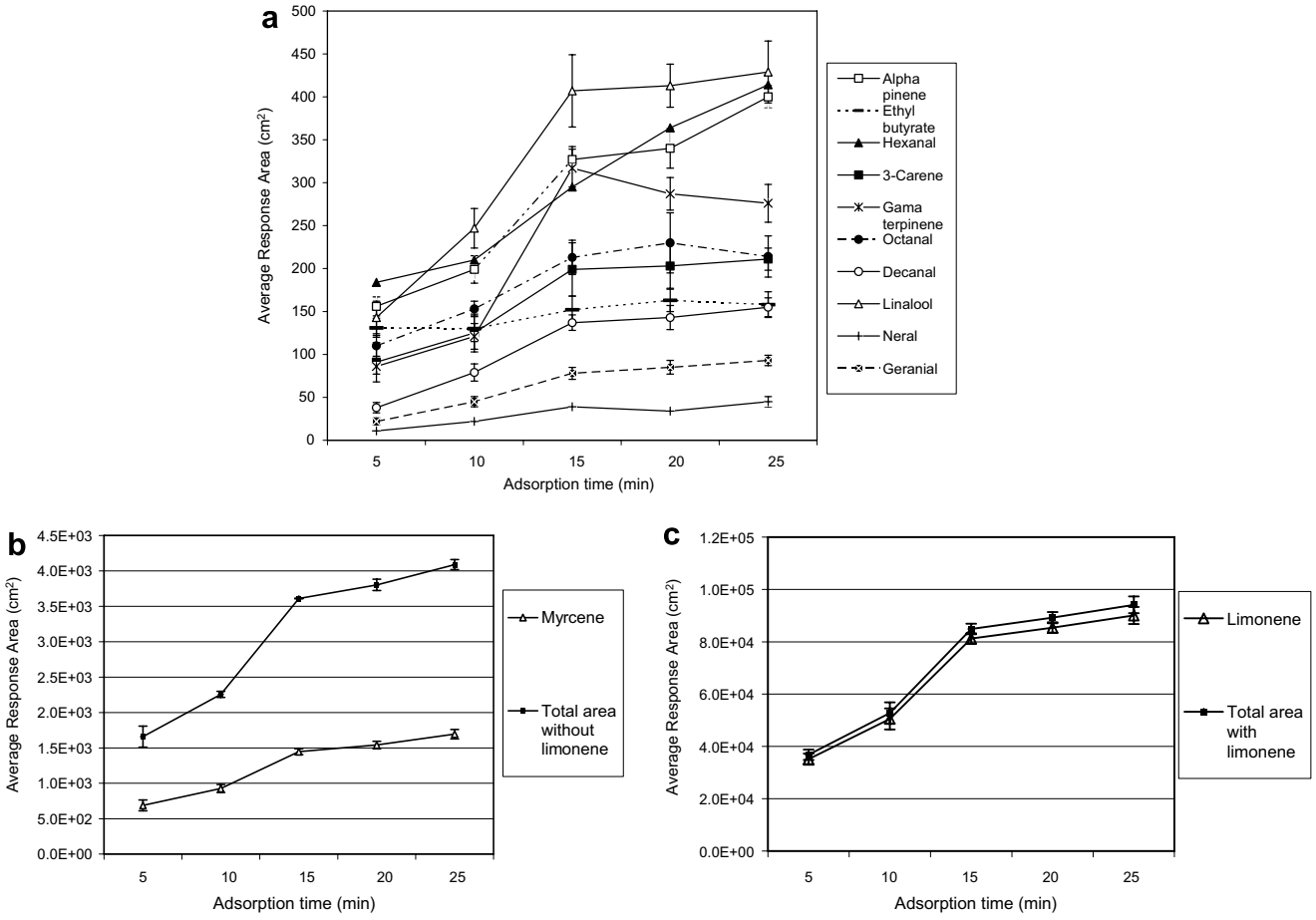

Fig. 2. Influence of exposure time on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene.

