



## Volatile Flavor Components of Ripe and Overripe Ki-mikans (*Citrus flaviculpus* Hort. ex Tanaka) in Comparison with Hyuganatsu (*Citrus tamurana* Hort. ex Tanaka)

Hyang-Sook CHOI and Masayoshi SAWAMURA\*

Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

Received May 31, 2000; Accepted August 3, 2000

The volatile flavor components of ripe and overripe ki-mikan (*Citrus flaviculpus* Hort. ex Tanaka) peel oil samples, which had been isolated by cold-pressing, were investigated by capillary GC and GC-MS, and compared with the Hyuganatsu (*Citrus tamurana* Hort. ex Tanaka) flavor. Limonene (ripe fruit, 82.44%; overripe fruit, 73.10%) was the most abundant compound in the ki-mikan oil, this being followed by  $\gamma$ -terpinene (8.83% and 13.74%), *trans*- $\beta$ -farnesene (1.76% and 3.12%) and myrcene (1.54% and 1.13%). The composition of overripe ki-mikan oil was characterized by higher amounts of aliphatic and sesquiterpene hydrocarbons, monoterpene and sesquiterpene alcohols, ketones and esters than that of ripe ki-mikan oil. Monoterpene hydrocarbons, especially limonene (84.78%), were predominant in Hyuganatsu oil. The CPO composition of ki-mikan was qualitatively similar to that of Hyuganatsu, but differed quantitatively. The content of sesquiterpene hydrocarbons was higher in the ki-mikan oil samples than in Hyuganatsu oil, while ketones showed the opposite predominance. These differences were more evident in the *trans*- $\beta$ -farnesene and *l*-carvone contents. The ratio of both these compounds could be used to distinguish ki-mikan oil from Hyuganatsu oil.

**Key words:** ki-mikan (*Citrus flaviculpus* Hort. ex Tanaka); cold-pressed peel oil; ripening stage; volatile flavor components; *trans*- $\beta$ -farnesene; *l*-carvone

Fruit has long been an essential dietary supplement, due to its nutritionally valuable components and pleasant flavor. Citrus fruits in particular have wide acceptance as a result of their nutritional value, attractive flavor and other intrinsic attributes. Citrus fruits and their products provide a number of nutrients such as ascorbic acid, citric acid, folacin, vitamin B<sub>6</sub> and flavonoid.<sup>1)</sup> These nutrients are widespread in the plant kingdom and comprise a large group of naturally occurring compounds found in fruits and vegetables, especially in the *Citrus* ge-

nus. A variety of aromatic plant materials are gathered or cultivated as a source of essential oils, many of which are of considerable commercial importance. Among them, citrus fruits are one of the most valuable materials. Many studies have focused on the composition of the essential oils of such *Citrus* fruits as yuzu,<sup>2-5)</sup> orange,<sup>6-9)</sup> lemon,<sup>10)</sup> pummelo,<sup>11,12)</sup> kabosu, daidai and yuko,<sup>13)</sup> mochiyuzu,<sup>14)</sup> naoshichi and Tahiti lime,<sup>15)</sup> and bergamot.<sup>16,17)</sup> In Japan, many kinds of *Citrus* fruits of unique flavor are cultivated that may be relatively unknown in other countries. Among them, ki-mikan (*Citrus flaviculpus* Hort. ex Tanaka), locally named ogon-kan, and Hyuganatsu (*Citrus tamurana* Hort. ex Tanaka), locally named konatsu, have been regarded as *Citrus* fruits with potential commercial value. Hyuganatsu is popular in Japan from early spring to summer. This fruit is mainly grown in Japan's Miyazaki and Kochi prefectures. Ki-mikan is thought to have been first found in Kagoshima prefecture about 100 years ago, and there is now small-volume production of ki-mikan at a few farms on Shikoku island. Ki-mikan is harvested in early spring, and Hyuganatsu from spring to summer. Most citrus fruits in Japan are usually harvested by the end of the year in order to avoid chilling injury and to maintain their commercial quality. Ki-mikan and Hyuganatsu fruits are, however, commonly left on the trees until the following spring; they are resistant to winter's severe cold. Both ki-mikan and Hyuganatsu have a yellow color and round shape, although the fruit size is different: ki-mikan has a diameter of 4–5 cm and weight of 60–80 g; Hyuganatsu has a larger diameter of 8–10 cm and weight of 180–200 g. The two fruits have similar pleasant aroma characters and tastes, but ki-mikan has a stronger sweet factor than Hyuganatsu. The flesh of these fruits is juicy and has a sweet and sour taste. The albedo layers of ki-mikan and Hyuganatsu are eaten together with the flesh. *Citrus* ki-mikan and Hyuganatsu are desirable products because of their flavor, taste and nutrients.<sup>18)</sup> Ki-mikan is sold from

\*To whom correspondence should be addressed. Tel: +81-88-864-5184; Fax: +81-88-864-5200; E-mail: sawamura@cc.kochi-u.ac.jp

