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ORIGINAL ARTICLE

Headspace Solid-Phase Microextraction Analysis of Volatiles in Floral Organs of *Nelumbo nucifera*

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Abstract The objective of this study was to identify the main floral scents and their relative contents in the floral organ of *Nelumbo nucifera*. *N. nucifera* flower, a traditional Chinese medicinal herb, is rich in volatile compounds. In this study, the volatile components of *N. nucifera* flowers were investigated by headspace solid-phase microextraction gas chromatography-mass spectrometry for each organ of the flower: petals, sepals, pistils, and stamens. In total, we identified 39 compounds, among which aliphatics were major constituents, representing more than 94% of petals and sepals volatiles, followed by sesquiterpenes representing more than 69% of pistils and stamens volatiles. Pentadecane, 1-pentadecene, 8-hexadecyne, 8-heptadecene, and β-caryophyllene characterize the scent of the *N. nucifera* flower. We identified 24 volatiles in petals and sepals, 25 volatiles in pistils, and 18 volatiles in stamens. Among the monoterpenes, 3-Isopropylidene-4-methylcyclohexene, isoterpinolene, *p*-Menth-2-en-7-ol, and methyl 2,6,6-trimethylcyclohex-1-enecarboxylate were analyzed and identified for the first time from the *N. nucifera* flower. This study demonstrates that *N. nucifera* flowers differ greatly in volatile composition depending on the floral organ of the plant.

Additional key words: alphatics, floral scents, gas chromatography-mass, petal, pistil, sepal, sesquiterpenes, stamen

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Introduction

Nelumbo nucifera (Gaertn.), a perennial aquatic herb, is cultivated on a large scale in Eastern Asia, particularly in China (Mukherjee et al. 2009). All parts of *N. nucifera*, including the leaves, stamens, flowers, seeds, and rhizomes, have been used as traditional Chinese medicine or vegetables for thousands of years. *N. nucifera* is distributed throughout Asia (Kim 1996). The flowers are solitary, large, 10 - 25 cm in diameter, white, pink or pinkish white, and fragrant and have peduncles arising from the nodes of the rhizome, and 1 - 2 cm long sheathing at the base. The sepals, petals, and stamens are spirally arranged, passing gradually into each other (Gupta and Ahluwalia 1979). *N. nucifera* flowers are rich in volatile compounds. Omata et al. (1991) identified seventy component as the major volatiles constituents, and more than 75% of the extract were hydrocarbons, such as 1,4-dimethoxybenzene, 1,8-cineole, terpinen-4-ol and linalool. However, this report deal with was only for the petals of *N. nucifera*. Xu et al. (2011) analyzed volatile components of five varieties of the *N. nucifera*. Therefore, our study was carried out to analyze the volatile components included in each organ of the flower, which as petals, sepals, pistils, and stamens using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS).

Headspace analysis can be used to analyze the volatile

composition of living natural materials and to obtain different and broader olfactory profiles (Schossmam 2009). Solid-phase microextraction (SPME) is a simple, fast, sensitive and convenient sample preparation technique, which minimizes solvent usage while integrating sampling and sample preparation steps prior to instrumental analysis (Zhu et al. 2013). Nowadays, SPME is widely applied to sampling and analysis of environmental, food, aromatic, metallic, forensic, biological, and pharmaceutical samples (Ouyang et al. 2011). Several types of coating fibers are currently available for the extraction of various analytics.

The objective of this study was to identifiy the main floral scents and their relative contents in floral organ from *Nelumbo nucifera*. In order to easily and extensively identify the volatile components of *N. nucifera* flowers, by the headspace solid-phase microextraction (HS-SPME) technique, with subsequent analysis used by gas chromatography-mass (GC-MS). Moreover, volatiles present in each floral organ of *N. nucifera* such as the petals, sepals, pistils, and stamens were analyzed and identified for the first time. This study demonstrates that *N. nucifera* flowers differ greatly in volatile composition depending on the floral organs of the plant, a finding that provides important theoretical references for flower appreciation studies on aromatic volatile composition.

