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Full Length Research Paper

Identification of compounds characterizing the aroma of oblate-peach fruit during storage by GC-MS

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Three analytical methods, including headspace solid-phase microextraction (HS-SPME), liquid–liquid extraction (LLE) and steam distillation extraction (SDE), were utilized to investigate the aroma profile characteristics of Xinjiang oblate-peach fruit during storage, and the characterizing compounds were detected by gas chromatography mass spectrometry (GC-MS). Silica fiber coated with (DVB-CAR-PDMS) was found to be more efficient for collecting the SPME headspace volatile compounds, and the extraction time of 40 min was preferred in this study. The SPME headspace volatile constituents present in oblate-peach before and after 4 weeks of cold storage (4±1°C) have been analysed, while some physical characteristics such as fruit weight, firmness, soluble solids content (SSC), and titratable acid were monitored during storage. The results show that the volatile compounds displayed different composition during storage, a total of 58 volatiles were identified, 52 prior to storage and 45 after 4 week's storage, the content of lactones and esters, characteristic compounds of peach aroma, were much lower after 4 weeks of storage. Thirteen of the pre-storage volatiles were not found after storage.

Key words: Oblate-peaches, aroma, gas chromatography mass spectrometry (GC–MS), headspace solid-phase microextraction (HS-SPME), liquid–liquid extraction (LLE), steam distillation extraction (SDE).

INTRODUCTION

Flavour, besides other parameters such as texture and appearance, plays a very important role in the quality assessment of fruits and vegetables. As such, this parameter affects the appreciation and the acceptance of horticultural products and, as a consequence, the purchasing behaviour of consumers (Harker et al., 2003; Kühn and Thybo, 2001; Peneau et al., 2006). Flavour is defined as the interaction of individual taste and aroma components with the human sensory system (Vermeir et al., 2009). Oblate-peach *(Prunus persica* L.*)*, an economically important variety plant grown in Xinjiang of China, which is famous for its crisp, juicy texture and sweet flavour, along with important nutrient contributions from its phytochemical constituents is highly favoured by consumers worldwide. However, oblate-peach has a very short shelf-life under normal ambient conditions. Low temperature and modified atmosphere storage is commonly used for peaches to delay fruit ripening, senescence, and textural changes, reduce activity of certain enzymes and other positive benefits, however, they often have little aroma which upon removal from storage, greatly diminish consumer acceptability (Hardenburg et al., 1986; Biale, 1960; Aubert et al., 2003).

Conventional sampling methods for fruit aromas in previous studies are mainly liquid-liquid extraction (LLE) and steam distillation extraction (SDE) (Aubert et al., 2007; Baldry et al., 1972). These conventional methods always require long extraction times, large amounts of solvents and multiple steps. Moreover, many unstable aroma volatiles may be thermally decomposed and degraded during thermal extraction or distillation. However, being simple and straightforward procedures, they were still extensively applied for fragrance-and-aroma characterization, either alone or combined with

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other sample-preparation procedures (Moser et al., 1980). Solid-phase microextracton (SPME) is a simple, solvent-free method for concentration of volatiles present in the headspace. This technique had been used to analyse the volatile compounds of different fruits (Zhang et al., 2007).

In this study, HS-SPME combined with LLE and SDE were used to sample aroma volatiles of oblate-peach fruit emanating quantitatively and qualitatively with 4 weeks storage (4±1°C), followed by GC-MS analysis. The type of SPME fibre coating of various polarities and extraction time were carried out to select the optimum condition for the analysis of the volatile compounds. The objective of our research was to evaluate the effectiveness of three different sampling methods combined with GC–MS, to identify the volatile compounds of oblate-peaches. In addition, fruit quality was evaluated from a consumer perspective. It is hoped that the study would provide a method for investigating the aroma profile characteristics of oblate-peach pulp during storage and some helpful clues for quality discrimination.

