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Differences in the Volatile Compositions of Ginseng Species (*Panax* sp.)

In Hee Cho, Hyun Jeong Lee, and Young-Suk Kim*

Department of Food Science and Engineering, Ewha Womans University, Seoul 120-750, South Korea

ABSTRACT: The volatile compositions in dried white ginseng according to species (*Panax ginseng*, *Panax notoginseng*, and *Panax quinquefolius*) were analyzed and compared by applying multivariate statistical techniques to gas chromatography−mass spectrometry data sets. Main volatile compounds of ginseng species in the present study were sesquiterpenes, such as bicyclogermacrene, (*E*)-*β*-farnesene, *β*-panasinsene, calarene, *α*-humulene, *β*-elemene, etc. In particular, *α*-selinene, *α*-terpinolene, *β*-bisabolene, *β*-phellandrene, *β*-sesquiphellandrene, zingiberene, germacrene D, limonene, *α*-gurjunene, (*E*)-caryophyllene, *δ*-cadinene, (*E*)-*β*-farnesene, *α*-humulene, bicyclogermacrene, longiborn-8-ene, *β*-neoclovene, and (+)-spathulenol were mainly associated with the difference between *P. ginseng* and *P. notoginseng* versus *P. quinquefolius* species. On the other hand, the discrimination between *P. ginseng* and *P. notoginseng* could be constructed by hexanal, 2-pyrrolidinone, (*E*)-2-heptenal, (*E*)-2-octenal, heptanal, isospathulenol, (*E*,*E*)-2,4-decadienal, 3-octen-2-one, benzaldehyde, 2-pentylfuran, and (*E*)-2-nonenal.

KEYWORDS: *Ginseng species (Panax sp.), volatile composition, gas chromatography−mass spectrometry, hierachical cluster analysis, principal component analysis*

INTRODUCTION

Ginseng (*Panax* sp.) is a perennial semi-shade plant of the family Araliaceae.[1] It is one of the most valuable medicinal plants in the Orient, in which almost every species of the genus has been employed as a source of medicine.[2] In particular, Koreans and Chinese have used it for more than 2000 years as a tonic agent, a stimulant, and an agent to foster fatigue- and stress-resistance.[1] Recently, the pharmaceutical effects of ginseng, such as anti-aging, anti-diabetic, anti-carcinogenic, analgesic, anti-pyretic, anti-stress, anti-fatigue, tranquilizing activities, and promotion of DNA, RNA, and protein synthesis activities, have been widely investigated.[3−8] On the other hand, it has also been used for a variety of culinary dishes, including salad, soup, stew, and steamed dishes, as well as tea and other beverage. Even though many methods have been developed to cook ginseng, it can also be consumed raw, preserving its original aroma and taste. Many studies have been carried out on the pharmaceutical effects of ginseng, but a few reports have focused on its aroma compounds.[9−12] Over 40 volatiles in ginseng were tentatively identified; sesquiterpene hydrocarbons and sesquiterpenyl alcohols were found to be the major volatile compounds, and methoxypyrazines were included as the key odorants.[9,10]

The quality and chemical composition of ginseng could vary widely and substantially depend upon the species, variety, geographical origin, cultivation, environment, harvesting, storage, and postharvest processing,[13−20] because it needs to grow for 4−6 years before being consumed. In particular, some studies have shown that the pharmaceutical effects of ginseng vary according to the species.[6,17−20] *Panax ginseng* has a "warm" property and is known to replenish the "vital energy", whereas *Panax quinquefolius* has a "cooling" effect and is mainly used to reduce the "internal heat" and promote the secretion of body fluids.[18,19] In addition, the distribution of ginsenosides (ginseng saponins), which are considered to be the main bioactive compounds in ginseng, varies with the species.[6,20] The ginsenosides Rg_1, Re, Ro, Rb_1, Rc, Rb_2, and Rd are present in both *P. ginseng* and *P. quinquefolius* but in different proportions, whereas the ginsenoside Rf is only found in *P. ginseng*. In contrast, *P. quinquefolius* contains 24-(*R*)-pseudoginsenoside F_{11}, an ocotillol-type triterpene, which is absent in *P. ginseng*.[20]

Ginseng commonly comprises of 13 *Panax* species, as listed in Table 1;[21] the 3 main species are *P. ginseng* C. A. Meyer (cultivated in Korea, Japan, China, and Russia and generally referred to as ginseng species), *Panax notoginseng* (Burkill) F.

Table 1. Ginseng Species[21]

	species	cultivation area
1	*Panax ginseng* C. A. Meyer	Korea, Japan, China, and Russia
2	*Panax japonicus* C. A. Meyer	Japan
3	*Panax major* Ting	
4	*Panax notoginseng* (Burkill) F. H. Chen	China's Yunnan province
5	*Panax omeiensis* J. Wen	
6	*Panax pseudoginseng* Wallich	Nepal and the eastern Himalayas
7	*Panax quinquefolius*	southern Canada and the United States
8	*Panax sinensis* J. Wen	
9	*Panax stipuleanatus* H. T. Tsai and K. M. Feng	
10	*Panax trifolius* L.	
11	*Panax wangianus* Sun	
12	*Panax zingiberensis* C. Y. Wu and K. M. Feng	
13	*Panax vietnamensis* Ha et Grushv.	Vietnam

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Table 2. Volatile Compounds Identified in Dried White Ginseng According to Species

