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Headspace analyses of *Gastrodia elata* blume by solid-phase microextraction

Sang-Yeol Ahn[1], Hyang-Sook Choi[2]*

[1]Banpo High School, 30-19, Banpo-Dong, Seocho-Gu, Seoul 137-041, (KOREA)

[2]Department of Food and Nutrition, Duksung Women's University, 419, Sangmun-Dong, Tobong-Gu, Seoul 132-714, (KOREA)

Tel.: +82 2 901 8380; fax: +82 2 901 8372.

E-mail : hschoi@duksung.ac.kr

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ABSTRACT

The volatile composition of the headspace from *Gastrodia elata* Blume was investigated. The volatile constituents were absorbed by a solid-phase microextraction fiber and directly transferred to a GC-MS. Volatile compositional changes of *Gastrodia elata* Blume prepared via different drying method (hot air, freeze, and shade drying methods) were also determined. A total 91 volatiles constituents were confirmed in the headspace from these samples. Acids were predominant in the headspace volatiles of *Gastrodia elata* Blume: fresh, 17.81%; hot air-dried, 24.45%; freeze-dried, 20.54%; and shade- dried, 39.18%. Hexadecanoic acid was the most abundant volatile component in all samples. Dehydro carveol, tetradecenal, cinnamyl alcohol, hexadecanoic acid, and hexadecenoic acid showed the greatest percent difference of all the volatile constituents from 4 *Gastrodia elata* Blume samples. Tetradecenol concentration in the hot air-dried sample was significantly increased.

KEYWORDS

Gastrodia elata blume;
Hheadspace composition;
Solid-phase microextraction;
Drying method.

INTRODUCTION

The importance of aromatic plants is considerable owing to their applications in folk medicine and their potential for commercial value in various fields as spices, beverages, perfumery, cosmetics, pharmaceutics, and aromatherapy[1]. *Gastrodia elata* Blume(Orchidaceae), which grown in the woods of Korea, Japan and the central province of China, has traditionally been used for the treatment of a variety of conditions including neuralgia, paralysis, lumbago, headache, and other neuralgic and nervous problems as raw and dried tubers[2]. *Gastrodia elata* Blume (GE) is an effective analygesic and antispasmodic agent to subdue the hyperactivity of the liver, relieve muscular and treat infantile convulsion[3-4].

There are many pharmacological and nutritional studies on GE[3-9]. GE influenced the elevation of gamma aminobutyric acid (GABA) concentration by inhibiting GABA shunt. GE also demonstrated other pharmacological activities such as anti-inflammatory and inhibitory activity on nitric oxide level in rat model[6,10]. Chung and Ji[7] reported the composition and functionality of GE, and Heo et al.[9] studied the antioxidant and antitumor activities of GE. Hong et al.[8] evaluated the quality characteristics of the beverage with GE. Many researchers have been tried to use of GE in food industries[10-11].

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GE is an important food and medicinal material in Korea (Cheonma in Korean) and China (Tianma in Chinese). Although many papers on functionality of GE are published, but there seem to have been a few reports concerned with volatile flavor composition[12-13]. The economic importance of edible plants and their usefulness in the food industries necessitate the acquisition of accurate compositional data.

Solid-phase microextraction (SPME) is a solvent-free extraction technique for organic compounds and flavor, in which analytes are absorbed directly from the sample into a fused-silica fiber coated with either thin film polymers in the stationary phase (e.g., Carbowax, divinylbenzene (DVB), polyacrylate (PA), polydimethylsiloxane (PDMS)) or a mixture of polymers blended with a porous carbon-based solid material (e.g., PDMS-Carboxen)[14-16]. SPME has been successfully utilized for the qualitative analysis of many food substances and flavors. However, the available fibers are not consistently responsive to all compounds. The extraction efficiency of solutes by SPME could be different when the headspace, composed of different classes of compounds, is quantitatively analyzed[16]. The fibers for SPME consist of a fixed liquid film or fixed solid sorbents, and their length and thickness depend on the coatings[17]. Several fiber coatings are commercially available for the extraction of volatile and semivolatile compounds. In PDMS and PA phases, volatiles are extracted via absorption by dissolving and diffusing them into the bulk of the coating. Carbowax-DVB, PDMS-Carboxen and PDMS-DVB, which are mixed coatings, extract via adsorption with analytes staying on the surface of the fiber. Extraction efficiency, precision and reproducibility by SPME technique can be affected by modifying the matrix of the fiber, extraction and desorption time, incubation temperature, sample volume, and other sample treatments[15,18-20].

