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The Study of the Aroma Characteristics of Chinese Mango Cultivars by GC/MS with Solid Phase Microextraction

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Abstract: In this study manual headspace Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) was used to study the aroma characteristics of three kinds of Chinese mango cultivars (xiangya, xiaoxiang and jidan mangoes). In total, twenty-four aroma volatiles, including alkene, alkane, alcohol, aldehyde, ketone and aromatic compound were identified. Aroma characteristics of different mango cultivars were specified by Principal Component Analysis (PCA). Three kinds of mango cultivars possessed the typical but different aroma characteristics. It is hoped that the work could provide helpful clues for the mango quality control and specimen discrimination.

Key words: Aroma characteristics, Chinese mango cultivars, SPME, PCA

Introduction

Mango (*Mangifera indica* L.) is a kind of important tropical fruits, originated from the Southeastern Asia (He, 1999). The aggregate yields of mango in the Asia-Pacific region are the largest in the world. The mango industry in China develops faster than any other countries, so China has been one of the major bases of mango industry in the world (Li, 2005). There are many kinds of mango cultivars popular in China. Most of the cultivars possess the strong and attractive aroma (Huang, 2000). Although most of the studies have been undertaken focusing on the aroma characteristics of mango cultivars from India (John *et al.*, 1999), Africa (Sakho *et al.*, 1985; Sakho *et al.*, 1997), Spain (Ibáñez *et al.*, 1998), Australia (Bartley and Schwede, 1987; Lalel *et al.*, 2003a; Lalel *et al.*, 2003b), United States (Malundo *et al.*, 1997; Beaulieu and Jeanne, 2003) and Brazil (Andrade, 2000), there are still few systematical reports focusing on the aroma characteristics of different Chinese mango cultivars. Fruit aroma could be considered as a kind of terminal metabolites (Feng and Zhao, 2001). Different mango cultivars show the obviously various aroma characteristics. The modern chromatographic methodologies such as gas chromatography- mass spectrometry (GC-MS) could interpret the fruit aroma characteristics effectively via corresponding aroma chromatograms.

Headspace method (Malundo *et al.*, 1997), solvent extraction (Li *et al.*, 1998; Ollé *et al.*, 1998; John *et al.*, 1999) and simultaneous distillation extraction (Andrade, 2000) are used as the common sampling methods for mango aroma. The consequent multiple steps such as clean-up make these classical sampling methods unsuitable for the rapid analysis. Supercritical fluid extraction (SFE) is an advanced sampling method (Tuan and Ilangantileke, 1997; Morales *et al.*, 1998) for the fruit aroma. However, lacking suitable solvents for polar analytes and the high expense for the SFE analysis still

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limit the application. In recent years, solid phase microextraction (SPME), developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Pawliszyn, 1995), has been considered as an excellent pre-sampling method, simple and solvent-saving. SPME has been widely used in the environmental (Peñalve *et al.*, 1999), biological (Mills and Walke, 2000), pharmaceutical (Ulrich, 2000) and the field analyses (Koziel *et al.*, 1999). Especially, headspace solid phase microextraction (HSSPME) has been considered as the suitable sampling method for the fruit aroma volatiles (Ibáñez *et al.*, 1998). Recently, HSSPME has been used to study the aroma volatiles of mangoes (Ibáñez *et al.*, 1998; Lalel *et al.*, 2003a; Beaulieu and Lea, 2003).

In this study HSSPME was used to sample aroma volatiles from three kinds of Chinese mango cultivars (xiangya, xiaoxiang and jidan mango) followed by GC-MS analysis. An original "chromatographic data processing system" based on Matlab 6.5 was programmed to manage the chromatographic data to specify the corresponding aroma characteristics based on the principal component analysis (PCA). It is hoped that the study would provide helpful clues for the mango quality control and specimen discrimination.