February to May in Japan, and during this period, there is no apparent difference in color and shape. However, there are some differences in the taste and flavor. Ki-mikan fruits harvested in February have been said to be of the best commercial quality at the market, having a good balance of sweet and sour and a refreshing aroma. The refreshing aroma of ki-mikan fruits deteriorates with maturation, although the taste becomes thicker. Hyuganatsu fruits, whose aroma is quite similar to that of ki-mikan, generally replace overripe ki-mikan fruits at the market in May. We therefore tried to elucidate the change in volatile flavor components of ripe and overripe ki-mikans, and of ripe Hyuganatsu. There is a substantial body of studies on the volatile flavor components of various citrus fruits.<sup>2-19)</sup> However, this paper is the first study on the volatile constituents of ki-mikan oil, while few studies of Hyuganatsu oil have been reported.<sup>20)</sup> In view of the commercial value and wide applications of ki-mikan, flavor researchers and consumers require detailed information, especially about the flavor quality of the essential oil of this fruit; therefore, the present study was undertaken in order to increase knowledge about the chemical composition of the essential oil of ki-mikan for its use in foods, beverages and other products. We report in this paper the volatile flavor components of cold-pressed oil samples of ripe and overripe ki-mikans, and compare them with the Hyuganatsu flavor.

## Materials and Methods

**Materials.** Ki-mikan fruits (*Citrus flaviculpus* Hort. ex Tanaka), which had been harvested in February (ripe fruit) and May (overripe fruit), were collected from a farm located in Kochi Prefecture. Ripe Hyuganatsu fruits (*Citrus tamurana* Hort. ex Tanaka), which had been harvested in May, were obtained from the Kochi Prefectural Fruit Tree Experimental Station, Kochi, Japan. All samples were grown in open fields.

Authentic chemicals for co-injection during gas chromatography and mass spectrometry were obtained from the following commercial sources: Tokyo Kasei Kogyo Co. (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), Sigma Chemical Co. (St. Louis, MO, U.S.A.), and Extrasynthèse S. A. (Genay, France). Some chemicals were provided by Ogawa & Co. (Tokyo, Japan).

**Preparation of the cold-pressed oil (CPO) samples.** Highest quality natural essential oil samples from ki-mikan and Hyuganatsu were prepared by means of the cold-pressing method described by Sawamura and Kuriyama.<sup>11)</sup> The fruits were sliced, and the mesocarp and albedo layers were peeled from the flavedo. The peel oil was extracted by hand-pressing

the flavedo, and 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, each resulting CPO being stored at -25°C until needed for analysis.

**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., 0.25 μm film thickness; J & W Scientific, Folsom, CA, U.S.A.) and a flame ionization detector (FID) were 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, and the injector and detector temperatures were 250°C. Nitrogen was used as the carrier gas at a flow rate of 2 ml/min. An oil sample of 1 μl was injected, the split ratio of the injector being 1:50. The retention index (RI) was calculated for each volatile component by using a homologous series of *n*-alkanes (C<sub>7</sub>-C<sub>29</sub>) under the same GC conditions. A non-polar column was also used for GC analysis, this being a DB-5 fused silica type (30 m × 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific, Folsom, CA, U.S.A.). Each peak area percentage (peak area %) shown is the mean value from three injections.

**Gas chromatography/mass spectrometry (GC/MS).** Gas chromatography combined with mass spectrometry (GC/MS) was used for identifying the volatile flavor components that had been detected. The analysis was carried out with a Shimadzu GC-17A linked with a Shimadzu QP-5000 at an 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 used for the GC-14A instrument. An oil sample of 0.2 μl was injected at a split ratio of 1:34, the carrier gas being helium at a constant flow of 1.0 ml/min.

**Identification of the components.** Each component was identified by comparing its GC retention index (RI) from the DB-Wax column, which had been determined relative to the retention time of a homologous series of *n*-alkanes with linear interpolation, with that of the authentic compound. The constituents were also identified by comparing their RI values with those of other essential oils which had been identified earlier and by comparing with the fragmentation pattern of the mass spectrum of each authentic compound. These data were confirmed by matching the mass spectra with those of reference compounds with a Compaq-ProLinea system (Compaq Co., U.S.A.; class 5 K software) connected to the QP-5000 mass spectrometer. The volatile flavor components were also matched by gas-co-

chromatography with authentic compounds.

## Results and Discussion

The volatile flavor components of ki-mikan and Hyuganatsu which had been grown in open fields are shown in Table 1, together with their approximate concentrations (peak area %). The components are listed in order of their elution from the DB-Wax column. The classification based on functional groups is summarized in Table 2.

