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Analysis and characterization of aroma-active compounds of *Schizandra chinensis* (omija) leaves

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Abstract: Volatile components from leaves of *Schizandra chinensis* (omija), a native plant of Korea, were extracted by simultaneous distillation–extraction (SDE) and analyzed by gas chromatography–mass spectrometry (GC-MS) using two types of capillary column with different polarities (DB-5MS and DB-Wax). The GC-MS analysis of volatile compounds obtained by SDE revealed that germacrene D is the most abundant compound (22.6%) in omija leaves, followed by β-elemene (17.4%), (*E*)-2-hexenal (8.7%), and (*E*)-β-ocimene (7.2%). Aroma-active compounds were determined by gas chromatography–olfactometry (GC-O) using the aroma-extract-dilution analysis method. (*E*,*Z*)-2,6-Nonadienal (cucumber) was the most intense aroma-active compound due to its higher flavor-dilution factor (243–729) than any other compound. (*Z*)-3-Hexenal (green/apple), (*E*)-2-hexenal (green/fruity), and (*E*)-β-ocimene (wither green/grass) were also identified as important aroma-active compounds by GC-O. In addition, the volatile compounds were extracted by solid-phase microextraction (SPME), and the quantitative analysis of the SPME samples gave slightly different results, depending on the type of SPME fiber, compared with those from SDE, However, the aroma-active compounds identified in SPME were similar to those in SDE.

Keywords: gas chromatography–olfactometry; aroma-extract-dilution analysis; (*E*,*Z*)-2,6-nonadienal; headspace solid-phase microextraction; *Schizandra chinensis* leaves; omija

INTRODUCTION

The family *Schizandraceae* comprises more than 22 species,[1] among which *Schizandra chinensis* (omija) is native to Korea.[2] Because omija fruits have a characteristic aroma and strong sour taste,[2] in Korea they have not only been used traditionally for medicinal purposes[3–6] but also recently for various applications in food products.[5–9] The commercial importance of omija fruit in Korea has lead to several studies into its volatile compounds using gas chromatography–mass spectrometry (GC-MS), which have revealed the presence of terpinen-4-ol, γ-terpinene, caryophyllene, calarene, β-elemene, α-ylangene and zingiberene.[3,10–12]

As the consumption of omija fruits is large, as much as 223 tons per year in Korea, omija leaves are abundantly generated as a byproduct. Therefore, to seek possible application of the omija leaf as an aromatic resource for food and medicinal products, in this study the volatile compounds of omija leaves extracted by both simultaneous distillation–extraction (SDE) and headspace solid-phase microextraction (HS-SPME) have been analyzed by GC-MS, and the aroma-active compounds have been characterized by gas chromatography–olfactometry (GC-O)[13] with successive dilution by a solvent and varying GC injector split ratios[14] for the extracts of SDE and SPME, respectively.

EXPERIMENTAL

Materials

Omija leaves were obtained from Bangtae Mt in Gangwon Province during September 2002. The leaves were stored in a plastic bag at −70 °C before analysis. Authentic flavor compounds were purchased from Aldrich Chemical (Milwaukee, USA), Seoul Aromatics (Seoul, Korea), and Borak (Seoul, Korea).

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Extraction of volatile compounds

For SDE, 80 g of omija leaves were ground using a commercial blender, and simultaneously distilled and extracted with 350 ml of distilled water and 50 ml of CH_2Cl_2, respectively for 2 h at atmospheric pressure. After SDE, the extract was dried over anhydrous Na_2SO_4 and concentrated to 300 µl using a gentle stream of N_2 gas. The entire SDE procedures were repeated four times.

For HS-SPME sampling, omija leaves (4 g) cut into small pieces were placed in a 40-ml glass vial equipped with an open-center screw cap and a Teflon/silicon septum (Supelco, Bellefonte, USA) for 1 h at 45 °C. Subsequently, the headspace volatiles were extracted by an SPME fiber, 100-μm polydimethylsiloxane (PDMS) or 75-µm Carboxen (CAR)-PDMS (Supelco) for 20 min with a needle length of 20 mm.

