Aroma-Active Compounds of Miniature Beefsteakplant (*Mosla dianthera* Maxim.)

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Volatile flavor compounds of miniature beefsteakplant (*Mosla dianthera* Maxim.) from Vietnam were analyzed through gas chromatography−mass spectrometry−olfactometry (GC-MS-O). Sixty-two compounds were identified by GC-MS. Of these, (±)-carvone and (±)-limonene were the most abundant, followed by (*Z*)-limonene oxide, *β*-caryophyllene, and α-humulene. Twenty aroma-active compounds were detected by aroma extract dilution analysis conducted on two GC columns of different polarities (DB-5MS and DB-Wax). The most intense aroma-active compounds were linalool (floral/sweet/lemon), (−)-carvone (spearminty), and 1-octen-3-one (mushroom/earthy). Other predominant aroma-active compounds included (*Z*)-3-hexenol (grassy/leafy/metallic), (*Z*)-limonene oxide (lemon/floral), myrcene (plastic/sweet), (+)-limonene (orange/lemon), α-thujene (soy sauce/grassy), and (*Z*)-dihydrocarvone (spearminty/pepperminty). On the basis of the aroma characteristics and intensity, it was concluded that (−)-carvone was responsible for the characteristic aroma of miniature beefsteakplant.

Keywords: *Miniature beefsteakplant; Mosla dianthera Maxim.; volatile flavor compounds; aroma extract dilution analysis; (−)-carvone*

INTRODUCTION

The Lamiaceae (Labiatae) family includes various culinary herbs such as basil (*Ocimum basilicum* L.), hyssop (*Hyssopus officinalis* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), and spearmint (*Mentha spicata* L.) (Duke, 1992; Tucker, 1986). Miniature beefsteakplant (*Mosla dianthera* Maxim.), native to Korea, China, Japan, and Vietnam, is an annual aromatic plant of the Lamiaceae family (Murata and Yamazaki, 1993). In Vietnam, it has been used as a spice in foods due to its characteristic aroma and as a medicinal plant against colds, headaches, and intestinal and skin diseases (Loi, 1995; Perry, 1980).

Several studies have reported on the volatile compounds of *Mosla*, otherwise known as *Orthodon*, species using gas chromatography−mass spectrometry (GC-MS). Dung et al. (1995) identified *β*-caryophyllene (20.3%), *β*-bisabolene (15.6%), (*Z*)-α-bergamotene (11.1%), and α-humulene (10.3%) as the major volatile components in essential oil of *M. calveriei* Level. from Vietnam. Lo et al. (1994) analyzed volatile compounds of *M. chinensis* Maxim. and identified thymol (52.1%), *p*-cymene (25.8%), and *γ*-terpinene (8.6%) as the major volatile compounds. However, volatile compounds and characteristic aroma components of miniature beefsteakplant are still unknown.

Gas chromatography−olfactometry (GC-O) analyses, such as aroma extract dilution analysis (AEDA) and CharmAnalysis, have been developed for the identification of aroma-active compounds from foods (Cunningham et al., 1986; Ullrich and Grosch, 1987). In AEDA, serial dilutions of a flavor extract are evaluated by GC-O to provide flavor dilution (FD) factors, which are proportional to the aroma values for aroma-active compounds (Ullrich and Grosch, 1987).

This study was conducted to identify volatile flavor compounds and to evaluate characteristic aroma-active compounds of miniature beefsteakplant through GC-MS-O.

MATERIALS AND METHODS

Materials. Miniature beefsteakplant (*M. dianthera* Maxim.) grown in the experimental garden of the Institute of Ecology and Biological Resources, Hanoi, Vietnam, was harvested in August 1998.

Authentic flavor compounds were supplied from Aldrich Chemical Co. (Milwaukee, WI) and French-Korean Aromatics (Seoul, Korea).

Steam Distillation. Within a few hours of collection, 200 g of fresh leaves and inflorescences was surface cleaned and extracted for 1 h in a steam distillation apparatus with distilled water (500 mL) under atmospheric pressure, whereby 0.458 g of extract was obtained.