Materials and Methods

Sample Collection and Preparation

Fresh ripe mangoes were booked from the settled stand of the local largest wholesale fruit market in Guangzhou. All the samples purchased were selected for uniformity in size and color. Blemished or diseased fruits were discarded. The samples were considered fresh as soon as they picked up from the market and analyzed within 24 h. For each measurement, the fruits of mangoes were randomly distributed into groups of 3 fruit. Before peeling mangoes were washed with tap water followed by rinsing with deionized water to get rid of the dirt on the surface and dried naturally. Then, fifty grams of mango pulp from one group were homogenized with 30 mL of NaCl solution (0.02 g mL^{-1}) using a commercial blender. After that, five grams of fruit tissue homogenate were put in a 15 mL glass vial followed by HSSPME for 1 h. Finally, aroma volatiles were thermally desorbed by inserting the fiber into the GC injector set at 250 °C in splitless mode for 5 min.

GC-MS Analysis

The Hewlett-Packard (HP) 6890 gas chromatography-HP 5973 mass detector system was used in the study. Chromatographic separation was performed with an HP-VOC (Agilent Scientific, USA) capillary column (60 m length×0.32 mm I.D.×1.8 µm film thickness) with the following instrumental conditions: Ultra-purified helium flow 1 mL min⁻¹; injector temperature 250 °C; transfer line temperature 280 °C; energy of electron 70 eV; oven temperature from 65 to 80 °C at ramp rate of 5 °C min⁻¹, 80 °C for 1 min, from 80 to 130 °C at ramp rate of 2 °C min⁻¹, 130 °C for 2 min, from 130 to 160 °C at ramp rate of 3 °C min⁻¹ and from 160 to 240 °C at ramp rate of 6 °C min⁻¹; The parameters of HP 5973 mass detector were: ion mass/charge ratio, 20-550 m/z; scan model.

Chromatographic Data Processing System

In this study, an original "chromatographic data processing system" based on the Matlab 6.5 was coded to manage the chromatographic data. Wavelet transform and polynomial smoothing were applied to smooth the chromatograms in this system. The original data of the aroma chromatograms acquired from the GC-MS were exported and transformed to an "m×2" matrix ("m" represented frequencies of MS data-collecting). The first column in this "m×2" matrix represented the time of MS
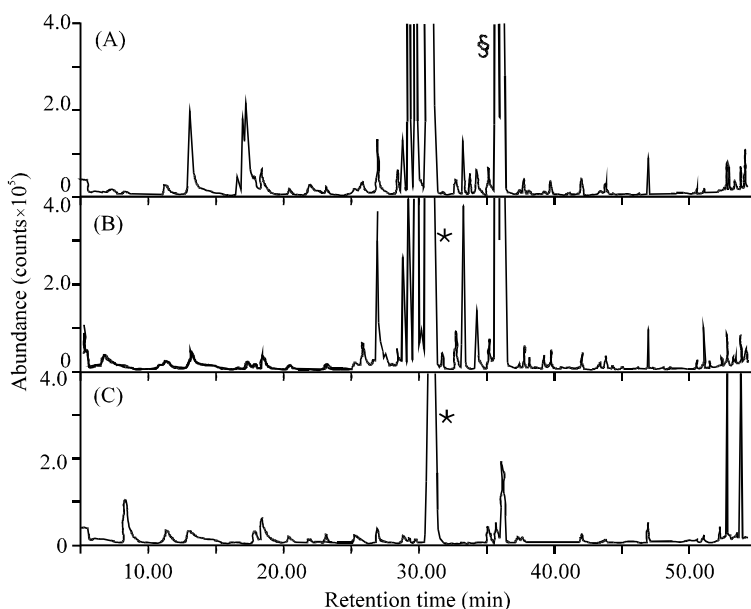
Fig. 1: The aroma chromatograms of xiangya (A), xiaoxiang (B) and jidan (C) mangoes. The marks, '✳' and '§', corresponded to the aroma volatiles with the highest fractions (*, D-Limonene; §, 1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene).

data-collecting, and the second column was on behalf of corresponding detector's responses. After normalization, the data of the total chromatograms of all the investigated samples were merged into an "m×n" matrix ("n" represented the numbers of the aroma chromatograms). Finally, PCA analysis was based on this "m×n" matrix. In brief, the data processing system was an important tool to distill the statistical information from the experimental data in this study.

