

Characteristic Aroma-Active Compounds of Korean Perilla (Perilla frutescens Britton) Leaf

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Aroma-active compounds from Korean perilla (*Perilla frutescens* Britton) leaf were extracted by solventassisted flavor evaporation (SAFE), liquid–liquid continuous extraction (LLCE), and hydrodistillation (HD) and analyzed by gas chromatography–mass spectrometry (GC-MS) and gas chromatography–olfactometry (GC-O). Thirty-three volatile compounds were identified by GC-MS. 1-(3-Furyl)-4-methyl-1-pentanone (perilla ketone) was found to be the most abundant volatile compound, followed in order by (*Z*)-3-hexenol and 1-octen-3-ol. Perilla ketone comprised 81% (93 ppm), 84% (120 ppm), and 95% (490 ppm) of the volatile compounds obtained from SAFE, LLCE, and HD, respectively. Thirteen aroma-active compounds were detected by GC-O. Perilla ketone, 1-(3-furyl)-4-methyl-3-penten-1-one (egoma ketone), and 1-(3-furyl)-4-methyl-2-penten-1-one (isoegoma ketone) were considered to be the characteristic aroma-active compounds of Korean perilla leaf. Perilla ketone, (*Z*)-3-hexenal (green), egoma ketone, and isoegoma ketone were the most intense aroma-active compounds in Korean perilla leaf. Other relatively intense odorants included (*Z*)-3-hexenol (green), (*E*)-2-hexenal (green), benzaldehyde (almond), 1-octen-3-one (metallic), 1-octen-3-ol (mushroom), phenylacetaldehyde (honeysuckle), linalool (lemon), and β -caryophyllene (woody).

KEYWORDS: Perilla leaf (*Perilla frutescens* Britton); gas chromatography-olfactometry; aroma-active compound; perilla ketone; egoma ketone; isoegoma ketone

INTRODUCTION

Perilla (*Perilla frutescens* Britton) is an annual herb with a distinctive aroma and taste that has been cultivated for centuries in Korea. Its leaves and extracted essential oil are often used as flavoring ingredients for soups, stews, and roasting (1). Perilla is also grown in many other countries including Japan, China, Vietnam, Turkey, and the United States. Although perilla is known to cause acute pulmonary edema in cattle and sheep due to perilla ketone (2), it is a popular leafy vegetable in Korea, which is generally consumed as a pickle or wrapping with roasted meats.

Korean perilla leaf, "Kkaennip", has a different appearance and flavor from Japanese perilla leaf, "Shiso" in that the former is larger, rounder, and flatter with a less serrated edge and a violet coloring on the reverse side and has an aroma reminiscent of apples and mint (3). Japanese perilla, which is used as a garnish and a red food coloring (4), has several botanical varieties, which differ in the composition of the volatile oils in leaves. Perilla plants are classified into seven chemotypes, such as perillaldehyde (PA), perilla ketone (PK), elsholtzia ketone (EK), citral (C), perillene (PL), piperitenone (PT), and phenylpropanoid (PP) types, based on the main components of the volatile oils in their leaves (5–7). Korean perilla leaf contains perilla ketone as a major volatile component (8–10). The perilla leaf is used as an edible colorant in Japan because it contains anthocyanin pigments, such as anthocyanins, flavones, and flavone glycosides (11). Choung et al. (12) isolated and detected anthocyanins, such as 3-O-(6-O-cis-p-coumaryl)- β -D-glucopyranoside, 5-O- β -D-glucopyranoside, and 5-O-(6-O-malonyl)- β -D-glucopyranoside, from perilla leaf and identified their chemical structures by spectrometry. The essential oil from perilla leaf has also been applied as an oral deodorant (1).

Korean perilla leaf is known to have several biological activities (13-16). Lee et al. (13) examined the inhibitory effects of phytol and methyl 11,14,17-eicosatrienoic acid separated from Korean perilla leaf on the growth of human cancer cells. Chung et al. (14) reported that Korean perilla leaf contained superoxide dismutase activity responsible for antioxidant activity. Methanol extracts of Korean perilla leaf showed antimutagenic and antioxidative activities (15). In addition, Kim et al. (16) found that enzymatic extracts from Korean perilla leaf possessed antioxidative and neuroprotective effects.

