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Characterization of Volatile Constituents of *Magnolia denudata Desr* Flowers by Gas Chromatography-Mass Spectrometry with Headspace Solid-Phase Microextraction

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In this study, the volatile constituents of *Magnolia denudata Desr* flowers, including budding flowers, blooming flowers and withered flowers, were investigated by headspace (HS) solid-phase microextraction(SPME) and gas chromatography-mass spectrometry (GC-MS). Different SPME parameters (extraction temperature and extraction time) and fibres were studied. There are 47 compounds, 32 compounds, 31 compounds detected in budding, blooming and withered flowers, respectively. The result showed that HS-SPME-GC-MS afforded a simple, rapid, solvent-free method suitable for analysis of volatile compounds emitted from *Magnolia denudata Desr* flowers in different florescence.

Key Words: *Magnolia denudata Desr* flowers, Solid-phase microextraction, GC-MS, Volatile organic compounds.

INTRODUCTION

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 under taken with the aim of identifying the substances responsible for characteristic aromas and flavors[1]. *Magnolia denudata Desr* is as ornamental plants in the Chinese regional massive cultivations. In March every year, *Magnolia denudata Desr* flowers open and release the aroma compounds, which are very pleasant to the human sensory system. Moreover, its flowers contain volatile oils, the magnolia alkali and so on many kinds of chemical compositions. The bud of *Magnolia denudata Desr*, also been called Flos Magnolia , is often used for treating diseases such as cold , headache, stuffy nose, running nose *etc.*, in China[2,3]. Many different analytical methods have been developed to determine volatile constituents present in the flowers. Usually flavors and fragrances of aromatic flowers are analyzed by using the corresponding volatile essential oils, which are complex mixtures of volatile substances generally present at low concentrations in flowers. Before such substance can be analyzed, they have to be extracted from the matrix. Various different methods have been used for the extraction of essential oils and fragrances from flowers for commercial purposes as well as for research. Earlier studies employed the classical flavor procedures of steam distillation or solvent distillation[4,5]. liquid-liquid extraction[6], simultaneous distillation and extraction[7], purge and trap headspace sampling[8] and headspace sampling[9]. Methods based on the use of solvents have several drawbacks, such as the possibility of sample contamination, the lost of analytes during the concentration process and environmental problems related to the use of large amounts of organic solvent[10]. Headspace sampling and purge and trap techniques are similar. These methods have others disadvantages, such as exhaustive concentration steps and time-consuming. Solid-phase micoextraction (SPME) is an extraction and simultaneous concentration, solvent-free sample technique, which is widely applied to food analysis[11,12], enviromet analysis[13-17], biological analysis[18-20], plants[21] and flowers analysis[22-24].

In the work, HS-SPME followed by gas chromatography-mass spectrometry (GC-MS) was developed for the analysis of volatile compounds present in *Magnolia denudata Desr* flowers.

EXPERIMENTAL

Budding, blooming and withered *Magnolia denudata Desr* flowers were collected in the same week (March 2012) from the same tree. The SPME fibers: 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinylbenzene (CW/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) and 85 μm polyacrylate (PA) were purchased from Supelco (Bellefonte, USA).

Gas chromatography-mass spectrometry: A Finnigan Voyager GC-MS was used in EI mode. The extracted compounds were separated on an HP-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film). Desorption of the SPME fibers was performed in splitless mode for 3 min. The column oven temperature was programmed to rise from an initial temperature of 40 ºC (3 min) to 280 ºC at 10 ºC /min, 280 ºC was maintained for 5 min. The injection temperature and ion source temperature were 250 and 230 ºC, respectively. Helium was used as the carrier gas with a flow rate of 1 mL /min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range of 41-450 amu. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA).

Optimization of the solid-phase microextraction conditions: The blooming flower sample (3.0 g) was ground and put into a 10 mL headspace vial and used to optimize the SPME conditions. Firstly, to obtain the optimum fiber, five commercial fibers were tested and used for the extraction of volatile constituents in its flowers at 60 ºC for 10 min. The extracted analytes on the fibers were desorbed in the GC injector in splitless mode at 250 ºC for 3 min, analyzed by GC-MS. The optimal fiber was determined by the peak areas of the main compound in the flower sample. Next, using the optimal fiber of the CAR-PDMS fiber, the extraction temperature and time was also studied by adsorption of the volatiles at different adsorption temperature (25, 40, 60 and 80 ºC) with different extraction times (5, 10, 15, 20 and 30 min) for each temperature. The analytes absorbed on the fibers were desorbed and analyzed by GC-MS.