There are some reports on the volatile oil composition from GE[12-13]. Despite the useful plant resource, no detailed analysis of headspace volatiles from GE have been reported. The objective of this study was to identify the headspace volatiles of *Gastrodia elata* Blume and to investigate the changes of volatiles effected by various drying methods by using SPME.

EXPERIMENTAL

Plant material

GE tuber was obtained from the NangSeong-Gastrodia farm of Chungbuk, Korea. It was harvested at its optimal edible stage (2007). Authentic chemicals for co-injection in gas chromatography and mass spectrometry were obtained from reliable commercial sources as follows: Aldrich Chemical Co. (WI, USA), Sigma Chemical Co. (MO, USA), PolyScience Co. (IL, USA), AccuStandard, Inc. (CT, USA), Theta Co. (PA, USA), and Wako Pure Chemical Industries (Osaka, Japan).

The water activity of each sample was tested by AquaLab (Decagon Devices Inc., Washington, USA, model series 3TE) at room temperature. GE samples were analyzed colorimetrically in three replications by using a Color and Color Difference Meter (Color Techno system Co., Ltd., Tokyo, Japan) coupled with a spectrophotometer (Shimadzu Co., Kyoto, Japan, UV-1601PC). The results were expressed as HunterLab L (whiteness/darkness), a (red/green), b (yellow/blue), and color difference (ΔE) with standard, white color.

Drying method

In this study three different drying methods were adopted in GE tuber. A sample of the fresh tuber was hot air-dried by using a domestic hot air-drying oven (Dongyang Science Co., Kwangju, Korea, model 1060) for 24 hours at 50^0C. A fresh GE sample from the same batch was dried in a freeze-dryer (Ilshin Laboratory Co. Ltd., Seoul, Korea, model FD 5505) at -54^0C and under vacuum (7 mm Torr) overnight. A third sample of fresh tuber was placed in shady place (~20^0C) that was well-ventilated for 3 days.

SPME

In this study, a 100 m PDMS fiber (Supelco, Inc., Bellefonte, PA) was used because this particular stationary phase material showed a higher affinity toward volatile compounds at low concentrations in previous tests. The SPME fiber was exposed to the headspace of samples varied from 30 to 70 min to establish the best extraction time, and 50 min was determined to be the optimal extraction time. A 3 g fresh sample and 0.5 g of each dried samples were hermetically sealed in a 10 mL vial from Supelco having a silicone septum and

an aluminum cap. The stainless steel needle containing PDMS fiber was inserted through the septum of the sample vial in order to sample the headspace for 50 min by using a Varian 8200 autosampler (Walnut Creek, CA).

Gas chromatography (GC)

After the extraction by the PDMS fiber, the volatile compounds were desorbed for 2 min into the injector of a GC (Agilent 6890N, CA, USA) equipped with a DB-Wax fused-silica capillary column (60 m × 0.25 mm i. d., film thickness 0.25 μm, J and W Scientific, Folsom, CA, USA) and a flame ionization detector. The column temperature was programmed from 70^{0}C (2 min for desorption) to 230^{0}C (20 min) at a rate of 2^{0}C/min. The injector and detector temperatures were both 250^{0}C. Nitrogen as a carrier gas was used at a flow rate of 1 mL/min. The headspace gas of this aromatic plant was injected into GC, and the splitless mode was adopted. The retention indices (RIs) were calculated for all volatile components using a homologous series of n-alkanes (C_7-C_{29}) under the same GC conditions.

