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Short communication

Rapid determination of volatile constituents of *Michelia alba* flowers by gas chromatography–mass spectrometry with solid-phase microextraction

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Abstract

The volatile constituents of *Michelia alba* flowers, including fresh flowers, frozen flowers and withered flowers, were investigated by GC–MS. The volatiles in a simulated natural environment were sampled by solid-phase microextraction (SPME), with a 100 μm polydimethylsiloxane fiber at $25 \pm 5^\circ\text{C}$ for 4 h. The fibers were desorbed in a GC injection liner at 250°C for 3 min. With headspace SPME–GC–MS analysis, 61 peaks were separated. The main compounds in headspace of fresh *Michelia alba* flowers included α -myrcene, (*S*)-limonene, (*R*)-fenchone, linalool, camphor, caryophyllene, germacrene D, etc., a greater number of compounds than for frozen flowers and withered flowers. At the same time, the biomarkers of fresh flowers were compared with the frozen flowers and withered flowers. In this study, headspace SPME–GC–MS afforded a simple and more sensitive sampling method for fresh *Michelia alba* flowers and other fresh flowers. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Michelia alba*; Volatile organic compounds

1. Introduction

Solid-phase microextraction (SPME) was introduced in 1990 by Arthur and Pawlitzyn [1]. The technique is proving increasingly useful in organic analytical chemistry because it is a rapid and simple procedure of extraction with a great capacity of concentration without the need for any organic solvent. Recently, there has been an increasing use of SPME for the extraction of organic compounds from several matrices: the volatile compounds of tomato juice [2], the sulfur compounds in beer [3], the

freshness of fish [4], the hydrocarbons in blood [5], the free volatile fatty acids in waste waters [6] the pesticide residues in fruits and fruit juices [7], water samples [8,9] or serum [10]. The fiber adsorbed organic compounds were usually transferred to the gas chromatograph or gas chromatograph–mass spectrometer to desorb at high temperatures. Alpendurada reviewed this technology [11].

Many flowers' volatiles are pleasant to the human sensory system and have potential application as components of perfumes. Thus, there is a never-ending need to characterize and synthesize new aroma compounds. Most studies have been undertaken with the aim of identifying the substances responsible for characteristic aromas and flavors

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[12]. However fewer papers used SPME to characterize the fragrance of fresh flowers.

The flowers of *Michelia alba* can be used to cure bronchitis, prostatitis, leucorrhea et al. The flowers' oil has a good essence. To the best of our knowledge, this is the first paper to deal with the volatile compounds of *Michelia alba* by headspace SPME–gas chromatography–mass spectrometry (GC–MS). Through headspace SPME of the aroma compounds of flowers in a simulated natural environment, the low concentration of analytes can be concentrated effectively, and the problems related to the use of large amounts of organic solvent and the environmental contamination could be avoided. We wished to identify and quantify the main volatile constituents of flowers of *Michelia alba* rapidly. At the same time, we analyzed the changes of characteristic aroma and biomarkers of fresh flowers, frozen flowers and withered flowers.

This work developed a method as simple as possible, involving a simulated natural environment, for analyzing the volatile compounds of fresh flowers based on headspace (HS) SPME–GC–MS.

2. Experimental

2.1. Materials

Fresh and withered *Michelia alba* flowers came from the campus of Fudan University directly. Some fresh flowers were frozen at -20°C for 10 days.

Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

A Finnigan Voyager GC–MS system was used, with a mass-selective detector with electron impact ionization. Analytes were separated using a HP-5MS capillary column of $30\text{ m}\times 0.25\text{ mm}$ with a phase thickness of $0.25\text{ }\mu\text{m}$ from Supelco, which was inserted directly into the ion source of the MS system. The temperature program used for analysis was as follows: the initial temperature was 50°C for 2 min, which was increased to 250°C at $5^{\circ}\text{C}/\text{min}$, the temperature of 250°C was maintained for 5 min. Helium (99.999%) was the carrier gas maintained at

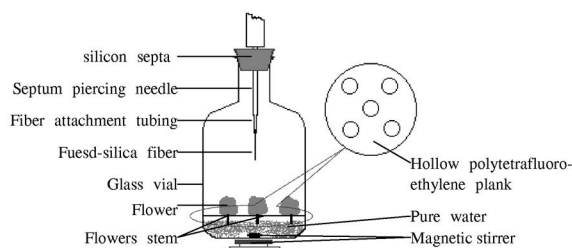


Fig. 1. Scheme of simulated natural environment.

a flow-rate of $1\text{ ml}/\text{min}$. The split flow ratio was 15:1.