			relative peak areas[a]						
			P. ginseng						
number[b]	RI[c]	volatile compounds	GH[d]	GS[e]	PG[f]	GAE[g]	*P. notoginseng*	*P. quinquefolius*	ID[h]
1	800	hexanal	0.4 ± 0.0 d	0.1 ± 0.0 a	0.2 ± 0.0 b	0.7 ± 0.0 e	1.1 ± 0.0 f	0.3 ± 0.0 c	A
2	889	2-heptanone	nd a	nd a	nd a	tr b	nd a	tr b	A
3	902	heptanal	tr a	tr a	tr a	tr a	0.1 ± 0.0 b	tr a	A
4	910	dihydro-2(3*H*)-furanone	nd a	nd a	tr b	tr b	tr b	tr b	A
5	935	*α*-pinene	tr a	tr a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	0.3 ± 0.0 c	A
6	953	camphene	nd a	nd a	tr b	tr b	tr b	0.1 ± 0.0 c	A
7	957	(*E*)-2-heptenal	tr a	tr a	tr a	tr a	0.1 ± 0.0 b	tr a	A
8	965	benzaldehyde	nd a	nd a	nd a	nd a	tr b	nd a	A
9	980	*β*-pinene	0.4 ± 0.0 c	0.2 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.0 b	0.5 ± 0.0 d	0.2 ± 0.0 a	A
10	987	*β*-myrcene	0.1 ± 0.0 b	nd a	nd a	0.1 ± 0.0 b	0.2 ± 0.0 c	0.1 ± 0.0 b	A
11	989	hexanoic acid	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.4 ± 0.0 b	0.4 ± 0.0 b	0.1 ± 0.0 a	A
12	990	2-pentyl-furan	0.1 ± 0.0 c	0.1 ± 0.0 c	0.1 ± 0.0 c	tr b	nd a	nd a	A
13	1003	octanal	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	A
14	1032	limonene	nd a	nd a	nd a	tr b	tr b	1.0 ± 0.0 c	A
15	1034	*β*-phellandrene	nd a	nd a	nd a	nd a	tr b	0.1 ± 0.0 c	B
16	1038	3-octen-2-one	nd a	nd a	nd a	nd a	tr b	nd a	A
17	1053	5-ethyldihydro-2(3*H*)-furanone	nd a	nd a	nd a	tr b	nd a	tr b	A
18	1059	(*E*)-2-octenal	nd a	nd a	nd a	nd a	tr b	nd a	A
19	1072	*α*-terpinolene[i]	nd a	nd a	nd a	nd a	nd a	0.1 ± 0.0 b	A
20	1074	2-pyrrolidinone	nd a	nd a	nd a	tr b	0.1 ± 0.0 c	tr b	A
21	1077	heptanoic acid	tr b	nd a	0.1 ± 0.0 c	tr b	tr b	nd a	A
22	1085	tetramethyl-pyrazine	0.1 ± 0.0 c	0.3 ± 0.0 d	tr b	nd a	nd a	tr b	A
23	1088	2-methoxy-3-(1-methylethyl)-pyrazine[i]	nd a	tr b	tr b	tr b	tr b	nd a	A
24	1104	nonanal	tr b	tr b	tr b	tr b	tr b	nd a	A
25	1111	3-hydroxy-2-methyl-pyran-4-one (maltol)	tr a	tr a	0.3 ± 0.0 b	tr a	tr a	tr a	A
26	1131	2,5-pyrrolidinedione (succinimide)	tr b	tr b	tr b	tr b	tr b	nd a	A
27	1142	2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	nd a	0.1 ± 0.0 b	0.6 ± 0.0 c	nd a	nd a	nd a	B
28	1156	(*E*)-2-nonenal	tr a	tr a	tr a	tr a	0.1 ± 0.0 b	0.1 ± 0.0 b	A
29	1171	octanoic acid	0.1 ± 0.0 b	tr a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	A
30	1226	1-isopropyl-2-methoxy-4-methyl-benzene	nd a	nd a	nd a	0.1 ± 0.0 b	0.1 ± 0.0 b	nd a	A
31	1316	(*E*,*E*)-2,4-decadienal	0.7 ± 0.0 b	0.1 ± 0.0 a	0.1 ± 0.0 a	0.6 ± 0.0 b	1.0 ± 0.0 c	0.2 ± 0.0 a	A
32	1331	bicycloelemene[i]	1.8 ± 0.0 e	nd a	0.1 ± 0.0 b	0.8 ± 0.0 d	0.6 ± 0.0 c	nd a	B
33	1336	5-penyl-2(5*H*)-furanone	0.1 ± 0.0 b	nd a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	B
34	1358	longiborn-8-ene[i]	0.4 ± 0.0 c	0.4 ± 0.0 c	0.4 ± 0.0 c	0.3 ± 0.0 b	0.3 ± 0.0 b	0.1 ± 0.0 a	B
35	1375	longifolen-v2[i]	0.7 ± 0.0 e	0.7 ± 0.0 d	0.9 ± 0.0 f	0.7 ± 0.0 c	0.4 ± 0.0 b	nd a	B
36	1387	*β*-panasinsene	8.4 ± 0.1 d	8.3 ± 0.1 d	9.3 ± 0.2 e	7.4 ± 0.1 c	5.8 ± 0.1 b	2.4 ± 0.0 a	B
37	1389	*β*-elemene	4.5 ± 0.1 e	2.4 ± 0.0 b	5.6 ± 0.2 f	3.80 ± 0.1 d	2.6 ± 0.0 c	0.8 ± 0.0 a	B
38	1391	4-hydroxy-3-methoxy-benzaldehyde (vanillin)	nd a	nd a	0.1 ± 0.0 c	tr b	0.2 ± 0.0 d	0.1 ± 0.0 c	A
39	1408	*α*-gurjunene	1.8 ± 0.0 d	1.2 ± 0.0 b	1.7 ± 0.1 c	1.8 ± 0.0 e	1.7 ± 0.0 c	0.1 ± 0.0 a	B
40	1419	(*E*)-caryophyllene	1.3 ± 0.0 c	1.2 ± 0.0 b	1.6 ± 0.0 d	1.6 ± 0.0 d	1.3 ± 0.0 c	0.4 ± 0.0 a	A
41	1430	calarene	6.7 ± 0.1 c	4.9 ± 0.2 b	7.5 ± 0.2 d	10.6 ± 0.1 f	9.2 ± 0.0 e	1.4 ± 0.0 a	B
42	1450	(*E*)-*β*-farnesene	11.3 ± 0.8 b	6.4 ± 0.0 a	10.8 ± 1.0 b	14.4 ± 0.5 c	11.6 ± 0.5 b	45.6 ± 0.3 d	B
43	1456	*α*-humulene	5.5 ± 0.0 c	5.6 ± 0.0 c	6.4 ± 0.3 c	6.1 ± 0.0 c	3.7 ± 0.0 b	tr a	A
44	1457	neoclovene	2.1 ± 0.1 c	5.0 ± 0.0 f	3.2 ± 0.2 e	2.9 ± 0.0 d	1.8 ± 0.0 b	1.5 ± 0.0 a	B
45	1477	*β*-neoclovene	1.7 ± 0.0 e	1.6 ± 0.0 d	1.4 ± 0.1 c	1.6 ± 0.0 de	1.2 ± 0.0 b	0.5 ± 0.0 a	B
46	1478	germacrene D	nd a	nd a	nd a	nd a	nd a	tr b	B
47	1487	*β*-selinene	1.0 ± 0.0 d	1.2 ± 0.1 f	1.1 ± 0.1 e	0.9 ± 0.0 c	0.8 ± 0.0 b	0.2 ± 0.0 a	B
48	1489	zingiberene[i]	nd a	nd a	nd a	nd a	nd a	0.4 ± 0.0 b	B
49	1493	*α*-selinene	nd a	nd a	nd a	nd a	nd a	0.1 ± 0.0 b	B
50	1495	*β*-bisabolene	nd a	nd a	nd a	nd a	nd a	0.7 ± 0.0 b	B
51	1496	bicyclogermacrene	26.2 ± 0.2 c	12.6 ± 0.4 b	22.7 ± 0.6 c	24.0 ± 0.0 c	17.4 ± 0.4 b	nd a	B
52	1514	*δ*-cadinene	0.5 ± 0.0 c	0.4 ± 0.0 b	0.4 ± 0.0 b	0.3 ± 0.0 a	0.4 ± 0.0 b	0.9 ± 0.0 d	B
53	1516	*β*-sesquiphellandrene	nd a	nd a	nd a	nd a	nd a	3.3 ± 0.0 b	B