MATERIALS AND METHODS

Plant materials

Oblate-peach (*P. persica* L.) CV. 'Ruipan' at commercial harvest maturity (SSC 12.5±1.0% and firmness 11.8±2.5 kg/m^2) was obtained from the orchard at Shihezi, Xinjiang Province, China, and quickly transported to the laboratory on the day of harvest. The fruits were selected for uniform size, maturity and appearance and free from defects and mechanical damage, transported to the laboratory and stored at 8 to 10°C overnight before processing.

Treatments

Oblate-peach were dipped for 2 min in 1.0 g/L sodium hypochlorite solution to control disease and dried at room temperature with air. The fruits were kept in hermetically sealed plastic drums (20 L) of low-density polyethylene (LDPE, 0.02 mm thickness). Three replicates per treatment were used and evaluations were made every 7 days for up to 28 days during cold storage (4±1°C)

HS-SPME procedure and sample preparation

Duplicate measurements of each sample were performed for the optimization of the HS-SPME conditions. The silica fibers and the manual SPME holder were purchased from Supelco (Bellefonte, Inc., PA, USA) Three fibers were tested and compared: polydimethylsiloxane (PDMS, 100 µm), carboxen-polydimethylsiloxane (CAR-PDMS, 65 µm) and divinylbenzene-carboxen-polydimethyl-siloxane (DVB-CAR-PDMS, 50/30 µm). The fibers were conditioned prior use according to the supplier's prescriptions. In this experiment, the different extraction times (20, 30, 40, 50 and 60 min) were evaluated to obtain the optimized sampling efficiency.

For sampling, seeds were removed, and 500 g of peaches were homogenized using a commercial blender, to which was added 10 g NaCl. For each measure, 8 g of puree was transferred into a 15 ml capped solid-phase microextraction vial. Vials were equilibrated during the equilibrium time (depending on the experimental design) in a thermostatic bath at desired temperature (depending on the experimental design). Experimental conditions were set before fiber screening study, as follows: extraction temperature 50°C, equilibrium time 15 min, extraction time 30 min. After sampling, desorption of the analytes from the fiber coating was made in the injection port of GC at 250°C during 3 min in splitless mode. Before sampling, each fiber was reconditioned for 1 min in the GC injector port at 250°C.

LLE and SDE procedure

In the LLE analysis, the extraction procedure was a modification of the method described by Aubert et al. (2005), 200 g fresh peach pulp free of seeds was cut, homogenized, and centrifuged at 800 rpm for 30 min. The filtered supernatant was subjected to continuous liquid-liquid extraction with 3×25 ml of dichloromethane.

In the SDE procedure, as described by Zhang et al. (2007), 200 g fresh peach pulp free of seeds was cut, homogenized, and subjected to hydrodistillation for 120 min. The obtained fraction was extracted using 3×25 ml of dichloromethane. The organic phase was dried over anhydrous sodium sulfate, concentrated to 1 ml using a rotor evaporator (Yarong Instrument, Shanghai, China); 0.5 µL concentrated organic phase from the SDE and SD samples were introduced to the GC-MS for subsequent analysis.

GC–MS analysis

Desorption and analysis of volatile components was carried out on a QP2010 GC-MS (Shimadzu, Tokyo, Japan). Chromatographic separation was performed with a DB-5MS capillary column (30.0 m ×0.25 mm i.d., 0.5 µm film thickness) under the following instrumental conditions. The carrier gas was helium with a flow rate of 1.0 ml/min. The injector temperature was set at 250°C. The GC oven temperature was maintained at 40°C for 2 min after injection, then programmed at 8°C /min to 200°C for 5 min, and then at 10°C /min to 250°C which was maintained for 5 min. The temperature of mass spectrometer was 230°C. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 20 to 600 amu.