The electron impact ionization conditions were: ion energy 70 eV and the mass range scanned was $40\text{--}450\text{ u}$ in the full-scan acquisition mode. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA).

The extraction bottle (500 ml) was made in our laboratory to create a simulated natural environment (SNE, Fig. 1).

2.3. Headspace solid-phase microextraction

An SPME holder (Supelco, Bellefonte, PA, USA) was used to perform the experiments. A fused-silica fibre, coated with a $100\text{ }\mu\text{m}$ layer of polydimethylsiloxane (PDMS), was chosen to absorb the volatile components of the flows.

For the HS-SPME process, some pure water was placed in the SNE bottle. The flowers were placed on the hollow polytetrafluoroethylene plank and the flowers' stems submerged in water. Firstly the fiber, desorbed at 250°C for 10 min in the GC injector, was exposed in the headspace of the stirred water at for $25\pm 5^{\circ}\text{C}$ for 4 h and then it was removed from the flowers headspace and introduced into the GC injector where the thermal desorption of the analytes at 250°C for 3 min was carried out.

3. Results and discussion

There are two balances of volatiles: headspace/flowers and fiber/headspace. Stirring of the water could increase the flow of headspace air. It would shorten the balance time, especially the headspace/

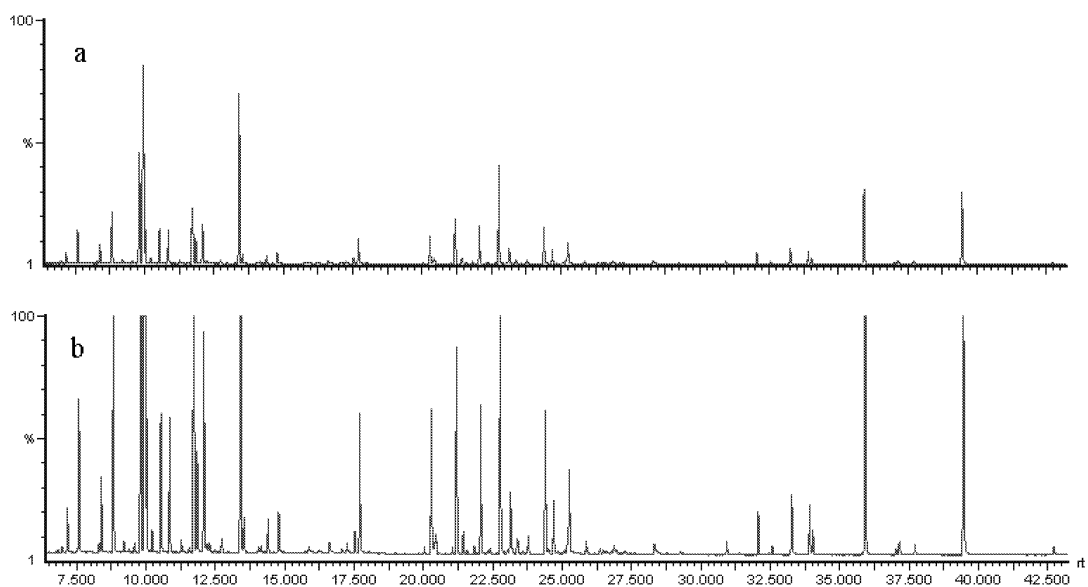


Fig. 2. Absorption effect of stirring or not with SNE-HS-SPME–GC–MS. Absorption at $25\pm 5^\circ\text{C}$ for 4 h under stirring; (a) without magnetic stirring, (b) with magnetic stirring. rt=Retention time in min.