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Table 2. continued

			relative peak areas[a]						
			P. ginseng						
number[b]	RI[c]	volatile compounds	GH[d]	GS[e]	PG[f]	GAE[g]	*P. notoginseng*	*P. quinquefolius*	ID[h]
54	1557	nerolidol	nd a	0.3 ± 0.0 c	0.3 ± 0.0 d	0.3 ± 0.0 c	0.2 ± 0.0 b	0.4 ± 0.0 e	A
55	1574	(+)-spathulenol	3.1 ± 0.1 c	3.7 ± 0.1 d	2.6 ± 0.1 b	4.5 ± 0.0 e	3.2 ± 0.0 c	nd a	B
56	1575	veridiflorol	nd a	nd a	nd a	0.3 ± 0.0 c	0.2 ± 0.0 b	nd a	B
57	1620	isospathulenol	nd a	nd a	nd a	0.3 ± 0.0 b	0.9 ± 0.0 c	nd a	B

[a]Average of relative peak areas to that of the internal standard ($n = 3$) ± standard deviation. Significant differences within the same row are shown by the different letters ($p < 0.05$). [b]Numbers correspond to those in Table 3. [c]Retention indices were determined using *n*-paraffins C_7−C_{22} as external references. [d]GH = Gangwha. [e]GS = Geumsan. [f]PG = Punggi. [g]GAE = Gaesung. [h]Volatile compounds were identified on the basis of the following criteria: A, mass spectrum and retention index were consistent with those of an authentic compound; B, mass spectrum and retention index were consistent with that of Wiley 7n mass spectrum database or by manual interpretation (tentative identification). [i]Volatile compounds were reported as new constituents of ginseng in this study.

H. Chen (usually known as "Sanchi ginseng" and found in the Yunnan province in China), and *P. quinquefolius* L. (grown in southern Canada and the United States).[1,2] The recent increase in the demand and consumption of ginseng has led to some species being frequently substituted and/or adulterated with other species because of their different market prices.[17] However, it is not easy to identify the origin of ginseng species because some are morphologically very similar (e.g., *P. ginseng* and *P. notoginseng* species) and many products are provided commercially in the form of a powder or shredded slices. This situation makes it necessary to determine the differences and/or similarities in the chemical compositions, qualities, and pharmaceutical effects between ginseng species. Very recently, some studies have assessed the qualities and pharmaceutical effects of several types of ginseng using multianalytical approaches, which apply multivariate statistical techniques to their instrumental data sets (e.g., ginsenoids, inorganic elements, and secondary derivatives).[17,20] However, no previous studies have applied a multianalytical approach to investigate the differences and/or similarities in the volatile compositions among different species of ginseng. The present study profiled and compared the volatile compositions of three ginseng species by applying multivariate statistical techniques to their complex gas chromatography−mass spectrometry (GC−MS) data sets to establish the differences and/or similarities between them.

MATERIALS AND METHODS

Solvent and Chemicals. Dichloromethane (≥99.9% purity) was obtained from Fisher Scientific Korea (Seoul, South Korea), and sodium sulfate, *n*-alkane standards (C_7−C_{22}), and hexyl acetate, an internal standard compound, were purchased from Sigma-Aldrich (St. Louis, MO). The stock solutions of 31 authentic standard compounds were prepared in dichloromethane. The authentic standards were obtained from various suppliers as follows (numbers correspond to those in Table 2): numbers 1−9, 11, 13−14, 16−18, 22−23, 25−26, 28−31, 38, 40, and 54 were from Sigma-Aldrich; numbers 9 and 43 were from Fluka (Buchs, Switzerland); numbers 19 and 24 were from Seoul Aromatics (Seoul, South Korea); and number 12 was from TCI (Tokyo, Japan).

Materials. The ginseng species (*P. ginseng*, *P. notoginseng*, and *P. quinquefolius*) used in the present study were 6-year-old dried white ginseng. *P. ginseng* species were cultivated from four different cultivation areas in Korea (Gaesung, Gangwha, Geumsan, and Punggi) and then collected directly from their local markets, except Gaesung, which is located in North Korea. *P. notoginseng* was cultivated in the Yunnan province in China, whereas *P. quinquefolius* was grown in the state of Wisconsin in the United States. These two species were acquired through a local market in Geumsan in Korea. The ginseng species used in the present study were harvested from their cultivation areas in 2009, peeled, and then dried to ≤14% water content with hot air (60 ± 5 °C) to obtain a yellowish-white color. They were wrapped in low-level-density polyethylene (LLD-PE) film and stored at −70 °C in a deep freezer until they were used for analysis.