Few studies have been carried out to characterize volatile flavor components of Korean perilla leaf (8-10) and Shiso grown in Korea (17-19), except a few major volatile compounds such as perilla ketone found in the Korean perilla leaf (8-10). Perilla ketone is a potent pulmonary edemagenic agent for laboratory animals and livestock (20). Kim et al. (9) reported that perilla ketone comprised 92% of the volatile compounds obtained by simultaneous steam distillation and solvent extraction, 86% by headspace analysis, and 62% by solvent extraction. Jang et al. (17) analyzed volatile components of Shiso (*Perilla folium*)

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Table 1. Volatile Compounds Identified from Korean Perilla Leaf

		RI ^b		concentration (ppm)			
no. ^a	compound	DB-5 ms	DB-Wax	SAFE ^c	LLCE ^d	HD ^e	identification
		Hydroca	rhons				
		Tiydroca	100113				
1	dodecane	1200	1200		0.14		$PI'(MS, ^{h}RI)$
2	eta-caryophyllene	1412	1583	0.12	0.35	7.4	PI (MS, RI, odor')
3	α -humulene	1446	1652	0.03	0.12	0.83	PI (MS, RI)
4	germacrene-D	1475	1742	0.02	0.04	0.88	PI (MS, RI⁄)
5	(Z,E) - α -farnesene	1494	1732	0.08	0.25	5.6	PI (MS, RI/)
6	δ -cadinene	1531	1724	0.01			TI ^{<i>g</i>} (MS, RI ^{<i>j</i>})
7	neophytadiene	1836			0.05		TI (MS)
		Alcoh	ols				
8	1-penten-3-ol	664	1135	0.10			PI (MS, RI)
9	(Z)-2-pentenol	746	1320	0.26	0.10		PI (MS, RI)
10	(Z)-3-hexenol	875	1390	7.7	0.30	0.21	PI (MS, RI, odor)
11	(E)-2-hexenol	879	1405	0.31	0.19		PI (MS, RI)
12	1-hexanol	881	1331	0.42			PI (MS, RI)
13	1-octen-3-ol	992	1460	6.5	6.1	1.1	PI (MS, RI, odor)
14	3-octanol	999	1394	0.30	0.28		PI (MS, RI)
15	linalool	1102	1540	0.84	0.87	0.78	PI (MS, RI, odor)
16	eugenol	1368	2171	2.2	0.84	0.73	PI (MS, RI [/])
17	nerolidol	1568	2004	0.04	0.02		TI (MS, RI)
18	α-cadinol	1670	2169	0.01			TI (MS, RI)
		Aldehy	des				
19	(E)-3-pentenal	733	1104	0.02			PI (MS, RI)
20	(Z)-3-hexenal	806	1137	0.23	0.50	0.31	PI (MS, RI, odor)
21	hexanal	808	1065	0.37	0.10	0.26	PI (MS, RI, odor)
22	(E)-2-hexenal	867	1219	2.0	0.28	0.16	PI (MS, RI, odor)
23	(E,E)-2,4-hexadienal	915	1368	1.1	1.6		PI (MS, RI, odor)
24	benzaldehyde	960	1520	0.07	0.13	0.16	PI (MS, RI, odor)
25	(E.E)-2.4-heptadienal	1005	1462	0.02			PI (MS, RI)
26	phenylacetaldehyde	1051	1648	0.13	0.30		PI (MS, RI, odor)
		Keton	es				
27	1-penten-3-one	669	1030	0.02			PI (MS, RI)
28	3-pentanone	678	958	0.01			PI (MS, RI)
29	1-octen-3-one	982	1295	trace			PI (MS, RI, odor)
30	1-(3-furyl)-4-methyl-1-pentanone (perilla ketone)	1289	1846	93	120	490	TI (MS)
31	1-(3-furyl)-4-methyl-3-penten-1-one (egoma ketone)	1316	1890	0.11	0.24	0.36	TI (MS)
32	1-(3-furyl)-4-methyl-2-penten-1-one (isoegoma ketone)	1321	1951	0.61	0.74	1.5	TI (MS ^{k,l})
		Fura	n				
33	perillene	1099				0.16	TI(MS)