GC-MS. GC-MS analyses were carried out using an HP 5890 Series II GC/HP 5972 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). A fused silica open tubular (FSOT) column, HP-5MS (30 m length × 0.25 mm i.d. × 0.25 *μ*m film thickness, Hewlett-Packard Co.) or a DB-Wax column (30 m length × 0.25 mm i.d. × 0.25 *μ*m film thickness, J&W Scientific, Folsom, CA) was used for GC-MS analyses. After 30 *μ*L of extract was diluted with 780 *μ*L of dichloromethane (27-fold dilution), 1 *μ*L of dilution was injected (splitless mode) into each column. The carrier gas was helium

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with a constant flow rate of 0.8 mL/min. The oven temperature was held for 5 min at 40 °C, then raised at 2 °C/min to 200 °C, and held for 20 min at 200 °C. The injector and detector temperatures were 200 and 250 °C, respectively. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33−550 amu; electron multiplier voltage, 1729 V; scan rate, 1.4 scans/s. Extract was analyzed twice.

GC-O. The GC-O system consisted of a Varian 3350 GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port. Effluent from the end of the GC column was split 1:1 between the FID and sniffing port using deactivated capillary columns (1 m length × 0.25 mm i.d.). AEDA was performed as previously described (Ullrich and Grosch, 1987). Serial dilutions (1:3) of extract were prepared using dichloromethane as a diluent. From each dilution, 1 μL was injected into an FSOT column (DB-5MS or DB-Wax, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific). The oven temperature was held for 5 min at 40 °C, then raised at 8 °C/min to 200 °C, and held for 20 min at 200 °C. Except for the oven temperature, the GC conditions were the same as those of GC-MS. GC-O was performed by two panelists familiar with miniature beefsteakplant aroma. FD chromatograms were based on one panelist's AEDA evaluations because all responses were essentially the same for predominant aroma-active compounds.

Identification and Quantification of Compounds. Retention indices (RI) were calculated using *n*-paraffins C_8-C_{22} as external references (van den Dool and Kratz, 1963). Positive identifications of compounds were based on comparison of mass spectra, RI, and aroma properties of unknowns with those of authentic standards. When standards were not available, compounds were positively identified with the aid of the Wiley mass spectral database (Hewlett-Packard Co., 1995), published RI (Kondjoyan and Berdagué, 1996), and aroma properties of the literature. Tentative identifications were based on matching mass spectra of unknowns with those in the Wiley mass spectral database. Concentrations of positively identified aroma-active compounds were estimated on the DB-Wax column using an external standard method (Reineccius, 1994).

RESULTS AND DISCUSSION

Volatile Flavor Compounds. Table 1 lists volatile flavor compounds identified in miniature beefsteakplant, the relative peak area, and RI of the compounds on HP-5MS and DB-Wax columns. A total of 62 compounds were identified, 57 of which were positively identified. Identified compounds included 23 terpene hydrocarbons, 18 alcohols, 2 aldehydes, 8 ketones, 4 esters, and 7 miscellaneous compounds. The volatile profile of miniature beefsteakplant essential oil was similar to that of spearmint oil (Canova, 1972; Lawrence, 1980), which is somewhat different from those of another two *Mosla* species (Dung et al., 1995; Lo et al., 1994).

Of the 23 identified terpene hydrocarbons, 13 compounds were monoterpenes and 10 were sesquiterpenes. In total, monoterpene hydrocarbons constituted ∼25% of total volatiles. (±)-Limonene (**7**) was the most abundant of these moneterpenes, followed by myrcene (**5**) and sabinene (**4**). These compounds have been reported as the major flavor constituents in various citrus fruits (Owusu-Yaw et al., 1986; Staroscik and Wilson, 1982; Wilson and Shaw, 1980). (±)-Limonene has been identified as a trace flavor compound in *M. calveriei* (Dung et al., 1995) and *M. chinensis* (Lo et al., 1994). (*Z*)-β-Ocimene (**8**), terpinolene (**11**) and β-phellandrene (**12**) have not been identified in other *Mosla* species (Dung et al., 1995; Lo et al., 1994). The contents of sesquiterpene hydrocarbons were low except for β-caryophyllene (**17**) and α-humulene (**18**), which are known as the most

Table 1. Volatile Compounds Identified from Miniature Beefsteakplant (*M. dianthera* Maxim.)