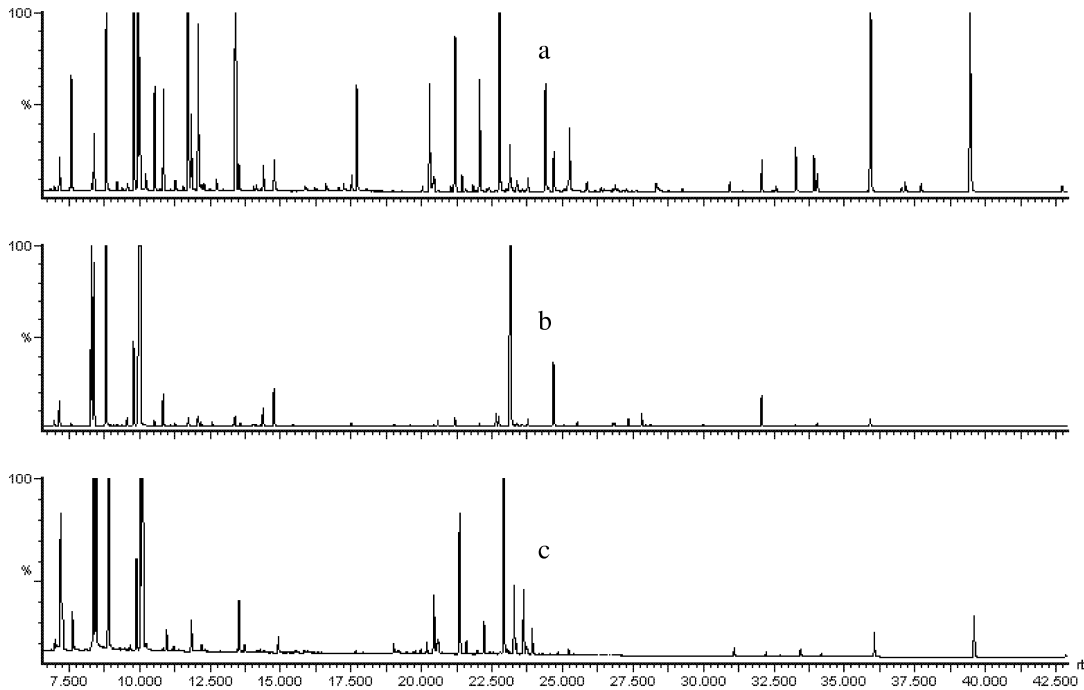


Fig. 3. Total ion current of headspace volatile compounds of *Michelia alba* flowers with SNE-HS-SPME–GC–MS. Absorption at $25\pm 5^\circ\text{C}$ for 4 h under stirring; (a) fresh flowers, (b) frozen flowers, (c) withered flowers. rt=Retention time in min.