^aNumbers correspond to those in **Table 2**. ^b Retention indices were determined on DB-5 ms and DB-Wax using C₆-C₂₂ as external references. ^c Solvent-assisted flavor evaporation. ^d Liquid—liquid continuous extraction. ^e Hydrodistillation. ^f Positive identification. ^g Tentative identification. ^h Mass spectrum was consistent with that of Wiley 7N mass spectrum database. ⁱ Identification was based on GC-O injection with an authentic compound. ^j From Kondjoyan and Bergague (*26*). ^k From Nitta et al. (*7*). ^I Identification was based on interpretation of mass spectrum.

grown in Korea and reported myristicin as a major component with perilla ketone as minor component. Choi and Min (18) and Choi (19) reported that perillaldehyde was the most abundant volatile compound of *P. frutescens* var. acuta Kudo, which is called "Chazugi" or "Soyup" in Korean.

Gas chromatography-olfactometry (GC-O) is widely used to determine aroma-active components in foods (21-23), which is a valuable tool for screening and identification of aroma-active compounds. Aroma extract dilution analysis (AEDA), which involves an analysis of a serially diluted flavor extract to obtain a flavor dilution (FD) factor, provides valuable information on characteristics of important aroma-active compounds.

Choi (19) reported that perilla ketone, terpinen-4-ol, α -humulene, perillaldehyde, humulene epoxide, and (*E*)-2-dodecenol were characteristic aroma components in *P. frutescens* var. acuta Kudo, which is a different variety from Korean perilla leaf, by AEDA. However, there is no report on the characteristic aromaactive compounds of Korean perilla leaf.

The aims of this study were to isolate the volatile flavor components from Korean perilla leaf using solvent-assisted flavor evaporation (24), liquid–liquid continuous extraction, and hydrodistillation and to evaluate the characteristic aroma-active compounds by GC-O.

MATERIALS AND METHODS

Materials. Korean perilla leaf (*P. frutescens* Britton), which had been grown and harvested at Chubu, Chungchongnamdo, Korea, was purchased from a local market in Cheonan, Korea. The perilla leaves have green on the front and purple on the back. After purchase, the perilla leaves were refrigerated at 4 °C until extraction. The extraction was performed within 10 days after harvest. The whole perilla leaf was surface-cleaned and ground in a Waring blender at a ratio of 1:1 with

deodorized distilled water prior to extraction, and then 100 mL of a saturated CaCl₂ (solubility = 74.5 g/100 g of water, 25 °C) solution was added to inactivate the lipoxygenase activity.

The standard flavor compounds dodecane, germarcrene-D, (*Z*,*E*)- α -farnesene, 1-penten-3-ol, (*Z*)-2-pentenol, (*E*)-2-hexenol, 1-hexanol, (*Z*)-3-hexenol, 3-octanol, 1-octen-3-ol, eugenol, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*,*E*)-2,4-hexadienal, (*E*)-3-pentenal, (*E*,*E*)-2,4-heptadienal, benzaldehyde, 1-penten-3-one, 3-pentanone, 1-octen-3-one, phenylacetaldehyde, and linalool were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI); β -caryophyllene and α -humulene were from Fluka Chemie (Buchs, Switzerland).

Solvent-Assisted Flavor Evaporation (SAFE). Volatiles were isolated by high-vacuum distillation using SAFE (24). SAFE was carried out with 100 g of ground Korean perilla leaves and internal standard (3-heptanol, 19.4 μ g) for 2 h at 35 °C under vacuum (10⁻⁶ torr). After distillation, the solution was extracted three times with 10 mL of dichloromethane. After extraction, water was removed by freezing out, and residual water was dried over 3 g of sodium sulfate. The extract was concentrated to 100 μ L under a gentle N₂ stream. Extractions were performed in duplicate.

Liquid–Liquid Continuous Extraction (LLCE). One kilogram of ground perilla leaves was placed into a liquid–liquid continuous extraction (1 L, catalog no. LG-6996-100, Lab Glass, Vineland, NJ) apparatus. 3-Heptanol (194.4 μ g) was added to the LLCE apparatus as an internal standard. The extraction was carried out for 12 h using 225 mL of dichloromethane as the solvent. After extraction, water was removed by freezing, and the extract was then dried over 3 g of anhydrous sodium sulfate. The extract was concentrated to 200 μ L under a gentle nitrogen stream. Extractions were performed in duplicate.