Quality evaluation

Fruit firmness was measured on two paired sides of 10 fruits from each replicate (skin removed) with a hand-held firm meter (GY-B, Jilin, P.R. China) with a 10 mm diameter probe at a speed of 1 mm s^{-1}. Data were expressed as kg/cm^2. Soluble solids content (SSC) was determined with a digital refractometer (Atago, Japan) and expressed as a percentage. Percentage of titratable acidity (TA) was determined by titration with 0.01 M NaOH and calculated as citric acid equivalents from 10 g of pulp obtained from 6 fruits. In each treatment, 50 fruits were selected for investigating the rot index of fruits. All fruits were classified in four ranks by the extent of rot: 0, fruits were not rotten; 1, the rotten surface was less than 1/3; 2, the rotten surface was between 1/3 and 2/3; 3, the rotten surface was more than 2/3. The rot index was expressed as the following equation:

$$\text{Rot index} = \sum (\text{Rank} \times \text{Quantity}) / (4 \times 50) \times 100\%.$$

Fruit appearance (visible structural integrity, off-aroma, color and flavor) was evaluated by six experienced panelists. The visual quality score was based on the following scale: 5, excellent; 4, very good; 3, good, limit of marketability; 2, fair, limit of usability; 1, poor, inedible.

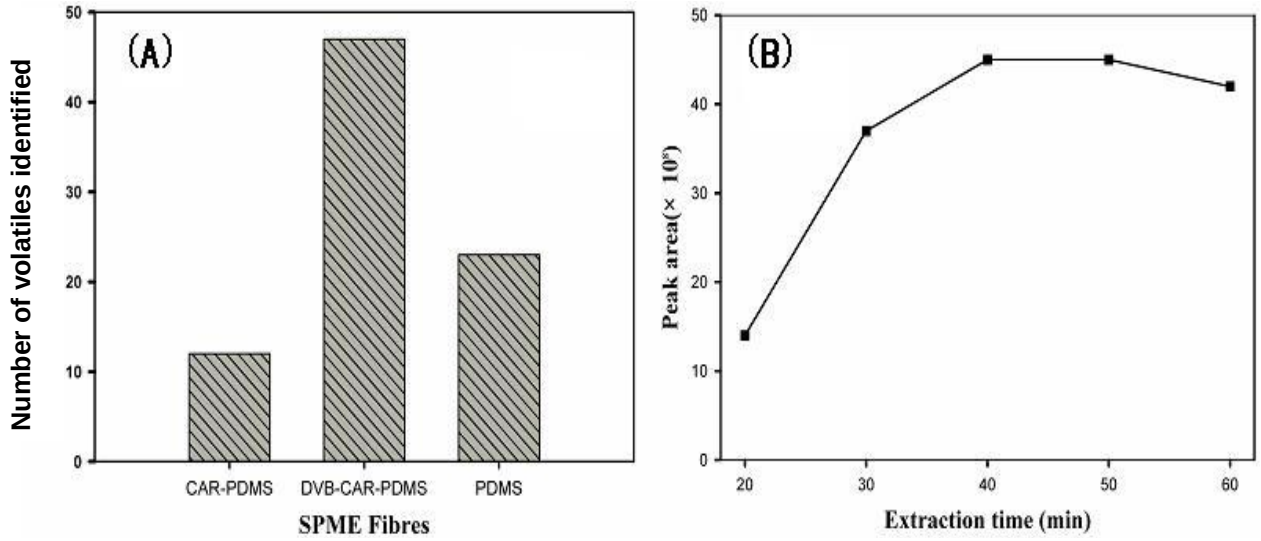

Figure 1. Effect of SPME fibre coatings (A) and extraction time (B) on sampling efficiency.

Statistical analysis

Each experiment was repeated three times and statistical analyses were performed using Microsoft Excel software and SPSS 13.0 software. P-values were determined by t-test. Data are presented as the mean ± standard error of the mean (S.E.M.). Data were treated for multiple comparisons by analysis of variance with least significant difference (L.S.D.) between averages determined at 5% level.

RESULTS AND DISCUSSION

The optimization of the HSSPME conditions

The type of SPME fibre coating used is crucial to sampling efficiency. Some useful and specific factors should be taken into consideration, including polarity, matrix, etc. Figure 1(A) shows desorption capacity of three different fiber coatings for extraction of oblate-peach volatile compounds. The results of the fiber screening confirmed that the DVB-CAR-PDMS fibers produced the best results for the compounds investigated. Of this, fiber had strong extraction capacity for esters, lactones, aldehydes, ketones, and acids. It seemed to be the best fiber for the analysis of volatile compounds in peach.