Table 1
SNE-HS-SPME–GC–MS identification of the main *Michelia alba* volatiles

Compound	M_r	Linear retention index		Electron impact mass spectrometric data	Relative content (%)
		Sample	Reference		
α -Pinene	136	941	942	93(100), 91(60), 92(45), 77(41), 121(18), 136(14)	0.611
Camphene	136	955	954	93(100), 121(73), 91(51), 79(45), 136(17)	2.128
Sabinene	136	972	976	93(100), 91(53), 77(37), 79(37), 136(16)	0.121
β -Pinene	136	979	981	93(100), 41(55), 91(42), 69(36), 136(11)	1.005
α -Myrcene	136	986	986	93(100), 69(88), 41(78), 91(35), 136(7)	4.891
α -Phellandrene	136	1000	1002	93(100), 91(79), 77(45), 119(23), 41(18), 136(24)	0.161
3-Carene	136	1008	1009	93(100), 91(68), 77(46), 41(27), 136(19)	0.093
α -Terpinene	136	1014	1016	121(100), 93(86), 91(80), 77(46), 136(53)	0.137
<i>p</i> -Cymene	134	1019	1017	119(100), 91(63), 117(38), 77(27), 134(65)	7.313
(<i>S</i>)-Limonene	136	1022	1025	68(100), 67(92), 93(88), 79(81), 53(66), 121(53), 136(61)	15.658
Eucalyptole	154	1025	/	43(100), 81(50), 71(40), 108(38), 139(30), 154(28)	2.118
(<i>Z</i>)- α -Ocimene	136	1029	1027	93(100), 91(62), 79(44), 41(32), 136(4)	0.296
β -Phellandrene	136	1035	1032	93(100), 91(61), 77(52), 41(46), 121(22), 136(9)	1.799
γ -Terpinene	136	1053	1053	93(100), 91(71), 77(46), 119(43), 41(21), 136(40)	1.872
Dihydromyrcenol	156	1058	1056	59(100), 43(40), 55(34), 58(30), 111(26)	0.201
Acetic acid 5-hexenyl ester	142	1070	/	43(100), 67(36), 82(24)	0.112
(<i>R</i>)-Fenchone	152	1078	1077	81(100), 69(72), 41(47), 152(20)	5.355
2-Nonanone	142	1090	1093	43(100), 58(89), 57(23), 71(21), 142(6)	1.379
Linalool	154	1094	1092	93(100), 41(66), 91(44), 69(41), 121(34), 136(17)	3.183
(<i>Z</i>)-Rose oxide	154	1099	1100	139(100), 69(72), 55(41), 41(38), 154(19)	0.107
Limonene-1,2-epoxide	152	1116	1119	81(100), 67(83), 55(76), 109(70), 152(52)	0.249
Camphor	152	1124	1126	95(100), 81(82), 108(80), 55(76), 152(45)	11.077
<i>p</i> -Menth-8-en-2-ol	154	1126	1124	93(100), 121(70), 43(53), 79(45)	0.492
Linalyl acetate	196	1234	1240	(93), 43(76), 69(70), 121(42)	0.480
Isolinyl acetate	196	1238	/	93(100), 91(63), 121(62), 68(62)	0.647
(<i>Z</i>)-Geraniol	154	1245	1243	41(100), 93(93), 69(91), 136(28)	0.111
Isogeranyl formate	182	1266	/	93(100), 41(94), 69(83), 91(64), 121(26), 136(19)	0.189
Cyclooctanol acetate	170	1279	1280	43(100), 54(35), 81(22), 110(18)	0.114
Isobornyl acetate	196	1282	1279	43(100), 95(93), 93(66), 121(51)	0.325
2-Undecanone	170	1288	1290	43(100), 58(94), 71(31), 170(5)	2.065
α -Cubebene	204	1360	1362	119(100), 105(96), 161(86)	0.099
1,2,3a,3b,4,5,6,6a,6b-Decahydro-1 λ -isopropyl-3a λ -methyl-6-methylene-cyclobuta[1.2:3.4]dicyclopentene	204	1374	/	81(100), 80(68), 123(53), 161(31)	2.045
γ -Elememne	204	1397	1400	93(100), 81(95), 67(94), 41(76), 107(68), 147(60), 161(56)	0.339
(<i>Z</i>)- β -Farnesene	204	1428	1426	119(100), 93(99), 41(63), 91(52)	0.106
Caryophyllene	204	1431	1428	91(100), 41(92), 79(79), 133(72), 105(70), 161(64)	3.626
Copaene	204	1445	1445	161(100), 91(51), 105(49), 119(38), 133(26)	0.324
Cedrene	204	1449	1446	161(100), 91(97), 105(82), 79(80), 119(68), 133(44)	0.144
α -Muurolene	204	1470	1475	93(100), 41(63), 69(62), 91(48)	2.191
Germacrene D	204	1484	1482	161(100), 105(61), 91(56), 119(38), 79(34), 204(21)	5.325
<i>n</i> -Pentadecane*	212	1500	1500		0.967
(<i>E,E</i>)- α -Farnesene	204	1505	1501	93(100), 41(86), 69(67), 91(55), 107(53), 119(48)	0.098
1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-(<i>S</i>)-cyclohexene	204	1509	/	69(100), 41(85), 93(83), 119(28), 161(24), 204(21)	0.288
Cadinene	204	1523	1518	161(100), 119(73), 105(69), 134(61), 91(56), 204(51)	0.247
Eudesma-4(14),11-diene	204	1580	/	93(100), 67(75), 81(70), 107(56), 121(55), 161(42), 189(28)	2.260
2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept	204	1598	/	69(100), 41(93), 93(93), 119(51), 79(48), 133(28), 161(20)	0.820
(<i>Z</i>)- α -Santalol	220	1658	1660	91(100), 79(85), 41(90), 105(65), 159(32), 187(28)	1.369