GC-mass spectrometry (GC-MS)

The GC was interfaced with a Varian Saturn 2000R MS to identify of the volatile compounds. SPME was injected into the GC-MS system using a Varian 8200 Autosampler (Walnut Creek, CA, USA). After the 50 min extraction time, the volatile compounds were desorbed for 2 min into the injector of a Varian Saturn 2000R 3800 GC (Walnut Creek, CA, USA) equipped with a DB-Wax fused silica capillary column (60 m × 0.25 mm i. D., film thickness 0.25 μm, J and W Scientific, Folsom, CA, USA). Helium was the carrier gas at a flow rate of 1.1 mL/min. The column temperature was programmed from 70^{0}C (2 min for desorptiom) to 230^{0}C (20 min) at a rate of 2^{0}C/min. The injector temperature was 250^{0}C and the injector split ratio was 34 to 1.

Identification of components

Components were identified by comparing their RIs and matching their mass spectra with those of reference compounds in the data system of Wiley library and NIST Mass Spectral Search Program (ChemSW. Inc., NIST 98 Version Database) connected to a Varian Saturn 2000R mass spectrometer. The volatile flavor components were also matched by co-injection with authentic compounds. The results were expressed as the average of the peak area percentages in duplicate.

TABLE 1: Water activities *Gastrodia elata* blume

Drying method	Water activity
Fresh	0.976/25.8^{0}C
Hot air-dried	0.260/25.2^{0}C
Freeze-dried	0.539/25.3^{0}C
Shade-dried	0.539/25.3^{0}C

TABLE 2: Color and color difference of *Gastrodia elata* blume

	L	a	b	ΔE
Fresh	86.04	-1.68	8.20	15.32
Hot air-dried	90.07	-1.27	10.48	13.95
Freeze-dried	94.78	-1.44	6.44	8.07
Shade-dried	90.31	-0.69	9.56	13.03

Statistical analysis

GC peak area percents of some volatile flavor compounds (carveol, geranyl butyrate, perilla acetate, dehydro carveol, tetradecenal, tetracosane, pentacosane, hexadecanoic acid, and hexadecenoic acid) were subjected to one-way analysis of variance ($p < 0.05$) using the Statistical System software package[21]. Significant differences between means by duplicate tests were determined by Duncan's multiple-range test.

RESULTS AND DISCUSSION

Headspace composition of fresh GE

The initial water activity of the GE tuber was 0.976 (TABLE 1). Hunter L, a, b, and ΔE values of fresh sample were 86.04, -1.68, 8.20, and 15.32, respectively. The identified volatile components and their peak area percents are given in TABLE 3. The components are listed in order of their elution on the DB-Wax column. A classification based on functional groups is summarized in TABLE 4. Eighty-six volatile flavor components, constituting 67.94% of the total volatile composition of the headspace, including 14 hydrocarbons (8.32 % peak area), 10 aldehydes (6.72%), 25 alcohols (13.87%), 3 ketones (2.01%), 12 esters (4.14%), 6 oxides and epoxides (2.12%) and 13 acids (17.81%), were identified from the GE tuber by SPME method (TABLES 3 and 4). Acids were predominant in fresh GE volatiles.

Hexadecanoic acid was the most abundant component (4.90%), followed by tetracosane (2.80%),

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TABLE 3: Volatile flavor components of *Gastrodia elata* blume