A combination of salt and temperature had positive effects on extraction yield of most orange flavor compounds except for ethyl butyrate, neral and geranial. As shown in Fig. 4a–c, the peak areas of analytes of interest increased significantly ($p < 0.05$) with an increase of temperature from 25 to 45 °C under dynamic sampling condition with salt. The highest extraction efficiency was observed using a combination 1 g salt and 45 °C under stirring condition. The results obtained at different temperature showed a positive effect of salt on the extraction efficiency. The average total areas of peaks obtained at 25 and 35 °C improved by 47% and 78% when salt was added to the sample. The total area achieved at 45 °C increased by 43% in the presence of salt. Comparison of the results obtained by extracting the volatiles at different temperatures with salt demonstrated that the average total peak areas increased by 107% and 164% with increasing temperature to 35 and 45 °C, respectively.

3.2.5. Effect of sample amount

The influence of sample amount on extraction efficiency was determined by varying the amount of samples (5, 7.5, 10 and 12.5 g) in a 20 ml vial. The results showed no significant differences ($p > 0.05$) between the sample amounts except for 12.5 g of sample. On the other hand, extraction efficiency decreased noticeably with an increase of sample amount from 10 to 12.5 g (data not shown). Therefore, a sample amount of 5 g was used for further experiments.

3.2.6. pH

The effect of four different pH values 2.5, 4 (original orange beverage emulsion pH), 7 and 9.5 were tested to determine effect of pH on extraction efficiency. The pH of sample was adjusted by adding citric acid or sodium hydrogen carbonate solutions (1 M). In general, it was found that changing the pH in the range of 2.5–9.5 did not lead to a significant effect ($p > 0.05$) on the extraction efficiency of the headspace volatile compounds of orange beverage emulsion using CAR/ PDMS fiber (data not shown).

3.2.7. Effect of salt type

Four different types of salt including potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3), sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) were selected to investigate the salt effect on extraction efficiency of headspace volatile compounds of orange beverage emulsion. The results showed that in all cases except geranial, the addition