Gas chromatography–mass spectrometry

For GC-MS analysis a mass-selective detector (HP 5890 Series II GC/5972, Hewlett-Packard, Palo Alto, USA) was equipped with a capillary column: DB-5MS or DB-Wax (30 m length × 0.25 mm id × 0.25 µm film thickness, J&W Scientific, Folsom, USA). A 1-µl aliquot of the extract was injected (splitless mode) into each column. The oven temperature was programmed to increase from 40 °C to 140 °C at a rate of 4 °C min^{-1}, followed by from 140 °C to 200 °C at 4 °C min^{-1} with initial and final hold times of 5 and 20 min, respectively. The flow rate of helium, the carrier gas, was 0.8 ml min^{-1}. The injector and detector temperatures were constant at 200 °C and 250 °C, respectively. Other conditions in the mass-selective detection were as follows: ionization energy, 70 eV; mass range, 33–550 amu; and scanning rate, 1.4 scans s^{-1}. Retention indices (RIs) were calculated using *n*-paraffins C_7–C_{22} as external references.[15]

In HS-SPME–GC-MS, only the DB-5MS column was used. Volatiles on each fiber were desorbed in a GC injector with a SPME inlet liner (0.75 mm id; Supelco) for 2 min at 200 °C with a needle length of 40 mm. Other conditions were identical with those in SDE.

Gas chromatography–olfactometry

For GC-O a Varian 3900 GC (Walnut Creek, USA) was equipped with a flame-ionization detector (FID) and a sniffing port (ODO II, SGE, Ringwood, Australia). At the end of the capillary column (DB-5MS or DB-Wax, 30 m length × 0.25 mm id × 0.25 µm film thickness), the effluent was split 1:1 by volume between FID and the sniffing port using deactivated capillary columns (0.5 mm length × 0.25 mm id).

The SDE extract (300 µl) was diluted stepwise with CH_2Cl_2 (1:3 by volume), and a 1-μl aliquot of this was injected into the capillary column. The oven temperature was initially held at 40 °C for 5 min, then raised at 10 °C min^{-1} to 200 °C and held there for 24 min. Other GC conditions were the same as those of GC-MS (see above). Flavor-dilution (FD) factors of the aroma-active compounds were determined by aroma-extract-dilution analysis (AEDA),[16] with three experienced panelists participating in the experiment.

Volatiles extracted by SPME were diluted stepwise by the method of varying the split ratio, which has been proven to be a suitable and reliable tool for the successive dilution of volatiles in HS-SPME–GC-O.[14] The split ratios used for aroma dilution were 1 (splitless), 2, 4, 8, 16, 32 and 64. In this case, FD factor was defined as the maximum split ratio at which a compound could be detected. Other GC conditions were the same as those in SDE.

Identification and quantification

Volatile compounds were identified by comparing their RIs and matching their mass spectra with those of authentic standards. When standards were not available, compounds were positively identified with the aid of the Wiley 275 mass-spectrum database (Hewlett-Packard, Palo Alto, CA, USA, 1995) and published RIs.[17,18] Aroma-active compounds were identified by comparing spectra, RIs, and aroma properties of unknowns with those of authentic standards.

RESULTS AND DISCUSSION

Volatile compounds by GC-MS

A total of 52 compounds were identified from omija-leaf extracts sampled by SDE and SPME, through analyses using two types of capillary column (DB-5MS or DB-Wax; Table 1). Volatile flavor compounds identified in omija leaves comprised 15 monoterpene hydrocarbons, 19 sesquiterpene hydrocarbons, 12 terpene oxygenates and 6 aliphatic aldehyde, alcohol and ketones.

In the SDE extract, monoterpene and sesquiterpene hydrocarbons represented 14 and 64% of total volatile compounds, respectively. In addition, the oxygenated derivatives of terpene comprised 11% of the extract. The main flavor compounds of omija leaves were germacrene D (No 26), β-elemene (No 19), (*Z*,*E*)-α-farnesene (No 28), (*E*)-2-hexenal (No 48), and (*E*)-β-ocimene (No 13). Unlike omija fruits, which predominantly have terpinen-4-ol, γ-terpinene, caryophyllene, β-elemene, α-ylangene and zingiberene,[3,10–12] in omija leaves sesquiterpene hydrocarbons were found to be the most abundant volatile compounds.