### *Volatile flavor components of ripe ki-mikan*

Ninety-seven volatile flavor components, constituting 99.29% of the total volatile composition of the oil, including 32 hydrocarbons (97.38% peak area), 11 aldehydes (0.44%), 27 alcohols (1.07%), 7 ketones (0.08%), 11 esters (0.28%), 7 oxides (0.03%) and 2 acids (0.01%), were identified in the CPO of ki-mikan harvested in February. Among these compounds, limonene (82.44%), a monoterpene hydrocarbon possessing little odor, was the most abundant compound, followed by  $\gamma$ -terpinene (8.83%), *trans*- $\beta$ -farnesene (1.76%) and myrcene (1.54%). Limonene is the major terpene in most citrus oils, often accounting for nearly 90% of the oil. The common characteristic of the essential oils of ki-mikan screened in this study was their high content of monoterpene hydrocarbons (94.72%). *trans*- $\beta$ -Farnesene (1.76%), which has a mildly sweet flavor, was the most abundant sesquiterpene hydrocarbon, its content in ki-mikan being higher than that in other citrus fruits such as yuzu (0.45%), lemon (0.02%), sudachi (0.65%) and mochiyuzu (0.39%).<sup>13-14</sup> The principal aldehyde was citronellal (0.20%) having a powerful green-citrusy odor. Monoterpene alcohols were the most abundant oxygenated compounds in ki-mikan, the major alcohols, arranged in decreasing order of quantity, being linalol (0.40%), *cis*-nerolidol (0.14%),  $\alpha$ -terpineol (0.11%) and citronellol (0.07%). While ketones are almost absent from citrus oil,<sup>13-15</sup> the total ketone content (0.08%) of CPO from ripe ki-mikan was higher than that of other citrus oils such as naoshichi, Tahiti lime, mochiyuzu, kabosu, daidai and yuko, while it was lower than that of sudachi.<sup>13-15</sup> The total ester content (0.28%) of ripe ki-mikan oil was higher than that of yuzu, kabosu, yuko, naoshichi, sudachi and mochiyuzu, while it was lower than that of Tahiti lime and daidai.<sup>13-15,19</sup> Oxides (0.03%) and acids (0.01%) were present in small quantities, seven kinds of oxides and 2 kinds of acids being found in ripe ki-mikan.

### *Volatile flavor components of overripe ki-mikan*

Ninety-four volatile flavor components, constituting 97.66% of the total volatile composition of the oil, including 31 hydrocarbons (94.28% peak area), 10 aldehydes (0.45%), 25 alcohols (2.07%), 6 ketones

(0.16%), 12 esters (0.64%), 8 oxides (0.05%) and 2 acids (0.01%), were identified in CPO of ki-mikan harvested in May. The principal components were limonene (73.10%),  $\gamma$ -terpinene (13.74%), *trans*- $\beta$ -farnesene (3.12%) and myrcene (1.13%). The ki-mikan oil samples at different stages of ripeness had almost the same composition, differing only quantitatively. The composition of overripe ki-mikan oil was characterized by higher amounts of aliphatic and sesquiterpene hydrocarbons, monoterpene and sesquiterpene alcohols, ketones and esters than that of ripe ki-mikan oil. However, ripe ki-mikan oil had a higher monoterpene hydrocarbon content. In contrast to the ripe ki-mikan oil, overripe ki-mikan oil had higher  $\gamma$ -terpinene, terpinolene, linalol, *trans*- $\beta$ -farnesene,  $\alpha$ -terpineol and *cis*-nerolidol contents. However, the situation was reversed with regard to the  $\alpha$ -phellandrene, limonene and valencene contents.

### *Volatile flavor components of Hyuganatsu*

One hundred and two components, including 33 hydrocarbons (96.78% peak area), 12 aldehydes (0.31%), 25 alcohols (1.18%), 7 ketones (0.38%), 14 esters (0.23%), 8 oxides (0.03%) and 3 acids (0.01%), were confirmed in CPO of Hyuganatsu fruits. As shown in Tables 1 and 2, monoterpene hydrocarbons, especially limonene (84.78%), were predominant in Hyuganatsu oil. *trans*- $\beta$ -Farnesene (0.58%) was the principal sesquiterpene hydrocarbon, although its content was much higher in the ki-mikan oil samples (ripe fruit, 1.76%; overripe fruit, 3.13%). The content of ketones in Hyuganatsu oil was higher than that of ki-mikan, while the number of ketones in Hyuganatsu oil was higher than that in the oil of other citrus fruit.<sup>13-15</sup> The content of *l*-carvone (0.34%), the most common diunsaturated monoterpene ketone, was higher in Hyuganatsu oil than in ki-mikan and other citrus fruits.<sup>13,19</sup> Isopiperitone, a rarely reported component in *Citrus* oil, was detected in the CPO of Hyuganatsu at a level of less than 0.01%.

Fourteen ester components (0.23%) were confirmed in Hyuganatsu oil. Esters are usually almost absent from *Citrus* oil, but various ester components were found in this study. The total ester level in Hyuganatsu oil was higher than that found in other citrus samples.<sup>13-15,19</sup> Kadota and Nakamura<sup>20</sup> have reported the essential oil composition of Hyuganatsu by the steam distillation method under reduced pressure. They identified 16 acids and 8 terpenes such as  $\alpha$ - and  $\beta$ -pinenes, myrcene,  $\alpha$ -phellandrene,  $\alpha$ - and  $\gamma$ -terpinenes, *p*-cymene, and *d*-limonene.