Determination of volatile compounds in *Magnolia denudata Desr* flowers by GC-MS following HS-SPME: The blooming flower sample with a mass of 3 g was ground and introduced into a 10 mL headspace vial. The volatile compounds in the sample were headspace extracted using the CAR-PDMS fiber at the optimal extraction conditions of 60 ºC for 15 min. The volatiles adsorbed on the fiber were desorbed at the GC injection port with a temperature of 250 ºC for 3 min and analyzed by GC-MS.

RESULTS AND DISCUSSION

Optimization of HS-SPME conditions: Several parameters that can affect the extraction efficiency, such as, fiber coating, extraction temperature and time were investigated. It is very important to investigate the SPME conditions. First, fiber coating was studied. The four compounds of β-myrcene, limonene, eucalyptol and pentadecane (Table-1) in the flower sample used for the determination of the optimal fiber coating Fig. 1 shows the peak areas of the four main compounds obtained by using the five different fibers. For the two compounds of β-myrcene and pentadecane, the CAR-PDMS fiber had better extraction efficiencies than the other fibers. The extraction amounts of limonene by the CAR-PDMS fiber were only less than those by the CW-DVB fiber. The extraction amounts of eucalyptol by the CAR-PDMS fiber were are very close to those by the PDMS and CW-DVB fiber. Comprehensively, the CAR-PDMS fiber was the optimal fiber and used for further

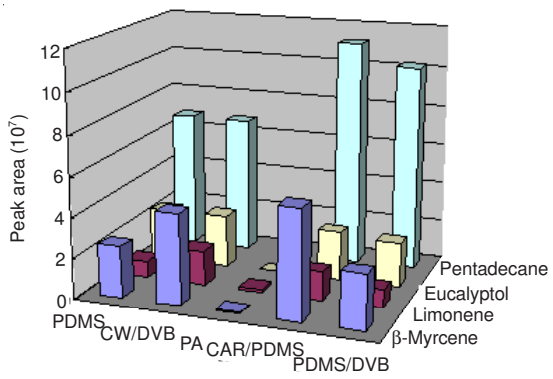

Fig. 1. Effect of fiber coating on the extraction efficiencies of β-myrcene, limonene, eucalyptol, pentadecane

work. However, the CAR-PDMS fiber can very strongly retain the analytes and causes a memory effect; it is necessary to make the carry-over desorbed by keeping the fiber on the inlet of the GC for 3 min after every run. Subsequently, the extraction temperature and time were also studied, using the CAR-PDMS fiber. Fig. 2 shows the effect of the extraction temperature and time on the peak area sum of the four compounds. It can be seen from Fig. 2 that the best extraction amount was obtained at 60 ºC and the extraction reached equilibrium after exposure for 15 min. Therefore, the optimal HS-SPME conditions are: CAR/PDMS fiber, extraction temperature of 60 ºC and time of 15 min.

