

The Compositions of Volatiles and Aroma-Active Compounds in Dried *Omija* Fruits (*Schisandra chinensis* Baillon) According to the Cultivation Areas

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ABSTRACT: Differences in the compositions of volatiles from dried *omija* fruits (*Schisandra chinensis* Baillon) cultivated in different areas (Mungyeong, Jangsu, Jechon, and Hoengseong) in South Korea were determined by applying principal component analysis to gas chromatography–mass spectrometry data sets. Quantitative assessments revealed that terpene hydrocarbons, such as germacrene D, β -selinene, α -ylangene, β -elemene, α -selinene, and (*E*)- β -farnesene, were the main volatiles in all *omija* fruit samples. On the other hand, (*E*)- β -ocimene, calarene, (*E*)- β -farnesene, β -selinene, nonanal, 2-methylbutanoic acid, benzoic acid, 2,3-butanediol, and phenethyl alcohol were the major volatile components that contributed to the discrimination between *omija* fruit samples from the four cultivation areas. In addition, aroma-active compounds in four dried *omija* fruits were investigated and compared by gas chromatography–olfactometry using aroma extract dilution analysis. (*E*)- β -Ocimene (floral and herbaceous), α -pinene (pine-like and woody), hexanal (cut grass-like), 5-methylfurfural (burnt sugar-like and sweet), and α -terpinene (minty, green, and fresh) were important aroma-active compounds in all *omija* samples. Interestingly, the flavor dilution factors of most aroma-active compounds were lower for *omija* sample cultivated in Hoengseong than for those cultivated in Mungyeong, Jangsu, and Jechon.

KEYWORDS: dried *omija* fruits (*Schisandra chinensis* Baillon), volatile component, aroma-active compound, principal component analysis, aroma extract dilution analysis

INTRODUCTION

The *Schisandra* genus (clusters of 6–8 cm with bright red berries) comprises 25 species, one of which, namely, *Schisandra chinensis* Baillon,¹ is known as “*omija*” in Korea. The literal meaning of *omija* in Korean is “berries with five distinctive flavor characteristics: sweetness, sourness, saltiness, bitterness, and astringency”, and the aqueous extract of this fruit has an unambiguous pinkish-red color and palatable flavors.² The consumption of *omija* has been increasing in recent years in Korea, mainly due to its exerting several biological effects in coughs, phlegm, bronchitis, hepatitis, alcohol detoxification, and fatigue,^{1,3,4} as well as its unique color and flavor, particularly for making both alcoholic and nonalcoholic beverages.^{5,6}

Most previous studies of *omija* flavor have focused on analyzing the volatile components of fruits, leaves, stems, and roots using gas chromatography (GC) and/or GC–mass spectrometry (GC–MS).^{2,7–9} Quantitatively, the main volatile components of *omija* fruits were found to be the monoterpenes and sesquiterpenes, including β -myrcene, γ -terpinene, β -elemene, γ -selinene, α -bergamotene, and α -ylangene.^{2,7,10} On the other hand, the main volatile components of *omija* leaves included germacrene D, β -elemene, (*Z,E*)- α -farnesene, (*E*)-2-hexenal, and (*E*)- β -ocimene.⁸ β -Pinene, 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, α -pinene, and 1,8-cineol were the most abundant volatile components in *omija* stems, whereas β -pinene, camphene, linalyl acetate, 1-methyl-4-(1-methylethyl)benzene, and linalool oxide were the main volatile compounds in *omija* roots.⁹ (*E,Z*)-2,6-Nonadienal (cucumber-like), (*Z*)-3-hexenal (green and apple-like), (*E*)-2-hexenal (green and fruity), and (*E*)- β -ocimene (wither green and grass-like) were also determined

to be important aroma-active compounds of *omija* leaves by GC–olfactometry (GC–O) using aroma extract dilution analysis (AEDA).⁸

Multivariate statistical analysis has been applied to evaluate changes or differences in diverse food components according to various factors such as origins, grades, and species.^{11,12} In particular, principal component analysis (PCA), which is an unsupervised clustering technique, can be a useful tool for arranging and visualizing large and complex data sets obtained from different samples.¹³ Furthermore, PCA can be used to investigate the components that contribute most to the variance among the various components in different samples.^{14–17} Accordingly, volatile profiling using a combination of GC–MS and PCA has been applied to classify and discriminate complex data sets in various foods, such as tomato,¹⁴ pine-mushroom,¹⁵ wine,¹⁶ and fermented soybean paste.¹⁷

While some studies have investigated the volatiles present in *omija* fruits, they have focused mainly on instrumental analyses, and no study has compared the compositions of volatiles and aroma-active compounds in *omija* fruits according to the cultivation areas. The aim of the present study was to determine whether the compositions of volatiles and aroma-active compounds of dried *omija* fruits vary with the cultivation area by applying a multivariate statistical method to GC–MS data sets, and AEDA using GC–O.

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Table 1. Volatile Components in Dried *Omija* Fruits According to the Cultivated Areas