Results and Discussion

The Optimization of HSSPME

The experimental conditions of HSSPME potentially influencing the extraction process included the type of SPME fiber coating, extraction time and ionic strength. Replicated measurements were performed to improve the sampling efficiency in the study. The type of SPME fiber coating was crucial to the sampling efficiency. Some useful and specific factors should be taken into consideration, such as polarity, matrix, etc. Comparing five common commercial SPME fiber coatings, 100 µm polydimethylsiloxane, 75 µm carboxen-polydimethylsiloxane (CAR-PDMS), 65 µm carbowax-divinylbenzene, 85 µm polyacrylate and 65 µm polydimethylsiloxane- divinylbenzene (Supelco, Inc., PA, USA), 75 µm CAR-PDMS fiber coatings could sample more species and amounts of aroma volatiles. Therefore, the recommended SPME fiber was 75 µm CAR-PDMS in the study. Secondly, extraction time was also highly influential to the sampling efficiency. In this experiment the different extraction times (15, 30, 45, 60 and 90 min) were performed to obtain the optimized sampling efficiency. Due to the complexity of aroma composition, the short sampling time (less than 60 min)

Table 1: Aroma volatiles of three kinds of Chinese mango cultivars

Aroma volatiles	Retention time (min)	Fit[a]	Fraction of aroma volatiles (%)[b] (n = 7)		
			Xiangya mango	Xiaoxiang mango	Jidan mango
Alkene					
α-Pinene	23.13	91	-	<0.01	0.40±0.01
β-Myrcene	26.92	92	0.94±0.02	1.33±0.03	0.67±0.02
cis-2,6-Dimethyl-2,6-octadiene	27.58	91	-	<0.01	-
[+]-2-Carene	28.38	97	0.36±0.01	0.13±0.01	-
α-Phellandrene	28.78	94	0.46±0.03	0.83±0.01	0.33±0.01
3-Carene	29.23	96	4.44±0.09	2.06±0.02	-
1-Methyl-4-(1-methylethylidene)-cyclohexene	29.71	96	-	-	0.18±0.005
(R)-1-Methyl-4-(1-methylethyl)-cyclohexene	30.21	94	<0.01	0.14±0.001	-
D-Limonene	30.84	94	35.42±0.52	40.07±0.62	87.07±1.30
β-Ocimene	31.63	91	-	0.11±0.01	<0.01
2-Carene	32.74	91	0.27±0.01	0.34±0.01	-
1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	33.28	94	0.35±0.02	1.15±0.04	-
4-Methyl-1-(1-methylethenyl)-cyclohexene	34.28	93	0.45±0.01	0.56±0.01	-
1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene	35.74	94	38.59±2.43	36.43±0.76	0.56±0.02
1,3,8-p-Menthatriene	37.79	91	0.17±0.003	0.13±0.003	-
α-Cubebene	51.11	89	<0.01	0.12±0.01	0.24±0.03
Caryophyllene	52.78	98	0.17±0.01	<0.01	3.56±0.12
α-Caryophyllene	53.81	98	0.20±0.01	0.12±0.01	2.72±0.12
Alkane					
7-Oxabicyclo [4.1.0] heptane	16.55	85	0.27±0.01	-	-
Alcohol					
[Z]-3-Hexen-1-ol	16.97	95	0.37±0.03	-	-
Aldehyde					
[E]-2-Hexenal	17.22	97	0.95±0.08	---	---
[E,E]-2,4-Hexadienal	21.95	90	0.74±0.07	---	---
Ketone					
4-Methoxy-2,5-dimethyl-3[2H]-furanone	33.77	85	0.37±0.04	---	---
Aromatic compound					
1-Methyl-4-(1-methylethenyl)-benzene	36.12	96	10.45±0.82	9.45±0.30	---

[a] Fit value was referred to what degree the target spectrum matched the standard spectrum in the NIST library (100 relates to a perfect fit).

