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Comparison of the Cold-Pressed Peel Oil Composition between Korean and Japanese Satsuma Mandarin (*Citrus unshiu* Marcov. forma *Miyagawa-wase*) by GC, GC-MS and GC-O

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Abstract

The comparison of the volatile flavor components from Korean and Japanese Satsuma mandarin (*C. unshiu* Marcov. forma *Miyagawa-wase*) peel oils, isolated by cold-pressing, was performed by gas chromatography, mass-spectrometry and gas chromatography-olfactometry (GC-O). Eighty-five volatile components were identified in each oil by GC and GC-MS. Forty-three components were detected in each oil by GC-O. The total amount of monoterpene hydrocarbons was 95.88% (Korean mandarin) and 95.29% (Japanese mandarin). Limonene, γ-terpinene, myrcene and α-pinene were the main components of the cold-pressed oils from the both samples. The volatile composition of the Japanese mandarin was characterized by a higher content of sesquiterpene hydrocarbons, especially bicyclogermacrene, α-humullene and valencene. The volatile composition of two samples can easily be distinguished by the percentages of aldehydes, ketones and esters, which were found at higher levels in the Japanese mandarin. The sweet and fruity flavor was stronger in the Korean mandarin oil while herbaceous flavor was stronger in Japanese sample. From GC-O data it is suggested that the sweet and fruity flavor of the Korean mandarin resulted from terpinolene and linalool, and the herbaceous note of the Japanese mandarin from α-humullene, neral, *l*-carvone and perill aldehyde.

Key words: Satsuma mandarin (*C. unshiu* Marcov. forma *Miyagawa-wase*), cold-pressed peel oil composition, GC, GC-MS, GC-O

INTRODUCTION

Citrus is the most produced fruit in the world. The origin of the *Citrus* genus is thought to be northeastern India, near Assam. It is suspected that cultivation of *Citrus* fruits started in China. The mandarin species was also born near Assam. It is said that the most primitive variety of the mandarin, *C. indica*, still grows in the forest of Assam. After migration to China it has been supposed that it was carried to Southeast Asian countries. The three major regions of *Citrus* cultivation in the subtropical latitudes of the northern hemisphere are North America, Mediterranean and East Asia (1,2). The most widely grown *Citrus* variety in Korea and Japan is *Citrus unshiu*, Satsuma mandarin. It is assumed that Satsuma mandarin originated on Nagashima island in Kagoshima Prefecture of Japan early in the 17th century. Nowadays, it is cultivated not only in Japan, but also in Korea, China and Spain. In Korea most Satsuma mandarin fruits are produced in Jeju, the southeast island of Korea. Many varieties of *C. unshiu*, 100 or more bud variations named, are cultivated in the world. Among them, *C. unshiu* Marcov. forma *Miyagawa-wase* (Miyagawa-wase unshiu) is the most popular cultivar in Korea and Japan. Miyagawa-wase unshiu originated in Fukuoka Prefecture in about 1915 in Japan as a mutant, and it has been assumed that it was then brought to Jeju in Korea (3). It matures earlier and has a better quality than other unshiu cultivars. It is used for fresh fruit, canning and frozen concentrate.

A number of studies on the Satsuma mandarin have been carried out (4-8). The chemical composition of the essential oils of Satsuma mandarin also has been extensively studied (9-13). A number of factors including climate, genetics, soil conditions are involved in determining citrus oil quality. The objective of this study was to investigate the differences of cold-pressed peel oil composition between Korean and Japanese Satsuma mandarin, miyagawa wase unshiu.

MATERIALS AND METHODS

Materials

Satsuma mandarin (*Citrus unshiu* Marcov. forma *Miyagawa-wase*), which were harvested in November 2000,

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were provided by the Citrus Experiment Station Rural Development Administration, Jeju, Korea, and the Kochi Prefectural Fruit Tree Experimental Station, Kochi, Japan, respectively. The climates between Jeju of Korea and Kochi Prefectures of Japan are similar. These regions are located at a similar latitude between 33.3° and 34.5° north, however, differ vastly in humidity and rainfall patterns. Satsuma mandarin samples were all of the highest quality level. These fruits were stored at 4°C until used for chemical analysis.