no. ^a	RI ^b	volatile components	rel peak area (mean ± SD) ^c				ID ^h
			MG ^d	JA ^e	JE ^f	HS ^g	
1	709	3-hydroxy-2-butanone (acetoin)	tr ⁱ	0.4 ± 0.0 c ^j	0.1 ± 0.0 a	0.2 ± 0.0 b	A
2	791	2,3-butanediol	nd ^k	nd	nd	0.2 ± 0.1 a	A
3	801	hexanal	tr	tr	tr	tr	A
4	830	furfural	0.2 ± 0.0 b	tr	0.1 ± 0.0 a	0.1 ± 0.0 a	A
5	848	2-methylbutanoic acid	nd	tr	tr	0.2 ± 0.0 a	A
6	906	γ-butyrolactone	nd	0.1 ± 0.0 a	tr	0.1 ± 0.1 a	A
7	925	α-thujene	0.2 ± 0.0 ab	0.2 ± 0.0 ab	0.2 ± 0.1 b	0.1 ± 0.0 a	B
8	932	α-pinene	0.4 ± 0.0 ab	0.7 ± 0.0 c	0.7 ± 0.2 bc	0.3 ± 0.0 a	A
9	949	camphene	tr	0.3 ± 0.1 b	0.3 ± 0.1 ab	0.1 ± 0.0 a	A
10	959	5-methylfurfural	tr	tr	tr	tr	A
11	972	sabinene	0.7 ± 0.0 b	0.9 ± 0.1 b	0.8 ± 0.2 b	0.4 ± 0.1 a	A
12	977	β-pinene	0.3 ± 0.0 a	0.6 ± 0.0 b	0.6 ± 0.0 b	0.3 ± 0.1 a	A
13	983	6-methyl-5-hepten-2-one	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	A
14	989	β-myrcene	1.1 ± 0.1 b	1.3 ± 0.2 b	1.2 ± 0.2 b	0.4 ± 0.1 a	A
15	1006	α-phellandrene	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	tr	A
16	1017	α-terpinene	0.2 ± 0.0 b	0.2 ± 0.1 a	0.1 ± 0.1 ab	0.1 ± 0.0 a	A
17	1024	p-cymene	0.3 ± 0.0 a	0.6 ± 0.0 bc	0.7 ± 0.2 c	0.5 ± 0.1 ab	A
18	1029	limonene	1.3 ± 0.0 c	0.3 ± 0.0 b	0.3 ± 0.1 b	0.2 ± 0.0 a	A
19	1032	1,8-cineole	0.2 ± 0.0 b	0.2 ± 0.0 b	0.2 ± 0.0 b	0.1 ± 0.0 a	A
20	1039	pantolactone	0.1 ± 0.0 a	nd	0.3 ± 0.1 b	0.5 ± 0.1 c	A
21	1046	(E)-β-ocimene	0.1 ± 0.0 a	0.2 ± 0.0 a	0.1 ± 0.0 a	tr	A
22	1058	γ-terpinene	0.3 ± 0.0 a	0.6 ± 0.1 b	0.6 ± 0.1 b	0.2 ± 0.1 a	A
23	1071	(Z)-sabinene hydrate	nd	nd	0.1 ± 0.0 a	0.1 ± 0.0 a	B
24	1079	methyl 2-furoate	1.5 ± 0.0 d	0.6 ± 0.0 a	1.3 ± 0.1 c	0.9 ± 0.1 b	A
25	1085	α-terpinolene	0.1 ± 0.0 a	nd	tr	tr	A
26	1104	nonanal	nd	nd	nd	0.1 ± 0.0 a	A
27	1112	phenethyl alcohol	nd	nd	nd	0.2 ± 0.1 a	A
28	1141	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	0.5 ± 0.0 c	0.2 ± 0.0 a	0.5 ± 0.0 c	0.4 ± 0.0 b	B
29	1168	benzoic acid	0.1 ± 0.0 a	0.1 ± 0.0 a	0.2 ± 0.0 b	0.5 ± 0.0 c	A
30	1181	terpinene-4-ol	0.3 ± 0.0 a	0.6 ± 0.0 c	0.4 ± 0.0 b	0.4 ± 0.0 b	A
31	1228	5-hydroxymethylfurfural (HMF)	6.9 ± 0.3 c	0.8 ± 0.1 a	8.3 ± 0.2 d	5.3 ± 0.2 b	B
32	1284	bornyl acetate	0.2 ± 0.0 a	0.4 ± 0.1 b	0.5 ± 0.1 b	nd	A
33	1337	δ-elemene	0.2 ± 0.0 ab	0.3 ± 0.1 b	0.2 ± 0.1 a	0.1 ± 0.1 a	B
34	1347	α-cubebene	0.5 ± 0.0 a	nd	nd	nd	B
35	1349	citronellyl acetate	0.5 ± 0.0 a	0.9 ± 0.1 b	1.2 ± 0.2 c	0.5 ± 0.1 a	A
36	1370	α-ylangene	1.7 ± 0.0 a	4.1 ± 1.5 a	7.5 ± 2.0 b	3.2 ± 2.2 a	B
37	1377	α-copaene	1.1 ± 0.1 b	1.1 ± 0.2 b	1.1 ± 0.1 b	0.5 ± 0.1 a	A
38	1384	β-bourbonene	0.4 ± 0.0 a	0.6 ± 0.1 b	0.4 ± 0.1 a	1.0 ± 0.1 c	B
39	1390	β-elemene	4.8 ± 0.1 c	3.7 ± 0.2 b	3.6 ± 0.4 b	1.0 ± 0.1 a	B
40	1420	β-caryophyllene	nd	0.5 ± 0.0 b	0.4 ± 0.0 a	nd	A
41	1429	calarene	0.3 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.0 a	nd	B
42	1450	(E)-β-farnesene	1.9 ± 0.1 b	2.1 ± 0.0 c	1.8 ± 0.1 b	0.3 ± 0.0 a	B
43	1455	α-humulene	0.5 ± 0.1 b	0.3 ± 0.1 b	0.4 ± 0.1 b	0.2 ± 0.1 a	A
44	1474	α-amorphene	1.4 ± 0.0 b	nd	1.5 ± 0.2 b	0.6 ± 0.0 a	B
45	1481	germacrene D	8.4 ± 0.6 d	6.5 ± 0.2 c	5.4 ± 0.4 b	0.8 ± 0.0 a	B
46	1489	β-selinene	7.4 ± 0.2 c	7.7 ± 0.7 c	6.3 ± 0.6 b	2.0 ± 0.0 a	B
47	1495	α-selinene	4.1 ± 0.1 c	3.3 ± 0.2 b	3.5 ± 0.3 b	1.4 ± 0.1 a	B
48	1502	β-himachalene	0.6 ± 0.1 a	1.5 ± 0.2 b	2.0 ± 0.7 b	0.6 ± 0.3 a	B
49	1507	germacrene A	1.7 ± 0.1 b	2.4 ± 0.3 c	1.5 ± 0.2 b	0.4 ± 0.1 a	B
50	1512	γ-cadinene	1.0 ± 0.1 d	0.6 ± 0.1 b	0.7 ± 0.0 c	0.5 ± 0.1 a	B
51	1517	δ-cadinene	2.2 ± 0.1 c	1.2 ± 0.1 b	0.9 ± 0.2 b	0.5 ± 0.1 a	B
52	1531	cadina-1,4-diene	0.3 ± 0.0 b	nd	0.1 ± 0.0 a	nd	B