Materials and Methods

Plant materials

The *N. nucifera* flowers were collected from Gwangsukje (35°18'25.2"N 127°36'49.0"E), Wanju, South Korea, on August 2015 and were identified by National Institute of Horticultural and Herbal Science, Rural Development Administration, South Korea. A voucher specimen (F20150810-01) was deposited in the National Institute of Horticultural and Herbal Science (NIHHS). The inflorescence of *N. nucifera* is a raceme that always exhibits inconsistent flowering. First, 20 g of raw flower materials of *N. nucifera* was collected at 9:00 - 11:00 a.m., on August 10, 2015. The collected flowers were moisturized and kept immediately to the laboratory. *N. nucifera* flowers were separated into petals, sepals, pistils, and stamens. Floral organs were sliced by using a knife into similarly thin slices and 1.0 g of sliced materials put into a headspace vial (20 mL) for 30 min at ambient room temperature.

Analysis of volatile components by HS-SPME-GC-MS

The fibers used in this assay were polydimethylsiloxane (PDMS) with film thickness 100 μm (Supelco, Bellefonte, PA, USA). The SPME device was inserted into the sealed vial by manually penetrating the silicone septum, and the fiber was exposed to the headspace of the sliced material after 30 min. The SPME fiber was exposed to each sample for 30 min at 50°C. After extraction, the needle on the SPME manual holder was set to 0.5 cm in the GC injector. The fiber was then directly desorbed for 10 min. An Agilent 7000C GC-MS system (Agilent Technologies, Wilmington, DE, USA), with a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 μm, Agilent Technologies.) was used under the following conditions: MS transfer line heater of 280°C, injector temperature of 250°C, splitless mode operating. The initial oven temperature was held at 60°C for 5 min, then programmed from 60°C to 250°C at 3°C/min, and finally maintained for 5 min at 280°C. Helium gas was used as a carrier at a flow rate of 1.0 mL/min. An Agilent 7000C mass spectrometer was operated in the electron ionization mode at 70 eV with a source temperature of 250°C, and a quadrupole set to 150°C, and scanned from m/z 30 to 500 in the full-scan mode.

Data analysis

The constituents were identified by matching their spectra with those recorded in a NITS 14 (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library and published data (NIST, http://webbook.nist.gov/chemistry/; Pubchem, http://pubchem.ncbi.nlm.nih.gov/; Flavornet, http://www. flavornet. org/; Chemspider, http://chemspider.com/). The major components were

finally identified by analysis of fragmentation data in MS spectrum. In addition, the constituents were confirmed by comparing the retention indices (RI) or GC retention time (r.t.) data with those of authentic standards or by publication literature. It is defined as Eq. (1):

$$RI = 100 \times n + [100 \times (tx - tn)] / (tn+1 - tn)$$

where RI is the retention index of the unknown compound x, *n* is the number of carbon atoms of the *n*-alkane eluted before x, *n+1* is the number of carbon atoms of the *n*-alkane eluted after x, *tx* is the retention time of x, *tn* the retention time of the *n*-alkane eluted before x, and, *tn+1* is the retention time of the *n*-alkane eluted after the x. All the indices were calculated by performing three replicated measurements by injecting pure compounds (Bianchi et al. 2007). The separated compounds were measured as the relative contents (%), and calculated automatically the peak areas obtained by the total ion chromatographic (TIC) analysis, using Eq. (2):

Relative contents (%) = (area under peak / total peak area) × 100

Results and Discussion

N. nucifera flowers were collected and separated into petals, sepals, pistils, and stamens. The volatiles in each organ of *N. nucifera* flowers were analyzed by using HS-SPME coupled with GC-MS (Fig. 1). Each peak was identified by matching their spectra with those recorded on a NITS 14 mass spectral library and published data as well analyzing the RI or GC retention time data. And they were confirmed through analysis of fragmentation pattern in mass spectra. In total, 39 floral scents were identified as major volatile components, comprising 99.28% of the volatile components in petals and sepals, 97.12% in pistils, and