Authentic chemicals for co-injection in gas chromatography and mass spectrometry were obtained from reliable commercial sources as follows: Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan); Wako Pure Chemical Industries (Osaka, Japan); Aldrich Chemical Co. (Milwaukee, WI, USA); Sigma Chemical Co. (St. Louis, MO, USA); Extrasynthese S. A. (Genay, France). Some chemicals were provided by Ogawa & Co., Ltd. (Tokyo, Japan).

Preparation of cold-pressed oil (CPO)

The oil samples from Korean and Japanese mandarin were prepared according to the cold-pressing method described by Sawamura and Kuriyama (14). The fruits were sliced and the mesocarp and albedo layers were peeled off from the flavedo. The peel oils were extracted by hand-pressing the flavedo, and were collected in a brine solution on ice. The oil extract was centrifuged at 4000 *g* for 15 min at 4°C. The supernatant was dehydrated with anhydrous sodium sulfate at 5°C for 24 h and filtered. The CPOs were stored at -25°C until analyzed.

Gas chromatography (GC)

A Shimadzu GC-14A gas chromatograph (GC) equipped with a DB-Wax fused-silica capillary column (60 m × 0.25 mm i. d., film thickness 0.25 μm, J & W Scientific, Folsom, CA, USA) and a flame ionization detector (FID) was used. Peak areas were integrated with a Shimadzu C-R6A Chromatopack integrator. The column temperature was programmed from 70°C (2 min) to 230°C (20 min) at a rate of 2°C/min. The injector and detector temperatures were 250°C, respectively. Nitrogen was the carrier gas at a flow rate of 2 mL/min. Authentic compounds of 1-heptanol and methyl myristate (Wako Pure Chemical Industries, Osaka, Japan) were used as internal standards for accurate quantification. 1-Heptanol was employed as one internal standard for quantitative analysis up to linalool, and methyl myristate as the other standard was used for the peaks after linalool. The ratio of cold-pressed oil (CPO) to the two internal standards was 150 : 1 : 1. The weight percent (weight %) of each peak was calculated according to the correlation factor to the flame ionization detector (15). An oil sample of 1 μL was injected and the split ratio was 1 : 50.

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography combined with mass spectrometry (GC-MS) was used for identification of the volatile flavor components detected. The analysis was carried out on a Shimadzu GC-17A linked with a Shimadzu QP-5000 at a MS ionization voltage of 70 eV, accelerating voltage of 1500 V, and ion source temperature of 250°C. The GC column and oven conditions were the same as those given above for the GC-14A. An oil sample of 0.2 μL was injected and the split ratio was 1 : 34. The carrier gas was helium at a constant flow of 1.0 mL/min.

Gas chromatography-olfactometry (GC-O)

A Shimadzu GC-8A gas chromatograph equipped with a DB-Wax fused-silica capillary column (60 m × 0.53 mm i. d., film thickness 1 μm, J & W Scientific, Folsom, CA, USA) and FID was employed for GC-O. The oven condition, and injector and detector temperatures were the same as those given above for the GC-14A. The flow rate of nitrogen carrier gas was 5 mL/min and the split ratio was 1 : 10. GC-O was performed with 1 μL of the cold-pressed oils which were evaluated by sniffing. At the exit of the capillary, the effluent was split into an FID and a sniffing port. Humid air was added to the effluent at the sniffing port.

Identification of components

Components were identified by comparing their GC retention indices (RI) on a DB-Wax column. Kováts retention indices (RI) were calculated for all volatile components using a homologous series of *n*-alkanes (C_9-C_{27}) under the same GC conditions. The constituents were also identified by matching their mass spectra with those of reference compounds in the data system of Compaq-ProLinea (Compaq Co., USA; Class 5K software), connected to a QP-5000 mass spectrometer. Whenever possible, the volatile flavor components were matched by co-gas chromatography with authentic compounds. Furthermore, the components of CPOs were confirmed by GC-O.