Citrus oil is characterized by having a high percentage of terpenoid hydrocarbons and a relatively low content of terpenoid oxygenated compounds; these two groups are mainly responsible for the fruit's aromatic profile. The oxygenated terpenes, often ac-

**Table 1.** Volatile Flavor Components Identified in the Cold-pressed Oil Samples of Ki-mikan and Hyuganatsu

No.	Component	Retention index		Peak area % in DB-Wax column			Identification	References
		DB-Wax	DB-5	Ripe ki-mikan	Overripe ki-mikan	Hyuganatsu		
1	ethyl acetate	904		tr <sup>a</sup>	tr	tr	RI <sup>c</sup>	13
2	$\alpha$ -pinene	1039	933	0.79	0.70	1.04	RI, MS, <sup>d</sup> Co-GC <sup>e</sup>	13–15, 19, 28
3	$\alpha$ -fenchene	1076		tr	tr	tr	RI, MS	21–23, 26
4	camphene	1085	953	tr	tr	tr	RI, MS, Co-GC	13–15, 19
5	undecane	1110		tr	tr	tr	RI, MS, Co-GC	15
6	$\beta$ -pinene	1126	981	0.44	0.48	0.50	RI, MS, Co-GC	13–15, 19
7	(+)-sabinene	1135	973	0.11	0.10	0.14	RI, MS, Co-GC	13–15, 19
8	$\delta$ -3-carene	1164	1011	—	—	0.31	RI, MS, Co-GC	13–15
9	myrcene	1170	991	1.54	1.13	1.84	RI, MS, Co-GC	13–15, 19, 27
10	$\alpha$ -phellandrene	1179	1006	0.01	0.02	0.08	RI, MS, Co-GC	13–15, 19, 27
11	$\alpha$ -terpinene	1195	1017	0.12	0.14	0.11	RI, MS, Co-GC	13–15, 19
12	limonene	1231	1039	82.44	73.10	84.78	RI, MS, Co-GC	13–15, 19
13	$\beta$ -phellandrene	1233		nq <sup>b</sup>	0.07	0.24	RI, MS	13–15, 19
14	<i>cis</i> - $\beta$ -ocimene	1243	1043	0.01	0.02	0.02	RI, MS, Co-GC	13–15, 19
15	$\gamma$ -terpinene	1263	1059	8.83	13.74	6.48	RI, MS, Co-GC	13–15, 19, 28
16	<i>p</i> -cymene	1281	1027	0.05	0.06	0.03	RI, MS, Co-GC	13–15, 19, 27
17	2-methylbutyl butyrate	1287		—	—	0.01	RI	26
18	terpinolene	1293	1084	0.38	0.71	0.33	RI, MS, Co-GC	13–15, 19, 27
19	octanal	1296	1002	0.02	—	—	RI, MS, Co-GC	13–15, 19
20	tridecane	1311	1291	0.01	0.01	0.01	RI, Co-GC	15, 26
21	6-methyl-hept-5-en-2-one	1361		tr	—	—	RI	
22	tetradecane	1400	1116	0.01	0.03	tr	RI, MS, Co-GC	13, 15, 19
23	tetradec-1-ene	1428		tr	—	—	RI	13
24	$\alpha$ -thujone	1433		tr	0.01	tr	RI, Co-GC	
25	$\beta$ -thujone	1449		tr	tr	tr	RI, Co-GC	13
26	<i>cis</i> -linalol furanoxide	1454	1070	tr	tr	tr	RI, Co-GC	14
27	(+)- <i>cis</i> -limonene oxide	1458	1138	tr	0.01	0.01	RI, MS, Co-GC	13, 14, 19
28	(-)- $\alpha$ -cubebene	1466	1345	tr	—	—	RI, MS, Co-GC	14, 15, 19
29	(+)- <i>trans</i> -limonene oxide	1470	1139	0.01	tr	tr	RI, MS, Co-GC	13, 14, 19
30	menthone	1476	1247	—	0.03	0.02	RI, Co-GC	13
31	<i>trans</i> -linalol furanoxide	1482	1172	0.01	0.02	tr	RI, Co-GC	13, 14
32	citronellal	1486	1161	0.20	0.26	0.22	RI, MS, Co-GC	13–15, 19
33	$\alpha$ -ylangene	1494		0.01	0.01	0.01	RI	25
34	(-)- $\alpha$ -copaene	1500	1376	0.05	tr	tr	RI, MS, Co-GC	13–15, 19
35	pentadecane	1504		0.07	0.22	0.05	RI, Co-GC	13, 15, 19
36	decanal	1509	1229	0.01	tr	tr	RI, MS, Co-GC	13–15, 19
37	<i>d</i> -camphor	1529	1149	tr	—	tr	RI, Co-GC	13, 19
38	borneol	1548	1283	0.04	tr	—	RI, MS, Co-GC	13–15, 19
39	$\beta$ -cubebene	1552	1018	tr	0.07	0.02	RI, MS, Co-GC	13–15, 19
40	linalol	1557	1098	0.40	1.05	0.80	RI, MS, Co-GC	13–15, 19, 28
41	octanol	1562	1072	0.05	0.04	0.04	RI, MS, Co-GC	13, 15, 19
42	linalyl acetate	1570	1261	0.01	0.06	0.02	RI, MS, Co-GC	13–15, 19
43	(-)- $\alpha$ -cedrene	1574		—	tr	0.01	RI, Co-GC	
44	nonyl acetate	1581	1302	tr	tr	0.01	RI, Co-GC	13, 15, 10
45	bornyl acetate	1592	1279	tr	—	0.07	RI, Co-GC	13–15, 19
46	$\beta$ -elemene	1596	1393	0.08	0.10	0.01	RI, MS, Co-GC	13–15, 19
47	$\beta$ -caryophyllene	1606	1428	0.03	0.04	0.07	RI, MS, Co-GC	13–15, 19
48	terpinen-4-ol	1613	1178	0.02	0.06	0.01	RI, MS, Co-GC	13, 15, 19, 28
49	citronellyl formate	1628	1275	0.01	0.01	0.01	RI, MS, Co-GC	
50	$\gamma$ -elemene	1633		—	—	tr	RI	14, 15, 19
51	<i>trans</i> - <i>p</i> -mentha-2,8-diene-1-ol	1641		tr	—	—	RI	15, 19
52	<i>trans</i> -2-decenal	1648	1253	—	—	tr	RI, MS, Co-GC	19
53	<i>l</i> -menthol	1652		tr	0.01	tr	RI, Co-GC	
54	<i>cis</i> - $\beta$ -farnesene	1657		tr	—	—	RI, Co-GC	14, 15
55	citronellyl acetate	1668	1357	0.09	0.20	0.01	RI, MS, Co-GC	13, 15, 19
56	<i>trans</i> - $\beta$ -farnesene	1673	1452	1.76	3.12	0.58	RI, MS, Co-GC	14, 15, 19
57	$\alpha$ -humulene	1678	1444	0.04	0.07	0.02	RI, MS, Co-GC	13–15, 19
58	$\delta$ -muurolene	1683		tr	0.01	tr	RI	13, 19
59	decyl acetate	1690	1408	0.01	0.02	0.01	RI, MS, Co-GC	13, 15
60	neral	1697	1235	0.08	0.03	tr	RI, MS, Co-GC	13, 14, 19

