Composition of the Essential Oil of *Citrus tamurana* Hort. ex Tanaka (Hyuganatsu)

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The composition of the essential oil of *Citrus tamurana* Hort. ex Tanaka (Hyuganatsu), isolated by the cold-pressing method, was investigated by capillary GC and GC−MS. The effects of harvesting time, degree of freshness, and size of fruits on the composition of Hyuganatsu peel oils were also determined. A total of 126 volatile constituents were confirmed in the Hyuganatsu oils. The Hyuganatsu oils contained hydrocarbons (95.95−96.95%), aldehydes (0.33−0.62%), alcohols (1.91%-2.64%), ketones (0.40−0.62%), esters (0.28−0.39%), oxides (0.04−0.06%), acids (0.01%), and trace amounts of fugenol methyl ether. Monoterpene hydrocarbons were predominant. Limonene (80.35−82.39%), γ-terpinene (7.71−9.03%), myrcene (2.11−2.28%), linalol (1.37−2.01%), and α-pinene (1.17−1.43%) were the most abundant components in Hyuganatsu oils. The principal sesquiterpene hydrocarbon was trans-β-farnesene (0.60−1.04%), and its content in Hyuganatsu oils was higher than in oils of other citrus fruits. The number of ketones and the content of *l*-carvone in Hyuganatsu oils were higher than in other citrus oils.

Keywords: *Citrus tamurana Hort. ex Tanaka (Hyuganatsu); cold-pressed oil; essential oil composition; gas chromatography/mass spectrometry*

INTRODUCTION

Essential oils from citrus peel are natural flavoring materials of commercial importance. Worldwide demand for essential oils, especially citrus oils, has increased during the past few years. Many species of *Citrus* fruits are cultivated in Japan and utilized as raw materials, processed foods, and sources of essential oils. Among them, *Citrus tamurana* Hort. ex Tanaka (Hyuganatsu), locally named "Konatsu", is mainly cultivated on Shikoku island, particularly in Kochi Prefecture. Hyuganatsu has been regarded as a citrus fruit with potential commercial value in Japan because of its attractive and pleasant flavor. According to Swingle (1943), it belongs to the species *C. sinensis*, along with Valencia and Tarocco oranges. Hyuganatsu originated in Miyazaki Prefecture in Japan at the beginning of the 19th century and has been cultivated in Miyazaki, Fukuoka, Kochi, and Shizuoka Prefectures (Iwamasa, 1976). The average weight of this fruit is 160−200 g. The flesh of Hyuganatsu is juicy and has a sweet and sour taste, and its peel is smooth and thin. In general, the albedo of Hyuganatsu is eaten together with the flesh. This fruit is harvested from March to May in Japan, so it is one of the predominant citrus crops in Japan from spring to early summer. In recent years this new citrus fruit has increased in value, especially in the countries where it is grown. Hyuganatsu is a desirable product because of its distinct aroma and taste impression; furthermore, it is rich in vitamins B_1 and C (MDP Publishing Co., Ltd., 1984). The commercial value of essential oils from plants depends on a number of factors including variety, size, peel thickness, degree of maturity, storage period, and postharvest treatment (Heath, 1986). A large number of reports have been published on the constituents of citrus essential oils (Moshonas and Shaw, 1971, 1983; Lund et al., 1981; Berry and Tatum, 1986; Sawamura et al., 1989; Charara et al., 1992; Mondello et al., 1998; Verzera et al., 1998; Simpkins et al., 1999). However, there have been few studies on Hyuganatsu oil (Kadota and Nakamura, 1971). In view of the commercial value and wide applications of Hyuganatsu, flavor researchers (and also 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 of chemical composition of the essential oil of Hyuganatsu for its use in foods, beverages, and other products. In this paper, we present both qualitative and quantitative analyses of the volatile constituents of cold-pressed Hyuganatsu oils, with varying harvesting times, degrees of freshness, and sizes of fruit.

MATERIALS AND METHODS

Materials. *Citrus tamurana* Hort. ex Tanaka (Hyuganatsu) cultivated in greenhouses at the Kochi Prefectural Fruit Tree Experimental Station, Kochi, Japan, was sampled in 3 groups (GI, GII, and GIII). Each group was further divided into 2 subgroups based on size of fruits: subgroup L (fruit weight: 160−200 g) and subgroup S (fruit weight: 90−130 g). The fruits of group I (GI-L and GI-S) were harvested in the middle of March and cold-pressed within 24 h of harvest. Three weeks later, the fruits of group II (GII-L and GII-S) were harvested and cold-pressed within 24 h of harvest. The fruits of group III (GIII-L and GIII-S) were harvested at the same time as group II, but they were cold-pressed after being stored at 5 °C for 3 weeks. All of the essential oils were injected in GC within 48 h of extraction.

Authentic chemicals were obtained from reliable commercial sources (Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), Wako

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Pure Chemical Industries (Osaka, Japan), Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or Extrasynthèse S. A. (Genay, France)), or they were provided by Ogawa & Co., Ltd. (Tokyo, Japan).