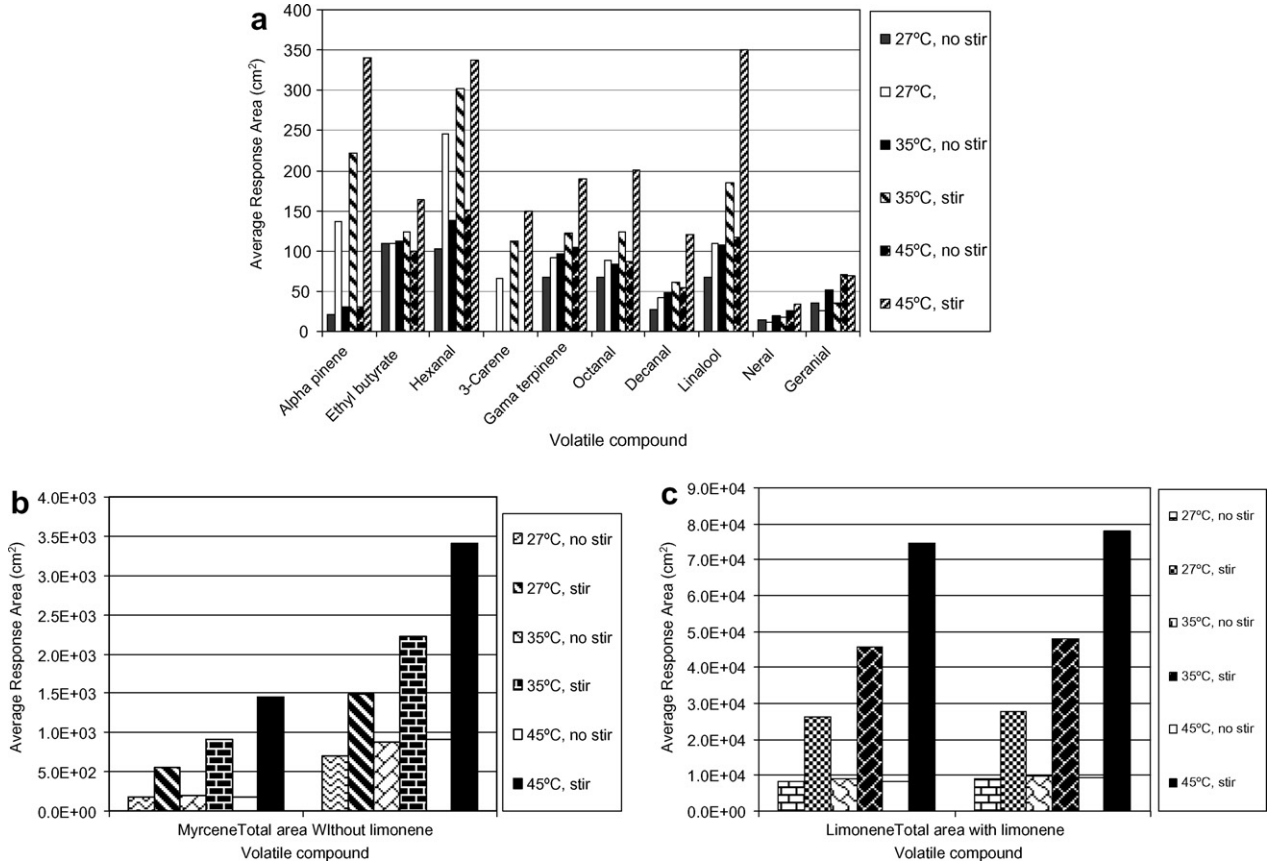

Fig. 3. Influence of absorption temperature and sample agitating on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene.

of NaCl resulted in the highest extraction yield. Hence, NaCl was used for further experiments (data not shown).

3.2.8. Effect of salt amount

The influence of the ionic strength of the sample matrix was studied by addition of different amount of NaCl (0–30% w/w). As shown in Fig. 5a–c, an increase of salt amount led to an increase in the overall extraction yield and better response was obtained by increasing the amount of salt. The effect of salt amount depended on type of analyte. In this study, the peak areas of α-pinene, ethyl butyrate, myrcene and limonene increased whereas octanal, decanal, linalool neral and geranial decreased with an increase in NaCl. These reverse effects have been also reported by Yang and Peppard (1994). The average of total peak areas improved by 11% and 17% as salt was added at 15% and 30%, respectively. However, no significant difference ($p > 0.05$) was found between the volatile extraction yields obtained by adding 15% and 30% NaCl. Therefore, 15% (w/w) of NaCl was used for the rest of experiments.

3.2.9. Effect of sample concentration

Six levels of sample concentration 1, 5, 10, 25, 50 and 100 (% w/w) were used to investigate the effect of matrix interference. The samples were diluted with deionized water. The results confirmed that sample concentration was a significant ($p < 0.05$) factor to increase recoveries, extraction yield and accuracy because the recovery values were significantly ($p < 0.05$) improved by diluting the samples. This may because matrix interference can be reduced by diluting the sample dilution. This observation was reported in beverage (Dong & Wang, 2006). As shown in Fig. 6a–c, the highest total peak area obtained by using a sample concentration of 1%. Conversely, undiluted sample showed the lowest extraction efficiency. Hence, sample concentration of 1% was chosen as the optimal concentration to quantify the headspace volatile compounds of orange beverage emulsion.

3.2.10. Summary of the set up conditions

To sum up, the optimized HS-SPME method can be described using the following parameters: 5 ml of diluted orange emulsion (1:100) was transferred into a 20 ml vial sealed with a PTFE septum were prepared and then 15% (w/w) of NaCl was transferred into the vial. Subsequently, the vial was immersed in a water bath at 45 °C. Extraction was performed with a 75 μm CAR/PDMS fiber for 15 min while the sample was being stirred. Finally, the fiber was immediately inserted into the injector for thermal desorption at 250 °C for 8 min.