Extraction of Volatiles. The ground ginseng sample (10.0 g) was directly extracted with 50 mL of dichloromethane that had been redistilled prior to being used. After 0.05 mL of 1000 ppm hexyl acetate (v/v, in dichloromethane) was added as an internal standard, the ground sample suspended in dichloromethane was magnetically stirred at 400 rpm for 30 min. The extract was dried over anhydrous sodium sulfate (Na_2SO_4) and then filtered (paper no. 41, Whatman, Maidstone, U.K.) under a vacuum. Volatiles were then placed under a gentle stream of nitrogen gas to obtain a final volume of 0.3 mL. All sample preparations were performed in triplicate.

GC−MS Analysis. GC−MS analysis was performed using an Agilent 6890N GC-5975 mass selective detector (GC−MSD) (Agilent Technologies, Palo Alto, CA) equipped with a DB-5 ms column (30 m length × 0.25 mm inner diameter × 0.25 mm film thickness, J&W Scientific, Folsom, CA). The carrier gas was helium at a constant flow rate of 0.8 mL/min. A total of 1 μL of the extract was injected in the split (1:10) mode. The oven temperature was held at 40 °C for 1 min, then raised to 120 °C at 10 °C/min, to 180 °C at 2 °C/min, and to 200 °C at 10 °C/min, and then held at 200 °C for 5 min. The injector and detector temperatures were 200 and 250 °C, respectively. The mass detector was operated in the electron impact mode with an ionization energy of 70 eV, a scanning range of 33−550 atomic mass units (amu), and a scan rate of 2.83 scans/s.

Identification and Quantitation. Volatile compounds were positively identified by comparing their mass spectra and retention indices (RIs) to those of their authentic compounds. When standards were not available, compounds were tentatively identified with the aid of the Wiley 7n mass spectral database (1995 version; Hewlett-Packard, Palo Alto, CA). The RIs of the compounds were calculated using *n*-alkanes C_7−C_{22} as external references.[22] The semi-quantitative analysis of volatile compounds was performed by comparing their peak areas to that of the internal standard compound (0.05 mL of 1000 ppm hexyl acetate in dichloromethane, v/v) on the GC−MS total ion chromatogram.

Statistical Analysis. To assess connectivity (or similarity) among ginseng species, hierachical cluster analysis (HCA) with a between-group linkage method was used in SPSS (version 18.0, Chicago, IL). Analysis of variance (ANOVA) was performed using the general line model procedure in SPSS to evaluate significant difference in each volatile compound of ginseng species. Duncan's multirange test was used when the samples exhibited significantly different peak areas of volatiles, with the level of significance set at $p < 0.05$. In addition, principal component analysis (PCA) was applied to relative peak area values of volatiles obtained on the GC−MS total ion chromatograms using both SIMCA-P (version 11.0, Umetrics, Umeå, Sweden) and

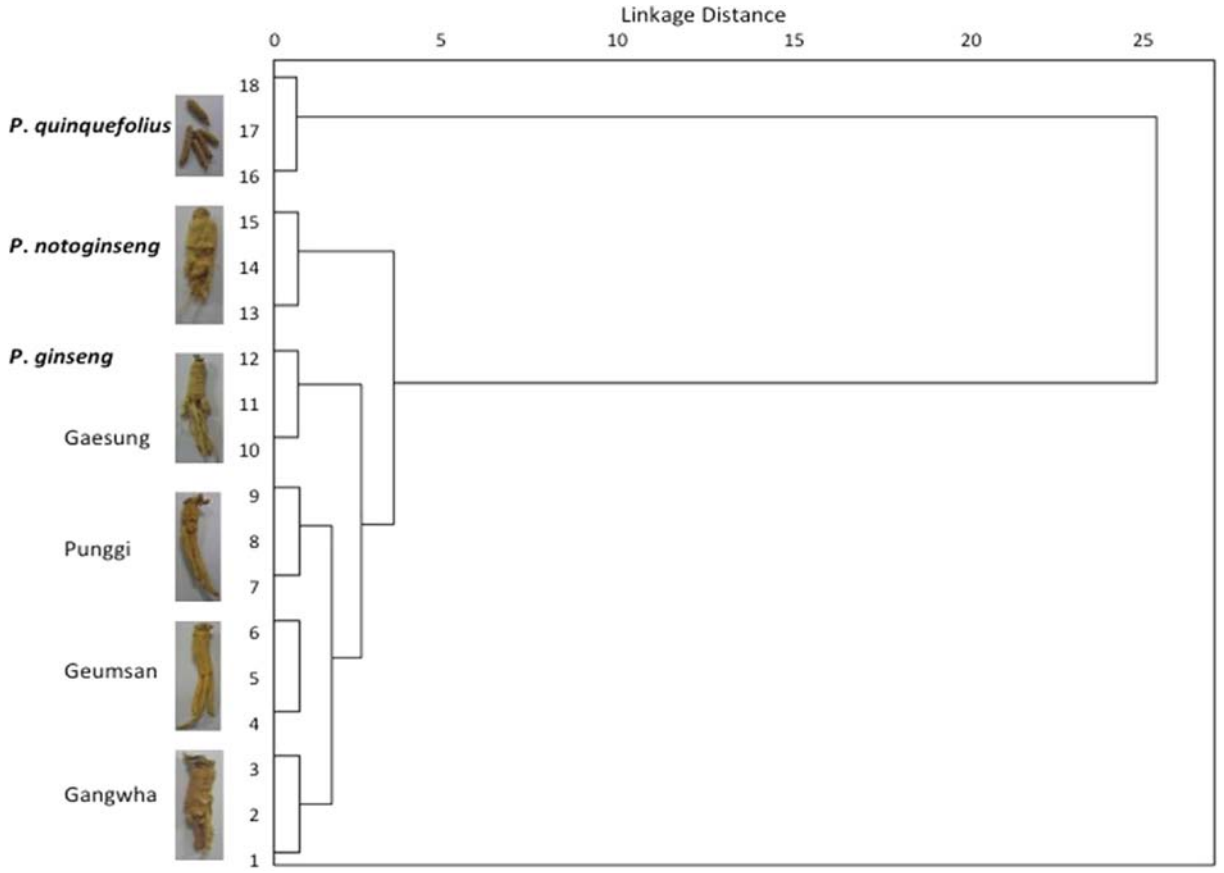

Figure 1. Dendrogram for dried white ginseng according to species generated by HCA with a between-group linkage method.

SPSS to clarify the relationship between the ginsengs and the volatile compounds present.