[b] Fraction of aroma volatiles (%) = $\dfrac{\text{Area of the peak of an aroma volatile}}{\text{Total area of all aroma volatile}}$ ±SD

The SDs of stable main aroma components (Fraction>0.01%) were calculated in the table

resulted in incomplete absorption; however, the longer sampling time (90 min) aroused competitive absorption and also caused lower sampling efficiency. Finally, the sampling time of 60 min was preferred in the work. Thirdly, ionic strength was believed to affect the extraction in HSSPME as analytes tended to be in the vapor phase. Increasing ionic strength in the solution could reduce the surface tension and make the analytes volatile to vapor phase easily. However, the overmuch ionic strength would make the analytes dissolved in the matrix solution. Compared with other concentrations (0, 0.08, 0.20 and 0.35 g mL^{-1}), 0.02 g mL^{-1} NaCl solution resulted in the best extraction efficiency.

The Detection of the Aroma Volatiles

The aroma volatiles were identified by matching sample mass spectra with those of the National Institute of Standards and Technology MS spectral library. Aroma volatiles were considered "identified", when their fit values of mass spectra were at the default value of 85 or above. The percentage of the area counts of the identified peaks in the aroma chromatogram was more than
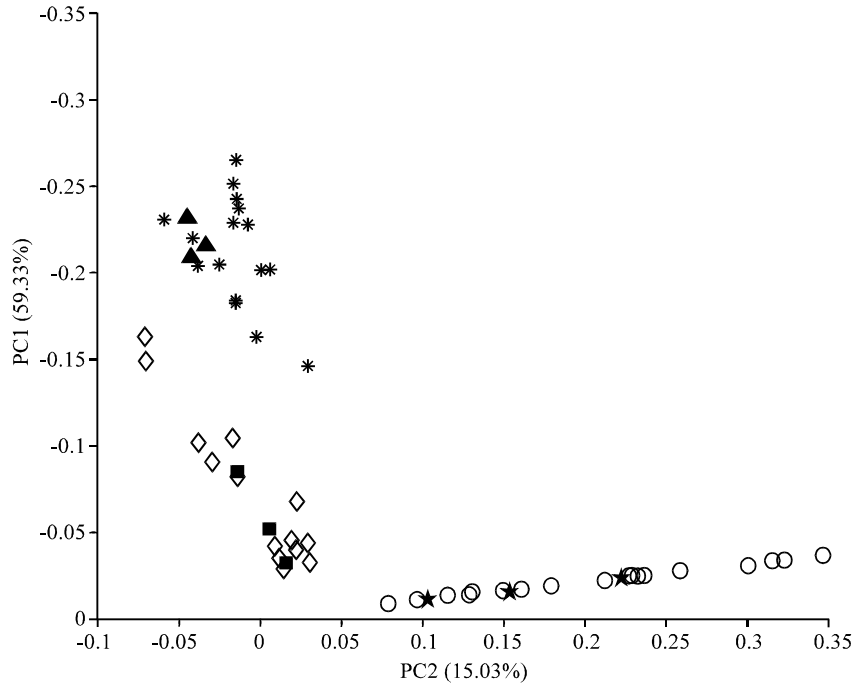
Fig. 2: PCA for the various aroma characteristics of xiangya ('◇'), xiaoxiang ('*') and jidan ('○')
mango cultivars. The marks '■', '▲' and '★' represented the corresponding unknown samples
of xiangya, xiaoxiang and jidan mango cultivars purchased from the local market

92.97% to the total area counts of the peaks in the TIC. Typical aroma chromatograms of three kinds
of Chinese mango cultivars were shown in Fig. 1.

