JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Aroma Evaluation of an Aquatic Herb, Changpo (*Acorus calamus* Var. *angustatus* Bess), by AEDA and SPME

HYANG-SOOK CHOI*

Department of Food Nutrition, Shinheung College, 117 Howon-Dong, Uijongbu-City, Kyungkido 480-701, South Korea

This study was conducted to determine the volatile flavor composition of fresh changpo (*Acorus calamus* var. *angustatus* Bess) leaves quantitatively and qualitatively by use of two internal standards and to determine which volatile compounds are primarily responsible for the aroma of this aquatic herb. The headspace composition of fresh changpo leaves was also analyzed by a solid-phase microextraction method. Aroma extract dilution analysis (AEDA) and sniffing test by gas chromatography−olfactometry were used for the detection of aroma-active compounds of this herb. According to the instrumental analyses of the changpo oil, octanoic acid (49.13%), α-cedrene (16.71%), α-phellandrene (4.46%), and γ-elemene (3.75%) were the most abundant compounds. *n*-Butylidene dihydrophthalide (8.61%), *trans*,*trans*-farnesyl acetate (7.29%), and *trans*-2-dodecenal (7%) were the main components of changpo headspace. *cis*-β-Farnesene was evaluated as the key aroma compound of this herb from results of AEDA and sniffing test.

KEYWORDS: Changpo (*Acorus calamus* var. *angustatus* Bess); aroma extract dilution analysis; solid-phase microextraction; aroma-active compounds; *cis*-β-farnesene

INTRODUCTION

Changpo (*Acorus calamus* var. *angustatus* Bess) is a perrenial aquatic herb belonging to the family of Araceae (*1*). The leaves of this aromatic plant have long been used as herb, seasoning, toiletry, and bath products, especially for hair rinse and soap due to its refreshing aroma. The roots of changpo have been used as a domestic folk medicine for the remedy of diarrhea, indigestion, and bronchopneumonia in Korea. Its use in the treatment of rheumatism was also reported in Iran (*2*). The roots of changpo were used as a tonic, insecticide, flavoring, and tea in Iran (*2*). The importance of aromatic plants is considerable owing to their applications in folk medicine and their potential for commercial value in various fields as spices, beverages, perfumery, cosmetics, pharmaceutics, and aromatherapy (*3*−*7*).

There have been few studies on the chemical composition (*8*), antimicrobial activity (*9*), narcotic effect (*10*), and nervous sedative effect (*11*) of changpo. Despite the pleasant flavor of this herb, no detailed analysis of the volatile components has been reported. In the present study, quantitative and qualitative determinations of the volatile compounds extracted by simultaneous steam distillation extraction (SDE) method and compositional analysis of the headspace from fresh changpo leaves were carried out by GC, GC-MS, and GC-O, and its aroma-active compounds were evaluated by aroma extract dilution analysis (AEDA) and sniffing test.

* Telephone +82-31-870-3410; fax +82-31-870-3415; e-mail hyangsookchoi@mail.shc.ac.kr.

MATERIALS AND METHODS

Materials. Fresh changpo leaves, harvested in May 2003, were collected from a farm located in Kyunggi province, South Korea. Authentic chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI), Fluka Fine Chemicals (Buchs, Switzerland), Funakoshi Co., Ltd. (Tokyo, Japan), Nacalai Tesque Inc. (Kyoto, Japan), PolyScience Co. (Nile, IL), Sigma Chemical Co. (St. Louis, MO), Theta Co. (Newtown Square, PA), Tokyo Kasei Kogyo Co. (Tokyo, Japan), and Wako Pure Chemical Industries (Osaka, Japan). Some chemicals were provided by Bolak Co., Ltd. (Osan, Korea) and French-Korean Aromatics (Youngin, Korea). Each of the sources of authentic chemicals was described in **Table 1**.

SDE. Fresh changpo leaves totaling 690 g were cutted into small size (0.5 × 0.5 × 0.5 cm). Two hundred and thirty grams of cut leaves was mixed with 1.6 L of water and then subjected to SDE with 125 mL of diethyl ether/pentane (1:1, v/v) as solvent for 2 h using a modified Likens−Nickerson apparatus described by Schultz et al. (*12*). The extraction was performed three times, and the total extract was dried by adding anhydrous sodium sulfate and then concentrated by evaporator. Finally, the oil sample was concentrated under reduced pressure at room temperature.

SPME. A 100-μm polydimethylsiloxane−divinylbenzene (PDMS−DVB) SPME fiber (Supelco Inc., Bellefonte, PA) was used. Two grams of small pieces of fresh changpo leaves was hermetically sealed in a 10-mL vial having a silicone septum and an aluminum cap. A stainless steel needle containing PDMS−DVB fiber was inserted through the septum of the sample vial for 40 min in a 40 °C water bath to sample the headspace (*13*).