Extraction time is another important factor to sampling efficiency. In this experiment, the different extraction times (20, 30, 40, 50 and 60 min) were evaluated to obtain the optimized sampling efficiency (Figure 1B). According to Zhang et al. (2007), an increase in sampling extraction time increases the headspace concentration of the volatile compounds, favoring their extraction. However, as the absorption of analytes by the fiber coating is an exothermic process, the partition coefficient decreases by increasing extraction time, negatively affecting the absorption of analytes. The results show that the best conditions to extract the volatile compounds of oblate-peach was extraction time close to 40 min.

Volatile compounds emanating from the peaches by HS-SPME

The aroma volatiles of oblate-peach pulp were identified according to the standard mass spectra of the National Institute of Standards and Technology (NIST) MS spectral library. Aroma volatiles were considered ‘identified’ when their mass spectral fit values were at the default value of 85 or above. When available, some aroma volatiles were further confirmed by comparing their retention times with standards. The variability of the retention times between the aroma volatiles and corresponding standards were within 0.05 min. Figure 2 shows the total ion chromatogram obtained for a pulp sample of oblate-peach before and after 4 weeks of cold storage (4±1°C) with the PDMS-CAR-DVB fibre at the optimal sampling conditions. The volatile compounds behaved differently during storage, a total of 58 volatiles included esters, lactones, alcohols, aldehydes, ketones, acids and hydrocarbons were identified in oblate-peach pulp, 53 prior to storage and 44 after 4 week’s storage (Table 1). Thirteen of the pre-storage volatiles were not found after storage.

A total of 53 volatile compounds emanating from intact peaches just prior to storage were identified: 11 esters, 5 lactones, 17 alcohols, 7 aldehydes, 3 ketones, 1 acid and 7 hydrocarbons (Figure 3; Table 1). Based on the relative proportion of the primary classes, aldehydes (61.91%) comprised the major components, followed by alcohols (11.96%), esters (6.31%), lactone (2.49%), and ketones (0.42%). Peach volatiles previously identified, lactones, in