Table 1 showed the 24 "identified" aroma volatiles from three various Chinese mango cultivars
(xiangya, xiaoxiang and jidan mangoes) thermally desorbed from the SPME fiber coating, which could
be divided into six groups such as alkene, alkane, alcohol, aldehyde, ketone and aromatic compound.
Alkenes consisted of the major aroma characteristics of mango. Jidan mango possessed the less
identified aroma volatiles desorbed from the SPME fiber than xiangya and xiaoxiang mangoes. Many
kinds of alkenes identified in the mango aroma composition, such as α-pinene, β-myrcene, carene, α-
phellandrene, caryophyllene, limonene, have been considered as the important terpenes from mango
aroma in the previous reports (Andrade *et al.*, 2000; Lalel *et al.*, 2003a). The major aroma volatiles
of three kinds of mangoes were different. Xiangya mango possessed the highest fraction of
1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene whereas xiaoxiang and jidan mangoes possessed the
highest fractions of D-limonene in the aroma characteristics. Limonene was a natural and functional
monoterpene and possessed a lot of physiological functions, especially its strong anticancer activity
(Wang, 2005). Also, limonene has been identified as one of the major aroma volatile of Brazilian mango
cultivars (Andrade *et al.*, 2000). Besides alkenes, there were several other aroma volatiles in the aroma
composition, such as 7-oxabicyclo[4.1.0]heptane and 4-methoxy-2,5-dimethyl-3[2H]-furanone, which
have not been identified before.

When the fractions of aroma volatiles were more than 0.01%, they possessed the stable present
frequencies. Therefore, the reproducibility of aroma characteristics could be evaluated from the
standard deviation (SD, n = 7). From the SDs listed in Table 1, the corresponding relative standard

deviations (RSDs) could be calculated with the satisfying range from 0.7% ((R)-1-methyl-4-(1-methylethyl)-cyclohexene of xiaoxiang mango) to 12.5% (α-cubebene of jidan mango). The fluctuation of the retention time of all the identified peaks was within 0.05 min.

PCA for the Various Aroma Characteristics of Three Mango Cultivars

Table 1 suggested that the aroma compositions of three kinds of mango cultivars were various. To statistically specify the difference of the aroma characteristics, a PCA model was established to study the chromatographic data of xiangya, xiaoxiang and jidan mangoes. In the PCA model the different clustering principles of three mango cultivars emerged, which suggested that the aroma characteristics of xiangya, xiaoxiang and jidan mangoes were obviously various. Due to the completeness of chromatographic data inducing to the PCA, the clustering variety could be considered caused by the variety of entire aroma characteristics but not individual aroma volatiles. Therefore, the aroma characteristics of different mango cultivars could be effectively specified by PCA. Figure 2 showed the PCA clustering rules of aroma characteristics of xiangya, xiaoxiang and jidan mangoes. After the PCA model was established, the fresh ripe mangoes were purchased from the local market and analyzed as the above procedure. The chromatographic data of three kinds of Chinese mango cultivars could fall into the corresponding PCA segregations. The preliminary results assumed that various aroma characteristics might reflect the diversity of xiangya, xiaoxiang and jidan mangoes and could be used as the potential markers for the mango quality control and specimen discrimination.

Conclusions

An HSSPME method was developed to study various aroma characteristics of three kinds of Chinese mango cultivars (xiangya, xiaoxiang and jidan mangoes) followed by GC-MS detection. In total, twenty-four aroma volatiles were identified. The aroma characteristics of xiangya, xiaoxiang and jidan mangoes were specified by PCA. Three kinds of Chinese mango cultivars possessed the different aroma characteristics. It is hoped that the work could provide helpful clues for the mango quality control and specimen discrimination. The next phase work would focus on quantifying the important aroma volatiles and further revealing the potential biomarkers of Chinese mango cultivars for the specimen discrimination.

Acknowledgements

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