GC and GC-MS. An Agilent 6890N gas chromatograph equipped with a DB-Wax (60 m × 0.25 mm i. d., film thickness = 0.25 μm) fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used. The column temperature was

10.1021/jf040239p CCC: $27.50
Published on Web 12/01/2004

Table 1. Volatile Flavor Components Identified in Changpo Essential Oil

no.	compound[k]	retention index	wt %, SDE	peak area %, SPME	FD factor[b]	odor description[c]	identification
1	3-methyl-2-butanone	929	tr[a]	tr			RI,[d] MS[e]
2	1,1-dimethylpropane	963	tr	tr			RI, MS
3	methyl phenyl acetate[1]	990	0.02				RI, MS, Co-GC[f]
4	decane[1]	1004	0.32	0.03			RI, MS, Co-GC
5	α-pinene[2]	1048	0.14	0.01	1	mild green	RI, MS, Co-GC, GC-O[g]
6	camphene[2]	1090	0.29	0.02	2	sweet, warm	RI, MS, Co-GC, GC-O
7	undecane[2]	1101	1.29	0.01			RI, MS, Co-GC
8	sabinene[2]	1133	0.20	0.03	2	green, herbaceous	RI, MS, Co-GC, GC-O
9	δ-3-carene[3]	1156	0.02	0.04	1	green, fresh	RI, MS, Co-GC, GC-O
10	myrcene[2]	1165	tr				RI, MS, Co-GC
11	α-phellandrene[3]	1177	4.46	0.08	2	green, gaseous	RI, MS, Co-GC, GC-O
12	α-terpinene[2]	1194	0.02	0.03	2	tree-like	RI, MS, Co-GC, GC-O
13	3-methyl-1-butanol[1]	1204	0.66	0.08		green, warm	RI, MS, Co-GC, GC-O
14	limonene[1]	1222	0.09	0.01	4	green	RI, MS, Co-GC, GC-O
15	γ-terpinene[4]	1257	0.01		4	green, tree-like	RI, MS, Co-GC, GC-O
16	*p*-cymene[1]	1275	0.01				RI, MS, Co-GC
17	terpinolene[5]	1292	0.02	1.11	2	green	RI, MS, Co-GC, GC-O
18	tridecane[1]	1309	tr				RI, MS, Co-GC
19	6-methyl-5-hepten-2-one[h]	1342	0.01				RI, MS
20	hexanol[6]	1351	0.03	4.14			RI, MS, Co-GC
21	(*E*)-3-hexen-1-ol[2]	1362	0.01	0.61	1	green, medicine-like	RI, MS, Co-GC, GC-O
22	(*Z*)-3-hexen-1-ol[2]	1368	0.01	0.86	1	woody	RI, MS, Co-GC, GC-O
23	*cis*-linalool (furan) oxide[7]	1423	0.01	0.03	2	green	RI, MS, Co-GC, GC-O
24	tetradec-1-ene[h]	1433	0.02				RI, MS
25	α-thujone[7]	1438	0.27	0.22	3	mild herbaceous	RI, MS, Co-GC, GC-O
26	1-heptenyl acetate[h]	1444	tr				RI, MS
27	β-thujone[7]	1447	0.01				RI, MS, Co-GC
28	*cis*-limonene oxide[2]	1451	0.01				RI, MS, Co-GC
29	*trans*-linalool (furan) oxide[7]	1459	0.04	0.02	1	lemon-like	RI, MS, Co-GC, GC-O
30	*trans*-sabinene hydrate	1463	0.01	0.02	1	gaseous, green	RI, MS, GC-O
31	α-cubebene[3]	1467	0.02		1	sweet, buttery	RI, MS, Co-GC, GC-O
32	*trans*-limonene oxide[2]	1472	0.02		1	sweet	RI, MS, Co-GC, GC-O
33	octyl acetate[1]	1479	0.01				RI, MS, Co-GC
34	citronellal[1]	1485	0.03	0.05	1	sweet, fruity	RI, MS, Co-GC, GC-O