to be continued

Table 1. Continued

No.	Component	Retention index		Peak area % in DB-Wax column			Identification	References
		DB-Wax	DB-5	Ripe ki-mikan	Overripe ki-mikan	Hyuganatsu		
61	terpinyl acetate	1701	1340	—	0.02	0.01	RI, Co-GC	13
62	$\alpha$ -terpineol	1708	1185	0.11	0.28	0.10	RI, MS, Co-GC	13–15, 19
63	dodecanal	1718	1401	0.07	0.06	0.01	RI, MS, Co-GC	13–15, 19
64	germacrene-D	1724	1476	—	0.10	0.07	RI, MS	13–15, 19
65	valencene	1728	1490	0.33	0.02	tr	RI, Co-GC	13
66	neryl acetate	1734	1367	0.09	0.19	0.03	RI, MS, Co-GC	13–15
67	<i>l</i> -carvone	1744	1245	0.07	0.08	0.34	RI, MS, Co-GC	
68	<i>cis</i> -linalol pyranoxide	1755		tr	tr	tr	RI, Co-GC	
69	<i>trans</i> -2-undecenal	1761	1371	—	0.01	0.02	RI, Co-GC	
70	geranyl acetate	1766	1383	0.06	0.12	0.04	RI, MS, Co-GC	13, 15, 19
71	citronellol	1772	1435	0.07	0.17	0.02	RI, MS, Co-GC	13, 14, 19
72	sesquiphellandrene	1777	1149	0.23	0.20	0.03	RI, MS	13–15, 19
73	perillaldehyde	1793	1271	0.01	0.02	0.01	RI, MS, Co-GC	14, 15
74	octadecane	1809		0.02	tr	tr	RI, Co-GC	13
75	carvone oxide	1817		tr	0.01	tr	RI	14
76	tridecanal	1822	1503	tr	—	tr	RI, MS, Co-GC	14, 15
77	geranyl propionate	1830	1471	—	0.01	tr	RI, Co-GC	13, 19
78	<i>p</i> -mentha-1-en-9-yl acetate	1832		tr	—	—	RI	13
79	isopiperitone	1838		tr	0.03	tr	RI	15
80	nerol	1851	1230	tr	tr	0.03	RI, MS, Co-GC	13–15, 19
81	<i>trans</i> -2-dodecanal	1867	1462	0.02	0.04	0.02	RI, Co-GC	
82	<i>trans</i> -carveol	1877	1211	—	tr	0.01	RI, MS, Co-GC	14, 19
83	tetradecanal	1929	1613	—	tr	tr	RI, Co-GC	13–15, 19
84	<i>p</i> -mentha-1-en-9-ol	1948		tr	0.01	tr	RI, Co-GC	
85	$\beta$ -ionone	1953	1486	0.01	0.01	tr	RI, Co-GC	
86	tetradecenal	1964		tr	—	tr	RI	19
87	caryophyllene oxide	2001	1573	tr	0.01	tr	RI, Co-GC	28
88	<i>cis</i> -nerolidol	2008	1565	0.14	0.26	0.03	RI, MS, Co-GC	14, 15
89	<i>trans</i> -dodec-2-enol	2036		tr	0.01	tr	RI	
90	<i>trans</i> -nerolidol	2050	1539	0.02	0.05	0.04	RI, MS, Co-GC	
91	globulol	2062		0.01	0.02	0.05	RI	13, 19
92	octanoic acid	2084	1192	—	—	tr	RI, Co-GC	14
93	elemol	2090	1547	0.01	0.01	tr	RI, Co-GC	19
94	viridiflorol	2108		0.02	tr	—	RI	14, 15, 19
95	cedrol	2112	1586	—	0.03	0.01	RI, Co-GC	13, 14
96	spathulenol	2121		tr	0.01	0.01	RI	14, 19
97	cedrenol	2142	1604	tr	—	—	RI, Co-GC	14, 15, 19
98	cedryl acetate	2150	1758	tr	tr	tr	RI	14, 19
99	eugenol	2172	1351	tr	—	—	RI, Co-GC	13, 19, 28
100	nonanoic acid	2194		0.01	0.01	tr	RI, Co-GC	14
101	$\gamma$ -eudesmol	2210		tr	—	—	RI	15
102	$\alpha$ -cadinol	2219		—	tr	—	RI, MS	15, 19
103	isothymol	2225	1295	tr	—	—	RI, Co-GC	13, 19
104	(–)- $\alpha$ -bisabolol	2229	1689	—	—	tr	RI, Co-GC	13, 14, 19
105	$\beta$ -sinensal	2239	1696	0.01	0.01	tr	RI, MS, Co-GC	24
106	$\beta$ -eudesmol	2246	1654	0.03	0.02	0.01	RI, Co-GC	13, 14, 19
107	isoeugenol	2271	1397	—	tr	tr	RI, Co-GC	13, 14
108	<i>trans</i> , <i>trans</i> - farnesyl acetate	2276	1839	—	0.01	0.02	RI, Co-GC	14, 15, 19
109	cinnamyl alcohol	2303	1312	0.06	0.01	tr	RI, Co-GC	14, 15, 19
110	<i>p</i> -mentha-1,8-dien-10-ol	2314	1288	tr	0.01	tr	RI	13
111	limonene-diol	2325		tr	—	0.01	RI	19
112	<i>cis</i> , <i>trans</i> -farnesol	2352	1735	0.02	0.02	tr	RI	19
113	<i>trans</i> , <i>trans</i> -farnesol	2368	1722	0.04	0.01	0.01	RI, Co-GC	14, 19
114	nerol oxide	2381		—	tr	tr	RI, Co-GC	
115	octadecanal	2390		0.01	0.01	tr	RI	15
116	undecanoic acid	2417	1490	tr	0.01	tr	RI, Co-GC	15
	Total			99.29	97.66	98.92		