No. compd.		RI	Peak area percent			
			Fresh	Hot air-dried	Freeze-dried	Shade-dried
1	Ethyl acetate	990	0.06	0.30	0.02	0.03
2	Camphene	1089	0.02	0.04	0.01	tr
3	2-Pentenal	1126	-	0.02	-	-
4	Sabinene	1134	-	-	0.01	-
5	δ-3-Carene	1157	0.01	-	0.04	-
6	α-Terpinene	1190	0.02	-	-	-
7	Limonene	1231	0.01	-	-	-
8	γ-Terpinene	1292	0.01	-	-	-
9	Terpinolene	1312	-	-	0.01	-
10	2-Hexenyl acetate	1332	0.02	-	-	-
11	Nonanal	1394	0.02	-	-	-
12	Tetradecene	1451	0.02	-	-	-
13	*cis*-Limonene oxide	1451	0.01	-	0.01	-
14	Linalool oxide isomer	1493	-	-	0.01	-
15	β-Cubebene	1540	0.09	-	-	-
16	cis-Limonene epoxide	1562	0.02	-	-	-
17	Nonyl acetate	1586	0.01	-	-	-
18	Myrcenol	1621	0.19	0.09	0.10	0.06
19	Trans-2-Decenol	1645	0.17	-	-	-
20	cis-β-Farnesene	1658	0.09	-	-	-
21	Trans-β-Farnesene	1672	0.09	-	-	-
22	Trans-Piperitol	1693	0.27	0.43	-	-
23	α-Terpineol	1702	0.12	-	-	-
24	Dodecanal	1716	0.22	0.39	-	-
25	Neryl acetate	1737	0.32	0.69	1.29	0.15
26	Carvone	1744	0.16	-	-	-
27	*cis*-Linalool pyran oxide	1753	0.31	-	-	-
28	Sesquiphellandrene	1778	0.61	1.42	0.46	
29	Cumin aldehyde	1810	0.53	0.80	0.57	0.20
30	Tridecanal	1810	0.56	1.19	0.65	0.29
31	Geranyl propionate	1826	0.39	-	-	-
32	Carveol	1841	0.79^{a}	2.37^{b}	1.27^{c}	0.75^{a}
33	*p*-Mentha-1,8-dien-10-ol	1858	0.84	1.44	0.79	0.47
34	Geranyl butyrate	1872	0.93^{a}	2.25^{b}	1.09^{c}	0.70^{d}
35	Dodecyl acetate	1891	1.06	-	1.00	0.66
36	Perilla acetate	1903	1.18^{a}	2.60^{b}	1.34^{c}	0.78^{d}
37	Tetradecanal	1929	1.35	-	-	-
38	Dehydro carveol	1939	1.48^{a}	6.20^{b}	4.11^{c}	2.19^{d}
39	β-Ionone	1955	1.04	-	-	-
40	Tetradecenal	1968	1.57^{a}	7.01^{b}	0.34^{c}	0.71^{d}
41	Hymullene epoxide	1972	0.74	2.03	0.26	2.20
42	*cis*-β-Caryophyllene	1979	2.25	-	0.69	2.65
43	*trans*-Caryophyllene epoxide	2007	0.59	0.84	0.38	3.47
44	Ledol	2020	0.41	-	-	-
45	*trans*-Dodec-2-enol	2029	0.55	1.10	0.68	-
46	*trans*-Nerolodol	2044	0.31	0.29	0.25	0.25
47	Octanoic acid	2053	0.31	0.28	0.22	0.34
48	Globulol	2061	0.24	0.18	0.20	0.09
49	Elemol	2091	0.34	0.25	0.28	0.14
50	Viridifloral	2098	0.28	0.16	0.19	0.10
51	Cedrol	2115	0.66	0.37	0.54	0.23
52	Spathulenol	2128	0.31	0.12	0.45	0.08
53	Cedrenol	2136	0.22	0.09	0.16	0.04
54	Cedryl acetate	2151	0.19	0.32	0.51	0.16
55	Cadinol	2161	0.51	0.16	0.52	0.10
56	Thymol	2174	0.59	0.21	0.46	0.12
57	Eugenol	2186	0.63	0.15	0.40	0.07
58	*p*-Vinyl guaiacol	2199	-	0.47	0.66	1.34
59	Nonanoic acid	2200	0.65	-	-	-
60	α-Bisabolol	2210	0.41	0.15	0.37	-
61	Isothymol	2223	0.62	0.13	0.44	0.01
62	β-Sinensal	2235	0.67	0.08	0.57	0.05
63	β-Eudesmol	2247	0.49	0.09	0.44	0.08
64	Isoeugenol	2257	0.50	0.09	0.44	0.04
65	Ethyl hexadecanoate	2267	0.56	0.09	0.54	0.06
66	*trans*,*trans*-Farnesyl acetate	2275	0.41	0.07	0.39	0.07
67	Cinnamyl alcohol	2301	2.51	-	-	-
68	Methyl heptadecanoate	2337	1.23	0.79	1.14	0.30
69	*cis*,*trans*-Farnesol	2343	0.35	0.22	0.31	-
70	*trans*,*trans*-Farnesol	2350	0.36	0.20	0.39	0.33
71	Methyl palmitate	2357	0.31	0.13	0.33	0.17
72	Ethyl heptadecanoate	2362	0.25	0.20	0.36	0.30
73	Decanoic acid	2367	0.55	0.48	0.67	0.12
74	Nerol oxide	2376	0.45	0.38	0.75	0.22
75	Octadecanal	2388	0.73	0.38	0.72	0.29
76	2-Hydroxy-4-methyl acetophenone	2400	0.81	0.47	0.83	0.27
77	Undecanoic acid	2414	0.76	0.55	0.83	-
78	Methyl ctadecanoate	2434	0.75	0.58	0.77	0.60
79	Undecanal	2446	0.79	1.34	0.83	0.70
80	Octadecanoic acid	2465	0.86	0.84	0.89	0.89
81	Dodecanoic acid	2470	0.84	0.86	0.86	3.52
82	Tetradecanoic acid	2487	1.01	1.03	1.07	3.41
83	Lauric acid	2492	0.77	0.83	0.80	3.02
84	Pentadecanoic acid	2500	1.14	1.14	1.20	1.66
85	Pentedecenoic acid	2521	1.02	0.82	1.08	1.43
86	Tricosane	2530	1.08	2.02	1.10	0.86
87	Tetracosane	2543	2.80^{a}	3.33^{b}	3.05^{c}	5.14^{d}
88	Pentacosane	2575	1.04^{a}	0.98^{b}	1.25^{c}	4.13^{d}
89	Hexadecanoic acid	2598	4.90^{a}	10.37^{b}	8.82^{c}	15.88^{d}
90	Hexadecenoic acid	2614	2.35^{a}	2.43^{b}	2.79^{c}	7.10^{d}
91	Heptadecanoic acid	2633	2.65	2.82	1.31	2.70
			67.94	81.40	67.36	81.52