Table 1. Continued

no. ^a	RI ^b	volatile components	rel peak area (mean ± SD) ^c				
			MG ^d	JA ^e	JE ^f	HS ^g	ID ^h
53	1540	α-calacorene	nd	0.5 ± 0.0 a	nd	nd	B
54	1547	germacrene B	0.8 ± 0.1 b	0.4 ± 0.0 a	nd	nd	B
55	1559	nerolidol	0.9 ± 0.0 c	0.7 ± 0.0 b	1.0 ± 0.1 d	0.4 ± 0.1 a	A
56	1575	spathulenol	0.8 ± 0.0 c	0.2 ± 0.0 a	0.5 ± 0.1 b	0.2 ± 0.0 a	B
57	1581	caryophyllene oxide	0.2 ± 0.0 b	nd	nd	0.1 ± 0.0 a	A
58	1654	α-cadinol	2.5 ± 0.1 c	1.4 ± 0.1 b	1.3 ± 0.1 b	0.7 ± 0.1 a	B

^a Numbers correspond to those in Table 2 and Figures 4–6. ^b Retention indices were determined using *n*-paraffins C₇–C₂₂ as external references.

^c Average of relative peak areas to that of internal standard (*n* = 3) ± standard deviation. ^d MG, Mungyeong. ^e JA, Jangsu. ^f JE, Jechon. ^g HS, Hoengseong.

^h Volatiles were identified on the basis of the following criteria: A, mass spectrum and retention indices were consistent with those of an authentic compound; B, mass spectrum was consistent with that of Wiley 275 mass spectral database. ⁱ Trace amount (<0.1). ^j There are significant differences (*P* < 0.05) among *omija* fruits using Duncan's multiple comparison test between the samples having a different letter in a row. ^k Not detected.

MATERIALS AND METHODS

Materials. *Omija* fruits, which had been cultivated and sun-dried for approximately two weeks in Mungyeong (MG), Jangsu (JA), Jechon (JE), and Hoengseong (HS) in South Korea in 2008, were obtained from local markets. The water contents of all the samples were determined to be in the range of 7.3–13.5%. The samples were stored in a plastic bag at –70 °C in a deep freezer until they were used for analysis.

Chemicals. Dichloromethane was obtained from J. T. Baker (Phillipsburg, NJ) and used for solvent extraction. Sodium sulfate, *n*-alkane standards (C₇–C₂₂), and an internal standard compound (hexyl acetate) were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions of 34 authentic standard compounds were prepared using dichloromethane. The authentic standards were obtained from various suppliers as follows: 2,3-butanediol, hexanal, 2-methylbutanoic acid, α-pinene, camphene, 5-methylfurfural, β-myrcene, α-terpinene, limonene, (*E*)-β-ocimene, methyl 2-furoate, benzoic acid, bornyl acetate, α-copaene, β-caryophyllene, and nerolidol were from Sigma-Aldrich; terpinene-4-ol and α-humulene were from Fluka (Buchs, Switzerland); 3-hydroxy-2-butanone, γ-butyrolactone, sabinene, β-pinene, 6-methyl-5-hepten-2-one, α-phellandrene, *p*-cymene, 1,8-cineole, pantolactone, γ-terpinene, α-terpinolene, nonanal, and caryophyllene oxide were from Seoul Aromatics (Seoul, Korea); fural and cirtoneily acetate were from Bolak (Seoul, Korea); phenethyl alcohol was from Junsei Chemical (Tokyo, Japan).

Extraction of Volatile Components. Three different dried *omija* samples (10.0 g) from each cultivated area were directly extracted with 50 mL of dichloromethane that had been redistilled prior to being used. After addition of 0.1 mL of an internal standard solution (hexyl acetate in dichloromethane, 1.0 mg/L), the sample was mixed using a magnetic stirrer at 300 rpm for 60 min. The extract was dried over anhydrous sodium sulfate (Na₂SO₄) and filtered on Advantec 110 mm filter paper (Tokyo Roshi Kaisha, Tokyo, Japan) before being concentrated to a final volume of 0.3 mL using a gentle stream of nitrogen gas.

Analysis by Gas Chromatography–Mass Spectrometry. 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-5MS column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness; J&W Scientific, Folsom, CA). Helium was run as a carrier gas at a constant column flow rate of 0.8 mL/min. The oven temperature was initially held at 40 °C for 1 min, raised to 140 °C at a rate of 5 °C/min, then further to 200 °C at a rate of 3 °C/min, and then to 280 °C at a rate of 10 °C/min, and was finally held at 280 °C for 20 min. One microliter of the extract was injected into the column with a split ratio mode (20:1). The temperatures of the injector and detector

transfer lines were 200 and 250 °C, respectively. The MSD was operated in the electron impact mode with an ionization energy of 70 eV, a scanning range of 35–550 amu, and a scan rate of 2.83 scans/s.