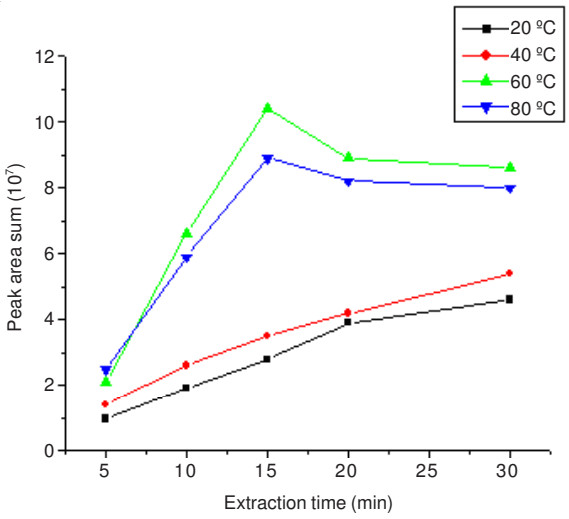

Fig. 2. Effect of the extraction temperature and time on extraction efficiency

Determination of volatile constituents in *Magnolia denudata Desr* flowers by GC-MS following HS-SPME in different flowering stages: The optimum extraction conditions were applied to extract and concentrate of volatile constituents of *Magnolia denudata Desr* flowers in different flowering stages and analyzed by GC-MS. The qualifications of every peak and their relative contents are shown in Table-1 and the total ion chromatograms of volatile compounds by SPME-GC-MS are shown in Fig. 3. A total of 47 volatile compounds were identified and the relative content was calculated by peak area ratio. From Table-1, we can find that the following results: there are 47 compounds, 32 compounds, 31 compounds detected in budding, blooming and withered flowers, respectively.