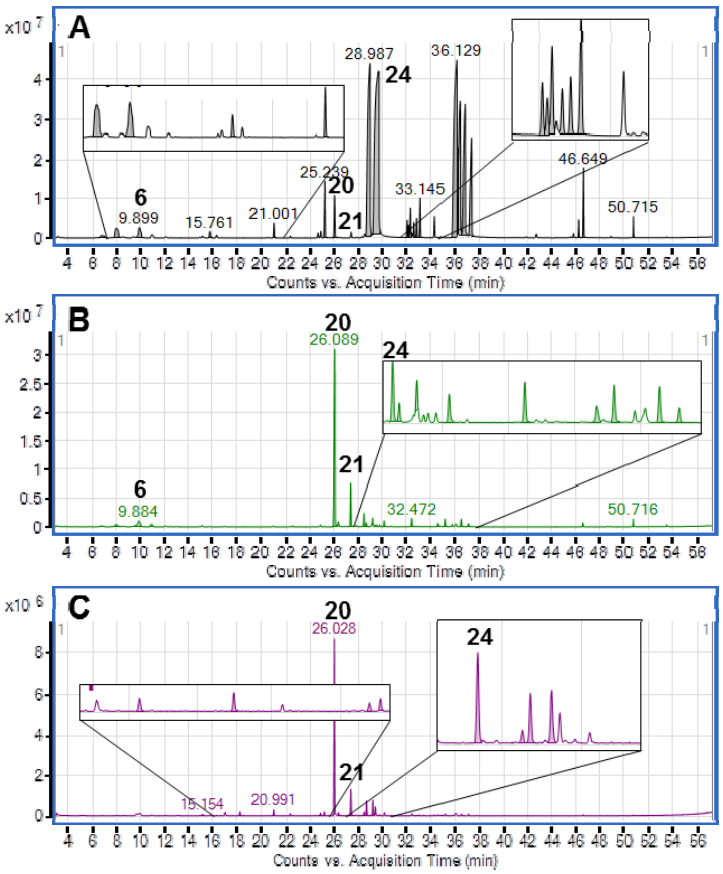

Fig. 1. Representative total ion chromatograms for petals and sepals (A), pistils (B), and stamens (C) of *N. nucifera* flower analyzed by HS-SPME-GC-MS.

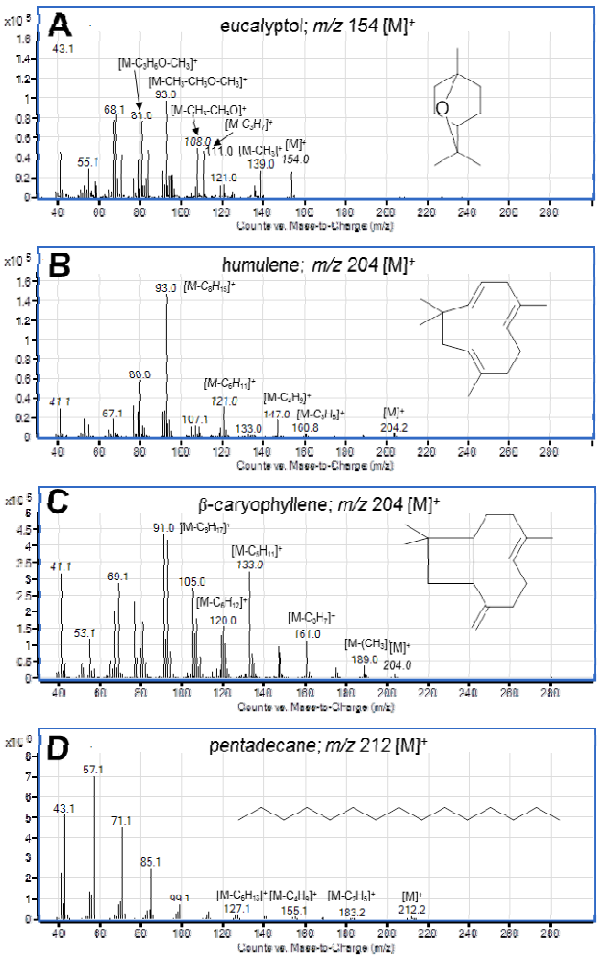

Fig. 2. Mass spectra of the major ion peaks. A: eucalyptol, B: *β*-caryophyllene, C: humulene, D: pentadecane.