Aroma Extract Dilution Analysis. GC–O was performed using an Agilent 7890A GC (Agilent Technologies) equipped with a flame ionization detector (FID), sniffing port (ODP, Gerstel, Mulheim and Ruhr, Germany) and a DB-5 column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness; J&W Scientific). Effluent from the end of the GC column was split equally 1:1 between the FID and the sniffing port. The injection and column oven conditions were the same as those used for GC–MS. The aroma descriptions and flavor dilution (FD) factors of each volatile component were determined by AEDA. The extracts (final volume = 0.3 mL) were diluted 1:1 (v/v) stepwise with dichloromethane by 2ⁿ until no odor was perceivable at the sniffing port. The FD factors corresponded to the highest dilution at which a compound could be perceived. Two experienced panelists determined the aroma descriptions, and the maximum value was considered to be the FD factor of that compound.¹⁸ The concentration ranges of each aroma-active compound in solvent extracts used for sniffing tests were determined using authentic compounds and considering their relative MS detector responses to that of the internal standard compound. The relative detector responses of some compounds such as α-phellandrene, δ-elemene, α-copaene, β-selinene, spathulenol, and α-cadinol, whose authentic compounds were not available, were assumed to be the same as that of the internal standard compound.

Identification and Quantification. Volatile components were positively identified by comparing their mass spectra and retention indices (RIs) with those of the authentic compounds. When standards were not available, compounds were tentatively identified based on the Wiley 7n mass spectral database (1995 version; Hewlett-Packard, Palo Alto, CA). The RI of each component was calculated using *n*-alkane C₇–C₂₂ as external references.¹⁹ The volatile components were semi-quantitatively analyzed by comparing their peak areas to that of the internal standard compound on the GC–MS total ion chromatograms. Aroma-active compounds perceived at the sniffing port were positively identified by comparing their mass spectra, RIs, and aroma properties with those of the authentic standards, while tentative identifications were based on matching these parameters with those in the literature.^{20,21}

Statistical Analysis. Analysis of variance (ANOVA) was performed using the general line model procedure in SPSS (version 10.1, SPSS, Chicago, IL) to evaluate significant differences in volatile components from dried *omija* fruits according to the cultivation areas. Duncan's multiple-range test was used when the volatiles in the samples exhibited significantly different peak areas, with the level of significance set at *p* < 0.05. PCA was performed based on the raw values (*n* = 3) of the relative peak areas obtained on the GC–MS total

Table 2. Aroma-Active Compounds Detected in Dried *Omija* Fruits According to the Cultivated Areas

no. ^a	RI ^b	aroma-active compds	aroma property ^c [concns in solvent extract used for sniffing (mg/L)] ^d	FD factor ^e			
				MG ^f	JA ^g	JE ^h	HS ⁱ
3	812	hexanal	cut grass-like [0.4–16.0]	32	32	16	8
4	833	furfural	sweet, fresh [2.9–54.0]	2	4	0	0
6	908	γ -butyrolactone	pungent, sweet [11.6–115.6]	2	4	0	2
8	925	α -pinene	woody [0.9–1.5] pine-like [1.7–88.2]	64	64	32	16
9	940	camphene	pine-like, camphoraceous [0.9–32.9]	4	2	2	4
10	975	5-methylfurfural	sweet [0.1–0.2] burnt sugar-like [0.2–12.6]	32	32	16	16
14	986	β -myrcene	green, fresh [5.5–237.5]	32	8	8	16
15	1003	α -phellandrene	minty, pine-like, spice-like [1.4–31.8]	16	8	2	1
16	1021	α -terpinene	minty, green, fresh [0.7–33.1]	32	32	16	16
21	1048	(<i>E</i>)- β -ocimene	floral, herbaceous [0.2–34.4]	64	128	64	32
25	1083	α -terpinolene	green, plastic-like [2.5–15.2]	2	2	2	2
27	1118	phenethyl alcohol	floral [3.7–14.7] gasoline-like [29.3]	0	0	0	8
30	1176	terpinene-4-ol	earthy, musty [15.7–62.9]	2	4	2	4
32	1284	bornyl acetate	woody [2.7–60.4]	8	4	8	4
33	1330	δ -elemene	herbaceous [7.7–25.3] <i>omija</i> -like, herbaceous [30.9–54.9]	2	4	4	4
35	1350	citronellyl acetate	dried leaf-like [23.1–64.6] woody, dusty [46.2–258.6]	4	8	4	4
37	1385	α -copaene	woody [28.1–125.0] woody, green tea-like [122.2–250.0]	4	2	2	4
40	1416	β -caryophyllene	woody [14.1–56.3]	4	4	4	2
43	1455	α -humulene	herbaceous [10.1–56.3]	2	2	2	2
46	1490	β -selinene	herbaceous [108.0–1661.6]	8	16	4	4
55	1552	nerolidol	woody [17.0–182.9]	8	8	8	4
56	1570	spathulenol	woody [8.4–169.1]	4	4	4	4
58	1654	α -cadinol	floral, herbaceous [37.4–71.8] floral, woody, herbaceous [74.7–558.7]	8	8	4	4

^a Numbers correspond to those in Table 1 and Figures 4–6. ^b Retention indices were determined using *n*-paraffins C₇–C₂₂ as external references.

^c Aroma properties perceived at the sniffing port by two trained panels. ^d The concentration ranges were determined in solvent extract used for sniffing considering detector responses of each compound, except for α -phellandrene, δ -elemene, α -copaene, β -selinene, spathulenol, and α -cadinol. ^e FD: flavor dilution factor. An FD factor <1 means that the respective compound was not detected during sniffing of the undiluted extract. ^f MG, Mungyeong. ^g JA, Jangsu. ^h JE, Jechon. ⁱ HS, Hoengseong.

ion chromatograms using SIMCA-P (version 11.0, Umetrics, Umeå, Sweden).