RESULTS AND DISCUSSION

Freshly isolated oils obtained by cold-pressing method from Korean and Japanese Satsuma mandarin were analyzed by GC, GC-MS, and GC-O. Eighty-five components were identified in each oil by GC and GC-MS (Fig. 1, 2). The identified components and their weight percentages are given in Table 1, where the components are listed in order of their elution on the DB-Wax column. A classification based on functional groups is summarized in Table 2.

Most components were common to both oils although the proportions differed. Limonene (Korean mandarin; 86.70%, Japanese mandarin; 87.58%), γ-terpinene (4.75%,

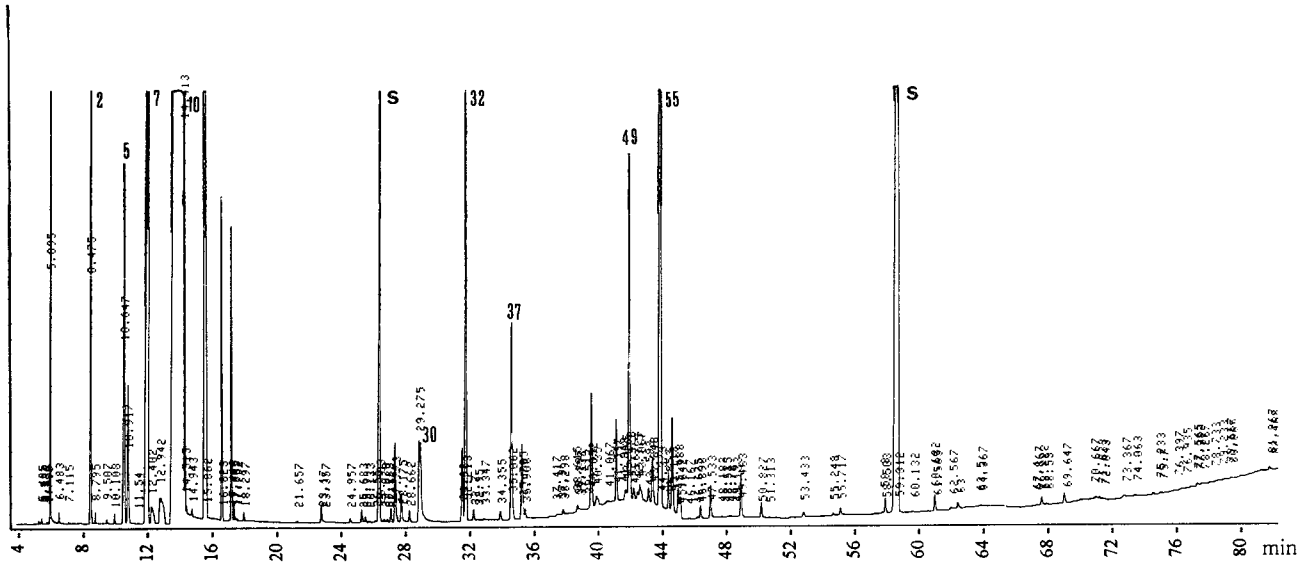

Fig. 1. Gas chromatogram of volatile components from Korean Satsuma mandarin peel oil (S: internal standard).