Germacrene D is known as the predominant sesquiterpene in various essential oils of *Pinus canariensis*,[19] *P peuce* and *P pinaster*[20] and *Piper nigrum*.[21] β-Elemene has also been reported in omija fruits,[3,11] and was identified as the main component of basil oil,[22] *Murraya koenigii*[23] and *Stachys swainsonii*

ssp *melangavica*.[24] (*E*)-2-Hexenal, the most common volatile compound in many flowers, is found in *Medicago sativa*[25] and in fruits of yellow cashew.[26] (*E*)-β-Ocimene is also commonly found in many plants including leaves of red cashew,[26] *Lathyrus azorica*,[27] inflorescences of *Origanum vulgare* ssp[28] and *L odoratus*.[29]

The volatile compounds extracted by HS-SPME using PDMS and CAR-PDMS fibers are also listed in Table 1. A total of 36 compounds were identified in the SPME extracts. (*E*)-2-Hexenal was the most abundant volatile component, followed by germacrene D, (*E*)-β-ocimene, and β-elemene. Therefore, the major compounds appeared to be the same as those in SDE extracts. However, the relative amounts of the flavor compounds differed with the fiber type. The nonpolar PDMS fiber was more efficient than the bipolar CAR-PDMS fiber for extracting total volatiles of omija leaves, since sesquiterpene hydrocarbons were the major

Table 1. Volatile compounds of *Schisandra chinensis* (omija) leaves extracted by simultaneous distillation–extraction and headspace solid-phase extraction