RESULTS AND DISCUSSION

The Volatile Compositions in Dried *Omija* Fruits According to the Cultivation Areas. Table 1 lists the volatile components identified in dried *omija* samples according to the cultivation areas, with their relative peak areas, and RIs on the DB-5 MS column. The following 58 volatile components were identified from extracts of *omija* fruits: 15 monoterpene hydrocarbons, 21 sesquiterpene hydrocarbons, 7 oxygenated terpenes, 10 carbonyls, 2 acids, and 3 miscellaneous compounds. Quantitatively, terpene hydrocarbons, such as monoterpenes and sesquiterpenes, were the main volatiles in all *omija* fruit samples. These components are commonly associated with the pleasant aroma note of citrus products, spices, and herbs.²² In particular, α -pinene

(no. 8), sabinene (no. 11), β -pinene (no. 12), β -myrcene (no. 14), *p*-cymene (no. 17), limonene (no. 18), and γ -terpinene (no. 22) were the main monoterpenes of *omija*, whereas α -ylangene (no. 36), β -elemene (no. 39), germacrene D (no. 45), β -selinene (no. 46), and α -selinene (no. 47) were the main sesquiterpenes in some samples. Germacrene D, which is reportedly one of the main flavor components in *omija* leaves,⁸ was most abundant in the *omija* fruits in this study. Some other terpene-type volatiles, such as β -selinene, α -selinene, and β -elemene, either were not detected or were detected only as minor components in *omija* fruits in previous studies.²³ However, they were quantitatively the most abundant volatile components of *omija* fruits in the present study. The difference between these findings might be attributable to the species of *omija* studied, the cultivation area, and the extraction methods used differing between the studies. Furan derivatives, 5-hydroxymethylfurfural (HMF) (no. 31) and methyl 2-furoate (no. 24), were also found to be

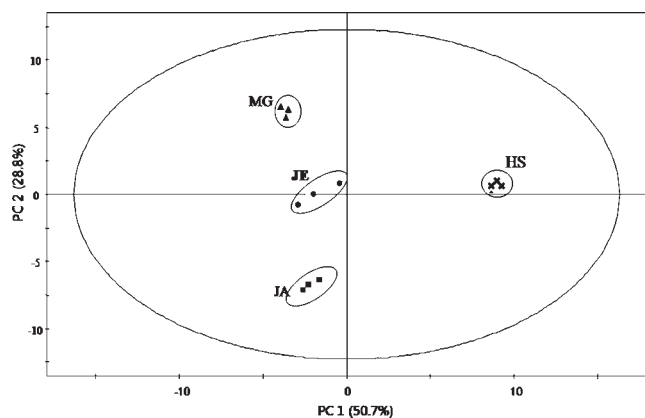


Figure 1. The PCA score plots for the extract of four dried *omija* fruits generated using combinations of PC 1 and PC 2.

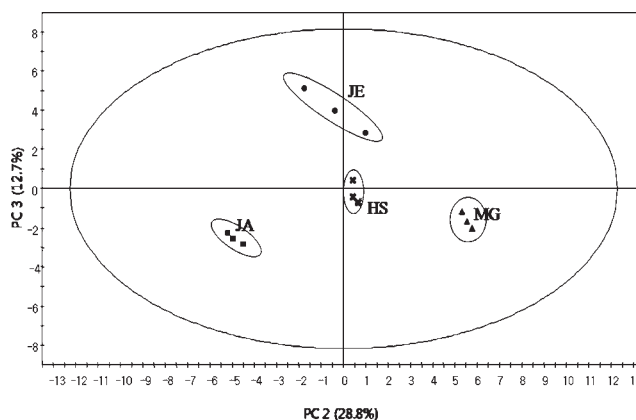


Figure 3. The PCA score plots for the extract of four dried *omija* fruits generated using combinations of PC 2 and PC 3.

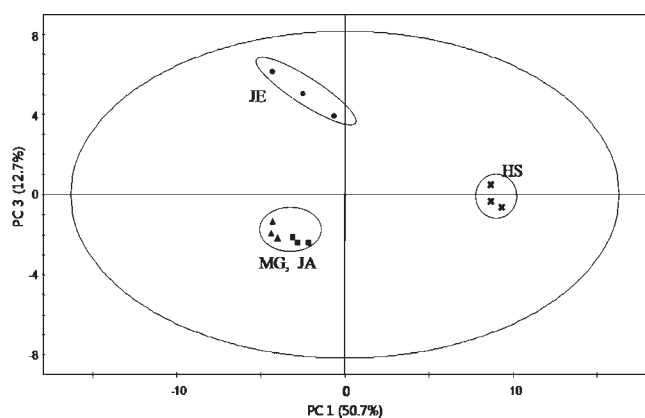


Figure 2. The PCA score plots for the extract of four dried *omija* fruits generated using combinations of PC 1 and PC 3.

present at high amounts in *omija* fruits. HMF is one of the characteristic flavor compounds in raisins, honey, fruit juice, and beer,²⁴ and can be formed via the Maillard reaction or caramelization of monosaccharides and disaccharides during thermal processing methods, such as drying.²⁵ Methyl 2-furoate, which is usually detected in oak wood, can be produced during the thermal degradation of glucose.²⁶ Furan derivatives in the present study can be formed by heat treatment during the drying process of *omija* fruits which contain considerable amounts of precursors for the Maillard reaction and caramelization. Xu et al.²⁷ reported a long heating time and a high temperature can increase the amount of HMF in *omija* fruits. However, these components have not been detected in *omija* leaves, stems, and roots,^{8,9} perhaps due to amounts of sugars being lower in these structures than in the fruits. In this study, the contents of most monoterpene and sesquiterpene hydrocarbons, except for (*Z*)-sabinene hydrate, were lower in *omija* sample from HS than in those from MG, JA, and JE. However, nonanal (no. 26), 2,3-butanediol (no. 2), and phenethyl alcohol (no. 27) were detected only in *omija* sample from HS. In contrast, α -cubebene (no. 34) and α -calacorene (no. 53) were found only in *omija* samples from MG and JA, respectively.