35	decanal[1]	1505	0.07	0.01	1	sweet	RI, MS, Co-GC, GC-O
36	benzaldehyde[8]	1519	0.57	0.09	2	sweet, herbaceous	RI, MS, Co-GC, GC-O
37	*d*-camphor[8]	1527	0.20	0.03	4	herbaceous, warm	RI, MS, Co-GC, GC-O
38	borneol[8]	1541	0.02		1	floral	RI, MS, Co-GC, GC-O
39	β-cubebene[3]	1545	0.02		1	sweet, herbaceous	RI, MS, Co-GC, GC-O
40	linalool[2]	1558	0.29	0.17	1	sweet, fruity	RI, MS, Co-GC, GC-O
41	α-cedrene[3]	1572	16.71	0.27	2	floral	RI, MS, Co-GC, GC-O
42	longifolene[2]	1577	0.06	0.15	1	green, floral	RI, MS, Co-GC, GC-O
43	α-bergamotene	1583	0.01				RI, MS
44	bornyl acetate[5]	1593	0.01				RI, MS, Co-GC
45	β-caryophyllene[5]	1598	0.02	0.36		green	RI, MS, Co-GC, GC-O
46	terpinen-4-ol[2]	1603	0.02			green, soap-like, pungent	RI, MS, Co-GC, GC-O
47	undecanal[9]	1611	0.01	0.33			RI, MS, Co-GC
48	(*E*)-2-decenal[3]	1615	0.01				RI, MS, Co-GC
49	citronellyl formate[10]	1625	0.06		1	green	RI, MS, Co-GC, GC-O
50	γ-elemene[7]	1636	3.75	0.32	4	green, oily	RI, MS, Co-GC, GC-O
51	*trans*-2-decenol	1648	0.02	0.06	3	green	RI, MS, GC-O
52	ethyldecanoic acid[9]	1654	0.02		3	green, floral	RI, MS, Co-GC, GC-O
53	*cis*-β-farnesene[7]	1658	0.04	0.97	6[i]	green, oily, floral	RI, MS, Co-GC, GC-O
54	nonanol[1]	1663	0.06		2	green, floral	RI, MS, Co-GC, GC-O
55	citronellyl acetate[5]	1669	0.54	0.04	8[j]	herbaceous	RI, MS, Co-GC, GC-O
56	α-humulene[7]	1677	0.02		3	floral	RI, MS, Co-GC, GC-O
57	α-murrolene	1680	0.03	0.09	1	green	RI, MS, GC-O
58	*trans*-piperitol	1889	0.03		2	sweet, fruity	RI, MS, GC-O
59	decyl acetate[1]	1692	0.02		2	mild green	RI, MS, Co-GC, GC-O
60	neral[10]	1696	0.03		2	sweet	RI, MS, Co-GC, GC-O
61	α-terpineol[1]	1709	0.04	0.77	7	fruity, floral	RI, MS, Co-GC, GC-O
62	dodecanal[9]	1719	0.07	0.62	1	fruity, perfume-like, citrus-like	RI, MS, Co-GC, GC-O
63	valencene	1727	tr				RI, MS
64	neryl acetate[5]	1733	0.01		1	floral	RI, MS, Co-GC, GC-O
65	*cis*-linalool (pyran) oxide[7]	1751	0.01	0.07	3	fruity, floral	RI, MS, Co-GC, GC-O
66	*trans*-2-undecenal[h]	1761	tr				RI, MS, Co-GC
67	geranyl acetate[1]	1768	0.01	0.27	3	floral	RI, MS, Co-GC, GC-O
68	citronellol[5]	1774	0.01		3	sweet, fruity, citrus-like	RI, MS, Co-GC, GC-O
69	*n*-decyl alcohol[5]	1778	0.01	0.22	3	floral	RI, MS, Co-GC, GC-O
70	cumin aldehyde[5]	1784	0.09	0.10	3	fruity, floral	RI, MS, Co-GC, GC-O
71	methyl laurate[1]	1814	0.03		3	sweet, fruit	RI, MS, Co-GC, GC-O
72	tridecanal[9]	1824	tr				RI, MS, Co-GC
73	geranyl propionate[1]	1830	0.01				RI, MS, Co-GC

