



Journal of
Plant Sciences

ISSN 1816-4951



Academic
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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 \times 0.32 mm I.D. \times 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 “ $m \times 2$ ” matrix (“m” represented frequencies of MS data-collecting). The first column in this “ $m \times 2$ ” matrix represented the time of MS

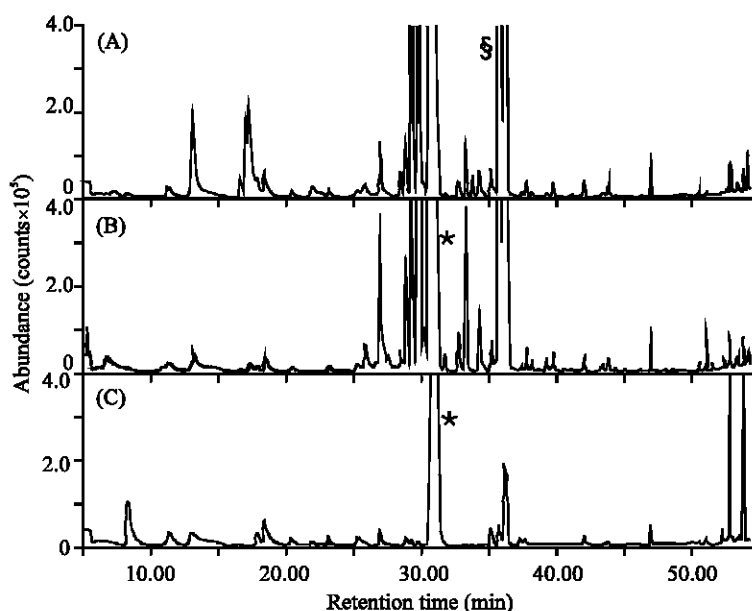


Fig. 1: The aroma chromatograms of xiangya (A), xiaoxiang (B) and jidan (C) mangoes. The marks, ‘*’ and ‘§’, corresponded to the aroma volatiles with the highest fractions (*, D-Limonene; §, 1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene).

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
<i>cis</i> -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	---

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

$$^b\text{Fraction of aroma volatiles (\%)} = \frac{\text{Area of the peak of an aroma volatile}}{\text{Total area of all aroma volatile}} \pm \text{SD}$$

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

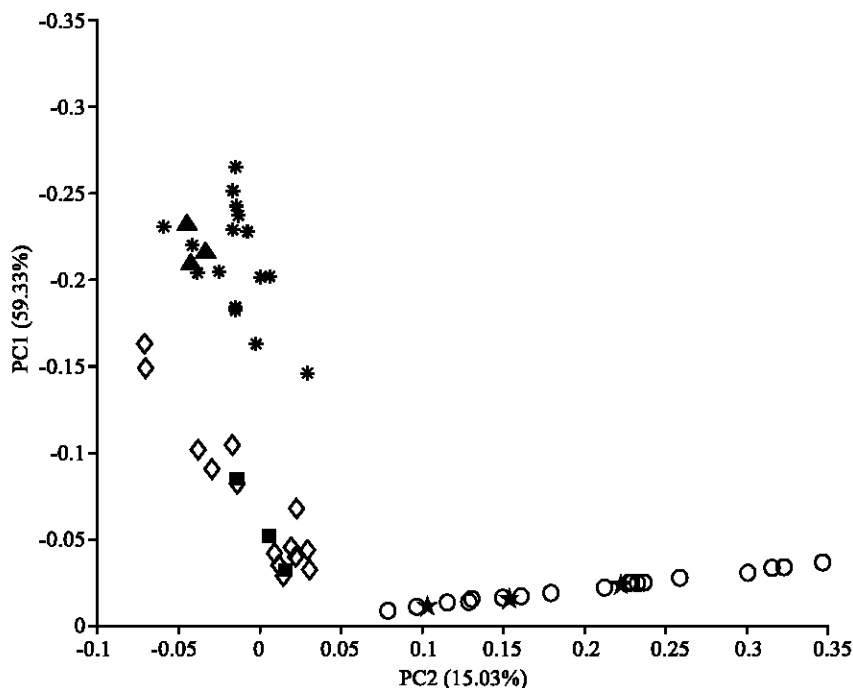


Fig. 2: PCA for the various aroma characteristics of xiangya (‘◇’), xiaoxiang (‘*’) and jidan (‘○’) mango cultivars. The marks ‘■’, ‘▲’ and ‘★’ represented the corresponding unknown samples of xiangya, xiaoxiang and jidan mango cultivars purchased from the local market

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

The authors would like to thank the National Natural Science Foundation of China for financially supporting the research under contact No. 20575081. Special thanks to Mr. Gui-Hua Ruan for his useful advices on the chemometric study.