Hydrodistillation (**HD**). One kilogram of fresh perilla leaves was extracted for 1 h in a Clevenger-type apparatus (consisting of a distilled flask, condenser, and separatory funnel) with 500 mL of deodorized distilled water to obtain the essential oil. The yield of essential oil was 0.52 g/kg.

Gas Chromatography–Mass Spectrometry (GC-MS). The GC-MS system consisted of an HP 6890N series GC-HP 5973 mass selective detector (MSD) (Agilent Co., Palo Alto, CA). One microliter of each extract or essential oil diluted at 1:100 was injected (splitless mode; 60 s valve delay) onto DB-5 ms (60 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness; J&W Scientific, Folsom, CA) and DB-Wax (60 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness: J&W Scientific) columns. The other conditions were the same as described previously (25). The analyses were performed in duplicate.

Gas Chromatography–Olfactometry (GC-O). GC-O was carried out using a Varian 3350 GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector and a sniffing port system (SGE Analytical Science, Austin, TX). All extracts used for GC-O were representative of the aroma of Korean perilla leaf. AEDA was carried out with serially diluted extracts (1:3) on a DB-5 ms column. Two panelists familiar with Korean perilla leaf aroma performed the GC-O. The GC-O procedure has been mentioned previously (25).

Compound Identification. Compounds were positively identified by a comparison of the retention indices (RI), mass spectra, and aroma properties as described previously (26). Tentative identification was made on the basis of matching mass spectra of the unknowns with those in the Wiley 7N mass spectral database (Agilent Co.) and the literature.

Quantification of Compounds. The concentration of a compound in the sample was calculated using the following equation:

concentration (ppm) =
$$\frac{\text{peak area ratio} \times \mu \text{g of 3-heptanol}}{\text{g of sample}}$$

RESULTS AND DISCUSSION

Volatile Compounds in the Korean Perilla Leaf. Table 1 lists the identified volatile compounds, their concentrations, and the RIs of the compounds on the DB-5 ms and DB-Wax columns by SAFE, LLCE, and HD. A total of 33 compounds were identified, which included 7 hydrocarbons, 11 alcohols, 8 aldehydes, 6 ketones, and 1 furan. Of the 7 hydrocarbons identified, 6 were

Table 2. Aroma-Active Compounds from Korean Perilla Leaf

				log ₃ FD			
no. ^a	compound	RI [♭]	aroma description	SAFE ^c	$LLCE^d$	HD ^e	
20	(Z)-3-hexenal	806	green	6	8		
22	(E)-2-hexenal	867	green + cucumber		4	0	
10	(Z)-3-hexenol	875	green + cucumber	4			
23	(E,E)-2,4-hexadienal	915	green	1			
24	benzaldehyde	960	bitter almond		3		
29	1-octen-3-one	982	metallic	2			
13	1-octen-3-ol	992	mushroom	2	2	2	
26	phenylacetaldehyde	1051	honeysuckle	4	5		
15	linalool	1102	lemon	5	4	3	
30	perilla ketone	1289	perilla leaf	7	6	6	
31	egoma ketone	1316	perilla leaf	7	8	2	
32	isoegoma ketone	1321	perilla leaf	2	6		
2	β -caryophyllene	1412	woody			3	

^{*a*} Numbers correspond to those in **Table 1**. ^{*b*} Retention indices were determined on DB-5 ms using C_7-C_{22} as external references. ^{*c*} Solvent assisted flavor evaporation. ^{*d*} Liquid-liquid continuous extraction. ^{*e*} Hydrodistillation.

sesquiterpenes. Volatile profiles were different depending on extraction methods. More volatile compounds were extracted by SAFE: 30, 23, and 16 volatile compounds were identified by SAFE, LLCE, and HD, respectively.

Perilla ketone (30), 1-(3-furyl)-4-methyl-1-pentanone, was the most abundant of all extracts. Perilla ketone was present at 93, 120, and 490 ppm, which comprised 81, 84, and 95% of the volatile compounds in SAFE, LLCE, and HD extracts, respectively. Perilla ketone has been reported to be a major flavor constituent in Korean perilla leaf (8-10). Choung et al. (8) and Kim et al. (9) extracted the volatile compounds from Korean perilla leaf by simultaneous steam distillation and solvent extraction (SDE). They reported that perilla ketone comprised 72.0 and 92.2% of the volatile compounds, respectively. Our result is similar to those in previous papers (8-10). However, Jang et al. (17) and Choi (19) reported that the abundance of perilla ketone was 5.2 and 0.1% in P. folium and P. frutescens var. acuta Kudo, which are different varieties of perilla leaves grown in Korea. Korean perilla leaf is classified as perilla ketone type on the basis of volatile composition. Different chemotypes have different aroma characteristics.