Cold-Pressed Oil (CPO) Preparation. The essential oils of Hyuganatsu were prepared according to the cold-pressing method described by Sawamura and Kuriyama (1988). 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 the peel oils were collected in 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 oils were stored at −25 °C until analyzed.

Gas Chromatography (GC). A Shimadzu GC 14-A gas chromatograph (GC) 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. 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 the program rate of 2 °C/min. The injector and detector temperatures were 250 °C. Nitrogen was the carrier gas at a flow rate of 2 mL/min. The closer retention time between one compound and a standard on a chromatogram is the more accurate for quantification. Thus, the two internal standards method ought to be more precise than the one internal standard method. 1-Heptanol was employed as one internal standard for quantitative analysis of cold-pressed oil, up to linalol, and methyl myristate as the other standard was used for the peaks after linalol. The ratio of cold-pressed oil for the two internal standards was 150:1:1. The weight percent of each peak was calculated according to the correlation factor to the flame ionization detector (Zheng, 1997). An oil sample of 1 μL was injected and the injector split ratio was 1:50. GC analyses were performed in triplicate.

Gas Chromatography−Mass Spectrometry (GC−MS). Gas chromatography combined with mass spectrometry (GC−MS) was used for identification of the 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 analysis. 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.

Identification of Components. Components were identified by means of comparison of their GC retention indices (RI) on a DB-Wax column, determined relative to the retention time of a homologous series of *n*-alkanes (C_7−C_{29}) with linear interpolation with those of authentic compounds. The constituents were also identified by comparison of their RI with those of other essential oils which had been identified earlier and by comparison with the fragmentation pattern of the mass spectra of authentic compounds being available in our laboratories. These measurements were confirmed 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 the QP-5000 mass spectrometer. Whenever possible, the constituents were matched by co-gas chromatography with authentic compounds.

RESULTS AND DISCUSSION

The identified components and their weight percents are given in Table 1. The data are mean values of triplicate analyses. 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.

Hydrocarbons. Monoterpene hydrocarbons were predominant in all samples and the contents in GI-L were higher than in the other samples. Monoterpene hydrocarbons accounted for 94.80−95.97% of the Hyuganatsu oils; limonene, γ-terpinene, myrcene, α-pinene, and β-pinene were the major components. Other monoterpene hydrocarbons occurred only in small amounts (less than 0.5%). The content of limonene in the Hyuganatsu oils was similar to that in the oils of Tosabuntan fruit (*C. grandis* Osbeck forma *Tosa*; pummelo) (Sawamura and Kuriyama, 1988). However, its content was higher than that of yuzu (*C. junos* Sieb. ex Tanaka; 78.13%), lemon (*C. limon* Burm. f. cv. *Lisbon*; 64.62%), kabosu (*C. sphaerocarpa* Tanaka; 75.48%), yuko (*C. yuko* Hort. ex Tanaka; 66.60%), sudachi (*C. sudachi* Hort. ex Shirai; 69.05%), mochiyuzu (*C. inflata* Hort. ex Tanaka; 77.17%), and Tahiti lime (*C. latifolia* Tanaka; 52.2%), and lower than that of daidai (*C. aurantium* Linn. var. Cyathifera Y. Tanaka; 94.68%) and naoshichi (*C. tagumasudachi* Hort. ex Tanaka; 90.5%) (Njoroge et al., 1994a,b, 1995, 1996). γ-Terpinene was the second major component, accounting for 7.71−9.03%. It is well-known that naturally occurring acyclic monoterpenes such as myrcene and cis-β-ocimene have pleasant sweet-refreshing odors. Their contents were 2.11−2.28% and 0.02−0.08%, respectively. Among the monoterpene hydrocarbons, six dienes, including limonene, α- and β-phellandrenes, α- and γ-terpinenes, and terpinolene, have the *p*-menthane skeleton which represents the most stable monoterpene structure. These 6 components were also identified in this study. The contents of α-pinene, δ-3-carene, and α-phellandrene were comparatively low in GII-S. The content of β-phellandrene was higher in the oils obtained from stored Hyuganatsu (group III) than in fresh Hyuganatsu oils (groups I and II). The sesquiterpene hydrocarbons, including trans-β-farnesene (0.60−1.04%) as the main component, accounted for 0.72−1.29%. The content of trans-β-farnesene, which has a mild sweet flavor, was higher than that found in other citrus fruits such as yuzu, lemon, sudachi, and lime (Njoroge et al., 1994b, 1995, 1996). The content of germacrene-D was higher in the oil from the stored Hyuganatsu than in oil from the fresh fruit. Except for trans-β-farnesene in all groups, no other sesquiterpene hydrocarbon surpassed 0.1% in Hyuganatsu oils.