Values with the different superscripts are significantly different($p < 0.05$)

TABLE 4: Constitution of functional groups in the headspace volatiles of *Gastrodia elata* blume

Functional group	Peak area percentage							
	Fresh		Hot air-dried		Freeze-dried		Shade-dried	
	Total no	Peak area %	Total no	Peak area %	Total no	Peak area %	Total no	Peak area %
Hydrocarbons								
Aliphatics	4	5.12	3	6.33	3	5.40	3	10.13
Monoterpenes	5	0.07	1	0.04	4	0.07	1	tr
Sesquiterpenes	5	3.13	1	1.42	2	1.15	1	2.65
Aldehydes								
Aliphatics	7	5.24	6	10.33	4	2.54	4	1.99
Terpenes	3	1.48	3	1.04	3	1.33	3	0.35
Alcohols								
Aliphatics	2	0.72	1	1.10	1	0.68	-	-
Monoterpenes	11	8.54	10	11.58	9	8.67	9	5.05
Sesquiterpenes	12	4.61	11	2.12	11	3.91	9	1.34
Ketones	3	2.01	1	0.47	1	0.83	1	0.27
Esters	12	4.14	11	8.02	12	8.78	12	3.98
Oxides and epoxides	6	2.12	3	3.25	5	1.41	3	5.89
Acids	13	17.81	12	24.45	12	20.54	10	39.18
Total	86	67.94	63	81.40	67	67.36	57	82.41

heptadecanoic acid (2.65%), cinnamyl alcohol (2.51%) and hexedecenoic acid (2.35%). Lee and Kim[12] reported the volatile flavor constituents of GC by a simultaneous steam distillation and extraction method. They identified 39 components in the fresh GE, and reported the hexadecanoic acid as the most abundant component.

Monoterpene alcohols were the abundant oxygenated compounds in fresh GE flavor, and cinnamyl alcohol was the most predominant component (2.51%), followed by dihydro carveol (1.48%), p-mentha-1,8-dien-10-ol (0.84%), carveol (0.79%) and thymol (0.59%). Cinnamyl alcohol is widely used in perfume compositions including many floral fragrances and soap perfumes, and in flavor compositions for imitation apricot, brandy, cinnamon, grape, raspsberry, strawberry and walnut for its warm-balsamic flavor[22]. The total amount of aldehydes in volatile composition was 6.72 %, with tetradecenal (1.57%) and tetradecanal (1.35%) being the main components. Ketone occurred in low level (2.01%), with β-ionone (1.04%) being the main component. Twelve ester components (4.14%) were confirmed in headspace flavor of this sample with methyl heptadecanoate (1.23%) being the main component.