Hydrocarbons identified in the SAFE extract were β -caryophyllene (2), α -humulene (3), germacrene-D (4), (Z,E)- α -farnesene (5), and δ -cadinene (6). All hydrocarbons were sesquiterpenes, and the compounds identified in this study were similar to those in other papers (9, 17). β -Caryophyllene and (Z,E)- α -farnesene, which are known as the most common sesquiterpenes found in various essential oils, have been identified as the most abundant volatiles in *Mosla calveriei* Level (27). Hydrocarbons identified in the LLCE extract were dodecane (1), β -caryophyllene (2), α -humulene (3), germacrene-D (4), (Z,E)- α -farnesene (5), and neophytadiene (7). β -Caryophyllene (2), α -humulene (3), germacrene (5) were detected in the HD extract.

Of the 11 alcohols identified in the SAFE extract, (*Z*)-3-hexenol (10) was the most abundant, followed by 1-octen-3-ol (13) and eugenol (16). (*Z*)-3-Hexenol, which is known as leaf alcohol, is considered to be formed in green leaves via lipoxygenase-mediated lipid oxidation (28). Linaool (15), 1-hexanol (12), (*E*)-2-hexenol (11), (*Z*)-2-pentenol (9), 3-octanol (14), 1-penten-3-ol (8), nerolidol (17), and α -cadinol (18) were found in low abundance. Eight and four alcohols were identified by LLCE and HD, respectively, of which 1-octen-3-ol (13) was the most abundant, followed by linalool (15) and eugenol (16). 1-Octen-3-ol has been identified as a major aroma-active compound in raw



Figure 1. Mass spectra of perilla ketone (A), egoma ketone (B), and isoegoma ketone (C) with a perilla leaf-like aroma from Korean perilla leaf.

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pine mushroom (29). (Z)-3-Hexenol (10), 3-octanol (13), (E)-2-hexenol (11), (Z)-2-pentenol (9), and nerolidol (17) were found in low abundance. The alcohols identified in Korean perilla leaf except for (Z)-2-pentenol (9) were also identified using the SDE method (9, 17).

Eight aldehydes were identified in Korean perilla leaf by SAFE. (*E*)-2-Hexenal (22) was the most abundant aldehyde followed by (*E*,*E*)-2,4-hexadienal (23) and hexanal (21). Aldehydes, such as (*Z*)-3-hexenal (20), hexanal (21), (*E*)-2-hexenal (22), and (*E*,*E*)-2,4-hexadienal (23), are considered to be biosynthesized in green leaves through lipoxygenase-mediated lipid oxidation, particularly from unsaturated fatty acids, such as linoleic and linolenic acid (30). Six aldehydes were identified by LLCE with (*E*,*E*)-2,4-hexadienal (23) the most abundant. (*Z*)-3-Hexenal (20), hexanal (21), (*E*)-2-hexenal (22), and benzaldehyde (24) were identified by HD.

Six ketones were identified in the SAFE extract. Perilla ketone (**30**) was the most abundant volatile compound, with 1-(3-furyl)-4-methyl-3-penten-1-one (egoma ketone, **31**) and 1-(3-furyl)-4-methyl-2-penten-1-one (isoegoma ketone, **32**) being detected in low abundance. Only three ketones, perilla ketone (**30**), egoma ketone (**31**), and isoegoma ketone (**32**), were detected by LLCE and HD. Baser et al. (*31*) reported that perilla ketone (**35**.6%) and isoegoma ketone (**35**.1%) were the main constituents of the essential oil of Turkish *P. frutescens* Britton.

Perillene (33), 3-(4-methyl-3-pentenyl) furan, which was known to be biosynthesized from (E)-citral (geranial) and oxidized to egoma ketone (32), was detected in low abundance by HD only.