TABLE-1
IDENTIFICATION OF VOLATILE COMPOUNDS FROM *Magnolia denudata Desr* FLOWERS IN DIFFERENT FLORESCENCE

No.	Retention time (min)	Compounds	m.w.	Mass spectra (relative abundance)	Relative content (%)			RSD (%)
					a	b	c	
1	6.78	2-Hexanal	98	55(100)69(90)83(77)98(25)	ND	1.09	6.49	2.1
2	7.05	2-Hexan-1-ol[E]	100	57(100)82(22)67(20)77(10)	ND	ND	1.73	3.5
3	7.09	Cyclopropene, propyl	84	56(100)69(38)84(5)45(4)	ND	ND	4.07	2.9
4	8.23	Bicyclo[3.1.0]hexane,4-methyl-1-[1-methylethyl]	136	93(100)77(41)136(10)65(5)	3.67	2.71	6.12	2.5
5	8.36	α-Pinene	136	93(100)77(35)121(18)105(15)	3.20	1.96	2.66	2.3
6	8.67	Camphene	136	93(100)121(78)79(40)107(38)	Tr	Tr	Tr	4.0
7	9.13	β-Phellandrene	136	93(100)77(40)136(20)69(10)	6.11	8.88	5.54	2.3
8	9.24	β-Pinene	136	93(100)69(32)79(30)121(18)	9.02	5.28	6.52	3.1
9	9.44	β-Myrcene	136	93(100)69(70)79(20)53(12)	24.73	24.90	23.43	2.8
10	9.70	α-Phellandrene	136	93(100)77(38)136(22)105(4)	0.48	Tr	Tr	3.7
11	9.83	3-Carene	136	93(100)77(39)121(24)136(22)	Tr	ND	ND	2.9
12	9.91	1,3-Cyclohexadiene,1-methyl-4 -[1-methylethyl]	136	121(100)93(91)136(55)77(36)	2.0	1.39	2.16	2.1
13	10.06	Benzene,1-methyl-4-[1-methylethyl]	134	119(100)134(30)91(25)115(8)	1.39	1.59	3.13	2.6
14	10.14	Limonene	136	68(100)93(90)77(44)121(36)	4.96	8.34	8.76	3.0
15	10.21	Eucalyptol	154	81(100)108(92)111(84)154(82)	6.71	9.65	10.36	4.0
16	10.44	1,3,7-Octatriene-3,7-dimethyl	136	93(100)79(42)105(20)121(18)	1.57	4.82	1.26	2.5
17	10.66	1,4-Cylcohexadiene,1-methyl-4-[1-methylethyl]	136	93(100)77(37)136(40)121(36)	4.77	3.03	4.56	2.8
18	10.84	*cis*-Sabinenehydrate	154	93(100)71(100)111(52)81(58)	0.11	0.56	Tr	3.9
19	11.17	Cyclohexene,1-methyl-4-[1-methylethylidene]	136	121(100)93(100)136(90)79(40)	1.13	0.97	1.21	2.2
20	11.55	(E)-4,8-Dimethyl-1,3,7-nonatrine	150	69(100)81(18)53(10)107(6)	1.31	3.37	Tr	2.3
21	12.17	Camphor	152	95(100)81(77)108(38)152(36)	0.022	Tr	Tr	4.2
22	12.65	3-Cyclohexen-1-ol,4-methyl-1-[1-methylethyl]	154	71(100)93(62)111(61)55(60)	0.011	Tr	Tr	4.9
23	12.84	3-Cyclohexene-1-methanol,α,α.4-trimethy	154	59(100)93(80)121(75)136(70)	0.97	1.18	Tr	2.6
24	14.23	Bicyclo[2,2,1]heptan-2-ol,1,7,7-trimeth	196	95(100)121(43)136(42)108(19)	Tr	ND	ND	3.8
25	14.81	Eudesma-4[14],11-Diene	204	81(100)93(64)105(46)123(46)	0.053	ND	ND	4.2
26	14.93	Aromadendrene	204	81(100)93(70)105(52)123(51)	0.28	ND	ND	3.6
27	15.20	α-Cubebene	204	161(100)105(92)119(90)91(38)	0.99	ND	ND	2.2
28	15.29	Exo-2-Hydroxycinede,acetate	212	108(100)93(50)126(54)71(52)	Tr	ND	ND	4.2
29	15.43	Ylangene	204	105(100)119(84)161(80)91(65)	0.48	ND	ND	3.2
30	15.51	Copaene	204	119(100)161(96)105(90)93(42)	1.37	Tr	Tr	2.9
31	15.60	Tetradecane	198	57(100)71(70)85(50)99(12)	0.68	ND	ND	2.5
32	15.69	1*H*-Cyclopropa[a]naphthalene,1a,2,3,4,5,6,7,7a,7b-oct	204	161(100)105(50)91(48)81(40)	Tr	ND	ND	3.1
33	16.05	1,7-Dirnethy-tricyclo[2,2,1,0[2,6]heptane	204	94(100)107(50)79(50)69(40)	Tr	ND	ND	2.7
34	16.14	Caryophyllene	204	93(100)133(98)79(72)69(70)	2.90	0.85	3.69	2.3
35	16.25	Octahydro-1*H*-cyclopenta[1,3]cycloprota[1,2]benzene	204	161(100)91(40)105(38)119(35)	0.34	Tr	ND	3.0
36	16.40	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene	204	69(100)93(64)133(43)79(28)	0.49	Tr	ND	3.1
37	16.57	α-Caryophyllene	204	93(100)121(34)80(35)147(20)	0.63	Tr	Tr	2.7
38	16.68	2-isopropyl-5-methyl-9-methyl-bicyclo[4,4,0]dec-1-ene	204	161(100)105(40)204(40)119(25)	0.37	Tr	Tr	4.1
39	16.81	Octahydro-7-methyl-4-naphthalene,1,2,3,4,4a,5,6,8a	204	161(100)119(48)105(50)91(42)	4.99	0.10	Tr	2.2
40	16.87	Pentadecane	212	57(100)71(80)85(60)99(19)	11.81	18.85	11.04	2.1
41	16.96	1.,3-Cyclohexadiene,5-[1,5-dimethyl-4-hexenyl]-2-me	204	161(100)105(30)91(29)119(72)	0.25	ND	ND	2.9
42	17.39	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1	204	161(100)134(57)119(60)204(60)	4.51	0.58	Tr	2.9
43	17.52	Naphthalene,1,2,3,4,4a,5,6,7-hexahydro-1,6-dimethyl-4	204	119(100)105(72)161(55)204(30)	0.42	Tr	ND	3.1
44	17.58	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1	204	105(100)161(66)91(28)204(30)	0.56	Tr	ND	3.4
45	17.68	Naphthalene,1,2-dihydro-1,1,6-trimethyl	172	157(100)142(48)200(35)115(10)	Tr	ND	ND	4.1
46	17.76	3,7,11-trimethy-1,6,10-dodecatrien-3-ol	222	69(100)93(78)107(42)55(35)	0.90	ND	ND	2.9
47	19.00	2-Naphthalenemethahol,decahdro-α,4a	222	59(100)149(70)95(50)109(43)	Tr	ND	ND	4.6

ND = not detection, Tr = the relative content less than 0.01 %.