Table 1 (Continued)

no.	compound[k]	retention index	wt %, SDE	peak area %, SPME	FD factor[b]	odor description[c]	identification
74	geraniol[1]	1852	0.05		3	sweet	RI, MS, Co-GC, GC-O
75	nerol[1]	1858	0.06		2	sweet	RI, MS, Co-GC, GC-O
76	*trans*-2-dodecenal[5]	1867	0.08	7.00	2	sweet, green	RI, MS, Co-GC, GC-O
77	dodecyl acetate[1]	1883	tr				RI, MS, Co-GC
78	isopentyl caproate[6]	1888	0.01	0.14	1	green	RI, MS, Co-GC, GC-O
79	perillyl alcohol[2]	1896	0.01	0.12	1	beany, oily	RI, MS, Co-GC, GC-O
80	dodec-2-en-4-one[h]	1905	0.02		1	beany, oily	RI, MS, GC-O
81	perillyl acetate	1916	tr		1	oily	RI, MS, GC-O
82	2-phentyl ethanol[1]	1927	0.01				RI, MS, Co-GC
83	tetradecanal[2]	1939	0.06	0.36	1	oily, green	RI, MS, Co-GC, GC-O
84	dehydrocarveol[7]	1949	0.28	0.17	7[j]	oily	RI, MS, Co-GC, GC-O
85	β-ionone[11]	1955	0.01	0.48			RI, MS, Co-GC
86	heptanoic acid[11]	1963	0.01	0.21			RI, MS, Co-GC
87	2-acetylpyrrole	1974	0.60	0.11	1	oily, green	RI, MS, GC-O
88	tetradecanal[9]	1982	0.01	0.05			RI, MS, Co-GC
89	*cis*-caryophyllene epoxide	1987	0.05	0.34	6[j]	green, pungent	RI, MS, GC-O
90	*cis*-nerolidol[5]	1997	0.06		1	oily, green	RI, MS, Co-GC, GC-O
91	caryophyllene oxide[3]	2004	0.07		1	oily, green	RI, MS, Co-GC, GC-O
92	*trans*-nerolidol[5]	2015	0.01				RI, MS, Co-GC
93	ledol	2022	0.01	0.39			RI, MS
94	methyl tetradecanoate[9]	2036	0.06	0.06	3	green, pungent	RI, MS, Co-GC, GC-O
95	*trans*-dodec-2-enol	2044	0.39	0.05	3	green, pungent	RI, MS, GC-O
96	octanoic acid[1]	2070	49.13	0.15	3	green, pungent	RI, MS, Co-GC, GC-O
97	elemol[7]	2088	0.07	0.07	3	green, pungent	RI, MS, Co-GC, GC-O
98	3-methylphenol[6]	2107	0.04	0.20			RI, MS, Co-GC
99	cedrol[7]	2119	0.01	0.12			RI, MS, Co-GC
100	2-pentadecanol[9]	2128	1.30	0.58	3	green, pungent	RI, MS, Co-GC, GC-O
101	cedryl acetate[3]	2143	1.28	2.76	2	green, pungent	RI, MS, Co-GC, GC-O
102	hexadecanol[6]	2152	0.02	0.18			RI, MS, Co-GC
103	ethyl pentadecanoate[9]	2161	0.02	0.52			RI, MS, Co-GC
104	eugenol[1]	2174	0.05	0.13	2	green	RI, MS, Co-GC, GC-O
105	muurolol	2180	0.01	0.14			RI, MS
106	γ-eudesmol[1]	2188	0.12	0.10	5	green	RI, MS, Co-GC, GC-O
107	methyl pentadecanoate[9]	2201	0.01	0.15			RI, MS, Co-GC
108	isoeugenol[1]	2213	0.05	0.67	2	green	RI, MS, Co-GC, GC-O
109	α-cadinol	2220	0.01				RI, MS
110	isothymol[5]	2227	0.02		2	mild green	RI, MS, Co-GC, GC-O
111	β-sinensal	2238	0.03				RI, MS
112	β-eudesmol[1]	2245	0.03	0.23			RI, MS, Co-GC
113	heptadecanal[9]	2249	0.07	0.23	3	mild green	RI, MS, Co-GC, GC-O
114	*trans*,*trans*-farnesyl acetate[2]	2259	0.62	7.29	6[j]	green, floral	RI, MS, Co-GC, GC-O
115	*trans*,*trans*-farnesol[7]	2283	0.01				RI, MS, Co-GC
116	*p*-mentha-1,8-dien-10-ol[h]	2292	0.02				RI, MS
117	cinnamic alcohol[8]	2302	0.54	1.73	3	mild green	RI, MS, Co-GC, GC-O
118	*cis*,*trans*-farnesol[7]	2321	0.02	0.25			RI, MS, Co-GC
119	octadecanol	2360	0.05	1.63			RI, MS
120	ethyl heptadecanoate[9]	2365	0.01	0.65			RI, MS, Co-GC
121	undecanoic acid	2419	0.39	0.64			RI, MS
122	14-hydroxy-β-caryophyllene	2445	0.06	0.43			RI, MS
123	isobutylidene phthalide	2563	0.57	6.41	4	green, oily	RI, MS
124	ligustilide	2629	1.93	1.55	4	green, oily	RI, MS
125	3-butyl dihydrophthalide	2643	0.57	1.98	4	green, oily	RI, MS
126	*n*-butylidene dihydrophthalide	2676	0.73	8.61	5	green, oily	RI, MS

[a] Trace, <0.005% (weight percentage). [b] Flavor dilution factor (3^n) of changpo leaf oil. [c] Odor description of changpo leaf oil by GC-O. [d] Identification based on retention index. [e] Identification based on comparison of mass spectra. [f] Identification based on co-injection with authentic compounds. [g] Identification based on gas chromatography–olfactometry. [h] Tentatively identified. [i] Most similar changpo-like odor compounds perceived at the sniffing port. [j] Changpo-like odor compounds perceived at the sniffing port. [k] Sources of authentic chemicals (1, Wako Pure Chemical Industries; 2, Aldrich Chemical Co.; 3, Fluka Fine Chemicals; 4, Funakoshi Co., Ltd.; 5, Tokyo Kasei Kogyo Co.; 6, PolyScience Co.; 7, Bolak Co.; 8, Nacalai Tesque Inc.; 9, Theta Co.; 10, French-Korean Aromatics; 11, Sigma Chemical Co.).

programmed from 70 (2 min) to 230 °C (20 min) at 2 °C/min. Injector and detector temperatures were 250 °C. Nitrogen was the carrier gas at a flow rate of 1 mL/min and a linear velocity of 22 cm/s. The linear retention indices were calculated for all volatile components using a homologous series of *n*-alkanes (C_7−C_{29}) under the same GC conditions. 1-Heptanol and methyl myristate were used as internal standards for quantitative analysis of changpo oil. The ratio of changpo oil for the two internal standards was 150:1:1. The weight percentage of each peak was calculated according to the correlation factor to the FID (*14*). One microliter of oil was injected, and the split ratio was 50:1. A headspace sample of this aromatic herb was also injected into the GC, and the injector split ratio was 50:1.