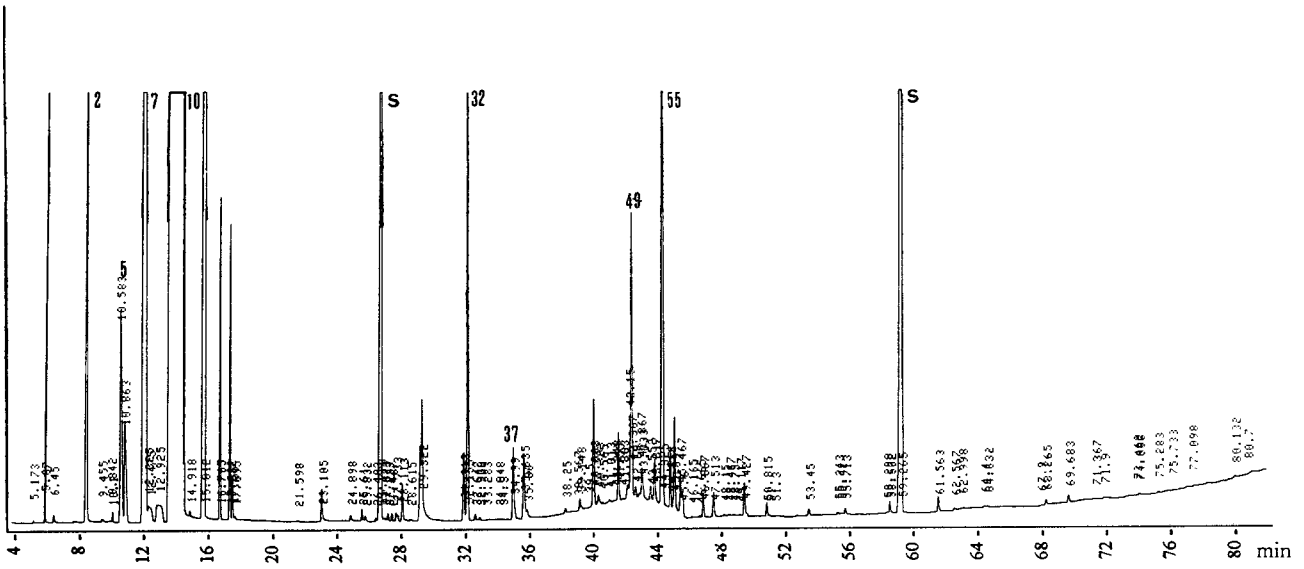

Fig. 2. Gas chromatogram of volatile components from Japanese Satsuma mandarin peel oil (S: internal standard).

3.71%), myrcene (2.08%, 2.12%) and α-pinene (0.86%, 0.73%) were the main components of the CPOs from the both samples.

Hydrocarbons

The principal component in the CPOs of Korean and Japanese Satsuma mandarin was limonene (Korean mandarin; 86.70%, Japanese mandarin; 87.58%), monoterpene hydrocarbon. As shown in Table 2, the total content of monoterpene hydrocarbons was 95.88% (Korean mandarin) and 95.29% (Japanese mandarin). There was a significant difference in the proportion of sesquiterpene hydrocarbons. The principal sesquiterpene hydrocarbons encountered in these fruits were bicyclogermacrene (Korean mandarin; 0.59%, Japanese mandarin; 1.24%), α-humullene (0.08%, 0.21%), β-caryophyllene (0.17%, 0.08%) and valencene (0.04%, 0.12%). The volatile composition of the Japanese mandarin was characterized by higher content of sesquiterpene hydrocarbons and it could be related to increase in the contents of bicyclogermacrene, α-humullene and valencene.

Aldehydes and alcohols

The CPOs of the Satsuma mandarin from Korea and Japan can easily be distinguished by the percentage of aldehydes. Total weight percent of aldehydes accounted for

Table 1. Volatile flavor components identified in the cold-pressed peel oils of *Citrus unshiu*