		RI[b]		Concentration (mean ± SD)	Area (%)[c]			
No[a]	Compound	DB-5MS	DB-Wax	($\mu g\,g^{-1}$)	$SDE_{CH_2Cl_2}$[d]	$SPME_{PDMS}$[e]	$SPME_{CAR\text{-}PDMS}$[f]	ID[g]
	Monoterpene hydrocarbons							
1	α-Thujene	925	—[h]	0.8 ± 0.2	0.1	0.3	0.1	B
2	α-Pinene	936	1009	5.2 ± 0.8	0.8	1.3	0.3	A
3	Camphene	945	—	0.4 ± 0.1	tr[i]	0.1	—	A
4	Sabinene	971	1108	4.1 ± 0.9	0.7	2.8	1.0	A
5	β-Pinene	975	1090	9.0 ± 0.8	1.4	2.7	0.7	A
6	Myrcene	989	1153	2.8 ± 0.6	0.4	1.0	1.5	A
7	α-Phellandrene	1004	1149	1.4 ± 0.4	0.2	0.1	0.2	A
8	α-Terpinene	1015	1163	1.7 ± 0.3	0.3	0.1	tr	A
9	*p*-Cymene	1023	1254	1.0 ± 0.3	0.2	0.8	0.9	A
10	Limonene	1026	1181	2.7 ± 0.4	0.4	0.5	0.5	A
11	β-Phellandrene	1028	—	0.33 ± 0.0	Tr	0.1	0.2	B
12	(*Z*)-β-Ocimene	1036	1218	6.7 ± 1.1	1.0	1.9	2.2	A
13	(*E*)-β-Ocimene	1046	1243	47.3 ± 12.2	7.2	10.5	18.4	A
14	γ-Terpinene	1057	1228	4.7 ± 0.5	0.7	0.5	0.5	A
15	α-Terpinolene	1089	1266	1.0 ± 0.1	0.2	0.2	0.2	A
	Sesquiterpene hydrocarbons							
16	δ-Elemene	1333	—	1.0 ± 0.3	0.2	—	—	B
17	α-Copaene	1374	1472	3.1 ± 0.5	0.5	1.4	0.9	B
18	β-Bourbonene	1385	1502	8.2 ± 0.8	1.3	1.9	1.0	B
19	β-Elemene	1388	1580	114.8 ± 27.1	17.4	8.2	4.3	B
20	α-Gurjunene	1413	—	7.8 ± 2.7	0.8	—	—	B
21	β-Caryophyllene	1417	—	4.0 ± 1.5	0.6	1.1	0.6	A
22	Calarene	1430	—	1.6 ± 0.3	0.3	0.3	tr	B
23	α-Humulene	1450	1649	15.6 ± 3.9	2.4	0.8	0.4	B
24	(*E*)-β-Farnesene	1453	—		—	3.84	1.1	B
25	α-Amorphene	1474	—		—	tr	0.5	B
26	Germacrene D	1480	1699	154.0 ± 40.3	22.6	26.3	6.7	B
27	β-Selinene	1482	—	0.4 ± 0.1	tr	tr	0.4	B
28	(*Z*,*E*)-α-Farnesene	1486	1720	67.2 ± 14.2	10.1	14.5	2.7	C
29	α-Muurolene	1499	1700	4.1 ± 0.7	0.5	—	—	B
30	(*E*,*E*)-α-Farnesene	1506	1715	5.6 ± 0.5	0.7	—	—	A
31	Germacrene A	1509	1738	10.7 ± 1.7	1.7	4.2	—	B
32	γ-Cadinene	1511	—	7.6 ± 1.1	1.2	1.5	1.0	B
33	δ-Cadinene	1516	1738	19.4 ± 4.4	3.0	0.7	0.7	B
34	α-Elemene	1638	—	5.4 ± 1.1	0.6	—	—	C
	Terpene oxygenates							
35	Linalool	1098	1530	0.9 ± 0.2	0.2	—	—	A
36	Terpine-4-ol	1178	1589	3.4 ± 0.5	0.6	tr	tr	A
37	α-Terpineol	1190	—	0.6 ± 0.2	0.1	—	—	A
38	Thymol methyl ether	1230	—	0.4 ± 0.1	tr	tr	—	B
39	Linalyl acetate	1251	—	0.7 ± 0.1	tr	—	—	A
40	Bornyl acetate	1285	—	0.4 ± 0.1	tr	—	—	A
41	Citronellyl acetate	1347	—	0.6 ± 0.1	0.1	—	—	A
42	(*E*)-Nerolidol	1558	2020	27.2 ± 9.3	4.1	0.6	0.1	A

(*continued overleaf*)

Table 1. Continued

No[a]	Compound	RI[b] DB-5MS	RI[b] DB-Wax	Concentration (mean ± SD) ($\mu g\,g^{-1}$)	Area (%)[c] $SDE_{CH_2Cl_2}$[d]	Area (%)[c] $SPME_{PDMS}$[e]	Area (%)[c] $SPME_{CAR\text{-}PDMS}$[f]	ID[g]
43	T-Muurolól	1640	2160	6.8 ± 1.9	1.0	—	—	B
44	δ-Cadinol	1643	2144	1.7 ± 0.2	0.2	—	—	B
45	α-Cadinol	1652	2170	29.1 ± 7.5	4.3	—	—	B
46	Phytol	2128	>2200	4.4 ± 1.9	0.7	—	—	B
	Aliphatic aldehydes, alcohols and ketones							
47	(*Z*)-3-Hexenal	803	1121	7.7 ± 2.6	1.2	1.9	2.9	A
48	(*E*)-2-Hexenal	855	1207	58.1 ± 18.0	8.7	9.6	46.0	A
49	(*E*,*E*)-2,4-Hexadienal	914	—	0.4 ± 0.1	tr	0.1	3.7	B
50	(*Z*)-3-Hexenol	—	1375	3.9 ± 0.1	0.4	—	—	B
51	Nonanal	1104	—	1.1 ± 0.2	0.2	—	tr	A
52	2-Undecanone	1290	—	1.7 ± 1.6	0.3	—	—	A