Gas chromatography combined with mass spectrometry was used for identifying the volatile components that had been detected. The analysis was carried out with a Varian Saturn 2000R 3800 GC (Walnut Creek, CA) linked with a Varian Saturn 2000R MS. The oven condition, injector and detector temperatures, and column were the same as those given above for the Agilent 6890N GC. An oil sample of 0.2 μL was injected, and the split ratio was 34:1. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s.

SPME injection into the GC-MS system was performed using a Varian 8200 autosampler (Walnut Creek, CA) under the same analysis

Table 2. Volatile Flavor Constitution of Functional Groups in the Changpo Leaves

	SDE		SPME	
functional group	total no.	wt %	total no.	peak area %
hydrocarbons				
aliphatics	5	1.63	3	0.04
monoterpenes	11	5.26	8	1.33
sesquiterpenes	12	20.74	7	2.59
aldehydes				
aliphatics	11	0.95	8	8.69
terpenes	4	0.18	2	0.15
alcohols				
aliphatics	13	2.61	11	8.61
monoterpenes	15	1.49	7	0.29
sesquiterpenes	11	0.36	7	1.30
ketones	7	0.52	4	0.73
esters	22	2.76	10	11.90
oxides and epoxides	7	0.21	4	0.46
acids	3	49.53	3	1.00
phthalides	4	3.80	4	18.55
miscellaneous	1	0.60	1	0.11
total	126	90.64	79	55.75

conditions. After 40 min of extraction, the volatile compounds were desorbed for 2 min into the injector of a Varian Saturn 2000R 3800 GC.

Identification of Components. Individual components were identified by comparing their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search Program (ChemSW Inc., NIST 98 version database) connected to a Varian Saturn 2000R MS. Other identifications were made by comparison of both mass spectrum and GC retention data with those of authentic compounds previously analyzed and stored in the data system. The volatile flavor components were also matched by coinjection with authentic compounds.

Sniffing Test by GC-O. An Agilent 6890N GC equipped with a DB-Wax fused-silica capillary column (60 m × 0.53 mm i. d., film thickness = 1 μm, J&W Scientific), FID, and olfactometer (Gerstel GmbH & Co., Mûlheim, Germany) including olfactory detector port, olfactory intensity device, and humidifier was employed for GC-O. The oven condition and injector and detector temperatures were the same as those given above for the GC. The flow rate of the nitrogen carrier gas was 2 mL/min, and the split ratio was 10:1.

AEDA. Changpo oil was stepwise (3-fold) diluted with acetone until the sniffer could not detect any significant odor in a run (*15*, *16*), and aliquots of the dilutions were evaluated by three assessors. Odor potencies of each volatile in the changpo oil were evaluated by sniffers, together with the odor description. The highest dilution at which an individual component could be detected was defined as the flavor dilution (FD) factor for that odorant (*15*). Mild herbaceous, herbaceous, mild green, green, beany, buttery, fruity, floral, fresh, gaseous, oily, sweet, warm, woody, pungent, tree-like, medicine-like, lemon-like, soap-like, perfume-like, and citrus-like were the terms used to describe the odorants. The lexicon was developed by sniffing the sample several times and selecting 21 of the most frequently used terms. On the basis of the AEDA results, relative flavor activity (RFA) was calculated using the following equation (*17*, *18*): RFA = $\log 3^n/S^{0.5}$, where n is the FD factor and S is the weight percentage of a component.

RESULTS AND DISCUSSION

Constituents of Changpo Leaf Oil. The detected constituents from changpo oil are listed in **Table 1**, together with their weight percentages. A classification based on functional groups is summarized in **Table 2**. The data are mean values of triplicates. The components are listed in order of their elution on the DB-Wax column.

One hundred and twenty-six components, representing 90.64% (weight percent) of the total oil, were identified in fresh changpo leaves. The leaf oil contained 28 hydrocarbons (27.63%), 15 aldehydes (1.13%), 39 alcohols (4.46%), 7 ketones (0.52%), 22 esters (2.76%), 7 oxides and epoxides (0.21%), 3 acids (49.53%), 4 phthalides (3.8%), and 1 miscellaneous component (0.6%). The leaf oil is characterized by a high percentage of acids (49.53%) and sesquiterpene hydrocarbons (20.74%). Octanoic acid (49.13%) and α-cedrene (16.71%) are the main components of this oil. Of the minor components the monoterpene hydrocarbon α-phellandrene (4.46%) and the sesquiterpene γ-elemene (3.75%) reached appreciable amounts.