No.	Compound	Retention index	Weight percent		Identification	Odor description
		DB-Wax	Korean mandarin	Japanese mandarin		
1	ethyl acetate	904	0.01	0.01	RI[2]	
2	α-pinene	1035	0.86	0.73	RI, MS[3], Co-GC[4], GC-O[5]	green
3	camphene	1082	tr[1]	tr	RI, MS, Co-GC, GC-O	green, sweet
4	undecane	1106	0.01	0.01	RI, MS, Co-GC, GC-O	green, dry
5	β-pinene	1124	0.40	0.30	RI, MS, Co-GC, GC-O	pungent green, waxy
6	(+)-sabinene	1132	0.15	0.14	RI, MS, Co-GC, GC-O	pungent green
7	myrcene	1167	2.08	2.12	RI, MS, Co-GC, GC-O	green, resinous
8	α-phellandrene	1177	0.04	0.01	RI, MS, Co-GC, GC-O	green, oily
9	α-terpinene	1192	0.10	0.07	RI, MS, Co-GC, GC-O	herbaceous, floral
10	limonene	1228	86.70	87.58	RI, MS, Co-GC, GC-O	green, citrus-like
11	β-phellandrene	1231	0.32	0.24	RI, MS, GC-O	green, floral
12	*cis*-β-ocimene	1241	0.01	0.01	RI, MS, Co-GC, GC-O	pungent, burnt-odor
13	γ-terpinene	1261	4.75	3.71	RI, MS, Co-GC, GC-O	green, resinous
14	*p*-cymene	1281	0.25	0.20	RI, MS, Co-GC, GC-O	green, citrus-like
15	terpinolene	1294	0.22	0.18	RI, MS, Co-GC, GC-O	fruity, citrus-like
16	octanal	1297	0.06	0.11	RI, MS, Co-GC, GC-O	green
17	tridecane	1300	tr	tr	RI, Co-GC	
18	heptyl acetate	1374	tr	tr	RI	
19	tetradecane	1399	0.02	0.03	RI, MS, Co-GC, GC-O	sweet, citrus-like
20	β-thujone	1432	0.01	0.01	RI, Co-GC	
21	1-hepten-1-yl acetate	1445	0.01	0.01	RI	
22	*cis*-linalool furanoxide	1448	0.01	tr	RI, Co-GC	
23	(+)-*cis*-limonene oxide	1459	tr	tr	RI, MS, Co-GC	
24	(-)-α-cubebene	1468	tr	tr	RI, MS, Co-GC	
25	(+)-*trans*-limonene oxide	1471	tr	0.01	RI, MS, Co-GC, GC-O	sweet, oily
26	menthone	1475	0.01	0.01	RI, Co-GC	
27	*trans*-linalool furanoxide	1479	0.09	0.01	RI, Co-GC	
28	citronellal	1485	0.03	0.04	RI, MS, Co-GC, GC-O	citrus-like, green
29	α-ylangene	1493	0.01	tr	RI	
30	pentadecane	1502	0.19	0.21	Ri, Co-GC, GC-O	green, tree-like
31	β-cubebene	1549	0.08	0.06	RI, MS, Co-GC	
32	linalool	1553	0.68	0.39	RI, MS, Co-GC, GC-O	green, citrus-like
33	octanol	1560	0.01	0.01	RI, MS, Co-GC, GC-O	sweet, citrus-like
34	*trans*-limonene epoxide	1564	tr	tr	RI	
35	linalyl acetate	1571	tr	tr	RI, MS, Co-GC, GC-O	sweet, citrus-like
36	β-elemene	1586	tr	tr	RI, MS, Co-GC, GC-O	herbaceous
37	β-caryophyllene	1597	0.17	0.08	RI, MS, Co-GC, GC-O	herbaceous
38	terpinen-4-ol	1608	0.04	0.12	RI, MS, Co-GC, GC-O	green, resinous, citrus-like
39	myrcenol	1611	tr	0.02	RI	
40	*trans*-*p*-mentha-2,8-diene-1-ol	1651	0.01	0.01	RI	
41	*cis*--farnesene	1657	0.02	0.02	RI, Co-GC	
42	citronellyl acetate	1666	0.01	0.03	RI, MS, Co-GC	
43	*trans*-β-farnesene	1671	0.02	0.03	RI, MS, Co-GC, GC-O	green, herbaceous
44	α-humullene	1679	0.08	0.21	RI, MS, Co-GC, GC-O	herbaceous
45	δ-muurolene	1684	0.03	0.07	RI	
46	decyl acetate	1695	0.06	0.02	RI, MS, Co-GC, GC-O	green, floral
47	neral	1703	0.09	0.16	RI, MS, Co-GC, GC-O	herbaceous
48	terpinyl acetate	1708	0.03	0.05	RI, Co-GC	
49	α-terpineol	1718	0.19	0.45	RI, MS, Co-GC, GC-O	citrus-like, sweet, oily
50	dodecanal	1723	0.04	0.11	RI, MS, Co-GC, GC-O	green, waxy
51	germacrene-D	1727	0.02	0.04	RI, MS	
52	valencene	1730	0.04	0.12	RI, Co-GC, GC-O	green, herbaceous
53	neryl acetate	1739	0.04	0.10	RI, MS, Co-GC	
54	*l*-carvone	1743	0.06	0.15	RI, MS, Co-G, GC-O C	floral, herbaceous
55	bicyclogermacrene	1752	0.59	1.24	RI	
56	*cis*-linalool pyranoxide	1761	0.03	0.11	RI, Co-GC	
57	*trans*-2-undecenal	1765	0.07	0.18	RI, Co-GC	
58	geranyl acetate	1771	0.02	0.08	RI, MS, Co-GC, GC-O	sweet, herbaceous
59	citronellol	1774	0.01	0.04	RI, Ms, Co-GC, GC-O	sweet, sour, floral