<sup>a</sup> Trace, less than 0.005% (peak area percentage)

<sup>b</sup> Not quantified

<sup>c</sup> Identification based on retention index

<sup>d</sup> Identification based on comparison of mass spectra

<sup>e</sup> Identification based on co-injection with authentic compounds

**Table 2.** Constitution of Functional Groups in the Cold-pressed Oil Samples of Ki-mikan and Hyuganatsu

Functional group	Ripe ki-mikan		Overripe ki-mikan		Hyuganatsu	
	Total No.	Peak area %	Total No.	Peak area %	Total No.	Peak area %
Hydrocarbons						
Aliphatics	6	0.12	5	0.27	5	0.06
Monoterpenes	14	94.72	14	90.26	15	95.91
Sesquiterpenes	12	2.54	12	3.75	13	0.81
Aldehydes						
Aliphatics	7	0.13	6	0.13	8	0.07
Terpenes	4	0.31	4	0.32	4	0.24
Alcohols						
Aliphatics	2	0.05	2	0.05	2	0.04
Monoterpenes	14	0.72	12	1.59	13	0.99
Sesquiterpenes	11	0.30	11	0.43	10	0.15
Ketones	7	0.08	6	0.16	7	0.38
Esters	11	0.28	12	0.64	14	0.23
Oxides	7	0.03	8	0.05	8	0.03
Acids	2	0.01	2	0.01	3	0.01
Total	97	92.29	94	97.66	102	98.92

counting for less than 5% of the oil, generally provide the characteristic flavor of different citrus species. Included in this group are the acyclic isoprenoid alcohols nerol, citronellol and farnesol which are present in citrus essential oil together with their corresponding aldehydes. The oxidoreductase system in citrus fruit is capable of mediating the interconversion of these acyclic aldehydes and alcohols.<sup>21)</sup> In the CPO of ki-mikan, the contents of nerol (ripe fruit, trace; overripe fruit, trace) and citronellol (0.07% and 0.17%), and of their corresponding aldehydes, neral (0.08% and 0.03%) and citronellal (0.20% and 0.26%), showed some variation. Cyclic terpenes also undergo structural changes and hydration during maturation, and acyclic monoterpenes can be converted to cyclic monoterpenes during maturation. A typical reaction for the formation of *p*-menthane structural monoterpenes involves the formation of limonene, terpinolene and  $\alpha$ -terpineol from neryl and linalyl pyrophosphates.<sup>22,23)</sup> It is presumed that these conversions would have resulted in the contents of terpinolene (ripe fruit, 0.38%; overripe fruit, 0.71%) and  $\alpha$ -terpineol (0.11% and 0.28%) in overripe ki-mikan being higher than those in ripe ki-mikan.