Headspace Composition of Changpo Leaves. Seventy-nine volatile flavor constituents, which make up 55.75% of the total volatile content of the headspace, were detected in the headspace of this herb, and *n*-butylidene dihydrophthalide (8.61%) was the most abundant component. The changpo headspace is characterized by high contents of *n*-butylidene dihydrophthalide (8.61%), *trans*,*trans*-farnesyl acetate (7.29%), *trans*-2-dodecenal (7%), isobutylidene phthalide (6.41%), and hexanol (4.14%). In comparison to the oil, the headspace composition of this herb is rich in phthalides (**Table 2**), which alone account for 18.55%. The contents of esters, alcohols, and aldehydes were also higher in the headspace than those of the oil. The contribution of acids and sesquiterpene hydrocarbons in the headspace was lower than those of the leaf oil and is due entirely to octanoic acid (0.15%) and α-cedrene (0.27%). The headspace approach simulates the odor of the headspace in a food product. The headspace concentration of a component would be related with the odor potency of the food. Therefore, compounds having high headspace concentration could be related to the aroma-active compounds of the food.

Aroma-Active Compounds of Changpo Leaves. The olfactory profile of changpo leaf oil was characterized by green, floral, and herbaceous top notes, slow fruity and woody notes, and an oily note in the lasting undertone. The odor activity of each compound in a mixture was determined by sniffing the GC effluent through a series of dilutions according to the AEDA technique. Each volatile component is separated by GC, and the odors are determined at a sniffing port of the GC−olfactometer. The FD factor was expressed as a power of 3. The sniffing test is used not only for AEDA but also for expressing the aroma character of each component. Odor descriptions for compounds detected with GC-O are given in **Table 1**. A comparison of the gas chromatogram obtained by GC and the corresponding FD chromatogram of the odor-contributing compounds is shown in **Figure 1**.

The range of the FD factors of each peak from changpo leaf oil was between 1 and 8 (**Table 1**). Although the contents of octanoic acid (peak 96) and α-cedrene (peak 41) of changpo oil were abundant, their FD factors were very low, 3 and 2, respectively. Furthermore, their headspace concentrations were low, 0.15 and 0.27%, respectively. They are of minor importance in the odor-active compounds of this aromatic herb. On the basis of their high FD factors (≥6), citronellyl acetate (FD factor = 8; herbaceous), dehydrocarveol (7; oily), *cis*-β-farnesene (6; green, oily, and floral), *cis*-caryophyllene epoxide (6; green and pungent), and *trans*,*trans*-farnesyl acetate (6; green and floral) showed to be the most odor-active among the 80 aroma compounds detected in changpo oil by AEDA.

The higher FD factors were often related to the aroma-active compounds. However, many researchers (*17*−*19*) have pointed out that a high FD factor of a compound may be caused by its high content in the sample. The FD factor for a compound is the ratio of its concentration in the initial extract to its concentration in the most diluted extract, in which the odor was