[a] Numbers correspond to those in Table 2.
[b] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references.
[c] Relative percentage of GC peak area ($n = 3$).
[d] SDE by CH_2Cl_2.
[e] SPME using a PDMS fiber.
[f] SPME using a CAR-PDMS fiber.
[g] Identification: A, mass spectrum and retention index were consistent with those of an authentic standard; B, mass spectrum was identical to that of the Wiley 275 mass-spectrum database (Hewlett-Packard, 1995), and retention index was consistent with that of literature;[14,15] C, mass spectrum was consistent with that of the Wiley 275 mass-spectrum database (tentative identification).
[h] Not detected.
[i] Trace amount (less than 0.1% of total peak area).

compounds. However, CAR-PDMS showed higher extraction efficiency for carbonyl compounds (Nos 47–49).

Aroma-active compounds by SDE–GC-O

In the AEDA of the SDE extract (Table 2), FD factors were based on the AEDA evaluations of one panelist, because the responses of all three panelists were very similar (the FD factors differed no more than twofold between the panelists). For better separation of aroma-active compounds, AEDA was conducted using two types of columns with different polarities (DB-5MS and DB-Wax).

Aroma-active compounds detected in omija leaves and their aroma properties are given in Table 2. (*E*,*Z*)-2,6-Nonadienal (No VII), with high FD factors (243–729), was the most intense aroma-active compound on both columns. This compound was identified by comparing its aroma quality and RIs with those of its authentic standard, since it was not detectable by either GC-MS or GC-FID. However, (*E*,*Z*)-2,6-nonadienal emitted a strong cucumber-like aroma at the sniffing port, probably due to its low threshold value ($0.0009–0.0025\,\mu g\,l^{-1}$)[30] and since it is known as the unique aroma of violet leaf,[31] cucumber and muskmelon.[32]

(*Z*)-3-Hexenal (No 47) and (*E*)-2-hexenal (No 48) were also identified as the key compounds contributing to the aroma of omija leaves, with a relatively high FD factor of 243. (*Z*)-3-Hexenal, exhibiting a green and apple-like aroma, has been identified as the most abundant volatile compound in tomato,[33] and also as the most potent aroma-active compound responsible for the green aroma in orange juice.[34] (*E*)-2-Hexenal, with a strong fruit, sweet-like aroma, has been reported as the important aroma-active compound of *M sativa* complex flowers.[25]

(*E*)-β-Ocimene (No 13) with high FD factors of 81–243, showing a wintergreen and grass-like aroma, was identified as another important aroma-active compound in omija leaves. β-Pinene (No 5) compound with a plastic and pine-like aroma showed a relatively high FD factor (81), and is the most potent aroma-active compound of the pine aroma of *Piper nigrum*.[21] β-Pinene was also identified as the most abundant volatile compound in resin oil of *Pistacia lentiscus*,[35] *Argyranthemum adauctum* (Link) Humphries[36] and *Sideritis bilgerana*.[37]

Hexanol (No I, green/grassy), methional (No II, boiled potato/roasty), myrcene (No 6, plastic/balsamic), γ-terpene (No 14, gasoline/herbaceous), α-terpinolene (No 15, plastic/petroleum), linalool (No 35, floral/fragrant), nonanal (No 51, citrus/refreshing), citronellal (No VI, fresh/green), and terpinen-4-ol (No 36, grass/musty) were detected with low FD factors in the range of 9–81. In addition, five compounds from SDE extracts were not identified (Nos III, IV, V, VIII and IX).

Aroma-active compounds by HS-SPME–GC-O

In HS-SPME–GC-O of omija leaves, 14 aroma-active compounds were detected with different FD factors, depending on the type of fiber used. Sesquiterpene hydrocarbons, which are preferentially extracted by the PDMS fiber, did not show relatively high FD factors. In contrast, aliphatic aldehydes such as (*Z*)-3-hexenal (No 47) and (*E*,*Z*)-2,6-nonadienal (No VII) exhibited relatively high FD factors (64). However, all

Table 2. Aroma-active compounds of *Schizandra chinensis* (omija) leaves extracted by simultaneous distillation–extraction and headspace solid-phase extraction