In general, geographical factors, such as altitude, temperature, rainfall, sun exposure time, and soil compositions, are known to

Table 3. The Climatic Conditions with Average Temperature ($^{\circ}\text{C}$), Average Daily Temperature Range ($^{\circ}\text{C}$), Total Rainfall (mm), and Total Sun Exposure Time (h) from May to October in 2008^a

location	av temp ($^{\circ}\text{C}$)	daily temp range ($^{\circ}\text{C}$)	total rainfall (mm)	sun exposure time (h)
MG	20.2	10.9	832.1	1064.0
JA	19.2	12.0	716.9	1037.1
JE	19.2	12.2	724.7	1017.8
HS	20.8	10.8	832.3	933.8

^aData were obtained from Korean Meteorological Administration (<http://www.kma.go.kr>).

influence the quality of agricultural products.²⁸ Many studies have investigated how the chemical composition and/or quality of various fruits and wines vary with the geographical factors.^{29,30} For example, grapes and wines of high quality are often produced only within highly restricted areas in many countries because of their geographical advantages. Moreover, several studies have shown that the formation of terpene hydrocarbons and esters is sensitive to temperature, sunlight, and rainfall in various species of grapes and apples.^{29,30} In the present study, the differences in the compositions of volatiles from *omija* fruits cultivated in four areas in South Korea were applied with PCA to GC–MS data sets. PCA is an unsupervised clustering method that does not require any knowledge of the data set and that reduces the dimensionality of multivariate data while preserving most of the variance therein.³¹ The original variables can be expressed as a particular linear combination of the PCs in the score plots, which account for a portion of the total variance of the data sets. Plotting the data in the space defined in this way provides a rapid means of visualizing similarities or differences in the data set,³² allowing for improved discrimination among samples. As shown in Figures 1–3, *omija* fruits could clearly be differentiated according to their cultivation area in score plots constructed by combining PC 1 with PC 2 (79.5%), PC 1 with PC 3 (63.4%), and PC 2 with PC 3 (41.5%), respectively. In particular, in the score plot combining PC 1 and PC 2 (Figure 1), *omija* sample from HS (positive PC 1 dimension) was well separated from other samples (negative PC 1 dimension) by PC 1, and *omija* samples from MG (positive PC 2 dimension) and JA (negative

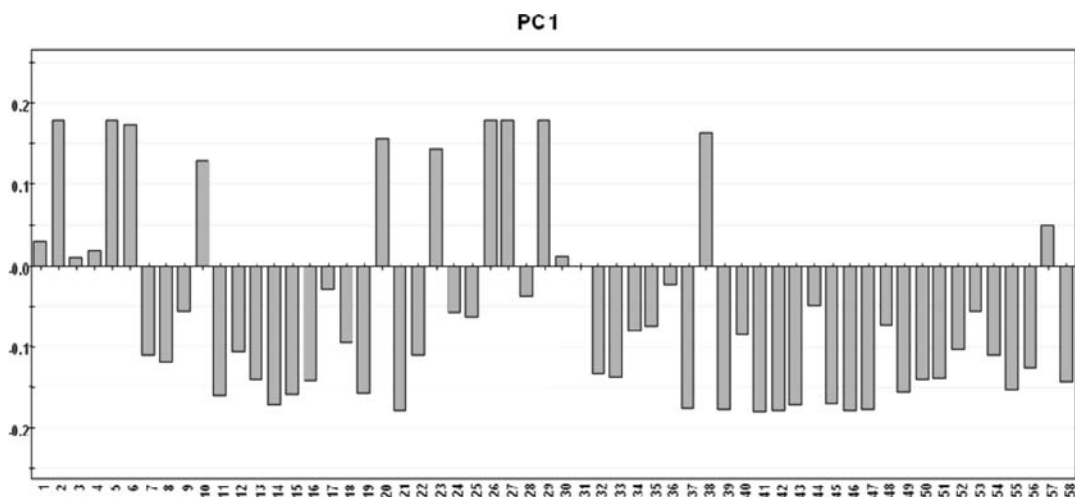


Figure 4. The PCA loading plots for the extract of four dried *omija* fruits associated with PC 1 (numbers correspond to those in Table 1).

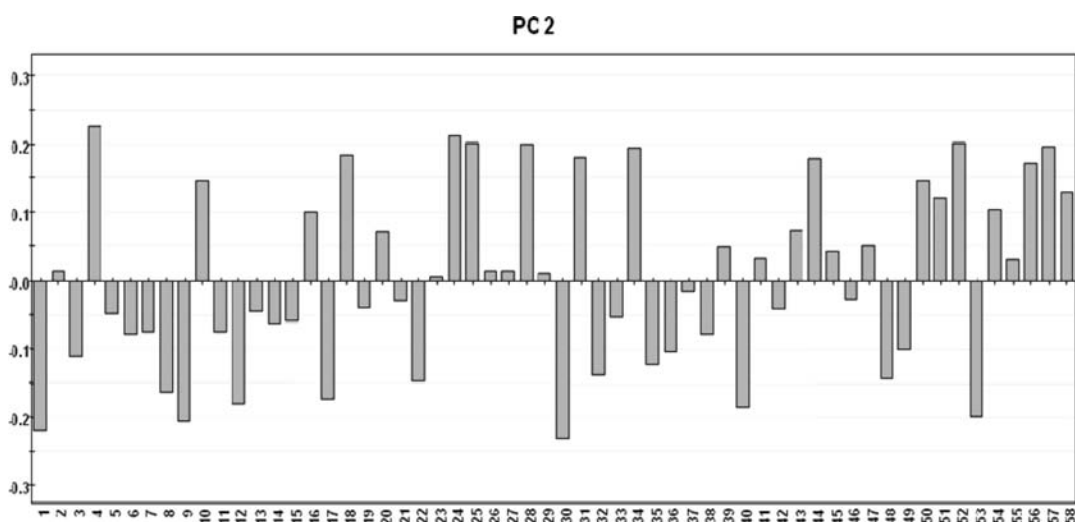


Figure 5. The PCA loading plots for the extract of four dried *omija* fruits associated with PC 2 (numbers correspond to those in Table 1).