Aroma-Active Compounds in Korean Perilla Leaf. Table 2 shows the aroma-active compounds detected from Korean perilla leaf by SAFE, LLCE, and HD. Of the 33 volatile compounds identified from Korean perilla leaf, 13 aroma-active compounds were detected in the SAFE, LLCE, and HD extracts by AEDA. Of these, perilla ketone (30) and egoma ketone (31) were the most intense aroma-active compounds with high $\log_3 FD$ factors (= 7) in the SAFE extract. Egoma ketone (31) was the most intense aroma-active compound with high log₃ FD factors (= 8) followed by perilla ketone (30) and isoegoma ketone (32) (log₃ FD factors = 6) in the LLCE extract. Perilla ketone (30), which was present in the highest amount, had a lower log₃ FD factor than egoma ketone (31) in the LLCE extract. Perilla ketone (30) was detected as the most intense aroma-active compound with a high $\log_3 FD$ factor (= 6) by HD. The aroma properties of perilla ketone (30), egoma ketone (31), and isoegoma ketone (32) were similar to that of Korean perilla leaf and are believed to be character-impact aroma-active compounds contributing to the aroma of Korean perilla leaf. Nishizawa et al. (32) reported that egoma ketone was oxidized from perillene. In addition, perilla ketone and isoegoma ketone are biosynthesized from egoma ketone. Egoma ketone and isoegoma ketone have not been identified as characteristic aroma-active compounds of Korean perilla leaf (*P. frutescens* Britton) in previous studies (8-10).

(Z)-3-Hexenal (20) was the second most intense aroma-active compound (\log_3 FD factors = 6) in the SAFE extract and the most intense one (\log_3 FD factors = 8) in the LLCE extract. (Z)-3-Hexenal is believed to impart a green and herbal note to Korean perilla leaf, which is biosynthesized in green leaves through lipoxygenase-mediated linolenic acid oxidation (27). Four aroma-active compounds, including linalool (15, lemon), phenylacetaldehyde (26), (E)-2-hexenal (22, green + cucumber), and (Z)-3-hexenol (10), were identified with relatively high \log_3 FD factors.

Morinaka et al. (33) pointed out that (E)-2-hexenal and (Z)-3-hexenol were important to the sensory attribute of perilla leaf

aroma. Linalool (15) has been identified as an aroma-active compound in the citrus hybrid Hallabong (34). Phenylacetalde-hyde (26), which has a honeysuckle, chocolate, and rose aroma, might be formed by enzymatic Strecker degradation from phenylalanine. 1-Octen-3-one (29, metallic), 1-octen-3-ol (13, mush-room), and (*E,E*)-2,4-hexadienal (23, green) were identified with relatively low log₃ FD factors. 1-Octen-3-one and 1-octen-3-ol have been identified as characteristic aroma-active compounds in raw pine mushroom (29). Benzaldehyde (24, bitter almond) and β -caryophyllene (2, woody) were identified with relatively low log₃ FD factors in the LLCE and HD extracts, respectively.

Figure 1 shows the mass spectra of perilla ketone (30), egoma ketone (31), and isoegoma ketone (32). The mass spectrum of isoegoma ketone (32) was not available from the mass spectral database in the GC-MS library. Therefore, the molecular structures were postulated from the mass spectral data (Figure 1C). The characteristic mass peak in the spectra of carbonyl compounds such as RCOR' is present as the RCO⁺ and R'⁺ type through α -cleavage reaction, and the major mass spectrum is represented as the RCO^+ form (35). The characteristic peaks of the mass spectrum of isoegoma ketone (32) were m/z 95, 135, and 164, which are almost the same as those of egoma ketone (31) except for the high abundance of m/z 135. The mass spectrum is also similar to that of perilla ketone (30) except for m/z 110 and 166. The peak at m/z 95 originates from the furyl ketone (RCO⁺ form), which is common to egoma ketone (31) and perilla ketone (30). For isoegoma ketone (32), unlike the mass spectrum of egoma ketone (31), a peak at m/z 135 was present in high abundance, indicating a different double-bond position (35). Therefore, this compound was postulated as 1-(3-furyl)-4-methyl-2-penten-1-one (isoegoma ketone) on the basis of the mass spectrum similar to that of egoma ketone and the high abundance of m/z 135. The mass spectral data in Figure 1C are similar to those presented for isoegoma ketone (7).