no.[a]	compound	RI[b] HP-5MS	RI[b] DB-Wax	rel %[c] HP-5MS	rel %[c] DB-Wax	ID[d]
	Monoterpene Hydrocarbons					
1	α-thujene	922	1022	tr[e]	tr	B
2	α-pinene	928	1017	0.14	0.11	A
3	camphene	941	1056	0.16	0.11	A
4	sabinene	969	1114	1.19	0.95	A
5	myrcene	990	1160	1.43	1.21	A
6	β-pinene	999	1099	tr	tr	A
7	(±)-limonene	1025	1189	21.70	21.84	A
8	(*Z*)-β-ocimene	1039	1231	0.14	0.11	B
9	(*E*)-β-ocimene	1048	1246	0.22	0.20	B
10	γ-terpinene	1055	1216	0.03	tr	B
11	terpinolene	1083	−[f]	tr	−	B
12	β-phellandrene	−	1203	−	0.28	B
13	*p*-cymene	−	1258	−	tr	B
	Sesquiterpene Hydrocarbons					
14	α-copaene	1370	1475	0.10	0.08	A
15	β-bourbonene	1378	1500	0.10	−	C
16	β-elemene	1388	−	0.10	−	B
17	β-caryophyllene	1419	1575	5.03	5.53	A
18	α-humulene	1452	1663	4.71	5.09	A
19	germacrene D	1473	1707	0.09	0.05	B
20	bicyclogermacrene	1489	1735	0.36	0.25	B
21	(*Z*,*E*)-α-farnesene	1496	1733	1.64	1.32	A
22	(*E*,*E*)-α-farnesene	1507	1743	0.62	0.83	A
23	δ-cadinene	1518	1708	0.06	tr	B
	Alcohols					
24	(*Z*)-3-hexenol	854	1382	0.08	0.14	A
25	(*E*)-2-hexenol	867	1406	tr	tr	A
26	1-octen-3-ol	981	1451	0.24	0.52	A
27	linalool	1100	1548	1.64	1.44	A
28	borneol	1160	1693	0.06	0.05	A
29	4-terpineol	1173	1593	tr	0.05	B
30	α-terpineol	1209	1701	tr	tr	B
31	3-octanol	1232	1397	tr	tr	C
32	(*Z*)-carveol	1258	1858	tr	0.04	C
33	geraniol	1274	1847	tr	0.02	A
34	eugenol	1357	2153	0.14	0.07	A
35	nerolidol	1561	2041	0.10	0.11	A
36	spathulenol	1581	2109	tr	tr	B
37	α-cadinol	1646	>2200	0.02	tr	B
38	phytol	2112	>2200	tr	0.06	B
39	1-hexanol	−	1355	−	tr	A
40	menthol	−	1636	−	0.19	A
41	nerol	−	1798	−	tr	A
	Aldehydes					
42	(*E*)-2-hexenal	851	1209	tr	tr	A
43	benzaldehyde	957	1502	tr	0.07	B
	Ketones					
44	acetophenone	1062	1629	0.04	0.06	B
45	camphor	1138	−	0.03	−	A
46	isomenthone	1148	−	0.02	−	C
47	(*Z*)-dihydrocarvone	1186	1589	0.15	0.22	A
48	(*E*)-dihydrocarvone	1193	1607	0.12	0.17	A
49	(±)-carvone	1251	1718	53.42	53.60	A
50	(*Z*)-jasmone	1397	1923	0.06	0.05	B
51	1-octen-3-one	−	1296	−	tr	A
	Esters					
52	methyl salicylate	1189	1755	0.09	0.08	B
53	octyl acetate	1213	1471	0.15	0.18	A
54	bornyl acetate	1290	1567	0.04	tr	B
55	nonyl acetate	1315	1573	0.04	tr	A
	Miscellaneous Compounds					
56	1,8-cineole	1037	1204	0.09	0.12	A
57	(*E*)-sabinene hydrate	1064	1462	0.05	0.06	B
58	(*Z*)-limonene oxide	1130	1433	2.53	2.36	A
59	(*E*)-limonene oxide	1134	1441	0.16	0.14	A
60	carvone oxide	1286	1837	0.68	−	C
61	caryophyllene oxide	1573	1955	0.16	0.12	A
62	humulene oxide	1598	2013	0.08	0.09	B

[a] Numbers correspond to those in Tables 2 and 3 and Figure 1. [b] Retention indices were determined using *n*-paraffins C_8-C_{22} as external references. [c] Average of relative percentage of total peak area ($n = 2$). [d] Compounds were identified on the basis of the following criteria: A, mass spectrum and retention index were consistent with those of an authentic standard; B, mass spectrum was identical with that of Wiley mass spectral database (Hewlett-Packard Co., 1995), and retention index was consistent with that of the literature (Kondjoyan and Berdagué, 1996); C, mass spectrum was consistent with that of Wiley mass spectrum database (tentative identification). [e] Trace amount <0.02% of total amount. [f] Not determined due to either low abundance or peak coelution.