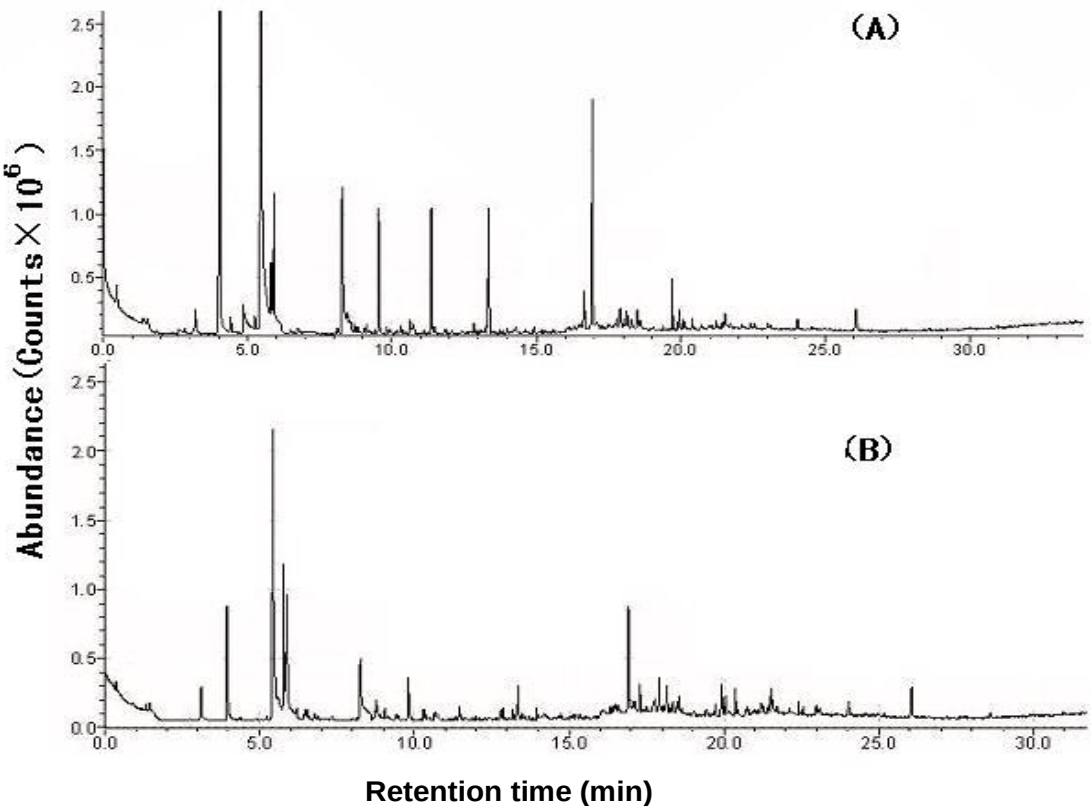

Figure 2. The aroma profile characteristics of oblate-peach pulp at before (A) and after (B) 4 weeks of cold storage (4±1°C) phases by HSSPME.

particular γ-C_8, C_{10}, δ-C10 and some unsaturated lactones have been reported as "character impact" compounds in peach aroma (Do et al., 1969). They act in association with other volatiles, such as C_6 aldehydes, C_6 alcohols and terpenoids, to produce the flavours specific to peach, and lactones contribute the "peachy" background whilst others contribute fruity and floral notes (Horvat and Chapman, 1990a).

In this study, δ-decalactone, which contributed only 0.18% of total volatiles (Table 1), has been identified as the major contributor to the overall aroma of peaches due to its low odor threshold and peach-like odor (Horvat and Chapman, 1990b). Pre-storage volatiles had significantly higher content of δ-decalactone than after storage. After 4 weeks of storage, the composition of volatile compounds varied quantitatively and qualitatively from the pre-storage composition (Table 1; Figure 2). The relative proportion of esters, alcohols, lactones, aldehydes, and ketones was 18.61, 23.48, 1.63, 35.62, and 0.9%, respectively. The relative proportion of alcohols and esters increased; however, the proportion of the esters of other classes decreased during storage. Volatile esters, which are formed by esterification of an alcohol and a carboxylic acid, play an important role in the characteristic aroma of many fruits (e.g., peach, pear, apple, apricots, and grapes); high content in esters should give a pleasant flavour in peaches (Narain et al., 1990; Takeoka et al., 1992; Holland et al., 2005; Greger Schieberle, 2007; Franco et al., 2004). Octanyl acetate, heptyl acetate, and allyl methacrylate, present in the peach prior to storage, were no longer found after 4 weeks in storage. However, 1 ester and 4 alcohol compounds that were initially absent were formed during storage.

Volatile compounds emanating from the peaches by LLE and SDE

Conventional LLE and SDE methods were also used to sample the aroma volatiles from oblate-peach pulp (Figure 3). Table 2 shows the results of LLE and SDE sampling. Twenty-four aroma compounds from peach pulp were isolated by LLE and twenty-nine aroma volatiles were sampled by SDE. Among 5 volatile esters identified in the LLE and SDE procedures, ethyl acetate and ethyl 3-methylbut-2-enoate made up the main aroma profile. HSSPME found more esters than the conventional methods. Generally speaking, HSSPME recovered more volatiles than LLE or SDE methods. It is possible that the degradation of some unstable compounds during the LLE and SDE procedures

Table 1. Volatile compounds of oblate-peaches before and after 4 weeks of cold storage (4±1°C) by HS-SPME.