97.35% in stamens. These volatiles, were grouped based on as monoterpenes, aliphatics, and sesquiterpenes by their biochemical synthetic pathways (Knudsen et al, 2006), are listed in Table 1. Eight volatiles were observed in three different parts of *N. nucifera* floral organs. The main volatile components were β-caryophyllene, humulene, eucalyptol, and pentadecane, which might dominate the flavor of *N. nucifera* (Fig. 2). It was reported that, β-caryophyllene has a woody and spicy odor; humulene has a woody odor (Yang et al. 1993); eucalyptol has a fresh camphor-like smell and a spicy, cooling taste (Bhowal and Gopal. 2015); and pentadecane has a waxy odor (Berdague et al. 1991).

In the petals and sepals, 24 volatile compounds were identified, which can be grouped as follows: aliphatics (94.15%), monoterpenes (2.97%), sesquiterpenes (2.08%), and benzenoids (0.08%). The most abundant compounds were pentadecane, (6*Z*,9*Z*)-6,9-pentadecadien-1-ol, 8-hexadecyne, 1-pentadecene, hexadecane accounting for about 86% of the total GC peak area, followed by (7*Z*)-7-hexadecane (1.90%), tetradecane (1.69%), nonadecane (1.76%), and β-caryophyllene (1.30%). In contrast, the relative contents of eucalyptol (0.89%) and β-caryophyllene (1.30%) at the petals and sepals than in other floral organs (Fig. 1A).

In the pistils, 25 volatile compounds were identified, belongs to the following cases: sesquiterpenes (78.36%), monoterpenes (12.41%), aliphatics (5.86%), and benzenoids (0.49%). The most abundant compound was β-caryophyllene, accounting for about 57% of the total GC peak area, followed by humulene (10.05%), eucalyptol (5.54%), and germacrene D (3.10%). The relative contents of pentadecane (1.80%), 8-(6*Z*,9*Z*)-6,9-pentadecadien-1-ol (2.03%), and

Table 1. Percentage of volatiles identified in three different floral organs of *N. nucifera* flower using HS-SPME-GC-MS.