PC 2 dimension) could also be discriminated from each other by PC 2. On the other hand, in the score plots using a combination of PC 1 and PC 3 (Figure 2), the *omija* sample from HS (positive PC 1 dimension) could be discriminated from other samples (negative PC 1 dimension) by PC 1, and *omija* sample from JE (positive PC 3 dimension) could also be separated from *omija* samples from JA and MG (negative PC 3 dimension) by PC 3. However, the extracts of *omija* from MG and JA still exhibited similar profiles of volatiles in this score plot. The discrimination of MG (positive PC 2 dimension) and JA (negative PC 2 dimension) from each other could be explained by PC 2 in the score plot combining PC 2 and PC 3 (Figure 3).

As shown in Table 3, the climatic conditions were varied according to the cultivated areas for *omija* fruits. For example, the rainfall was higher in MG and HS than in JA and JE, whereas the sun exposure time was lowest in HS. These differences in climatic conditions (especially the sun exposure time) might affect the volatile compositions of *omija* fruits. On the other hand, coefficients by which the original variables are multiplied to obtain the PC are called loadings, whose numerical values indicate how

similar each variable is compared to that component.³³ Thus, it was possible to determine the main volatile components that allowed *omija* fruits from different geographical origins to be discriminated by analyzing the PCA loading plots (Figure 4–6). As shown in Figure 4, β -myrcene (no. 14), (*E*)- β -ocimene (no. 21), α -copaene (no. 37), β -bourbonene (no. 38), β -elemene (no. 39), calarene (no. 41), (*E*)- β -farnesene (no. 42), α -humulene (no. 43), germacrene D (no. 45), β -selinene (no. 46), α -selinene (no. 47), γ -butyrolactone (no. 6), nonanal (no. 26), 2-methylbutanoic acid (no. 5), benzoic acid (no. 29), 2,3-butanediol (no. 2), and phenethyl alcohol (no. 27) were associated with the differentiation by PC 1. In particular, *omija* sample from HS contained much higher amounts of β -bourbonene (no. 38), γ -butyrolactone (no. 6), nonanal (no. 26), 2-methylbutanoic acid (no. 5), benzoic acid (no. 29), 2,3-butanediol (no. 2), and phenethyl alcohol (no. 27) with relatively higher positive values, whereas β -myrcene (no. 14), (*E*)- β -ocimene (no. 21), α -copaene (no. 37), β -elemene (no. 39), calarene (no. 41), (*E*)- β -farnesene (no. 42), β -selinene (no. 46), and α -selinene (no. 47), exhibiting relatively higher negative

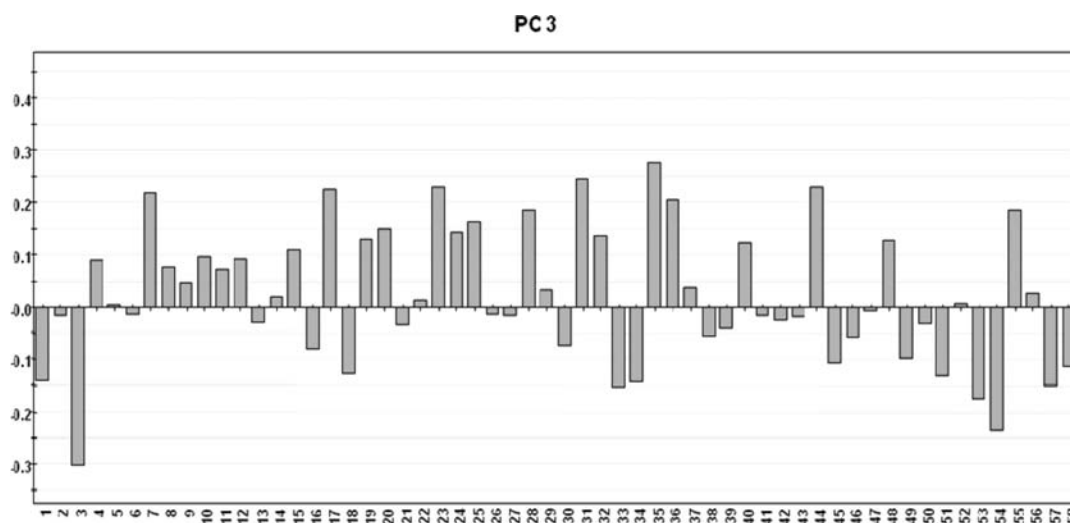


Figure 6. The PCA loading plots for the extract of four dried *omija* fruits associated with PC 3 (numbers correspond to those in Table 1).

values, occurred in higher proportions in *omija* fruit samples from MG, JA, and JE. On the other hand, as shown in Figure 5, terpinene-4-ol (no. 30), furfural (no. 4), 3-hydroxy-2-butanone (no. 1), methyl 2-furoate (no. 24), camphene (no. 9), calacorene (no. 53), α -terpinolene (no. 25), 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (no. 28), and cadina-1,4-diene (no. 52) were mainly related to discrimination by PC 2. *Omija* fruits from MG and JA could be also distinguished from *omija* fruit from JE in the score plots of PC 1 versus PC 2, and PC 2 versus PC 3, respectively (Figures 1 and 3). The proportions of terpinene-4-ol (no. 30), 3-hydroxy-2-butanone (no. 1), camphene (no. 9), and α -calacorene (no. 53), exhibiting relatively higher negative values in the PC 2 loading plot, were higher in *omija* fruit sample from JA, whereas that from MG was found to contain more furfural (no. 4), methyl 2-furoate (no. 24), α -terpinolene (no. 25), 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (no. 28), and cadina-1,4-diene (no. 52) with relatively higher positive values in the PC 2 loading plot. The main compounds associated with separation according to PC 3 were hexanal (no. 3), citronellyl acetate (no. 35), HMF (no. 31), germacrene B (no. 54), (*Z*)-sabinene hydrate (no. 23), *p*-cymene (no. 17), α -amorphene (no. 44), and α -thujene (no. 7) (Figure 6). On the other hand, the amounts of citronellyl acetate (no. 35), HMF (no. 31), (*Z*)-sabinene hydrate (no. 23), *p*-cymene (no. 17), α -amorphene (no. 44), and α -thujene (no. 7) with relatively higher positive values in PC 3 were substantially higher in *omija* sample from JE than in those from MG and JA. In contrast, the amounts of hexanal (no. 3) and germacrene B (no. 54), exhibiting relatively higher negative values in the PC 3 loading plot, were greater in *omija* samples from MG and JA than in that from JE.