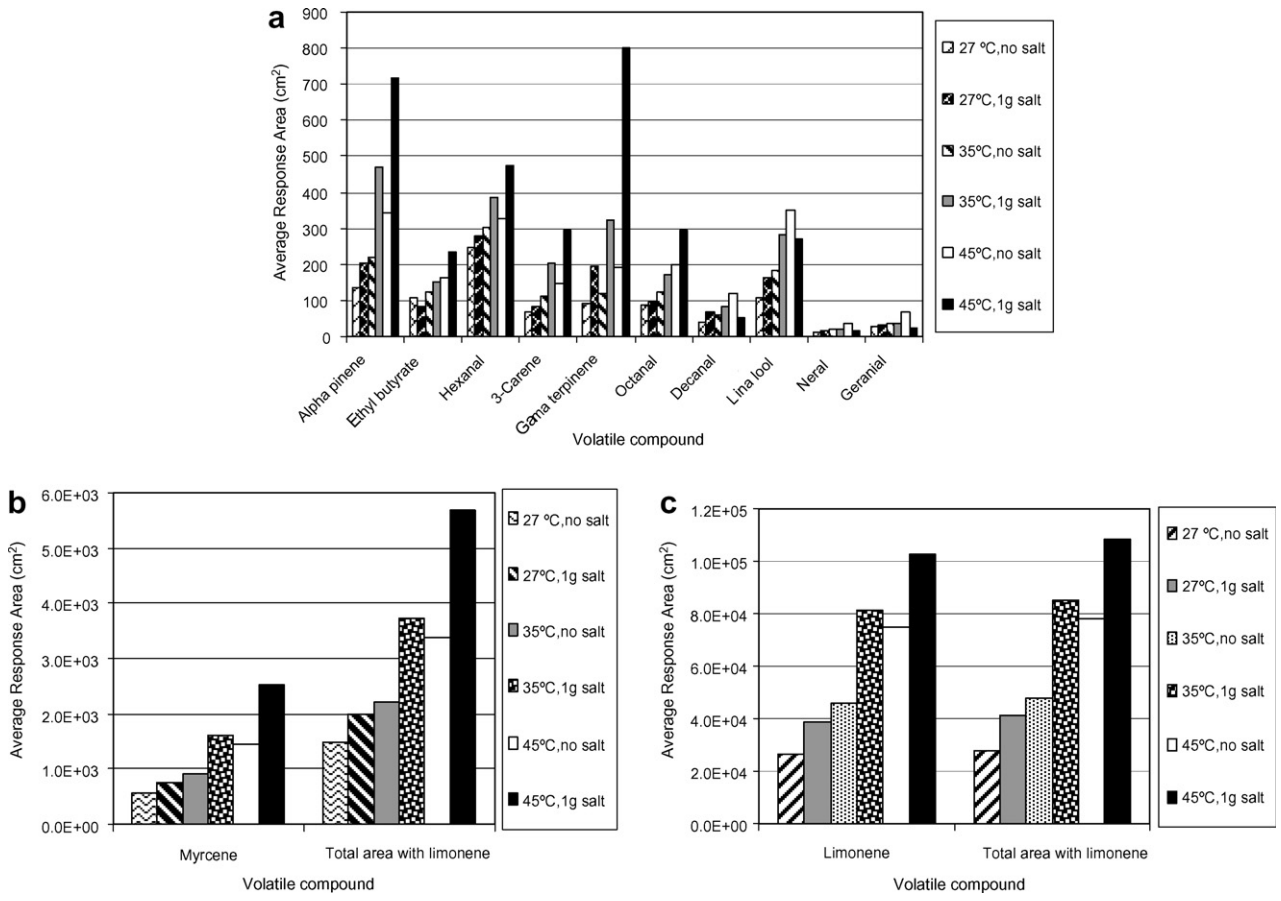

Fig. 4. Influence of absorption temperature and salt addition on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene.

3.3. Performance characteristics

In this study, the method validation was performed by determining linearity, recovery, reproducibility and limit of detection (LOD) for all volatile compounds. The performances were determined, according to the optimized conditions described, by using standard solutions and butyl acetate as an internal standard.