Headspace composition of dried GE

Volatile Compositional changes of GE on the different drying methods [hot air-drying (HD), freeze-drying (FD) and shade-drying (SD)] were also investigated. The final water activity and the Hunter L, a, b, and ΔE values of each dried samples were shown in TABLES 1 and 2. Comparison of the volatile flavor profiles of the fresh and dried GE samples revealed distinct qualitative and quantitative differences (TABLES 3 and 4). The headspace of dried GE was composed of hydrocarbons (HD, 7.79%; FD, 6.62%; SD, 12.78%), aldehydes (HD, 11.37%; FD, 3.87%; SD, 2.34%), alcohols (HD, 14.80%; FD, 13.26%; SD, 6.39%), ketones (HD, 0.47%; FD, 0.83%; SD, 0.27%), esters (HD, 8.02%; FD, 8.78%; SD, 3.98%), oxides and epoxides (HD, 3.25%; FD, 1.41%; SD, 5.89%) and acids (HD, 24.45%; FD, 20.54%; SD, 39.18%).

Acids were prominent in headspace volatiles. Hexadecanoic acid was the most abundant volatile component of hot air-, freeze-, and shade-dried samples (10.37, 8.82, and 15.88%, respectively). Dehydro carveol, tetradecenal, cinnamyl alcohol, hexadecanoic acid, and hexadecenoic acid showed the greatest percent difference of all the volatile constituents from 4 GE samples. The content of aliphatic hydrocarbons, oxides and epoxides, and acids were the highest in the shade-dried sample, where aliphatic and terpene aldehydes, monoterpene and sesquiterpene alcohols, ketones, and esters showed the opposite predominance. The oxygenated compounds are important contributors to plants flavor. α-Terpineol is a desirable flavor in many fruits, whereas in others it is perceived as an off-

flavor described as terpentine-like, camphoraceous, stale, musty and pungent[23-24]. It is a degradation product of essential oil components in some fruit juice. It occurs in aged orange juice. Therefore it has been proposed as a quality indicator of essential oil components in some fruit juices[25]. α-Terpineol content was found only in fresh sample (0.12%).

Fresh GE tuber had the greatest total number of volatile flavor compounds. The newly identified compounds in hot air-dried sample in comparison with fresh one were 2-pentenal, and *p*-vinyl guaiacol. Sabinene, terpinolene, and linalool oxide isomer were the compounds identified in only freeze-dried sample. The freeze-dried sample had a low hydrocarbon, and oxides and epoxides. Shade-dried GE had the highest acid content.

TABLE 3 shows the effect of drying methods on the abundance of nine volatile compounds marked with superscripts. Concentration of compounds, carveol, geranyl butyrate, perilla acetate, dehydro carveol, tetradecenal, tetracosane, pentacosane, hexadecanoic acid, and hexadecenoic acid showed significantly different patterns depending on the method used to dry the tuber. However, the level of carveol in shade-dried sample was not significantly different from level found in the fresh sample. The headspace of freeze-dried GE was characterized by its high contents of carveol, geranyl acetate, perilla acetate, dehydro carveol, and tetra decenol. Tetracosane, pentacosane, hexadecanoic acid and hexadecenoic acid concentrations in shade-dried sample were significantly increased upon drying by shade-drying method. The amount of geranyl acetate, perilla acetate and tetradecenal were decreased in shade-dried sample, but not in the hot air-dried sample. The significant increase of tetradecenol concentration in the hot air-dried sample was noteworthy.

CONCLUSION

Some of the differences between the volatile compounds profiles of plants materials using different drying methods were able to be detected instrumentally. Due to both economic importance and academic interest, research has been carried out on the identification of volatile flavor compounds of foods on various processing conditions. The effect of drying methods on volatile components is often regarded as a critical factor of food flavor quality. An understanding of the flavor quality according to drying methods requires that a relatively complete quantitative and qualitative database be developed. From these experiments it is concluded that the headspace volatiles of GE was found to be a rich source of acids. Hexadecanoic acid was the most abundant component. Comparing the volatile components of the fresh and three differently dried samples showed distinct qualitative and quantitative differences. An organoleptic comparison of the volatile flavor components from fresh and dried samples will provide more detailed information about the odor-active components of these samples.

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