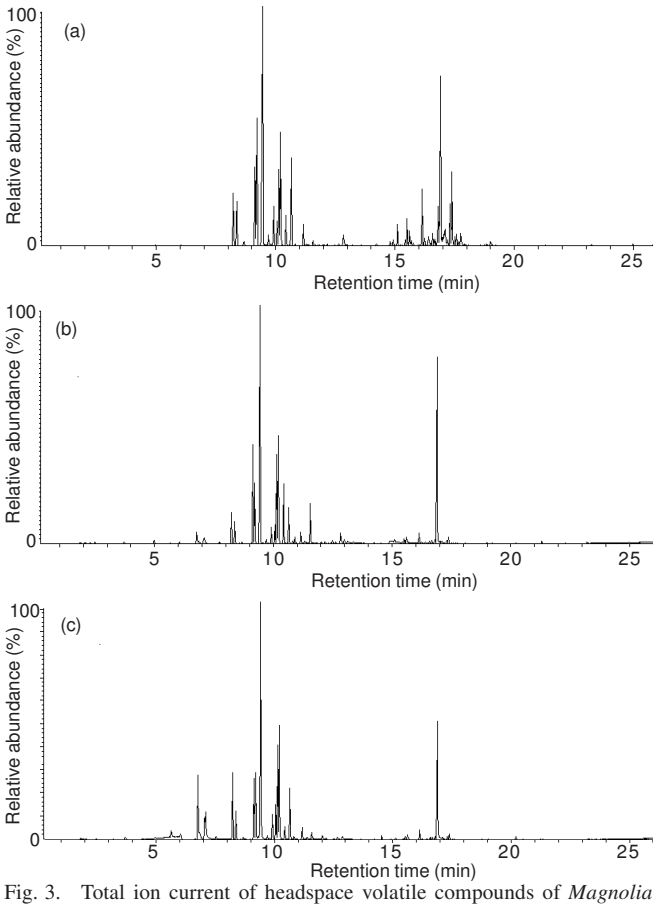

Fig. 3. Total ion current of headspace volatile compounds of *Magnolia denudata Desr* flowers with SNE-HS-SPME-GC-MS. (a) budding flowers, (b) blooming flowers, (c) withered flowers

The major compounds released from *Magnolia denudata Desr* flower in different florescence are same, which mainly include α-pinene, β-phellandrene, β-pinene, β-myrcene, limonene, eucalyptol, caryophyllene, pentadecane, *etc*. But their quantities and proportions change in different flowering stages. Some compounds only exist in the budding flower, sush as maromadendrene, α-cubebene, ylangene, tetradecane, *etc*. This indicated that volatile constituents in *Magnolia denudata Desr* flowers relating to florescence.

The volatile compounds produced by plants and other live species comprise a large number of organic substances, including isoprene and isoprenoid compounds, alkanes, alkenes, carbonyl compounds, alcohols and esters[25]. These volatile compounds are responsible for multiple interactions between plants and other organisms, such as pollinating animals, herbivores and the predators of herbivores[26]. Furthermore, because some volatile compounds have antipathogenic properties, they are produced and emitted by plants as a defence mechanism against attack by herbivores[27]. Frequently, the biological activity of essential oils is due to the presence of a mixture of compounds and not to a single one. However, characterization of the volatile components of *Magnolia denudata Desr* flowers has also provided significant insight into its practical medicinal uses, *e.g*. Myrcene has been shown to possess potent analgesic activityin rat, which was the most abundant identified volatile in the budding, blooming and withered *Magnolia denudata Desr* flowers[28]. Another monoterpene found in this plant, limonene, has been shown to inhibit rat mammary carcinogenesis[29] and it has also been shown to induce the activity of a detoxifying enzyme, indicating its potential as an anticarcinogenic agent[30].

In order to study the precision of the method, five replicate analysis of the volatile compounds of budding flowers were carried out. The relative standard deviation (RSD) values of the peak areas have shown the excellent repeatability of the method (Table-1).

Conclusion

HS-SPME-GC-MS is a simple, rapid no organic solvent method for the determination volatile compounds in plant flowers. Using this technique we have identified the most important compounds from *Magnolia denudata Desr* flowers in different flowering stages.

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