RESULTS AND DISCUSSION

Volatile Compositions of Ginseng Species. Table 2 lists the volatile compounds identified in dried white ginseng according to species, with their relative peak areas, and RIs on the DB-5 ms column. A total of 57 volatile compounds, including 10 aldehydes, 6 ketones, 4 alcohols, 3 acids, 4 furans and furan derivatives, 2 pyrazines, 27 terpenes, and 1 miscellaneous compound, were analyzed in dried white ginseng species. According to previous studies, sesquiterpene hydrocarbons and sesquiterpenyl alcohols were major volatile compounds in the neutral fraction of fresh ginseng extracts.[10,11] In the present study, the following sesquiterpenes were the main volatile compounds in most ginseng species: bicyclogermacrene (**51**), (*E*)-*β*-farnesene (**42**), *β*-panasinsene (**36**), calarene (**41**), *α*-humulene (**43**), and *β*-elemene (**37**). In general, sesquiterpenes ($C_{15}H_{24}$), together with monoterpenes ($C_{10}H_{16}$), are strongly associated with the flavor of plants.[23] Most ginseng species examined in the present study exhibited a large percentage of sesquiterpenes (51.699−77.152) and smaller proportions of monoterpenes (0.211−1.696). In particular, the levels of main sesquiterpenes (e.g., bicyclogermacrene, *β*-panasinsene, calarene, *α*-humulene, and *β*-elemene) identified in the present study, except for (*E*)-*β*-farnesene, were higher in *P. ginseng* and *P. notoginseng* than in *P. quinquefolius*. On the other hand, the total level of monoterpenes was lowest in *P. ginseng*. It is also notable that bicyclogermacrene was dominant in two of the ginseng species but was not detected in *P. quinquefolius*. On the other hand, methoxypyrazine derivatives could contribute to the characteristic earthy and green aroma notes of ginseng.[9,24,25] In particular, 2-methoxy-3-(1-methylethyl)-pyrazine (**23**), which is known to be the characteristic vegetative odorant in wine,[26,27] was detected in almost all of the ginseng species. Although many pyrazines had been previously found in ginseng species,[9,10] only two pyrazines, tetramethyl-pyrazine (**22**) and 2-methoxy-3-(1-methylethyl)-pyrazine (**23**), were detected in the present study. These disparate findings might be attributable to the sample preparation, extraction methods, and other factors differing between the studies. In particular, the steam-distillation extraction method, which had been previously used, could render more pyrazines detectable.

Comparison of the Volatile Compositions of Different Ginseng Species. HCA (in particular, a between-group linkage method) was applied in the present study to distinguish ginseng species according to similarity in volatile composition. HCA is a statistical technique that assigns a set of objects into groups (called clusters) such that objects in the same cluster exhibit greater similarity to each other (in some sense) than to those in other clusters.[28] A HCA dendrogram revealed that *P. quinquefolius* was clearly separated from two other ginseng species, *P. ginseng* and *P. notoginseng*, with a linkage distance of 25, whereas *P. ginseng* and *P. notoginseng* were differentiated from each other with a linkage distance of only 4 (Figure 1). Separation of *P. ginseng* was also performed according to the different cultivation areas with a linkage distance of 0.5. Thus, HCA was used to successfully discriminate different ginseng species.

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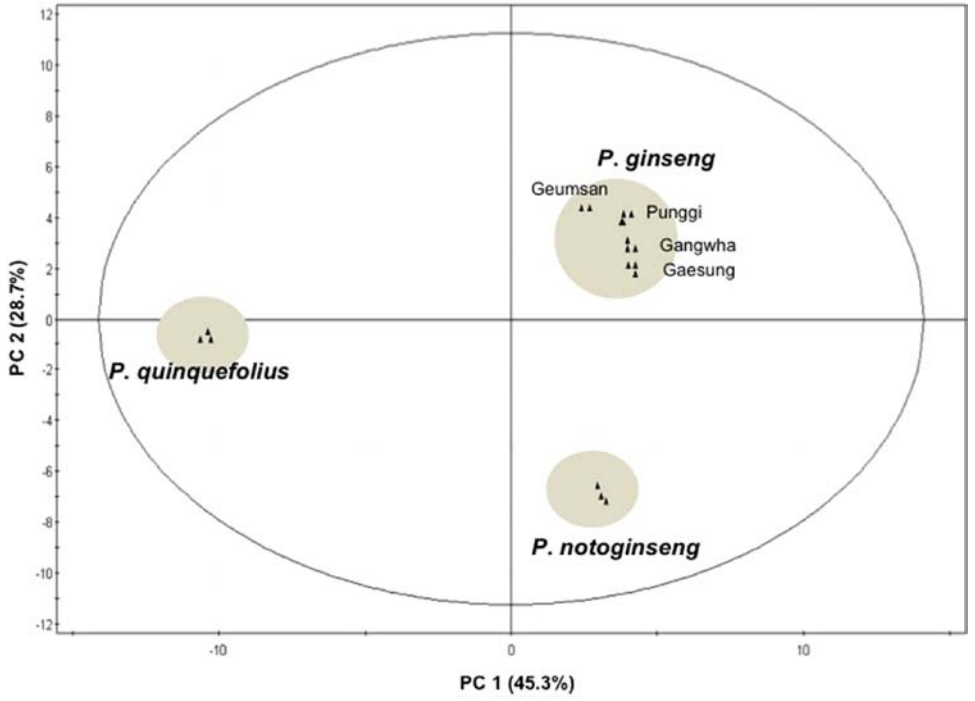

Figure 2. PCA score plots for dried white ginseng according to species generated using a combination of PC 1 and PC 2.