Table 1 Continued

Compound	M_r	Linear retention index		Electron impact mass spectrometric data	Relative content (%)
		Sample	Reference		
Caryophyllene oxide	220	1687	/	91(100), 67(94), 41(92), 109(70), 121(42), 138(39)	0.168
Aristolene	204	1713	/	91(100), 189(93), 105(76), 133(68), 79(64), 161(62), 204(52)	0.098
β -Patchoulene	204	1744	/	161(100), 105(96), 91(95), 81(78), 119(52), 204(48), 189(38)	0.100
(<i>E,E,E</i>)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	272	1781	/	69(100), 41(85), 93(77), 119(40), 107(32), 161(15), 204(9)	0.338
6,10,14-Trimethyl-2-pentadecanone	268	1855	/	43(100), 58(50), 71(33), 55(33), 85(27), 95(26), 109(22)	0.201
<i>n</i> -Nonadecane*	268	1900	1900		0.639
Palmitic acid methyl ester	270	1925	/	74(100), 87(64), 43(62), 143(12), 227(10), 270(9)	0.137
Palmitic acid, ethyl ester	284	1993	/	88(100), 101(55), 43(52), 55(40), 157(18), 284(9)	0.776
<i>n</i> -Eicosane*	282	2000	2000		0.338
<i>n</i> -Heneicosane*	296	2100	2100		5.382
Linoleic acid ethyl ester	308	2151	/	67(100), 81(80), 55(77), 95(52), 109(24), 263(12), 308(18)	0.094
Linolenic acid methyl ester	292	2168	/	79(100), 67(78), 55(66), 95(60), 108(32)	0.224
<i>n</i> -Docosane*	310	2200	2200		0.177
<i>n</i> -Tricosane*	324	2300	2300		4.934
<i>n</i> -Pentacosane*	352	2500	2500		0.150

Absorption at $25 \pm 5^\circ\text{C}$ for 4 h under stirring. Relative content was calculate from area ratio.

*Biomarker.

fiber balance of volatiles. Thus stirring was applied to increase the efficiency of extracting free volatiles. Fig. 2 shows the effect of stirring.

Fig. 3 shows the total ion GC–MS patterns of the HS-SPME extracts of fresh flowers, frozen flowers and withered flowers. This figure shows that the main volatiles of fresh flowers were existent in withered flowers. But the concentration of mono- or sesquiterpene hydrocarbons were much lower. The oxidates, such as alkene alcohol, alkene ketone, were present in traces or not at all. At the same time, the levels of *n*-alkanes were low. There were merely several main volatiles of fresh flowers to be separated from frozen flowers. Not only the most oxidates but also the mono- or sesquiterpene hydrocarbons and *n*-alkanes were present at trace levels or absent.

By this method, 61 volatile compounds were separated from fresh flowers by using the NIST Mass Spectral Search Program. Moreover, the linear retention indices were calculated against *n*-alkane standards (C_9 – C_{18} , Alltech Associates) as reference. The identity of the components was confirmed by comparing the retention indices with standard values of authentic samples (Table 1) [13–15]. The relative

content was calculated from the area ratio. The total area of 61 identified volatile compounds was more than 99.98% of the total chromatographic area. The main compounds of fresh flowers included α -myrcene, (*S*)-limonene, eucalyptole, (*R*)-fenchone, linalool, camphor, α -muurolene, germacrene D and their isomers. In addition, there are many kinds of low concentration terpenes and esters in the main volatile compounds of fresh flowers. These made the fragrance of *Michelia alba* pleasant and suave, and could be used to prepare a lot of flower essence.