Table 1. Continued

No.	Compound	Retention index	Weight percent		Identification	Odor description
		DB-Wax	Korean mandarin	Japanese mandarin		
60	sesquiphellandrene	1782	tr	tr	RI, MS	
61	cumin aldehyde	1791	tr	tr	RI, MS	
62	perill aldehyde	1794	0.02	0.09	RI, MS, Co-GC	herbaceous
63	octadecane	1805	0.02	0.04	RI, Co-GC	green, sweet
64	germacrene B	1817	tr	tr	RI	
65	tridecanal	1823	tr	tr	RI, MS, Co-GC	perfume-like, oily
66	geranyl propionate	1828	tr	tr	RI, Co-GC	
67	*cis*-carveol	1834	tr	tr	RI	
68	nerol	1840	0.03	0.06	RI, MS, Co-GC	
69	*p*-cymen-8-ol	1864	0.01	0.02	RI	
70	perill acetate	1910	tr	0.01	RI, MS,	
71	*p*-mentha-1-en-9-ol	1943	tr	tr	RI, Co-GC	
72	β-ionone	1952	tr	0.01	RI, Co-GC	
73	caryophyllene oxide	1998	tr	tr	RI, Co-GC	
74	*cis*-nerolidol	2001	0.01	0.02	RI, MS, Co-GC, GC-O	sweet, fruity, citrus-like
75	globulol	2061	0.01	0.02	RI, GC-O	sweet, herbaceous
76	octanoic acid	2080	tr	tr	RI, Co-GC	
77	elemol	2088	0.01	tr	RI, Co-GC, GC-O	warm, herbaceous
78	cedrol	2115	tr	tr	RI, Co-GC	
79	nonanoic acid	2192	tr	0.01	RI, Co-GC, GC-O	sweet, oily
80	α-cadinol	2222	0.01	0.01	RI, MS	
81	isoeugenol	2268	0.01	tr	RI, Co-GC	
82	cinnamyl alcohol	2299	tr	tr	RI, Co-GC, GC-O	sweet, herbaceous
83	*p*-mentha-1,8-dien-10-ol	2315	tr	tr	RI	
84	*cis, trans*-farnesol	2350	tr	tr	RI, MS, Co-GC	
85	undecanoic acid	2416	0.01	tr	RI, Co-GC	

[1)]Trace, less than 0.005% (weight percent).
[2)]Identification based on retention index.
[3)]Identification based on comparison of mass spectra.
[4)]Identification based on co-injection with authentic compounds.
[5)]Identification based on gas chromatography-olfactometry.

Table 2. Constitution of functional groups in the cold-pressed peel oils of Satsuma mandarin

Functional group	Korean mandarin		Japanese mandarin	
	Total no.	Weight percent	Total no.	Weight percent
Hydrocarbons				
Aliphatics	5	0.24	5	0.28
Monoterpenes	13	95.88	13	95.29
Sesquiterpenes	14	0.97	14	1.97
Aldehydes				
Aliphatics	4	0.17	4	0.41
Terpenes	4	0.15	4	0.29
Alcohols				
Aliphatics	1	0.01	1	0.01
Monoterpenes	13	1.00	13	1.11
Sesquiterpenes	6	0.03	6	0.06
Ketones	4	0.09	4	0.18
Esters	11	0.17	11	0.33
Oxides	7	0.14	7	0.15
Acids	3	0.01	3	0.01
Total	85	98.86	85	100

0.70% from the Japanese-grown mandarin, whereas made up 0.32% of the oil from the Korean-grown mandarin. The difference between Korean and Japanese mandarins with regard to the weight percentage of aldehydes could be due to octanal (Korean mandarin; 0.06%, Japanese mandarin; 0.11%), neral (0.09%, 0.16%), dodecanal (0.04%, 0.11%), *trans*-2-undecenal (0.07%, 0.18%) and perill aldehyde (0.02%, 0.09%).