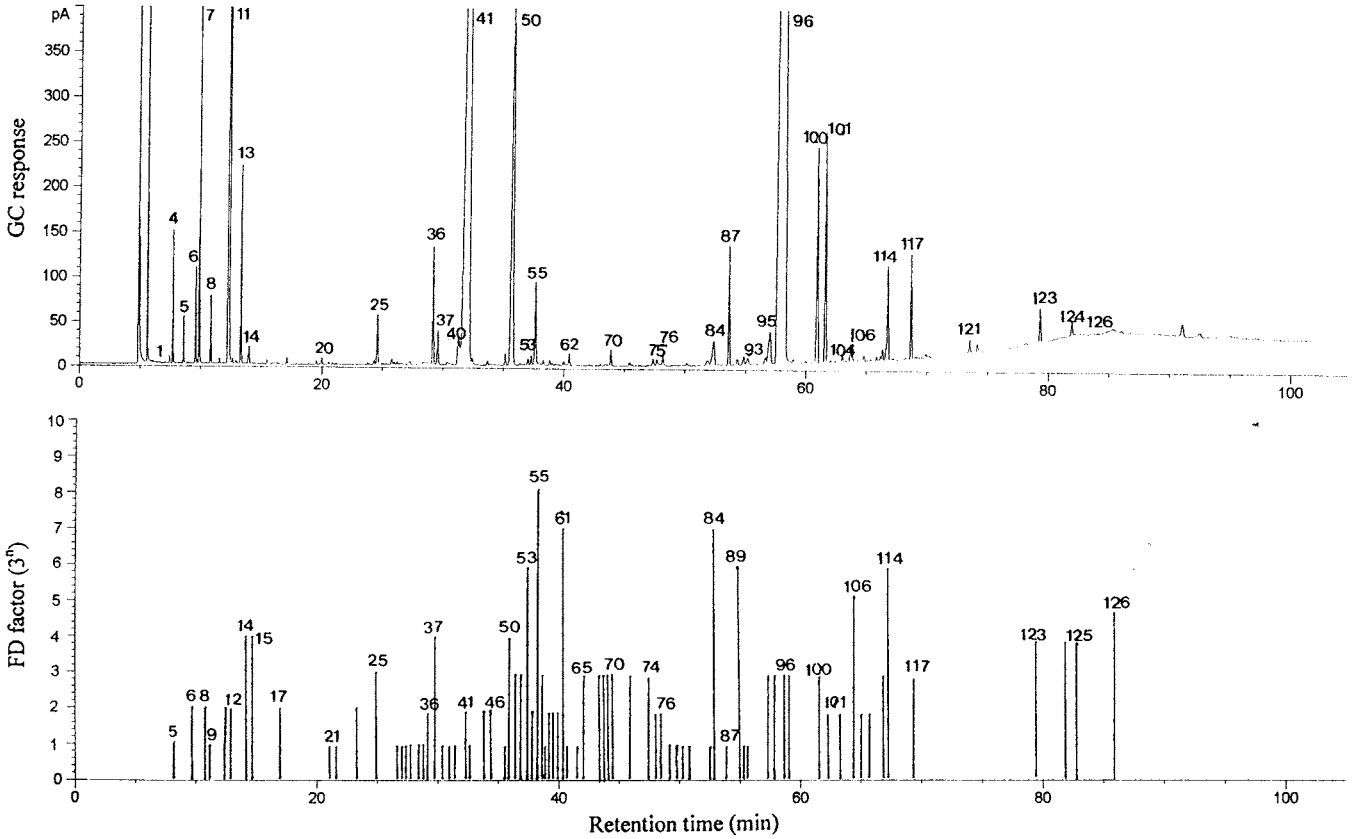

Figure 1. Gas chromatogram (top) and FD chromatogram (bottom) of changpo leaf oil.

Table 3. Most Aroma-Active Volatiles (FD ≥ 5) in the Changpo Leaf Oil As Detected by GC-O

peak[a]	compound	concn (μg/kg of fresh wt)	FD factor (3^n)	RFA
53	*cis*-β-farnesene	98	6[b]	14.3
55	citronellyl acetate	1322	8[c]	5.2
61	α-terpineol	98	7	16.7
84	dehydrocarveol	686	7[c]	7.2
89	*cis*-caryophyllene epoxide	122	6[c]	12.8
106	γ-eudesmol	294	5	6.9
114	*trans,trans*-farnesyl acetate	1518	6[c]	3.6
126	*n*-butylidene dihydrophthalide	1788	5	2.8

[a] Peak numbers correspond with peak numbers in **Table 1**. [b] Most similar changpo-like odor compound perceived by GC-O. [c] Changpo-like odor compounds perceived by GC-O.

detected by the GC-O. Therefore, a GC-O technique such as AEDA is based on the determination of odor-threshold values of the volatile components eluted from the GC column (*20*, *21*). In addition to the FD factor, the author determined the RFA (**Table 3**). Recently the concept of RFA has been employed in a wide range of flavor investigations (*17*, *18*). The RFA of aroma-active compounds (FD factor ≥ 5) of changpo leaf oil is shown in **Table 3**. Although the FD factors of citronellyl acetate and dehydrocarveol were high at 8 and 7, their relative flavor activities were low at 5.2 and 7.2, respectively. The RFA of *n*-butylidene dihydrophthalide, which was the main component of changpo headspace, was very low at 2.8. As a consequence of this finding it could be assumed that the high FD factors of citronellyl acetate, dehydrocarveol, *trans,trans*-farnesyl acetate, and *n*-butylidene dihydrophthalide and the high headspace concentration of *n*-butylidene dihydrophthalide might be due to their high concentrations (1322, 686, 1518, and 1788 μg/kg of fresh wt, respectively) in the sample. With regard to the RFA (>10), only *cis*-β-farnesene, α-terpineol, and *cis*-caryophyllene epoxide contribute essentially to the important aroma compounds of the changpo leaves.

The sniffing test of the original essential oil by on-line GC is an effective means of determining the key aroma compounds of food together with the FD factor. As shown in **Table 1**, *cis*-β-farnesene, citronellyl acetate, dehydrocarveol, *cis*-caryophyllene epoxide, and *trans,trans*-farnesyl acetate were estimated as having a changpo-like aroma by the sniffing test, and *cis*-β-farnesene gave the most aroma-active character of changpo leaves. α-Terpineol was not regarded as a characteristic odor of changpo leaves by the sniffing test, although its FD factor was high at 7 and it showed a high RFA of 16.7 (**Table 3**). Results reported here suggest that *cis*-β-farnesene was evaluated as the most aroma-active compound of changpo leaf oil from results of FD factor, RFA, and sniffing test by GC-O.