References

- Andrade, E.H.A., J.G.S. Maia and M. Das G.B. Zoghbi, 2000. Aroma volatile constituents of Brazilian varieties of mango fruit. *J. Food Compos. Anal.*, 13: 27-33.
- Arthur, C.L. and J. Pawliszyn, 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.*, 62: 2145-2148.

- Bartley, J.P. and A. Schwede, 1987. Volatile flavour components in the headspace of the Australian or "Bowen" mango. *J. Food Sci.*, 52: 353-355.
- Beaulieu, J.C. and J.M. Lea, 2003. Volatile and quality changes in fresh-cut mangos prepared from firm-ripe and soft-ripe fruit, stored in clamshell containers and passive MAP. *Postharvest Biol. Technol.*, 30: 15-28.
- Feng, S.Q. and Y.M. Zhao, (Eds.), 2001. The Change of Postharvest Physiology of Fruits and Vegetables. In *The Keeping-fresh Techniques and Routine Testing Methods for Fruits and Vegetables*, Chemical Industry Press, Beijing, China. pp: 1-15.
- He, P., 1999. The advances on the study of mango breeding. *Trop. Agric. Sci.*, 2: 60-63.
- Huang, G.D., 2000. The actual state and developing trend of mango breeding in China. *China Fruits*, 3: 47-49.
- Ibáñez, E., S. López-Sebastián, E. Ramos, J. Tabera and G. Reglero, 1998. Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem.*, 63: 281-286.
- John, K.S., L.J.M. Rao, S.G. Bhat and U.J.S.P. Rao, 1999. Characterization of aroma components of sap from different Indian mango varieties. *Phytochemistry*, 52: 891-894.
- Koziel, J., M.Y. Jia, A. Khaled, J. Noah and J. Pawliszyn, 1999. Field air analysis with SPME device. *Anal. Chim. Acta*, 400: 153-162.
- Lalel, H.J.D., Z. Singh and S.C. Tan, 2003a. Aroma volatiles production during fruit ripening of 'Kensington Pride' mango. *Postharvest Biol. Technol.*, 27: 323-336.
- Lalel, H.J.D., Z. Singh and S.C. Tan, 2003b. Glycosidically-bound aroma volatile compounds in the skin and pulp of 'Kensington Pride' mango fruit at different stages of maturity. *Postharvest Biol. Technol.*, 29: 205-218.
- Li, Q.C., L. Qiu and J. Xiong, 1998. Analysis of aromatic components in mangos from Nujiang Basin of Yunnan Province. *Chinese J. Chromatogr.*, 16: 268-270.
- Li, Y., 2005. The prediction for the yield and requirement of the major tropical fruits in the world. *World Trop. Agric. Inform.*, 8: 4-6.
- Malundo, T.M.M., E.A. Baldwin, M.G. Moshonas, R.A. Baker and R.L. Shewfelt, 1997. Method for the rapid headspace analysis of mango (*Mangifera indica* L.) homogenate volatile constituents and factors affecting quantitative results. *J. Agric. Food Chem.*, 45: 2187-2194.
- Mills, G.A. and V. Walke, 2000. Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J. Chromatogr. A.*, 902: 267-287.
- Morales, M.T., A.J. Berry, P.S. McIntyre and R. Aparicio, 1998. Tentative analysis of virgin olive oil aroma by supercritical fluid extraction-high-resolution gas chromatography-mass spectrometry. *J. Chromatogr. A.*, 819: 267-275.
- Ollé, D., R.L. Baumes, C.L. Bayonove, Y.F. Lozano, C. Sznaper and J.M. Brillouet, 1998. Comparison of free and glycosidically linked volatile components from polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *J. Agric. Food Chem.*, 46: 1094-1100.
- Pawliszyn, J., 1995. New directions in sample preparation for analysis of organic compounds. *Trends Anal. Chem.*, 14: 113-122.
- Peñalve, A., E. Pocurull, F. Borrull and R.M. Marcé, 1999. Trends in solid-phase microextraction for determining organic pollutants in environmental. *Trends Anal. Chem.*, 18: 557-568.
- Sakho, M., J. Crouzet and S. Seck, 1985. Volatile components of African mango. *J. Food Sci.*, 50: 548-550.

- Sakho, M., D. Chassagne and J. Crouzet, 1997. African mango glycosidically bound volatile compounds. *J. Agric. Food Chem.*, 45: 883-888.
- Tuan, D.Q. and S.G. Ilangantileke, 1997. Liquid CO₂ extraction of essential oil from star anise fruit (*Illicium verum* H.). *J. Food Eng.*, 31: 47-57.
- Ulrich, S., 2000. Solid-phase microextraction in biomedical analysis. *J. Chromatogr. A.*, 902: 167-194.
- Wang, W.J., 2005. Recent advances on limonene, a natural and active monoterpene. *China Food Additives*, 1: 33-37.