3.3.1. Calibration

3.3.1.1. Linearity. Five levels of concentration of each analyte were prepared for plotting standard calibration curves. The calibration curves were constructed to test the linearity range for each flavor compound. The (volatile compound/internal standard) peak area ratio obtained for each compound was interpolated into the calibration curves. In this study, 10 μl of butyl acetate standard solution (1000 mg/l) was used as internal standard. The concentration ranges, regression equations, R^2 values, recoveries, RSDs and LODs for the target flavor compounds are shown in Table 2. The HS-SPME procedure showed a good linear behavior in the concentration ranges studied. As shown in Table 2, ethyl butyrate showed the best linearity ($R^2 = 0.993$). Conversely, the least linearity was obtained for octanal ($R^2 = 0.97$).

3.3.2. Accuracy

Recovery tests were performed to study the accuracy of the method. As shown in Table 2, known quantities of the standard solution were added to the orange beverage emulsion and model emulsion at five concentration levels. The slope of the lines obtained for each target volatile compounds was compared with the corresponding slope obtained with standards in the model beverage emulsion. In general, no significant differences ($p > 0.05$) were found between those slopes. As shown in Table 2, the average recoveries of volatile compounds ranged from 88.3% to 121.7%. Therefore, the results demonstrated that the method was applicable for the analysis of headspace volatile compounds of orange beverage emulsion.

3.3.3. Precision

The repeatability was determined to check the precision of method. The repeatability of the experimental procedure was evaluated at five concentration levels by calculating the relative standard deviation (RSD) of three replicates of each concentration level. The RSDs results are summarized in Table 2. As shown in Table 2, the average RSD% for all analytes ranged from 2.94% to 13.30%. The low average RSDs for the target flavor compounds in orange beverage emulsion indicated that the analytical conditions of the