The volatile flavor components of the ki-mikan and Hyuganatsu oil samples exhibited a high percentage of limonene and were also characterized by the presence of  $\gamma$ -terpinene in appreciable proportions. The qualitative composition of the CPOs of ki-mikan and Hyuganatsu was found to be quite similar, although a marked difference was apparent in the quantitative composition of the oils from these two species. The ki-mikan oil samples at different stages of ripeness had almost the same volatile flavor components, varying only in their proportions. Limonene (ripe fruit, 82.44%; overripe fruit, 73.10%),  $\gamma$ -terpi-

nene (8.83% and 13.74%), *trans*- $\beta$ -farnesene (1.76% and 3.12%) and myrcene (1.54% and 1.13%) were the principal components in the ki-mikan oil samples. Comparing the ripe and overripe ki-mikan oil samples, the major differences were the limonene,  $\gamma$ -terpinene, linalol and *trans*- $\beta$ -farnesene contents. Limonene (84.78%),  $\gamma$ -terpinene (6.48%), myrcene (1.84%) and  $\alpha$ -pinene (1.04%) were the most abundant components in Hyuganatsu oil. The flavor pattern of ripe Hyuganatsu was more similar to that of ripe ki-mikan than of overripe ki-mikan, this being shown by the contents of  $\alpha$ -pinene, myrcene, limonene,  $\gamma$ -terpinene, terpinolene, citronellal, *trans*- $\beta$ -farnesene and citronellol. The CPO of ki-mikan had *trans*- $\beta$ -farnesene as a characteristic component, different from other citrus fruit oil samples. The CPO of Hyuganatsu was characterized by the presence of a higher percentage of *l*-carvone than that present in other citrus fruits.

Carvone has been found in spearmint and peppermint oils. The carvone series of terpenes (*e.g.*, limonene, carvone and dihydrocarvone) and pulegone series of terpenes (*e.g.*, terpinolene, piperitenone, pulegone and menthone) are formed from neryl pyrophosphate. It has been postulated that  $\alpha$ -terpineol or the corresponding carbonium ion may occur as an intermediate in the biosynthetic pathway.<sup>24)</sup> In the ki-mikan fruits, the content of limonene (ripe fruit, 82.44%; overripe fruit, 73.10%), which is a carvone series of terpene, was lower in the overripe fruits, while the pulegone series of terpenes such as terpinolene (ripe fruit, 0.38%; overripe fruit, 0.71%), menthone (0% and 0.03%) and isopiperitone (trace and 0.03%) were higher in the overripe fruits. Therefore, the pathway from neryl pyrophosphate (a monoterpene precursor) seems to pro-

ceed to the pulegone series of terpenes rather than to the carvone series during the maturation of ki-mikan.

The presence of significant amounts of *trans*- $\beta$ -farnesene in ki-mikan and of *l*-carvone in Hyuganatsu further distinguished these respective oil samples from other citrus fruit oils such as yuzu (0.45% and 0%, respectively), lemon (0.02% and trace), mochiyuzu (0.39% and 0%), and Tahiti lime (0.1% and 0%).<sup>13-15,19</sup> The composition of the oil from each of these two species was similar, with only small variation in the content of sesquiterpene hydrocarbons and ketones. Sesquiterpene hydrocarbons were higher in the ki-mikan oil samples than in the Hyuganatsu oils, while ketones showed the opposite predominance. These differences were more evident in respect of the *trans*- $\beta$ -farnesene and *l*-carvone contents, and the ratio of *trans*- $\beta$ -farnesene and *l*-carvone could be used to distinguish ki-mikan oil from Hyuganatsu oil. We investigated in this study the volatile flavor components of ripe and overripe ki-mikan, and of Hyuganatsu, and this data may be of use by the food and perfume industries. An organoleptic comparison of the volatile flavor components from ki-mikan and Hyuganatsu oils will provide more detailed information about the odor-active compounds of these fruits.

### Acknowledgments

We thank Mr. Y. Higuchi at the Kochi Prefectural Fruit Experimental Station for kindly providing the Hyuganatsu fruits. We also thank Mr. Y. Kuniyoshi of Kagami-cho in Kochi for providing the ki-mikan fruits. H. S. Choi is grateful for the post-doctoral fellowship provided by the Korea Science and Engineering Foundation.