Volatile compounds	Retention time (min)	Aroma volatiles	Normalized amounts of aroma volatiles (%)	
			Pre-storage	Post-storage
Alcohols	0.474	Methanol	0.45	0.41
	3.101	Pentanol	0.63	0.6
	4.432	Phenethyl alcohol	0.3	ND
	5.774	trans-2-Hexen-1-ol	1.27	13.8
	6.544	Hexanol	ND	0.46
	8. 462	Geraniol	0.83	ND
	8.707	cis-3-Octen-1-ol	0.23	0.25
	9.033	5-Methyl -heptan-1-ol	ND	0.35
	9.114	5-Ethyl-heptan-2-ol	0.19	0.3
	10.631	trans-2-Nonen-1-ol	0.21	ND
	11.379	Linalool	2.93	ND
	12.830	Menthol	0.23	0.39
	13.352	Terpineol	3.01	1.4
	17.284	Tetradecan-4-ol	ND	0.67
	17.770	Nonane-1,9-diol	ND	0.46
	17.906	trans-2-Decene-1-ol	0.62	1.89
	18.279	2-Decanol	0.15	ND
	20.38	Dodecan-4-ol	0.24	0.61
	21.215	Decan-2-ol	0.14	0.37
	21.514	2- Butyl-octan -1-ol	0.38	0.99
	22.422	trans-2-Nonen-1-ol	0.15	0.53
Esters	5.876	Hexyl formate	3.62	10.76
	6.198	Heptyl formate	ND	0.58
	8.807	Vinyl hexanoate	0.16	0.51
	9.819	Octyl propionate	0.15	1.55
	13.191	Salicyateformate	0.11	0.38
	13.292	Linalyl isobutyrate	0.21	0.49
	13.723	Allyl methacrylate	0.07	ND
	19.922	Diethyl phthalate	0.6	1.55
	20.132	Heptyl acetate	0.08	ND
	20.703	Octanyl acetate	0.22	ND
	24.047	Dipropyl phthalate	0.3	0.81
	26.05	Dibutyl phthalate	0.79	1.98
Lactones	10.181	γ-Hexalactone	0.2	0.36
	14.92	γ-Heptalactone	0.15	ND
	18.492	γ-Dodecalactone	0.4	0.25
	18.542	δ-Decalactone	0.55	0.18
	19.707	γ-Undecalactone	1.19	0.84
Aldehydes	4.025	Hexanal	20.68	7.47
	4.873	Furfural	1.14	ND
	5.466	trans-2-Hexen-1-al	38.96	27.29
	8. 138	cis-2-Hepten-1-al	0.15	ND
	8. 575	Benzaldehyde	0.64	ND
	11.464	n-Nonanal	0.13	0.47
	18.337	Glutaraldehyde	0.21	0.39

Table 1. Contd.

Ketones	16.5	α-Ionone	0.11	ND
	18.633	2-Dodecanone	0.15	0.31
	20.071	3-Methylheptan-4-one	0.16	0.59
Acids	8. 271	Benzaldehyde	6.46	4.24
Hydrocarbons	16.642	β-Myrcene	0.92	ND
	18.007	β-Caryophyllene	5.28	0.3
	16.920	Decene	0.17	0.9
	18.143	Trideca ne	0.52	11.79
	21.575	Hexadecane	0.13	0.41
	22.545	1-Bromodecane	0.16	0.36
	22.992	Nonadecane	0.12	0.36
Others	9.547	Maltol	3.05	0.74
	13.991	Benzothiazine	0.1	0.66

[a] ND= not detected.

[b] Normalized amounts of aroma volatiles (%) = $\frac{\text{Peak area of an aroma volatile}}{\text{Total peak area of all aroma volatiles}}$.