The reports in the literature on the high concentration of *n*-alkanes in volatile compounds of fresh flowers are few. To the best of our knowledge, only one paper had noticed the *n*-alkanes during analysis of the volatiles of *Gardenia* flower by SPME–GC–MS [16], but they were considered the effect of pollution. Through headspace SPME–GC–MS in a simulated natural environment, the *n*-alkanes content had the relation: fresh flowers \gg withered flowers \gg frozen flowers ≈ 0 . The Carbon Preference Index was higher than 10, which is close to the work of Pan [17]. The vascular plant wax component (Wax Cn) was indented. These results explained that the *n*-alkanes, as the biomarkers of

living flowers, came from flowers, not from pollution. This should be noted in further environmental research.

The fresh flowers in a simulated natural environment could be considered as alive within the time of SPME. Thus they were metabolites when the fibers were exposed in the headspace of the fresh flowers. Fig. 3 told us that the mono- or sesquiterpene hydrocarbons and *n*-alkanes come from the metabolism of fresh flowers, and the sesquiterpene hydrocarbons, the oxidates and the *n*-alkanes have lower retention times than the monoterpene hydrocarbons.

4. Conclusions

The simulated natural environment, combined with SPME–GC–MS, is a simple and more sensitive sampling method for fresh *Michelia alba* flowers and other fresh flowers. There is a lot of excellence, such as high effectual adsorption, low pollution, and simple operation, without the need for any organic solvent. At the same time, the simulated natural environment avoided the loss of biomarkers. Moreover, this technology, which is much cheaper than headspace SPME, can gain ground quickly.

References

- [1] C. Arthur, J. Pawliszyn, *Anal. Chem.* 62 (1990) 2145.
- [2] M. Servili, R. Selvaggini, A. Taticchi, A.L. Begliomoni, G.F. Montedoro, *Food Chem.* 71 (2000) 407.
- [3] P.G. Hill, R.M. Smith, *J. Chromatogr. A* 872 (2000) 203.
- [4] A. Béné, A. Hayman, E. Reynard, J.L. Luisier, J.C. Villettaz, *Sensors Actuators B* 72 (2001) 204.
- [5] J. Liu, K. Hara, S. Kashimura, M. Kashiwagi, T. Hamanaka, A. Miyoshi, M. Kageura, *J. Chromatogr. B* 748 (2000) 401.
- [6] M. Ábalos, J.M. Bayona, J. Pawliszyn, *J. Chromatogr. A* 873 (2000) 107.
- [7] A.L. Simplício, L.V. Boas, *J. Chromatogr. A* 833 (1999) 35.
- [8] C. Aguilar, S. Penalver, E. Pocerull, F. Borrull, R.M. Marce, *J. Chromatogr. A* 795 (1998) 105.
- [9] M. Natangelo, S. Tavazzi, R. Fanelli, E. Benfenati, *J. Chromatogr. A* 859 (1999) 193.
- [10] A. Namera, T. Watanabe, M. Yashiki, Y. Iwasaki, T. Kojima, *Forensic Sci. Int.* 103 (1999) 217.
- [11] M.F. Alpendurada, *J. Chromatogr. A* 889 (2000) 3.
- [12] J. Adedeji, T.G. Hartman, R.T. Rosen, C. Ho, *J. Agric. Food Chem.* 39 (1991) 1494.
- [13] W. Jennings, T. Shibamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Academic Press, 1980.
- [14] L. Haochun, *Analytical Chemistry Manual*, Chemical Industry Press, 1999.
- [15] A.A. Swigar, R.M. Silverstein, *Monoterpenes*, Aldrich, Milwaukee, WI, 1981.
- [16] B.Z. Liu, Y. Yun, *Chin. J. Chromatogr.* 18 (1999) 452.
- [17] X. Pan, M.Sc. Thesis, Fudan University, 1999.