Table 2. Aroma-Active Compounds of Miniature Beefsteakplant (*M. dianthera* Maxim.)

no.[a]	compound	RI[b] DB-5MS	RI[b] DB-Wax	aroma property	ID[c]
I	unknown I	<800		paint, chemical	
II	unknown II	825	1093	grassy, bitter	
24	(*Z*)-3-hexenol	859	1384	grassy, leafy, metallic	A
1	α-thujene	913	1029	soy sauce, grassy	B
3	camphene	941		camphoraceous	A
51	1-octen-3-one	981	1301	mushroom, earthy	A
5	myrcene	990	1161	plastic, sweet	A
7	(+)-limonene	1028	1195	orange, lemon	A
9	(*E*)-β-ocimene	1062	1254	warm, floral	C
27	linalool	1105	1548	floral, sweet, lemon	A
58	(*Z*)-limonene oxide	1145	1441	lemon, floral	A
47	(*Z*)-dihydrocarvone	1195	1595	spearminty, pepperminty	A
49	(−)-carvone	1254	1725	spearminty	A
17	β-caryophyllene	1425	1584	woody	A
22	(*E*,*E*)-α-farnesene	1512	1749	woody, grassy	A
35	nerolidol	1564		woody, apple	A
III	unknown III	1708	>2200	spearminty	
6	β-pinene		1104	paint, chemical	A
12	β-phellandrene		1210	pepperminty, grassy	B
IV	unknown IV		1898	pepperminty	

[a] Numbers correspond to those in Tables 1 and 3 and Figure 1. Roman numerals represent unknown compounds. [b] Retention indices were determined using *n*-paraffins C_8-C_{22} as external references. [c] Compounds were identified on the basis of the following criteria: A, retention index and aroma property were consistent with those of an authentic standard; B, retention index was consistent with that of GC-MS, and aroma property was similar to that of previous literature (Pino et al., 1995); C, retention index was consistent with that of GC-MS.

common sesquiterpenes found in various essential oils (Gaydou et al., 1986, 1989; Le Quere and Latrasse, 1990; Senanayake et al., 1978). Dung et al. (1995) identified β-caryophyllene (20.3%) and α-humulene (10.3%) as the most abundant volatiles in *M. calveriei* essential oil. β-Elemene (**16**), bicyclogermacrene (**20**), (*E*,*E*)-α-farnesene (**22**), and δ-cadinene (**23**) were not reported in *M. calveriei* (Dung et al., 1995) and *M. chinensis* (Lo et al., 1994).

Among the 11 alcohols identified in miniature beefsteakplant, linalool (**27**) was relatively abundant. This substance has been used in the formation of many fruit flavors, particularly apricot and peach flavors (Krueger, 1995). A number of essential oils contain high contents of linalool (Gaydou et al., 1986; MacLeod et al., 1988). This compound was reported to be present in *M. calveriei* (0.4%) (Dung et al., 1995) and *M. chinensis* (<0.05%) (Lo et al., 1994). In addition to linalool, 1-octen-3-ol (**26**), the aliphatic alcohol with mushroom-like aroma (Whitfield and Last, 1991), was detected in miniature beefsteakplant essential oil, whereas thymol, the most abundant compound (52.1%) in *M. calveriei* oil (Dung et al., 1995), was not found.

Ketones were present in high contents in miniature beefsteakplant essential oil. (±)-Carvone (**49**) was the most abundant compound in miniature beefsteakplant, whereas this compound was not found in other *Mosla* species (Dung et al., 1995; Lo et al., 1994). It was reported as the most abundant volatile compound in spearmint (*Mentha spicata* L.) oil, which has a spearminty aroma (Canova, 1972; Lawrence, 1980; Leitereg et al., 1971). Carvone derivatives such as (*Z*)- and (*E*)-dihydrocarvones (**47** and **48**, respectively) were also present in miniature beefsteakplant. Besides several monoterpene ketones, acetophenone (**44**), (*Z*)-jasmone (**50**), and 1-octen-3-one (**51**) were found in low abundance.

Two aldehydes (**42** and **43**), four esters (**52**−**55**), and seven miscellaneous compounds (**56**−**62**) were also detected in low abundance. Of these, (*Z*)-limonene oxide (**58**) showed relatively high abundance on HP-5MS and DB-Wax columns.