In conclusion, perilla ketone, egoma ketone, and isoegoma ketone were identified as character-impact compounds of Korean perilla leaf. Although perilla ketone has been suggested in Korean perilla leaf as aroma-active, egoma ketone and isoegoma ketone are reported for the first time as aroma-active compounds in Korean perilla leaf as well as other varieties. In addition, (*Z*)-3-hexenal, (*E*)-3-hexenol, (*E*)-2-hexenal, phenylacetaldehyde, and β -caryophyllene have a significant effect on the aroma of Korean perilla leaf.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; FD, flavor dilution; GC-MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; SAFE, solvent-assisted flavor evaporation; LLCE, liquid-liquid continuous extraction; HD, hydrodistillation.

LITERATURE CITED

- Lee, B. H.; Lee, S. T.; Kim, Y. S. References review for the scientific researches on perilla. *RDA J. Ind. Crop Sci.* **1998**, *40*, 80–112.
- (2) Tucker, A. O.; Maciarello, M. J. Some toxic culinary herbs in North America. In *Food Flavors: Formation, Analysis and Packaging Influences*; Contis, E. T., Ho, C.-T., Mussinan, C. J., Parliment, T. H., Shahidi, F., Spanier, A. M., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1998; pp 401–414.
- (3) Lee, J. K.; Ohnishi, O. Geographic differentiation of morphological characters among *Perilla* crops and their weedy types in East Asia. *Breeding Sci.* **2001**, *51*, 247–255.
- (4) Brenner, D. M. Perilla: botany, uses and genetic resources. In *New Crops*; Janick, J., Somon, J. E., Eds.; Wiley: New York, 1993; pp 322–328.

- (5) Koezuka, Y.; Honda, G.; Tabata, M. Genetic control of the chemical composition of volatile oils in *Perilla frutescens*. *Phytochemistry* **1986**, 25, 859–963.
- (6) Ito, M.; Toyoda, M.; Kamakura, S.; Honda, G. A new type of essential oil from *Perilla frutescens* from Thailand. J. Essent. Oil Res. 2002, 14, 416–419.
- (7) Nitta, M.; Kobayashi, H.; Ohnishi-Kameyama, M.; Nagamine, T.; Yoshida, M. Essential oil variation of cultivated and wild *Perilla* analyzed by GC/MS. *Biochem. Syst. Ecol.* 2006, 34, 25–37.
- (8) Choung, M. G.; Kwon, Y. C.; Kwak, Y. C. Test of components related to quality in perilla leaves: II. Test of volatile flavor components in perilla leaves. *RDA J. Agric. Sci.* 1998, 40, 127–132.
- (9) Kim, K. S.; Ryu, S. N.; Song, J. S.; Bang, J. K.; Lee, B. H. Comparison of analytical methods for volatile flavor compounds in leaf of *Perilla frutescens. Korean J. Crop Sci.* **1999**, *44*, 154–158.
- (10) Lim, S. U.; Seo, Y. H.; Lee, Y. G.; Baek, N. I. Isolation of volatile allelochemicals from leaves of *Perilla frutescens* and *Artemisia* asiatica. Agric. Chem. Biotechnol. **1994**, 37, 115–123.
- (11) Tamura, H.; Fujiwara, M.; Sugisawa, H. Production of phenylpropanoids from cultured callus tissue of leaves of Akacirimen-Shiso. *Agric. Biol. Chem.* **1989**, *53*, 1971–1978.
- (12) Choung, M. G.; Kwak, Y. H.; Kwon, Y. C. Test of components related to quality in perilla leaves: I. Test of purple pigment in perilla leaves. *RDA J. Agric. Sci.* **1998**, *40*, 119–126.
- (13) Lee, K. I.; Rhee, S. H.; Park, K. Y. Anticancer activity of phytol and eicosatrienoic acid identified from perilla leaves. *J. Korean Soc. Food Sci. Nutr.* **1999**, *28*, 1107–1112.
- (14) Chung, I. M.; Yun, S. J.; Kim, J. T.; Gwag, J. G.; Sung, J. D.; Suh, H. S. Test of superoxide dismutase characteristics and antioxidant activity in perilla leaves. *Korean J. Crop Sci.* **1995**, *40*, 504–511.
- (15) Lee, K. I.; Rhee, S. H.; Kim, J. O.; Chung, H. Y.; Park, K. Y. Antimutagenic and antioxidative effects of perilla leaf extracts. *J. Korean Soc. Food Sci. Nutr.* **1993**, *22*, 175–180.
- (16) Kim, E. K.; Lee, S. J.; Lim, B. O.; Jeon, Y. J.; Song, M. D.; Park, T. K.; Lee, K. H.; Kim, B.; Lee, S. R.; Moon, S. H.; Jeon, B. T.; Park, P. J. Antioxidative and neuroprotective effects of enzymatic extracts from leaves of *Perilla frutescens* var. japonica. *Food Sci. Biotechnol.* 2008, 17, 279–286.
- (17) Jang, H. J.; Park, J. Y.; Kim, Y. T. Volatile components of *Perilla folium. Korean J. Food Sci. Technol.* **1991**, *23*, 129–132.
- (18) Choi, H. S.; Min, K. C. Headspace analysis of *Perilla frutescens* var. acuta Kudo by solid-phase microextraction using diverse fibers. *Food Sci. Biotechnol.* **2003**, *12*, 409–414.
- (19) Choi, H. S. Characteristic odor components of *Perilla frutescens* var. acuta Kudo oil by aroma extract dilution analysis. *Food Sci. Biotechnol.* 2004, *13*, 279–284.
- (20) Wilson, B. J.; Garst, J. E.; Linnabary, R. D.; Channell, R. B. Perilla ketone: a potent lung toxin from the mint plant, *Perilla frutescens* Britton. *Science* 1977, 197, 573–574.