Table 3. Contribution of Volatile Variances Identified in Dried White Ginseng According to Species

number[a]	PC 1	PC 2	number	PC 1	PC 2	number	PC 1	PC 2
15	−0.994	−0.060	35	0.862	0.476	31	0.408	−0.842
50	−0.994	−0.024	5	−0.857	−0.406	16	0.519	−0.836
48	−0.994	−0.024	6	−0.844	−0.298	8	0.535	−0.820
53	−0.994	−0.024	41	0.833	−0.455	12	0.430	0.817
49	−0.994	−0.024	23	0.810	−0.055	28	−0.349	−0.802
19	−0.994	−0.024	37	0.759	0.275	56	0.336	−0.788
46	−0.993	−0.024	24	0.744	−0.636	30	0.336	−0.787
14	−0.993	−0.041	26	0.736	−0.517	11	0.363	−0.780
39	0.971	−0.144	17	−0.732	−0.230	9	0.483	−0.773
40	0.961	−0.016	29	−0.690	−0.403	33	−0.160	−0.689
52	−0.960	0.049	2	−0.672	−0.247	44	0.399	0.658
42	−0.960	−0.140	13	−0.654	−0.445	22	0.154	0.646
43	0.925	0.316	1	0.281	−0.955	4	0.107	−0.065
51	0.910	−0.030	20	0.036	−0.939	25	0.192	0.481
34	0.907	0.369	18	0.309	−0.908	27	0.239	0.489
45	0.903	0.210	7	0.326	−0.908	21	0.592	−0.134
55	0.901	−0.086	10	0.110	−0.906	38	0.129	−0.542
36	0.885	0.456	3	−0.134	−0.897	54	−0.460	0.201
47	0.868	0.459	57	0.254	−0.811	32	0.435	−0.269

[a]Numbers correspond to those in Table 2.

In addition, the relationship between the ginseng species and the volatile compounds present was investigated by applying PCA to the complex GC−MS data sets. PCA is an unsupervised clustering statistical method that does not require any knowledge of the data sets and reduces the dimensionality of multivariate data while preserving most of the variance therein.[29] The original variables can be expressed as a particular linear combination of the principal components (PCs) in the score plots, which account for a certain amount of the total variance of the data sets. Plotting the data in the space defined in this way facilitates the rapid visualization of similarities or differences in the data sets, allowing for improved discrimination among samples.[30] Figure 2 shows that ginseng could clearly be discriminated according to species in the score plots constructed by combining PC 1 (45.3%) with PC 2 (28.7%). *P. quinquefolius* was preferentially separated from *P. ginseng* and *P. notoginseng* by PC 1 in this score plot, whereas *P. ginseng* and *P. notoginseng* were separated from each other by PC 2, as the result of the HCA. Sohn et al. reported that the overall gas chromatograph pattern of the headspace volatiles was similar for *P. ginseng* and *P. notoginseng*.[12] However, the two species could be discriminated from each other in the present study.

The coefficients by which the original variables are multiplied to obtain the PC are called as loadings, and their numerical

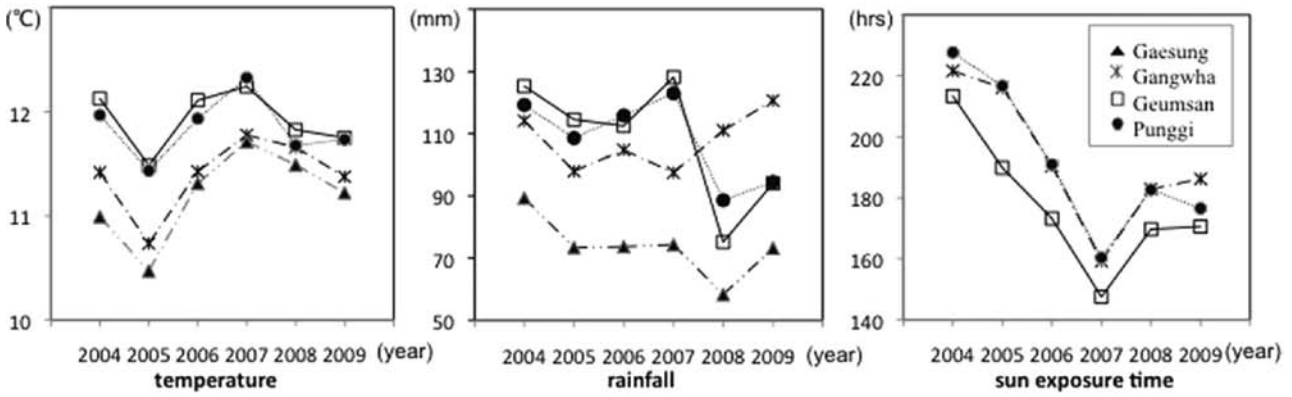

Figure 3. Climatic conditions (average temperature, total rainfall, and sun exposure time) of different cultivation areas of *P. ginseng* species from 2004 to 2009. The data were provided by the Korean Meteorological Administration (www.kma.go.kr) (the data of sun exposure time of the Gaesung area could not be obtained).

values indicate how similar each variable is to that component (Table 3).[30] The application of ANOVA to the GC−MS data set also demonstrated that the mean values of the relative peak areas of most of the volatile compounds identified in ginseng differed significantly with the species ($p < 0.05$) (Table 2). Thus, it was possible to determine the main volatile compounds that allowed ginseng to be discriminated according to species by analyzing the PCA loading plots. α-Selinene (**49**), α-terpinolene (**19**), β-bisabolene (**50**), β-phellandrene (**15**), β-sesquiphellandrene (**53**), zingiberene (**48**), germacrene D (**46**), limonene (**14**), α-gurjunene (**39**), (*E*)-caryophyllene (**40**), δ-cadinene (**52**), (*E*)-β-farnesene (**42**), α-humulene (**43**), bicyclogermacrene (**51**), longiborn-8-ene (**34**), β-neoclovene (**45**), and (+)-spathulenol (**55**) were significantly associated with differentiation by PC 1, with contributions excceding 90% (Table 3). In particular, *P. ginseng* and *P. notoginseng* contained higher levels of α-gurjunene, (*E*)-caryophyllene, α-humulene, bicyclogermacrene, longiborn-8-ene, β-neoclovene, and (+)-spathulenol with higher positive values in the PC 1 loading plot, whereas α-selinene, α-terpinolene, β-bisabolene, β-phellandrene, β-sesquiphellandrene, zingiberene, germacrene D, limonene, δ-cadinene, and (*E*)-β-farnesene exhibiting higher negative values in the PC 1 loading plot occurred in higher proportions in *P. quinquefolius*. In general, all terpenes are derived from isopentenyl diphosphate (IPP), which, following isomerization to dimethylallyl diphosphate (DMAPP), is sequentially elongated to geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP).[31] This biosynthetic pathway could suggest that, as the levels of (*E*,*E*)-germacradienyl cation and/or *trans*-humulyl cation rings increase in a certain ginseng species, there could be a corresponding decrease in the formation of bisabolyl cation-membered rings. Thus, the high level of bicyclogermacrene in *P. ginseng* and *P. notoginseng* could be related to the increased formation of the (*E*,*E*)-germacradienyl cation ring (Table 2). Similarly, the significantly higher level of (*E*)-β-farnesene in *P. quinquefolius* than in the other species could be attributable to the greater generation of the bisabolyl cation-membered ring. On the other hand, hexanal (**1**), 2-pyrrolidinone (**20**), (*E*)-2-octenal (**18**), (*E*)-2-heptenal (**7**), heptanal (**3**), isospathulenol (**57**), (*E*,*E*)-2,4-decadienal (**31**), 3-octen-2-one (**16**), benzaldehyde (**8**), 2-pentylfuran (**12**), and (*E*)-2-nonenal (**28**), whose contributions exceeded 80%, were mainly related to discrimination by PC 2 (Table 3). The levels of hexanal, 2-pyrrolidinone, (*E*)-2-octenal, (*E*)-2-heptenal, heptanal, (*E*,*E*)-2,4-decadienal, 3-octen-2-one, benzaldehyde, isospathulenol, and (*E*)-2-nonenal exhibiting higher negative values in the PC 2 loading plot were higher in *P. notoginseng*, whereas *P. ginseng* was found to contain more 2-pentylfuran, with higher positive values in the PC 2 loading plot. Recent studies have demonstrated that significant variation in ginsenoside compositions among *P. quinquefolius* roots is highly associated with plant geographic origin.[32,33] Thus, it is quite likely that the differences in volatiles between ginseng species could be affected by geographic factors.