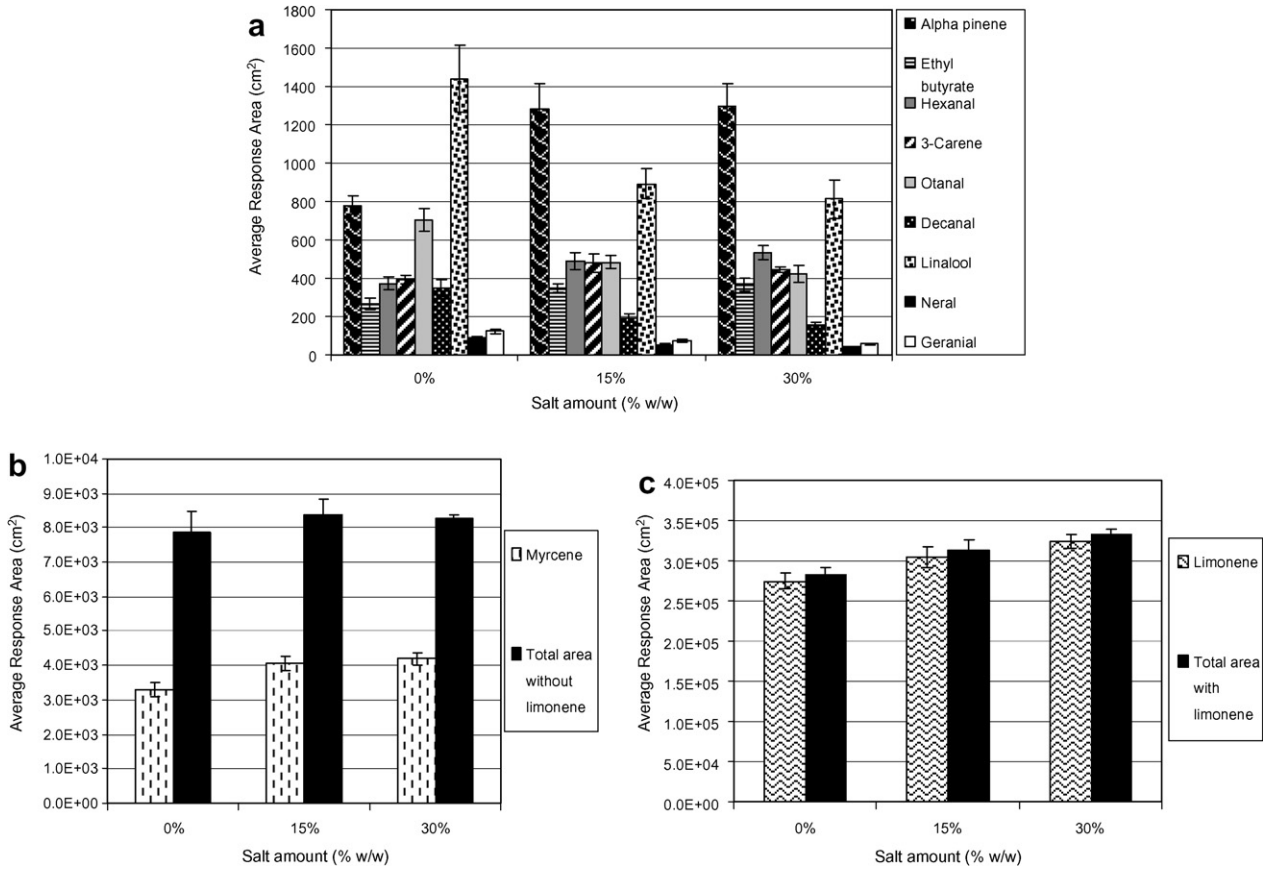

Fig. 5. Influence of salt amount on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene.