Aroma-Active Compounds of Dried *Omija* Fruits According to the Cultivation Areas. Aroma-active compounds in the *omija* extracts from the four areas were evaluated by AEDA. In total, 23 aroma-active compounds were detected in all *omija* samples (Table 2). (*E*)- β -Ocimene was the most potent aroma-active compound in all *omija* extracts, having the highest FD factor, followed by α -pinene, hexanal, 5-methylfurfural, and α -terpinene. (*E*)- β -Ocimene (floral and herbaceous), which could be derived from geranyl diphosphate,³⁴ is reportedly a characteristic odorant of *omija* leaves.⁸ α -Pinene, which has pine-like and woody aroma notes, was identified as the characterizing

compound of *Pinus densiflora* needles,³⁵ and also as an important precursor for various terpene derivatives.³⁶ This compound exhibited high FD factors (16–64) due to its relatively high content in four *omija* samples, although it had a relatively high threshold value (0.062 $\mu\text{g/L}$ in air).³⁷ Two other aroma-active compounds, hexanal (cut grass-like) and 5-methylfurfural (burnt sugar-like and sweet), also had relatively high FD factors. Hexanal, which is an important aroma-active compound in various fruits and leaves, could be derived from the oxidation of unsaturated fatty acids in plants.³⁸ In the present study, it exhibited relatively high FD factors of 8–32. 5-Methylfurfural, one of the furan derivatives, could be formed via Maillard reactions between reducing sugars and amino acids or caramelization.³⁹ This compound exhibited high FD factors of 16–32 in *omija* fruits, although it had a relatively high threshold value (6 mg/L in air).³⁷ In addition, α -terpinene, which is perceived as minty, green, and fresh aroma notes, exhibited relatively high FD factors of 16–32.

Nearly all of the aroma-active compounds detected in *omija* samples from MG, JA, and JE were also present in that from HS, although some compounds, such as hexanal, α -pinene, and α -phellandrene, were at lower FD factors. This probably reflects the effects of geographical factors on the aroma-active compounds in *omija* fruits, similar to those on the volatile compositions as explained above. Different climatic conditions can affect the levels of precursors and the activities of related enzymes, thereby leading to changes in fruit flavor development.⁴⁰ Many researchers have reported that variations in the emissions of diverse plant and fruit aromas were statistically related to the climatic conditions under which they were grown.⁴¹ In the present study, (*E*)- β -ocimene had the highest FD factor in *omija* sample from JA (FD factor 128), much higher than in that from HS (FD factor 32). On the other hand, α -pinene, which was the second most intense aroma-active compound in all *omija* extracts, had higher FD factors in *omija* samples from MG and JA (FD factor 64) than in that from HS (FD factor 16). The FD factors of hexanal (cut grass-like) were found to be higher in *omija* samples from MG and JA (FD factor 32) than in those from HS (FD factor 8). β -Myrcene (green and fresh) exhibited higher FD factors in *omija* samples from MG (FD factor 32) than in those from JA and JE (FD factor 8). However, α -phellandrene (minty and pine-like)

was found to be higher in *omija* samples from MG (FD factor 16) than in those from HS and JE (FD factor 1–2). β -Selinene (herbaceous) exhibited higher FD factors in *omija* samples from JA (FD factor 16) than in those from JE and HS (FD factor 4). These aroma-active compounds could be considered to be important contributors to the fresh and sweet aroma notes of *omija* fruits. However, phenethyl alcohol (floral and gasoline-like) was detected only in *omija* sample from HS, whereas furfural (sweet and fresh) was found only in *omija* samples from MG and JA. In contrast, γ -butyrolactone (pungent and sweet) could not be detected only in *omija* samples from JE.

In summary, PCA analysis applied to GC–MS data sets may represent a relatively simple and efficient method of differentiating *omija* cultivated in different areas. In addition, our findings clearly demonstrate that terpene hydrocarbons, such as germacrene D, β -selinene, α -ylangene, β -elemene, α -selinene, and (*E*)- β -farnesene, were major volatiles contributing to the difference in the volatile compositions in *omija* fruit samples. Interestingly, the *omija* sample from HS could be easily separated from the other samples by PC 1 in the score plots. GC–O was also employed to determine the aroma-active compounds of *omija* fruits from different cultivation areas using AEDA. (*E*)- β -Ocimene (floral and herbaceous), α -pinene (pine-like and woody), hexanal (cut grass-like), 5-methylfurfural (burnt sugar-like and sweet), and α -terpinene (minty, green, and fresh) were the most intense aroma-active compounds in all *omija* extracts. The FD factors of aroma-active compounds were generally much lower in *omija* sample from HS than in those from the other cultivation areas. Our results for the compositions of both volatiles and aroma-active compounds could clearly discriminate different *omija* fruits according to their region of cultivation.

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ABBREVIATIONS USED

GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; GC–O, gas chromatography–olfactometry; AEDA, aroma extract dilution analysis; PCA, principal component analysis; MG, Mungyeong; JA, Jangsu; JE, Jechon; HS, Hoengseong; GC–MSD, gas chromatography–mass selective detector; FID, flame ionization detector; FD, flavor dilution; RIs, retention indices; ANOVA, analysis of variance; HMF, hydroxymethylfurfural

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