In the present study, the separation of *P. ginseng* species according to their cultivation areas (Gwangwha, Geumsan, Punggi, and Gaesung) revealed clustering in both HCA and PCA (Figures 1 and 2). Some previous studies found that the chemical composition and/or quaility of ginseng vary according to climatic and geographical conditions.[14,16,17] In particular, the formation of terpenes, which are major volatile compounds in ginseng, is sensitive to factors, such as temperature, sun exposure time, and rainfall.[34,35] As shown in Figure 3, the climatic conditions varied with the cultivation area of the *P. ginseng* species. These differences in climatic conditions might affect the composition of volatiles in *P. ginseng* species by cultivation areas.

In summary, the difference in the volatile compositions of ginseng according to species could be clearly demonstrated in the present study. *P. ginseng* and *P. notoginseng* were separated from *P. quinquefolius*, and two *P. ginseng* and *P. notoginseng* species were discriminated from each other. In particular, the levels of sesquiterpenes, such as α-selinene, α-terpinolene, β-bisabolene, β-phellandrene, β-sesquiphellandrene, zingiberene, germacrene D, limonene, α-gurjunene, (*E*)-caryophyllene, δ-cadinene, (*E*)-β-farnesene, α-humulene, bicyclogermacrene, longiborn-8-ene, β-neoclovene, and (+)-spathulenol, which have been known to be the main volatile compounds of ginseng species, could significantly affect the similarities and/or differences between *P. ginseng* and *P. notoginseng* versus *P. quinquefolius*. Conversly, the similarities and/or differences between *P. ginseng* and *P. notoginseng* species could be influenced by the levels of certain carbonyls, in particular hexanal, 2-pyrrolidinone, (*E*)-2-heptenal, (*E*)-2-octenal, heptanal, isospathulenol, (*E*,*E*)-2,4-decadienal, 3-octen-2-one, benzaldehyde, 2-pentylfuran, and (*E*)-2-nonenal, which were quantitatively minor volatile compounds of ginseng species.

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AUTHOR INFORMATION

Corresponding Author

*Telephone: +82-2-3277-3091. Fax: +82-2-3277-4213. E-mail: yskim10@ewha.ac.kr.

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ABBREVIATIONS USED

GC−MS, gas chromatography−mass spectrometry; GC−MSD, gas chromatography−mass selective detector; RI, retention index; HCA, hierachical cluster analysis; ANOVA, analysis of variance; PCA, principal component analysis; PC, principal component; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate

REFERENCES

(1) Park, M. J.; Kim, M. K.; In, J.-G.; Yang, D.-C. Molecular identification of Korean ginseng by amplification refractory mutation system−PCR. *Food Res. Int.* **2006**, *39*, 568−574.

(2) Cho, Y. S. *Understanding of Korean Ginseng: Korean Ginseng Contains Various Effective Components*; The Society for Korean Ginseng: Seoul, South Korea, 1995 (in Korean).

(3) Angelova, N.; Kong, H.-W.; van der Heijden, R.; Yang, S.-Y.; Choi, Y. H.; Kim, H.-K.; Wang, M.; Hankermeier, T.; van der Greef, J.; Xu, G.; Verpoorte, R. Recent methodology in the phytochemical analysis of ginseng. *Phytochem. Anal.* **2008**, *19*, 2−16.

(4) Bhattacgarya, S. K.; Mitra, S. M. Anxiolytic activity of *Panax ginseng* root: An experimental study. *J. Ethnopharmacol.* **1991**, *34*, 87−92.

(5) Han, B. H.; Park, M. H.; Han, Y. N.; Shin, S. C. Studies on the antioxidant activity components of Korean ginseng-(IV)-antifatigue activity components. *Yakhak Hoeji* **1984**, *28*, 232−235.

(6) Hu, C.; Kitts, D. D. Free radical scavenging capacity as related to antioxidant activity and ginsenoside composition of Asian and North American ginseng extracts. *J. Am. Oil Chem. Soc.* **2001**, *78*, 249−255.

(7) Kitts, D. D.; Wijewickreme, A. N.; Hu, C. Antioxidant properties of North American ginseng extracts. *Mol. Cell. Biochem.* **2000**, *203*, 1−10.

(8) Woo, H.-C.; Shin, B.-K.; Cho, I.; Koo, H.; Kim, M.; Han, J. Anti-obesity effect of carbon dioxide supercritical fluid extracts of *Panax ginseng* C.A. Meyer. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 738−743.

(9) Iwabuchi, H.; Yoshikura, M.; Ikawa, T.; Kamisako, W. Studies on the sesquiterpenoids of *Panax ginseng* C.A. Mayer. Isolation and structure determination of sesquiterpene alcohols, panasinsanols A and B. *Chem. Pharm. Bull.* **1987**, *37*, 509−513.