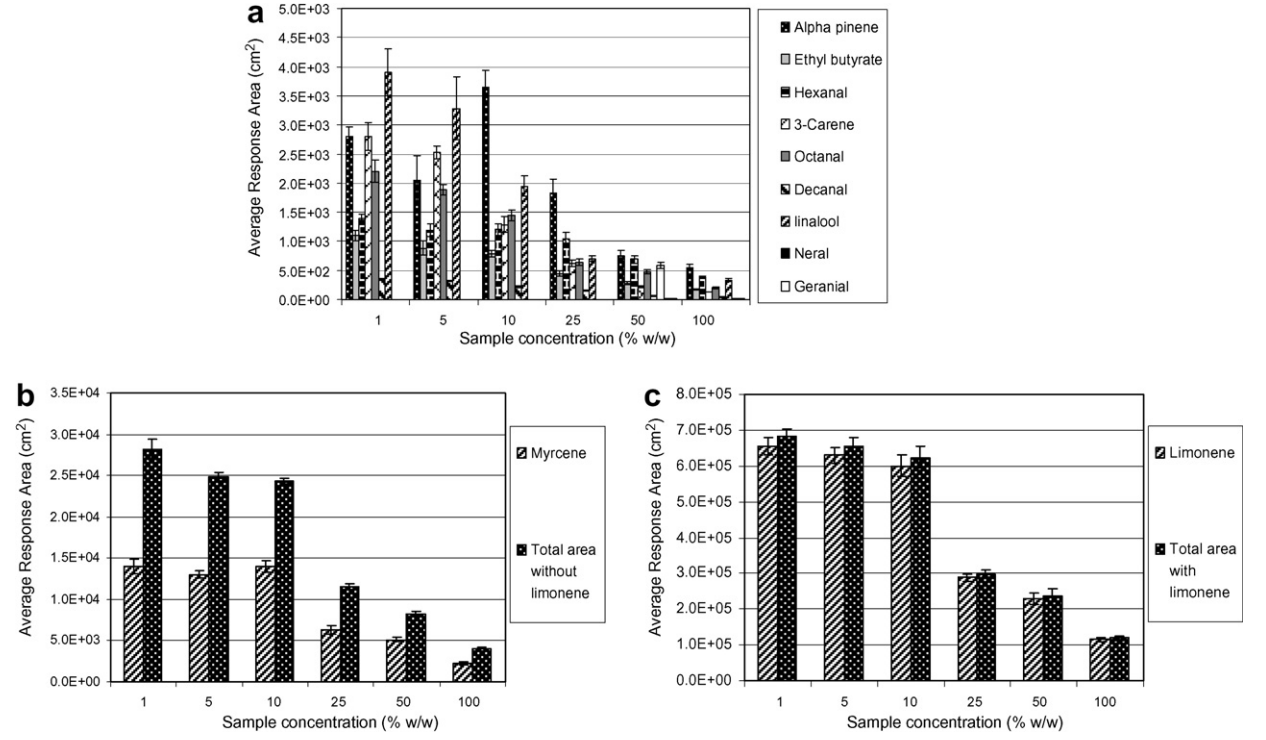

Fig. 6. Influence of sample concentration on extraction recovery of (a) all volatile compounds except myrcene and limonene; (b) myrcene and total flavor compounds without limonene and (c) limonene and total flavor compounds with limonene, made for the same sample amount.

Table 2
The concentration range, regression equations, R^2, recovery, LOD and RSD for the orange flavor compounds

Compound	Concentration range (mg/l)	Regression equation	R^2	Recovery range %	RSD range % (average RSD %)	LOD (mg/l)
α-Pinene	2.04–20.24	$y = 50.9x - 94.3$	0.992	77–113 (101.0)	1.58–16.60 (7.43)	1.17
Ethyl butyrate	1.74–17.27	$y = 36.7x + 105.8$	0.993	60–115 (96.6)	1.00–5.82 (2.94)	0.16
β-Pinene	2.07–20.60	$y = 76.3x - 164.3$	0.991	71–125 (102.7)	1.07–8.80 (4.23)	0.98
Myrcene	3.64–36.50	$y = 80.9x$	0.990	70–105 (89.0)	4.80–20.80 (10.07)	1.16
Limonene	28.25–280.26	$y = 90.6x$	0.974	71–124 (88.3)	2.91–19.60 (9.62)	2.27
γ-Terpinene	2.25–22.29	$y = 90.1x - 161.7$	0.973	68–128 (98.6)	4.88–21.70 (13.30)	2.05
Octanal	1.84–18.30	$y = 78.9x + 484.8$	0.970	62–161(106.8)	2.80–10.80 (7.37)	0.23
Decanal	2.2–21.82	$y = 114.9x$	0.985	94–160 (121.7)	1.80–13.60 (7.38)	0.18
Linalool	2.00–19.62	$y = 132.6x + 359.5$	0.977	63–164 (106.2)	4.60–12.80 (9.60)	0.08
Citral	1.78–17.64	$y = 168.7x + 431.6$	0.972	96–121 (107.7)	3.90–15.20 (9.00)	0.06

Y: The (volatile compound/internal standard) peak area ratio.
X: The (volatile compound/internal standard) concentration ratio.

Table 3
Concentration of volatile flavor compounds in five commercial beverage emulsions

Compound	Concentration ± SD (mg/l, $n = 3$)				
	Emulsion 1	Emulsion 2	Emulsion 3	Emulsion 4	Emulsion 5
α-Pinene	4.06 ± 0.22	3.73[a] ± 0.36	3.83[a] ± 0.34	3.34[a] ± 0.14	4.21 ± 0.31
Ethyl butyrate	0.39[a] ± 0.019	nd	1.98 ± 0.05	1.83 ± 0.17	0.19[a] ± 0.04
β-Pinene	3.62 ± 0.12	3.42 ± 0.22	3.74 ± 0.18	3.62 ± 0.21	3.58 ± 0.21
3-Carene	nd	nd	nd	nd	nd
Myrcene	8.23 ± 0.51	7.51 ± 0.45	7.32 ± 0.43	6.24 ± 0.31	6.34 ± 0.32
Limonene	232.88 ± 43	275.06 ± 39	286.10 ± 54	282.65 ± 28	228.24 ± 41
γ-Terpinene	1.79[a] ± 0.17	1.68[a] ± 0.19	2.44[a] ± 0.28	2.32[a] ± 0.07	1.78[a] ± 0.07
Octanal	3.17 ± 0.26	3.45 ± 0.21	nd	4.64 ± 0.27	nd
Decanal	2.93 ± 0.21	3.43 ± 0.12	4.31 ± 0.25	6.42 ± 0.36	1.14 ± 0.05
Linalool	1.63 ± 0.07	nd	0.65 ± 0.06	5.92 ± 0.24	nd
Citral	0.74 ± 0.05	nd	0.56 ± 0.05	0.89 ± 0.04	nd