### References

- 1) Araujo, P. E., Role of citrus fruit in human nutrition. In "Citrus Science and Technology," Vol. 1, eds. Nagy, S., Shaw, P. E., and Veldhuis, M. K., The AVI Publishing Co., Inc., Westport, Connecticut, USA, p. 1 (1977).
- 2) Shinoda, N., Shiga, M., and Nishimura, K., Constituents of yuzu (*Citrus junos*) oil. *Agric. Biol. Chem.*, **34**, 234-242 (1970).
- 3) Tajima, K., Tanaka, S., Yamaguchi, T., and Fujita, M., Analysis of green and yellow yuzu peel oils (*Citrus junos* Tanaka). Novel aldehyde components with remarkably low odor thresholds. *J. Agric. Food Chem.*, **38**, 1544-1548 (1990).
- 4) Njoroge, S. M., Ukeda, H., and Sawamura, M., Changes in the volatile composition of yuzu (*Citrus junos* Tanaka) cold-pressed oil during storage. *J. Agric. Food Chem.*, **44**, 550-556 (1996).
- 5) Song, H. S., Sawamura, M., Ito, T., and Ukeda, H., Chemical compositions of the volatile part of yuzu (*Citrus junos* Tanaka) peel cold-pressed oils from Japan and Korea. *Flavour Fragr. J.*, **14**, 383-389 (1999).
- 6) Coleman, R. L. and Shaw, P. E., Analysis of Valencia orange essence and aroma oils. *J. Agric. Food Chem.*, **19**, 520-523 (1971).
- 7) Moshonas, M. G. and Shaw, P. E., Quantitative and qualitative analysis of tangerin peel oil. *J. Agric. Food Chem.*, **22**, 282-284 (1974).
- 8) Shaw, P. E. and Coleman, R. L., Quantitative composition of cold-pressed orange oils. *J. Agric. Food Chem.*, **22**, 785-787 (1974).
- 9) Owusu-Yaw, J., Matthews, R. F., and West, P. F., Alcohol deterpenation of orange oil. *J. Food Sci.*, **51**, 1180-1182 (1986).
- 10) Staroscik, J. A. and Wilson, A. A., Seasonal and regional variation in the quantitative composition of cold-pressed lemon oil from California and Arizona. *J. Agric. Food Chem.*, **30**, 835-837 (1982).
- 11) Sawamura, M. and Kuriyama, T., Quantitative determination of volatile constituents in the pummelo (*Citrus grandis* Osbeck forma Tosa-buntan). *J. Agric. Food Chem.*, **36**, 567-569 (1988).
- 12) Sawamura, M., Shichiri, K. I., Ootani, Y., and Zheng, X. H., Volatile constituents of several varieties of pummelos and characteristics among citrus species. *Agric. Biol. Chem.*, **55**, 2571-2578 (1991).
- 13) Njoroge, S. M., Ukeda, H., Kusunose, H., and Sawamura, M., Volatile components of the essential oils from kabosu, daidai, and yuko, Japanese sour *Citrus* fruits. *Flavour Fragr. J.*, **9**, 289-297 (1994).
- 14) Njoroge, S. M., Ukeda, H., Kusunose, H., and Sawamura, M., Japanese sour *Citrus* fruits. Part III. Volatile constituents of sudachi and mochiyuzu oils. *Flavour Fragr. J.*, **10**, 341-347 (1995).
- 15) Njoroge, S. M., Ukeda, H., Kusunose, H., and Sawamura, M., Japanese sour *Citrus* fruits. Part IV. Volatile compounds of naoshichi and Tahiti lime essential oils. *Flavour Fragr. J.*, **11**, 25-29 (1996).
- 16) Mondello, L., Verzera, A., Previti, P., Crispo, F., and Dugo, G., Multidimensional capillary GC-GC for the analysis of complex samples. 5. Enantiomeric distribution of monoterpene hydrocarbons, monoterpene alcohols, and linalyl acetate of bergamot (*Citrus bergamia* Risso et Poiteau) oils. *J. Agric. Food Chem.*, **46**, 4275-4282 (1998).
- 17) Sawamura, M., Poiana, M., Kawamura, A., Itoh, T., Song, H. S., Ukeda, H., and Mincione, B., Volatile components of peel oils of Italian and Japanese lemon and bergamot. *Ital. J. Food Sci.*, **11**, 121-130 (1999).
- 18) MDP Publishing Co. Ltd., "Databook on Composition of Japanese Food," 4th ed., MDP Publishing Co. Ltd., Tokyo, Japan (1984).
- 19) Njoroge, S. M., Ukeda, H., Kusunose, H., and Sawamura, M., Volatile components of Japanese yuzu and lemon oils. *Flavour Fragr. J.*, **9**, 159-166 (1994).
- 20) Kadota, R. and Nakamura, T., Studies on the "Hyuga-natsu" a kind of summer orange, *Citrus Tamurana*. *Nippon Shokuhin Kogyo Gakkaishi*, **18**, 569-573 (1971).
- 21) Eskin, N. A. M., Terpenoids and flavonoids. In "Plant Pigments, Flavors and Textures," Academic Press Inc., London, UK, p. 65 (1981).

- 22) Sawamura, M., Citrus flavor. *Kagaku to Seibutsu*, **32**, 114-119 (1994) (in Japanese).
- 23) Francis, M. J. O., Monoterpene biosynthesis. In "Aspects of Terpenoid Chemistry and Biochemistry," ed. Goodwin, T. W., Proceedings of the Phytochemical Society Symposium, April 1970, Academic Press Inc., London, UK, p. 30 (1971).
- 24) Loomis, W. D., Biosynthesis and metabolism of monoterpenes. In "Terpenoids in Plants," ed. Pridham, J. B., Academic Press Inc., London, UK, p. 59 (1967).