(10) Iwabuchi, H.; Yoshikura, M.; Obata, S.; Kamisako, W. Studies on the aroma constituents of crude drugs. I. On the aroma constituents of ginseng radix. *Yakugaku Zasshi* **1984**, *104*, 951−958.

(11) Kim, M.-W.; Park, J.-D. Studies on the volatile flavor components of fresh ginseng. *Korean J. Ginseng Sci.* **1984**, *8*, 22−31.

(12) Sohn, H.-J.; Heo, J.-N.; Nho, K.-B.; Kim, M.-W. A comparison of the composition of the major headspace volatiles between the Korean ginseng and the Chinese ginseng. *Korean J. Ginseng Sci.* **1997**, *21*, 196−200.

(13) Ko, S. K.; Cho, O. S.; Bae, H. M.; Im, B. O.; Lee, O.-H.; Lee, B.-Y. Quantitative analysis of ginsenosides composition in flower buds of various ginseng plants. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 154−157.

(14) Lee, M. J.; Choi, J. S.; Cha, S. W.; Lee, K. S.; Lee, Z. W.; Hwang, G. S.; Lee, S. H.; Kamal, A. H. M.; Jung, Y. A.; Seung, N. S.; Woo, S. H. Variation in the ginsenoside profiles of cultivated ginseng (*Panax ginseng* CA Mayer) landraces in Korea. *Process Biochem.* **2011**, *46*, 258−264.

(15) Qu, C.; Bai, Y.; Jin, X.; Wang, Y.; Zhang, K.; You, J.; Zhang, H. Study on ginsenosides in different parts and ages of *Panax quinquefolius* L. *Food Chem.* **2009**, *115*, 340−346.

(16) Lee, E.-J.; Shaykhutdinov, R.; Weljie, A. M.; Vogel, H. J.; Facchini, P. Quality assessment of ginseng by ^{1}H NMR metabolite fingerprinting and profiling analysis. *J. Agric. Food Chem.* **2009**, *57*, 7513−7522.

(17) Lee, A.-R.; Gautam, M.; Kim, J.; Shin, W.-J.; Choi, M.-S.; Bong, Y.-S.; Hwang, G.-S.; Lee, K.-S. A multianalytical approach for determining the geographic origin of ginseng using strontium isotopes, multielements, and ^{1}H NMR analysis. *J. Agric. Food Chem.* **2011**, *59*, 8560−8567.

(18) Shanghai College of Traditional Chinese Medicine. *Zhongcayaoxue (Chinese Materia Medica)*; Commerce Press: Hong Kong, 1975; pp 511−515.

(19) Shanghai College of Traditional Chinese Medicine. *Zhongcayao Linchuang Shouce (Clinical Manual of Chinese Medicine)*; Shanghai People's Publishing House: Shanghai, China, 1977; pp 357−359.

(20) Chan, T. W. D.; But, P. P.; Cheng, S. W.; Kwok, I. M. Y.; Lau, F. W.; Xu, H. X. Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS. *Anal. Chem.* **2000**, *72*, 1281−1287.

(21) Yun, T. K. Brief introduction of *Panax ginseng* C.A. Meyer. *J. Korean Med. Sci.* **2001**, *16*, S3−S5.

(22) van den Dool, H.; Kratz, P. D. A generalisation of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.

(23) Reineccius, G. *Flavor Chemistry and Technology*, 2nd ed.; CRC Press (Taylor and Francis Group): Boca Raton, FL. 2007.

(24) Kaneko, S.; Kumazawa, K.; Nishimura, O. Studies on the key aroma compounds in soy milk made from three different soybean cultivars. *J. Agric. Food Chem.* **2011**, *59*, 12204−12209.

(25) Suriyaphan, O.; Drake, M. A.; Chen, X. Q.; Cadwallader, K. R. Characteristic aroma components of British Farmhouse Cheddar cheese. *J. Agric. Food Chem.* **2001**, *49*, 1382−1387.

(26) Allen, M. S.; Lacey, M. J.; Boyd, S. J. Methoxypyrazines in red wines: Occurrence of 2-methoxy-3-(1-methylethyl)pyrazine. *J. Agric. Food Chem.* **1995**, *43*, 769−772.

(27) Belancic, A.; Agosin, E. Methoxypyrazines in grapes and wines of *Vitis vinifera* cv. Carmenere. *Am. J. Enol. Vitic.* **2007**, *58*, 462−469.

(28) Ketchen, D. J.; Shook, C. L. The application of cluster analysis in strategic management research: An analysis and critique. *Strategic Manage. J.* **1996**, *17*, 441−458.

(29) Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Wold, S. *Multi- and Megavariate Data Analysis*; Umerics Academy: Umeå, Sweden, 2001.

(30) Masart, D. L.; Vandeginste, B. G. M.; Deming, S. N.; Michotte, Y.; Kauffman, L. *Chemometrics: A Textbook*; Elsevier: Amsterdam, The Netherlands, 1998.

(31) Davis, E. M.; Croteau, R. Cyclization enzymes in the biosynthesis of monoterpens, sesquiterpenes, and diterpenes. *Top. Curr. Chem.* **2000**, *209*, 53−95.

(32) Sun, J.; Chen, P. Differentiation of *Panax quinquefolius* grown in the USA and China using LC/MS-based chromatographic fingerprinting and chemometric approaches. *Anal. Bioanal. Chem.* **2011**, *399*, 1877−1889.

(33) Schlag, E. M.; McIntosh, M. S. Ginsenoside content and variation among and within American ginseng (*Panax quinquefolius* L.) populations. *Phytochemistry* **2006**, *67*, 1510−1519.

(34) Macaulay, L. E.; Morris, J. R. Influence of cluster exposure and winemaking processes on monoterpenes and wine olfactory evaluation of golden muscat. *Am. J. Enol. Vitic.* **1993**, *44*, 198−204.

(35) Lee, H. J.; Cho, I. H.; Kim, Y.-S. The compositions of volatiles and aroma-active compounds in dried *omija* fruits (*Schisandra chinesis* Baillon) according to the cultivation areas. *J. Agric. Food Chem.* **2011**, *59*, 8338−8346.