- (21) Blank, I. Gas chromatography-olfactometry in food aroma analysis. In *Flavor, Fragrance, and Odor Analysis*; Marsili, R., Ed.; Dekker: New York, 2002; pp297–331.
- (22) Cunningham, D. C.; Acree, T. E.; Barnard, J.; Butts, R. M.; Braell, P. A. Charm analysis of apple volatiles. *Food Chem.* 1986, 19, 137–146.
- (23) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavor compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* 1987, 184, 277–283.
- (24) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavor evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, 209, 237–241.
- (25) Seo, W. H.; Baek, H. H. Identification of characteristic aroma-active compounds from water dropwort (*Oenanthe javanica* DC.). J. Agric. Food Chem. 2005, 53, 6766–6770.
- (26) Kondjoyan, N.; Berdague, J. L. A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds; Laboratoire faveur, Station de Recherches sur la Viande: Clermont-Ferrand, France, 1996.
- (27) Dung, N. X.; Moi, L. D.; Nam, V. V.; Cu, L. D.; Leclercq, P. A. Volatile constituents of the aerial parts of *Orthodon calveriei* Level from Vietnam. *J. Essent. Oil Res.* **1995**, *7*, 111–112.
- (28) Hotanaka, A. The fresh green odor emitted by plants. *Food Rev. Int.* 1996, 12, 303–350.
- (29) Cho, I. H.; Choi, H. K.; Kim, Y. S. Difference in the volatile composition of pine-mushrooms (*Tricholoma matsutake* Sing.) according to their grades. J. Agric. Food Chem. 2006, 54, 4820–4825.
- (30) Reineccius, G. Flavor formation in fruits and vegetables. In *Flavor Chemistry and Technology*, 2nd ed.; Reineccius, G., Ed.; Taylor and Francis: Boca Raton, FL, 2006; pp 73–101.
- (31) Baser, K. H. C.; Demirci, A.; Donmez, A. A. Composition of the essential oil of *Perilla frutescens* (L.) britton from Turkey. *Flavour Fragrance J.* 2003, 18, 122–123.
- (32) Nishizawa, A.; Honda, G.; Tabata, M. Genetic control of perillene accumulation in *Perilla frutescens*. *Phytochemistry* 1989, 22, 423–425.
- (33) Morinaka, Y.; Fukuda, N.; Takayanagi, K. Evaluation of perilla (*Perilla frutescens*) aroma – statistical comparison between analytical and sensory data of perilla leaf aroma. J. Jpn. Soc. Hortic. Sci. 2002, 71, 424–433.
- (34) Choi, H. S.; Min, K. C. Headspace-SPME analysis of *Citrus* hybrid, Hallabong. *Food Sci. Biotechnol.* 2004, 13, 126–129.
- (35) McLafferty, F. W. Interpretation of Mass Spectra; University Science Books: Mill Valley, CA, 1980.

Received for review February 16, 2009. Revised manuscript received October 19, 2009. Accepted October 23, 2009. This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.