Aroma-Active Compounds. Aroma-active compounds detected in miniature beefsteakplant and their aroma properties are given in Table 2. A total of 20 aroma-active compounds were detected through AEDA. To better resolve aroma-active compounds, AEDA was conducted on two GC columns of different polarities (DB-5MS and DB-Wax). Although two FD chromatograms revealed different $\log_3$ FD factors for certain aroma-active compounds and some compounds (**3**, **6**, **12**, **35**, **I**, and **IV**) were detected on only one column, FD chromatograms on both columns were similar for potent aroma-active compounds (Figure 1).

Linalool (**27**), (−)-carvone (**49**), and 1-octen-3-one (**51**) were the most intense aroma-active compounds with high $\log_3$ FD factors (6−8) on both columns. Linalool, which had a floral/sweet/lemon-like aroma note, exhibited the highest $\log_3$ FD factors ranging from 7 (DB-5MS) to 8 (DB-Wax). This compound has been identified as a key compound responsible for the orange-like aroma in orange juice with a threshold value of 3.8 ppb in water (Ahmed et al., 1978). In our study, linalool was believed to play an important role in the aroma of miniature beefsteakplant due to its high $\log_3$ FD factors and characteristic aroma note. (−)-Carvone, which had a spearminty aroma note, was detected with the second highest $\log_3$ FD factors, ranging from 6 (DB-5MS) to 7 (DB-Wax). Its aroma property was most similar to that of miniature beefsteakplant. Leitereg et al. (1971) have reported that (+)- and (−)-limonene could be converted to (−)- and (+)-carvone, respectively. They described that (−)-carvone had a spearminty odor with a threshold value of 2 ppb in water, whereas (+)-carvone had a caraway-like odor with a higher threshold value (85−130 ppb in water). Although (−)-carvone was found to have lower $\log_3$ FD factors than those of linalool in our study, it is regarded as a key compound contributing to the aroma of miniature beefsteakplant on the basis of its aroma characteristics, low threshold value, and high

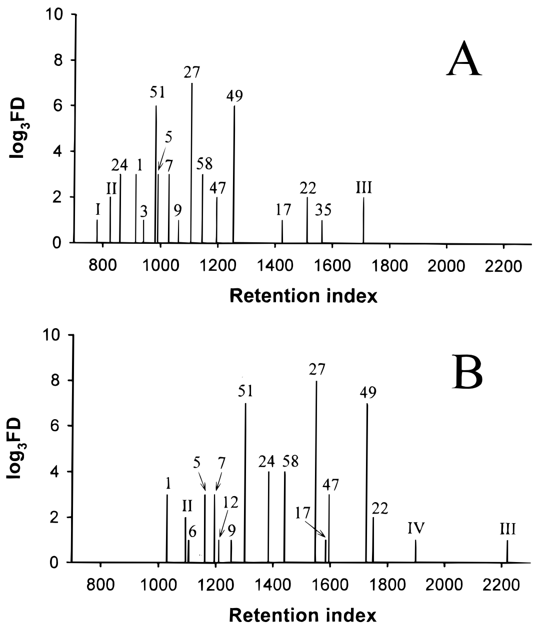

Figure 1. FD chromatograms of miniature beefsteakplant on the DB-5MS (A) and DB-Wax columns (B). Numbers correspond to those in Tables 1−3.

Table 3. Concentrations and Aroma Values for Identified Aroma-Active Compounds in Miniature Beefsteakplant (*M. dianthera* Maxim.)

no.[a]	compound	concn[b] (ppb)	threshold[c] (ppb)	aroma value[d]
24	(*Z*)−3-hexenol	4200	70[e]	60
1	α-thujene	na[f]		
3	camphene	5900		
51	1-octen-3-one	85	0.089[g]	960
5	myrcene	43000	42[h]	1000
7	(+)-limonene	460000	210[h]	2200
9	(*E*)-*β*-ocimene	na		
27	linalool	29000	3.8[h]	7600
58	(*Z*)-limonene oxide	57000		
47	(*Z*)-dihydrocarvone	4200		
49	(−)-carvone	900000	2.0[i]	450000
17	*β*-caryophyllene	68000	160[j]	430
22	(*E*,*E*)-α-farnesene	8400	160[j]	53
35	nerolidol	1300		
6	*β*-pinene	78	140[k]	0.56
12	*β*-phellandrene	na		