	RI[b]				SDE (FD factor[c])		SPME (FD factor[d])	
No[a]	DB-5MS	DB-Wax	Compound	Odor	DB-5MS	DB-Wax	PDMS[e]	CAR-PDMS[f]
47	799	1125	(*Z*)-3-Hexenal	Green, apple	243	243	64	32
48	857	1209	(*E*)-2-Hexenal	Fruity, sweet	243	243	8	64
I	869	1338	Hexanol	Green, grassy	27	81	—[g]	—
II	911	1450	Methional	Boiled potato, roasty	27	9	4	4
III	927	1327	Unknown	Green, roasted, savory	27	9	—	—
5	980	1086	β-Pinene	Plastic, pine, green	81	81	8	32
6	990	1150	Myrcene	Plastic, balsamic	9	9	1	2
13	1044	1240	(*E*)-β-Ocimene	Wither green, grass	243	81	2	8
14	1054	—	γ-Terpinene	Gasoline, herbaceous	9	—	1	—
IV	1079	1586	Unknown	Grass, dry	9	81	—	—
15	1091	1263	α-Terpinolene	Plastic, petroleum	81	27	2	1
35	1099	1528	Linalool	Floral, fragrant	27	81	—	2
51	1103	1370	Nonanal	Citrus, waxy, refreshing	9	27	—	—
V	1128	1368	Unknown	Green, dusty	81	27	—	—
VI	1148	—	Citronellal	Fresh, green	9	—	2	2
VII	1153	1560	(*E*,*Z*)-2,6-Nonadienal	Cucumber	729	243	64	64
VIII	1158	1514	Unknown	Grass	81	243	8	8
36	1180	1591	Terpinen-4-ol	Grass, musty	27	27	—	2
IX	1191	1637	Unknown	Dry, dust, sweat	9	27	2	2

[a] Numbers correspond to those in Table 1. Roman numerals represent compounds that were not detected by GC-MS.
[b] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references.
[c] Flavor-dilution factor for SDE by CH_2Cl_2.
[d] Flavor-dilution factor for SPME on DB-5MS column.
[e] SPME using a PDMS fiber.
[f] SPME using a CAR-PDMS fiber.
[g] Not detected.

other aroma compounds extracted using the PDMS fiber had relatively low FD factors in the range 1–8.

Among the volatiles extracted using the CAR-PDMS fiber, which has high absorption efficiency for aliphatic aldehydes, (*E*)-2-hexenal (No 48) and (*E*,*Z*)-2,6-nonadienal (No VII) resulted in a FD factor (64) that was higher than those of other aroma compounds. Also, (*Z*)-3-hexenal and β-pinene showed a relatively high FD factor (32). The FD factors of all other aroma compounds extracted by the CAR-PDMS fiber ranged from 1 to 8.

In the analysis using HS-SPME–GC-MS, significant differences were shown in the relative abundances of volatile compounds between the SPME fibers used, probably due to the different selectivity of the SPME fibers. In HS-SPME–GC-O, the identified aroma-active compounds were similar regardless of the SPME fiber type. It is additionally noted that most of the aroma-active compounds identified by HS-SPME–GC-O agreed well with those from SDE.

CONCLUSIONS

Volatile compounds of omija (*Schizandra chinensis*) leaves extracted using the two methods of SDE and SPME were analyzed by GC-MS, and their aroma-active compounds were identified by GC-O. The SDE–GC-MS analysis revealed that germacrene D and β-elemene were the major volatile compounds of omija leaves. In the GC-O analysis of the volatile compounds, (*E*,*Z*)-2,6-nonadienal, (*Z*)-3-hexenal and (*E*)-2-hexenal were identified as the major aroma-active compounds, on the basis of their high FD factors and their aroma properties being similar to the overall aroma of the plant regardless of extraction method (SDE or SPME). (*E*)-β-Ocimene and β-pinene were also important to the overall aroma of omija leaves. These results imply that the predominant volatile compounds are not necessarily the same as the aroma-active compounds.

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