nd, non-detected.
[a] Values lower than LOQ.

HS-SPME were found to be acceptable for the analysis of the target volatile compounds in the orange beverage emulsion.

3.3.4. Limit of detection (LOD)

The limit of detection (LOD) was calculated from the calibration curves constructed for each volatile compound. LODs (three times the relative standard deviation of the analytical blank values) was calculated from the calibration curve (Table 2). LOQ is almost 3.33 times LOD; therefore, LOQs ranges are not shown in Table 2. As shown in Table 2, the LOD ranged from 0.06 to 2.27 mg/l for the all volatile compounds. The results confirmed that the limit of detection (LOD) and limit of quantification (LOQ) were low enough to determine the target orange flavor compounds in real orange beverage emulsion.

3.4. HS-SPME analysis for commercial orange beverage emulsions

The optimized HS-SPME method was used to determine the content of the target orange flavor compounds in five commercial orange beverage emulsions. The mean results obtained are shown in Table 3. The ranges (mg/l) of the target orange flavor compounds in commercial orange beverage emulsions studied were: α-pinene (3.34–4.06), ethyl butyrate (not detected–1.98), β-pinene (3.42–3.74), myrcene (6.24–8.23), limonene (228.24–286.10), γ-terpinene (1.68–2.44), octanal (not detected–4.64), decanal (1.14–6.42), linalool (not detected–5.92) and citral (not detected–0.89). For all orange beverage emulsions, the highest responses were observed for limonene and myrcene, respectively (Table 3). The results obtained from the analysis of commercial orange beverage emulsions also confirmed that the quantification of volatile flavor compounds should be carefully carried out depending on the sample matrix.

4. Conclusion

The proposed HS-SPME procedure provides a convenient and powerful tool for the extraction and determination of headspace volatile compounds of orange beverage emulsion. The optimum condition of HS-SPME for extracting the target volatile flavor compounds was obtained using a 75 μm CAR/PDMS fiber. The best results were obtained by the HS-SPME at original pH (pH 4) and 5 g of diluted form (1:100) with 15% NaCl at 45 °C. With

the proposed method, only 15 min was needed to isolate 85% of total flavor compounds using SPME. This study showed that the matrix interference must be considered as the most important factor for headspace analysis of the complex system because the results confirmed that the responses were influenced more significant ($p < 0.05$) by matrix complexity than by the other factors. The linearity of the method was very good in the concentration range from 1.5 mg/l to 20 mg/l for all volatile compounds. The average recovery of all volatile compounds that ranged from 88.3% to 121.7% indicated reasonable accuracy of HS-SPME for headspace analysis of orange beverage emulsion. The average RSDs (less than 14%) obtained for the analytes also confirmed the feasibility of HS-SPME for headspace analysis of volatile flavor compounds of orange beverage emulsion. The LODs and LOQs were low enough for qualitative and quantitative analysis of headspace volatile compounds of orange beverage emulsion diluted greater than 10 times. The HS-SPME method also offered a solventless extraction procedure with much less labor intensity than the conventional extraction techniques. After optimization of the operating conditions, first application of SPME to the analysis of five commercial orange beverage emulsions demonstrated that the proposed analytical method was appropriate and reliable for the determination of volatile flavor compounds a complex system like the orange beverage emulsion. Internal standard calibration accompanied by aqueous standard solutions could be useful for semi quantification analysis in the complex system.

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