[a] Numbers correspond to those in Tables 1 and 2 and Figure 1. [b] Average of concentrations determined using external standards on DB-Wax column ($n = 2$). [c] Aroma threshold in water. [d] Aroma value = compound concentration divided by threshold. [e] Buttery et al. (1971). [f] Not available. [g] Swoboda and Peers (1977). [h] Ahmed et al. (1978). [i] Leitereg et al. (1971). [j] Meilgaard (1982). [k] Frazzalari (1978).

content (Table 3). In addition to linalool and (−)-carvone, 1-octen-3-one had high $\log_3$ FD factors with a mushroom-like/earthy aroma note. It has been reported as the aroma-active compound of milk products (Schieberle et al., 1993; Schnermann and Schieberle, 1997) and oysters (Josephson et al., 1985). The threshold value of this compound (0.089 ppb; Swoboda and Peers, 1977) was notably low compared with those of other aroma-active compounds in miniature beefsteakplant. This compound may have been generated via lipoxygenase-mediated lipid oxidation (Lindsay, 1985).

Six aroma-active compounds (**1**, **5**, **7**, **24**, **58**, and **47**) were detected on both columns with relatively high $\log_3$ FD factors (3−4). (*Z*)-3-Hexenol (**24**), which is known as leaf alcohol, was described as grassy/leafy/metallic. This compound is considered to be biosynthesized in green leaves via lipoxygenase and hydroperoxide lyase-mediated lipid oxidation (Hatanaka, 1996). (*Z*)-Limonene oxide (**58**) possesses a lemon/floral aroma note and was found in grapefruit oil (Wilson and Shaw, 1980). α-Thujene (**1**, soy sauce/grassy), myrcene (**5**, plastic/sweet), and (+)-limonene (**7**, orange/lemon) had the same $\log_3$ FD factor on both columns ($\log_3$ FD = 3). These monoterpene hydrocarbons were identified as aroma-active compounds in dill herb with warm/resinous, fragrant/fresh, and citrus-like/fresh aromas, respectively (Pino et al., 1995). (+)-Limonene was found in GC-O with relatively low $\log_3$ FD factors compared to its high content in miniature beefsteakplant. This result can be explained by its high threshold value of 210 ppb (Ahmed et al., 1978). It showed odor characteristics similar to those of (*Z*)-limonene oxide. Its enantiomer, (−)-limonene, has a turpentine-like aroma, which is different from that of (+)-limonene (Berger, 1995). Another minty aroma compound, (*Z*)-dihydrocarvone (**47**), was evaluated on both columns. Pino et al. (1995) reported that (*Z*)-dihydrocarvone had a cooling/fresh/minty aroma, which was different from the caraway-like/sour aroma of (*E*)-dihydrocarvone.

Seven aroma-active compounds were identified in GC-O with relatively low $\log_3$ FD factors ranging from 1 to 2. (*E*,*E*)-α-Farnesene (**22**, woody/grassy), *β*-caryophyllene (**17**, woody), and (*E*)-*β*-ocimene (**9**, warm/floral) were detected on both columns. Camphene (**3**, camphoraceous) and nerolidol (**35**, woody/apple) were detected only on the DB-5MS column, whereas *β*-pinene (**6**, paint/chemical) and *β*-phellandrene (**12**, pepperminty/grassy) were found only on the DB-Wax column.

Four unknown aroma-active compounds (**I**−**IV**) were detected with low $\log_3$ FD factors. Of these, unknowns **II** (grassy/bitter) and **III** (spearminty) were found on both columns with aroma characteristics similar to those of (*Z*)-3-hexenol and (−)-carvone, respectively. Unknown **I** (paint/chemical) was detected only on the DB-5MS column and unknown **IV** (pepperminty) only on the DB-Wax column.

Quantification of Aroma-Active Compounds. Table 3 shows concentrations of positively identified aroma-active compounds and their aroma values calculated on the basis of published threshold data. (−)-Carvone had the highest aroma value (450000), followed by linalool (7600). Quantitative data were consistent with the results of AEDA except that the aroma value of (−)-carvone was higher than that of linalool, and 1-octen-3-one had a lower aroma value than expected. Frijters (1978) stated that dilution analyses such as AEDA and CharmAnalysis have a limitation of a nonlinear relationship between perceived intensity of an odorant and its concentration.

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