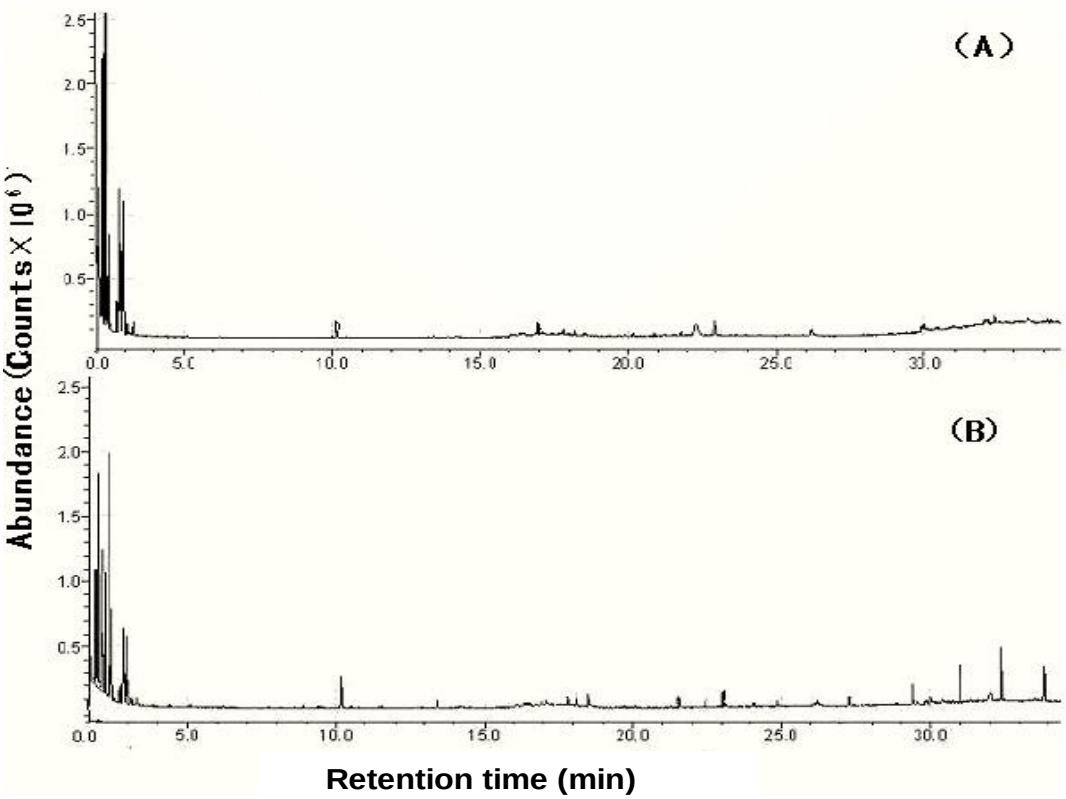

Figure 3. The aroma profile characteristics of oblate-peach pulp by LLE (A) and SDE (B).

produced some artifacts not found by the SPME method. The sample changed greatly during the LLE and SDE processes because of the complex extraction or distillation procedures.

Table 2. Volatile compounds of oblate-peach before and after 4 weeks of storage (4±1°C) by LLE and SDE.

Volatile compounds	Retention time (min)	Aroma volatiles	Normalized amounts of aroma volatiles (%)	
			LLE	SDE
Alcohols	2.864	Salicyl alcohol	9.6	7.54
	4.432	Phenethyl alcohol	ND	3.2
	17.284	Tetradecan-4-ol	ND	0.35
	17.906	trans-2-Decene-1-ol	0.21	0.53
	29.404	Nonadecanol	ND	2.29
	29.902	Oleyl Alcohol	0.25	0.47
Esters	2.363	Ethyl acetate	16.45	16.5
	2.465	Ethyl 3-methylbut-2-enoate	14.3	11.42
	3.111	Isoamyl hexanoate	0.67	0.51
	22.932	p-Methoxyphenyl acetate	0.59	ND
	33.483	Decyl butyrate	0.56	1
Lactones	10.181	γ-Hexalactone	0.85	0.2
	18.542	δ-Decalactone	ND	0.43
	18.092	γ-Octalactone	ND	0.53
Aldehydes	2.244	Isovaleraldehyde	17.75	11.53
Ketones	2.761	Heptane-4-one	1.95	1.43
Acids	26.195	Tetradecanoic acid	0.89	0.8
Hydrocarbons	2.046	Butane	8.58	5.21
	2.139	Pentane	8.89	5.47
	2.91	Hexane	12.57	8.91
	3.296	Heptane	0.82	0.63
	13.415	Nonane	0.18	0.58
	16.456	Undecane	0.34	1.69
	16.525	Dodecane	0.66	0.38
	21.575	Hexadecane	0.19	1.02
	22.375	Octadecane Tridecane	1.7	0.23
	22.992	Nonadecane	0.56	2.12
	29.942	Allylcyclohexane	1.37	ND
	31.028	Docosane	ND	3.47
	32.015	Tricosane	ND	1.87
	32.375	Tetracosane	ND	5.65
	33.817	Heptacosane	0.28	4.04

[a] ND= not detected.