With regard to the composition of volatiles, there is significant difference between the oil and the headspace of this aquatic herb. Whereas higher amounts of octanoic acid, α-cedrene, α-phellandrene, and γ-elemene were found in the oil of changpo leaves, *n*-butylidene dihydrophthalide, *trans,trans*-farnesyl acetate, *trans*-2-dodecenal, isobutylidene phthalide, and hexanol were found to be in higher amounts in the headspace of this herb. According to the AEDA and sniffing test, *cis*-β-farnesene was regarded as the key aroma compound of this herb.

ACKNOWLEDGMENT

I thank J. U. Kim, B. Y. Kim, and J. H. Song for supporting sniffing test.

LITERATURE CITED

(1) Lim, W. K.; Park, S. K.; Lyu, J. W.; Sa, D. M.; Lee, M. S.; Lim, K. O. *Resources Plants*; Seil Publishing: Seoul, Korea, 1986; p 331.

(2) Duke, J. A. *CRC Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, 1985; pp 14−15.

(3) Lee, M. S.; Choi, H. S. Volatile flavor components of *Dioscorea japonica*. *Kor. J. Food Sci. Technol.* **1994**, *26*, 68−73.

(4) Lee, M. S.; Choi, H. S. Volatile flavor components in various edible portions of *Calystegia japonica* (Thunb) Chois. *Kor. J. Food Sci. Technol.* **1994**, *26*, 359−364.

(5) Lee, M. S.; Choi, H. S. Volatile flavor components of *Commelina communis* L. as influenced by drying methods. *Kor. J. Food Sci. Technol.* **1995**, *27*, 380−386.

(6) Choi, H. S.; Lee, M. S. The effect of dispersion medium on intensity of volatile flavor components and recovery of essential oil from *Capsella bursa-pastoris* by steam distillation. *Kor. J. Food Sci. Technol.* **1996**, *28*, 827−833.

(7) Chung, T. Y.; Eiserich, J. P.; Shibamoto, T. Volatile compounds isolated from edible Korean chamchwi (*Aster scaber* Thunb). *J. Agric. Food Chem.* **1993**, *41*, 1693−1697.

(8) Kim, H. J.; Kim, S. W.; Shin, C. S. Analysis of chemical composition in leaf and root of *Acrorus calamus* L. *Kor. J. Food Sci. Technol.* **2000**, *32*, 37−41.

(9) Park, J. H. Studied on parasiticidal action of the crude drugs to *Eurytrema pancreaticum* in vitro. *Res. Rev. Kyungpook Natl. Univ.* **1976**, *21*, 289−303.

(10) Park, J. H. Some pamacological action of essential oil of *Acorus gramineus* Soland. *Res. Rev. Kyungpook Natl. Univ.* **1978**, *26*, 637−642.

(11) Kim, Y. H.; Park, J. H. Effect of essential oil of *Acorus rhizoma* on motility of isolated rabbit jejunum segment. *Agric. Res. Bull. Kyungpook Natl. Univ.* **1992**, *10*, 19−33.

(12) Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **1977**, *25*, 446−449.

(13) Choi, H. S. Headspace analysis of *Robus coreanus* berry by solid-phase microextraction and its sniffing test by gas chromatography-olfactometry. *Food Sci. Biotechnol.* **2002**, *11*, 355−360 (in Korean).

(14) Zheng, X. H. Studies on the chemotaxonomy of the *Citrus* genus. Ph.D. thesis, Ehime University, Ehime, Japan, 1997.

(15) Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797−800.

(16) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfadyen) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 1358−1363.

(17) Choi, H. S.; Kondo, Y.; Sawamura, M. Characterization of the odor-active volatiles in citrus Hyuganatsu (*Citrus tamurana* Hort. Ex Tanaka). *J. Agric. Food Chem.* **2001**, *49*, 2404−2408.

(18) Song, H. S.; Sawamura, M.; Ito, T.; Ido, A.; Ukeda, H. Quantitative determination and characteristic flavor of daidai (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka) peel oil. *Flavour Fragrance J.* **2000**, *15*, 323−328.

(19) Minh Tu, N. T.; Onishi, Y.; Choi, H. S.; Kondo, T.; Mitiku Bassore, S.; Ukeda, H.; Sawamura, M. Characteristic odor components of *Citrus sphaerocarpa* Tanaka (Kabosu) cold-pressed oil. *J. Agric. Food Chem.* **2002**, *20*, 2908−2913.

(20) Blank, I.; Grosch, W. Evaluation of potent odorants in dill seed and dill herb (*Anethum graveolens* L.) by aroma extract dilution analysis. *J. Food Sci.* **1991**, *56*, 63−67.

(21) Friedrich, J. E.; Acree, T. E. In *Flavor Chemistry: Industrial and Academic Research*; Risch, S. J., Ho, C. T., Eds.; American Chemical Society: Washington, DC, 2000; pp 124−132.

Received for review May 17, 2004. Revised manuscript received September 24, 2004. Accepted October 2, 2004.

JF040239P