Alcohols constituted the largest group of oxygenated compounds identified in mandarin oils, with 20 components were identified. Each oil has a characteristic volatile flavor pattern associated with the presence of linalool (Korean unshiu; 0.68%, Japanese unshiu; 0.39%), terpinen-4-ol (0.04%, 0.12%) and α-terpineol (0.19%, 0.45%). α-Terpineol, which is a major flavor component of *Eucalyptus* species (16), has a citrus-like odor, sweet and oily odor (Table 1) and is a first cyclic monoterpene alcohol formed in nature by an intramolecular alkylation of double bond of the acyclic precursor such as neryl pyrophosphate (17). The other alcohols were found at lower levels. Farnesol is well known as a natural flavor component and contributes considerably to the champagne flavor and it has been used in various drinks (18). In this study, *cis, trans*-farnesol was found in trace content.

Ketones and esters

The total content of ketones in Japanese Satsuma mandarin oil was 2 times higher than in Korean mandarin oil as shown in Table 2. *l*-Carvone was the main component of the ketones and its content of Japanese-grown mandarin (0.15%) was higher than in Korean-grown mandarin (0.06%). The other components, β-thujone, menthone and β-ionone were identified in small quantities.

Eleven ester components were confirmed in mandarin oils. Esters are usually almost absent from *Citrus* oil, but various ester components were found in this study. As for total esters content, there was significant difference between Korean and Japanese samples. The total ester content in Japanese mandarin oil was higher than that found in Korean samples. The content of neryl acetate was higher in Japanese mandarin oil (0.1%) than in Korean mandarin oil (0.04%).

Oxides and acids

Oxides (0.14%, 0.15%) and acids (0.01%) found in at low levels. Seven kinds of oxides and 3 kinds of acids were found in this study. The content of *trans*-linalool furanoxide was higher in Korean mandarin whereas *cis*-linalool pyranoxide was shown vise versa.

GC-O

Forty-three components were detected in each oil as shown in Table 1.

α-Humullene (peak no. 44) and valencene (peak no. 52), which were dominant hydrocarbons in Japanese mandarin CPO, showed herbaceous flavor by GC-O. However, β-caryophyllene (peak no. 37) having herbaceous flavor was abundant in Korean sample. Octanal (peak no. 16) showed green note, and neral (peak no. 47) and perill aldehyde (peak no. 62) revealed herbaceous flavor by GC-O. These aldehydes also were predominant components in Japanese sample than in Korean samples. Linalool (peak no. 32), which content was higher in Korean mandarin, showed green and citrus-like flavors. Terpinen-4-ol (peak no. 38) showed green, resinous and citrus-like flavors, and α-terpineol (peak no. 49) showed sweet, oily and citrus-like flavors. Their contents were higher in the Japanese mandarin CPO. *l*-Carvone (peak no. 54), which was the main components of the ketones from two samples, showed floral herbaceous flavor, and its content was higher in the Japanese sample. The herbaceous flavor was stronger in the Japanese sample. From GC-O data it is suggested that the sweet and fruity flavor of Korean mandarin resulted from terpinolene and linalool, and the herbaceous note of Japanese mandarin from a-humullene, neral, *l*-carvone and perill aldehyde. The fresh Korean and Japanese mandarin fruits showed sweet and herbaceous organoleptic characteristics, respectively. It seems that the flavor difference between Korean and Japanese Satsuma mandarin probably results from the differences in the ratio of these components.

Citrus oil is characterized by having a high percentage of terpenoid hydrocarbons and a relatively low content of terpenoid oxygenated compounds (19). The volatile flavor components of the miyagawa-wase unshiu from Korea and Japan also exhibited a high percentage of terpenoid hydrocarbons. The qualitative composition of the CPOs between two samples was found to be quite similar, varying in their proportions. Further research on non-volatile aroma components is necessary to obtain a quality index between Korean and Japanese Satuma mandarins.

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