[b] Normalized amounts of aroma volatiles (%) = $\frac{\text{Peak area of an aroma volatile}}{\text{Total peak area of all aroma volatiles}}$.

On the other hand, LLE and SDE were suitable for the sampling of stable compounds, such as hydrocarbons, isovaleraldehyde, and isoamyl hexanoate, which were not found by HS-SPME. Although different sampling methods resulted in a different sampling efficiency for some typical aroma volatiles, based on the normalized amounts, the main aroma volatiles were the same compounds in both HS-SPME and conventional sampling methods, such as phenethyl alcohol, γ-hexalactone, 3-methylheptan-4-one, γ-undecalactone, trans-2-decene-1-ol and so on. Our results support the idea that the methods are complementary for sampling the aroma volatiles of oblate-peach pulp. Similar information was reported on the aroma of the durian pulp (Zhang et al.,

Table 3. Physical characteristics of the oblate-peaches before and after 4 week's storage.

Parameter	Storage time (weeks)				
	0	1	2	3	4
Fruit appearance	5.0±0.3[a]	4.7±0.2[b]	4.3±0.3[c]	4.1±0.2[c]	3.5±0.2[d]
SSC (%)	12.5±1.0[c]	12.8±1.2[b]	11.5±0.8[d]	14.7±0.6[a]	10.3±0.2[e]
Rot index (%)		0[d]	1.8±0.5[c]	14.11±0.6[b]	22. 63±2.4[a]
Weight loss (%)		0.25±0.08[d]	0.37±0.07[c]	0.49±0.11[b]	0.67±0.08[a]
Firmness (kg/m^2)	11.8±2.5[a]	9.2±0.2[b]	7.5±0.5[c]	6.8±3.3[d]	5.5±1.8[e]
Acidity (%)	0.430±0.012[a]	0.391±0.007[b]	0.266±0.007[c]	0.252±0.002[d]	0.232±0.003[e]

Different letter in a row indicate a significant difference ($p < 0.05$). Values = means of standard deviations (n = 3).

2007).

Physical characteristics of the oblate-peaches

Physical characteristics of oblate selection are presented in Table 3. Appearance is a major criterion for determining the acceptability of products. As shown in the table, visual quality scores decreased after 4 weeks to 3.5 (good and limit of marketability) for the sample. Table 3 also shows that slight differences of SSC existed over the storage time. Fruit weight loss is mainly associated with respiration and moisture evaporation through the skin (Sanz et al., 1997).

The thin skin of peach fruits makes them susceptible to rapid water loss, resulting in shrivelling and deterioration. In our study, the fruits demonstrated a gradual loss of weight during storage. Throughout storage, the loss of weight of control fruit was significantly greater than that of the treated fruit. At the end of storage, fruits showed 2.81% loss in weight. It was found that the peach fruits started to rot after 2 weeks storage.

Conclusions

A combination of sampling methods (HS-SPME, LLE and SDE) was developed to study the aroma profiles of oblate-peach pulp during storage, followed by GC–MS detection. Silica fiber coated with (DVB-CAR-PDMS) was found to be more efficient for collecting the SPME headspace volatile compounds, and the extraction time of 40 min was preferred in this study. The SPME headspace volatile constituents present in oblate-peach fruit before and after 4 weeks of cold storage (4±1°C) have been studied, as well as some physical characteristics such as weight, firmness, soluble solids content (SSC), and titratable acidity.

The volatile compounds behaved differently during storage, a total of 58 volatiles were identified, 53 prior to storage and 44 after 4 weeks storage, the content of lactones and esters, characteristic compounds of peach aroma, were much lower after 4 weeks of storage. Potential bio-markers were looked for, based on the aroma profile characteristics by common model strategy. The combination of HS-SPME and the conventional methods provided the most representative aroma information for oblate- peach pulp during storage.

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