Peak	RI[z]	Compounds	Relative contents (%)[y] ± SD[x]		
			Petals and sepals	Pistils	Stamens
		Monoterpenes	2.97	12.41	8.83
1	936	α-pinene		0.88 ± 0.06	
2	948	2-norpinene, 3,6,6-trimethyl-	0.29 ± 0.01		
3	972	(-)-sabinene	1.12 ± 0.07	2.06 ± 0.13	
4	980	β-pinene		0.51 ± 0.05	
5	1023	3-Isopropylidene-4-methylcyclohexene		0.29 ± 0.02	
6	1031	eucalyptol	0.89 ± 0.05	5.54 ± 0.16	3.68 ± 0.47
7	1056	γ-terpinene	0.23 ± 0.01	1.67 ± 0.08	0.62 ± 0.02
8	1083	terpinolene		0.44 ± 0.04	
9	1090	isoterpinolene	0.07 ± 0.01		
11	1180	terpinen-4-ol	0.27 ± 0.03		
12	1190	α-terpineol	0.1 ± 0.02		
13	1254	trans-p-menth-2-en-7-ol		0.25 ± 0.02	1.51 ± 0.32
14	1282	methyl 2,6,6-trimethylcyclohex-1-enecarboxylate			1.82 ± 0.03
16	1343	α-elemene		0.26 ± 0.02	
17	1366	β-elemene		0.51 ± 0.40	1.18 ± 0.16
		Aliphatics	94.15	5.86	18.09
15	1313	tridecane	0.43 ± 0.03	0.23 ± 0.01	2.17 ± 0.05
18	1391	(5E)-5-tetradecene	0.31 ± 0.02		
19	1397	tetradecane	1.69 ± 0.04		1.58 ± 0.13
23	1490	1-pentadecene	16.1 ± 0.15	0.91 ± 0.60	4.68 ± 0.14
24	1500	pentadecane	24.07 ± 0.16	1.80 ± 0.58	8.52 ± 0.30
27	1545	7-tetradecyne	0.8 ± 0.02		
28	1550	5-undecyne	0.34 ± 0.10		
29	1564	(7*Z*)-7-hexadecene	1.9 ± 0.02		
31	1600	hexadecane	6.8 ± 0.85		
35	1664	8-hexadecyne	19.53 ± 0.46		0.66 ± 0.03
36	1705	(6Z,9Z)-6,9-pentadecadien-1-ol	19.86 ± 0.54	2.03 ± 0.08	0.48 ± 0.02
37	1811	5-octadecene	0.05 ± 0.01		
38	1880	9-nonadecene	0.51 ± 0.02		
39	1900	nonadecane	1.76 ± 0.04	0.82 ± 0.09	
		Sesquiterpenes	2.05	78.36	69.66
20	1428	β-caryophyllene	1.30 ± 0.03	57.51 ± 3.13	56.78 ± 1.31
21	1451	humulene	0.18 ± 0.01	10.05 ± 0.47	8.43 ± 0.99
22	1474	germacrene D		3.10 ± 0.02	1.50 ± 0.16
25	1512	γ-muurolene		1.14 ± 0.10	0.57 ± 0.07
26	1541	δ-cadinene		1.44 ± 0.12	1.28 ± 0.03
30	1580	caryophyllene oxide		2.11 ± 0.06	1.10 ± 0.01
32	1611	γ-eudesmol	0.60 ± 0.02		
33	1640	τ-cadinol		1.03 ± 0.20	
34	1654	α-cadinol		1.98 ± 0.05	
		Benzenoids	0.08	0.49	0.77
10	1165	1,4-dimethoxybenzene	0.08 ± 0.03	0.49 ± 0.04	0.77 ± 0.06
	Total		99.28	97.12	97.35

[z]Retention indices calculated against *n*-alkanes (C9 – C20).
[y]Relative contents (%) = (area under peak / total peak area) × 100.
[x]All data are presented as mean ± standard deviation (n = 3).

1-pentadecene (0.98%) in the pistils were significantly lower than that in the petals and sepals, and that of 8-hexadecyne was significantly lower than that in the other floral organs (Fig. 1B).

In the stamens, 18 volatile compounds were identified that can be grouped as follows: sesquiterpenes (69.66%), aliphatics (18.09%), monoterpenes (8.83%), and benzenoids (0.77%). The major compound was β-caryophyllene, accounting of about 57% for the total GC peak area, followed by pentadecane (8.52%), humulene (8.43%), 1-pentadecene (4.68%), and eucalyptol (3.68%). In contrast, the relative contents of pentadecane (8.52%) and 1-pentadecene (4.68%) were significantly lower in the stamens than the petals and sepals, but higher than that the pistils (Fig. 1C).

In accordance with the report of Xu et al. (2011), the petals of the five varieties of *N. nucifera* were analyzed by HS-SPME-GC-MS. In total, 74 compounds were identified in these five varieties, mainly consisting of aliphatics and sesquiterpenes exceeding 90%. Also, our results similar to indicated aliphatics as the main components in petals (94.15%) and sesquiterpenes to be the main components in pistils and stamens (78.36 - 69.66%).

Monoterpenes, 3-Isopropylidene-4-methylcyclohexene, isoterpinolene, *p*-Menth-2-en-7-ol, and methyl 2,6,6-trimethylcyclohex-1-enecarboxylate were identified as the volatiles of the *N. nucifera* flower in this research for the first time.

Representatively, 3-Isopropylidene-4-methylcyclohexene can be found in the essential oils of *Ligustrum robustum* (Wu et al. 2012), *Angelica sinensis* (Huang et al. 2004), and *Artemisia herba* (Nezhadali et al. 2008). Moreover, isoterpinolene can be reported in the essential oils of *Anthemis maritima* (Ciccarlli et al. 2016), *Buddleja polystachya* (Ati HYA et al. 2014), and *Cananga odorata* (Qin et al. 2014).

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