Aldehydes. The main aldehydes were citronellal (0.25−0.27%), dodecanal (0.02−0.15%), and octanal (0−0.11%). Citronellal has a powerful green-citrusy odor rather than a sweet and fruity odor (Arctander, 1969). Sinensal is a farnesane-type sesquiterpenoid. It has alpha and beta isomers which are present in tangerine and orange oils (Moshonas and Shaw, 1974), but only the beta isomer was identified in Hyuganatsu oils, at a level less than 0.01%. The apha isomer has been reported as an important aroma component of Valencia orange oil (Wolford et al., 1971) and the beta isomer has been identified in naoshichi oil (Njoroge et al., 1996).

Alcohols. Monoterpene alcohols were the most abundant oxygenated compounds in all groups, and linalol was the most predominant component (1.37−2.01%). The weight percent of linalol was slightly lower in subgroup L (GI-L; 1.37%, GII-L; 1.54%) than in subgroup S (GI-S; 1.64%, GII-S; 2.01%) in the case of fresh Hyuganatsu (group I and II). The contents of three naturally occurring acyclic alcohols, linalol, geraniol, and nerol, were 1.40% (GI-L), 1.70% (GI-S), 1.59% (GII-L), 2.05% (GII-S), 1.75% (GIII-L), and 1.52% (GIII-S). No significant differences were observed in the content of nerol between group I and II, but the cold-pressed oil of GIII-L was characterized by a high level of nerol. The content of α-terpineol was high in stored Hyuganatsu.

Table 1. Composition of the Essential Oils of *Citrus tamurana* Hort. ex Tanaka

			% (w/w)[a,b]							
			group I		group II		group III			
no.	component	retention index (DB-Wax)	L	S	L	S	L	S	identification[c,d,e]	references[f]
1	ethyl acetate	904	tr	tr	tr	tr	tr	tr	RI	8
2	α-pinene	1039	1.33	1.43	1.24	1.17	1.33	1.22	RI, MS, Co-GC	7−10
3	α-fenchene	1076	tr	tr	tr	tr	tr	tr	RI, MS	1−3, 6
4	camphene	1085	0.01	0.01	0.01	0.01	0.01	0.01	RI, MS, Co-GC	7−10
5	undecane	1110	tr	tr	tr	tr	tr	tr	RI, MS, Co-GC	10
6	*β*-pinene	1126	0.77	0.89	0.80	0.85	0.90	0.63	RI, MS, Co-GC	7−10
7	(+)-sabinene	1135	0.19	0.21	0.19	0.18	0.20	0.17	RI, MS, Co-GC	7−10
8	*δ*−3-carene	1164	0.55	0.41	0.47	0.28	0.36	0.37	RI, MS, Co-GC	8−10
9	myrcene	1170	2.28	2.20	2.23	2.13	2.11	2.26	RI, MS, Co-GC	7−10
10	α-phellandrene	1179	0.11	0.10	0.09	0.05	0.09	0.10	RI, MS, Co-GC	7−10
11	α-terpinene	1195	0.16	0.17	0.16	0.17	0.18	0.16	RI, MS, Co-GC	7−10
12	limonene	1231	82.39	81.01	81.28	80.63	80.35	82.17	RI, MS, Co-GC	7−10
13	*β*-phellandrene	1233	nq	nq	nq	nq	0.25	0.25	RI, MS	7−10
14	cis-*β*-ocimene	1243	0.02	0.03	0.02	0.03	0.02	0.08	RI, MS, Co-GC	7−10
15	*γ*-terpinene	1263	7.71	8.31	8.00	9.03	8.58	8.01	RI, MS, Co-GC	7−10
16	*p*-cymene	1281	0.05	0.05	0.05	0.04	0.04	0.04	RI, MS, Co-GC	7−10
17	2-methylbutyl butyrate	1287	0.01	0.01	0.01	0.01	0.01	0.01	RI	6
18	terpinolene	1293	0.39	0.37	0.38	0.40	0.39	0.39	RI, MS, Co-GC	7−10
19	octanal	1296	0.11	0.11	0.08	0.10	0.02	-	RI, MS, Co-GC	7−10
20	tridecane	1311	tr	tr	tr	tr	tr	0.01	RI, Co-GC	6, 10
21	6-methyl-hept-5-en-2-one	1361	tr	-	tr	tr	-	-	RI	
22	heptyl acetate	1392	-	tr	-	-	-	-	RI, Co-GC	7
23	tetradecane	1400	0.01	0.01	0.01	0.01	0.01	tr	RI, MS, Co-GC	7, 8, 10
24	tetradec-1-ene	1428	tr	tr	tr	tr	tr	-	RI	8
25	α-thujone	1433	tr	tr	tr	tr	tr	tr	RI, Co-GC	
26	*β*-thujone	1449	tr	tr	tr	tr	tr	tr	RI, Co-GC	8
27	*cis*-linalol furanoxide	1454	tr	tr	tr	tr	tr	tr	RI, Co-GC	9
28	(+)-*cis*-limonene oxide	1458	0.01	0.01	0.01	0.01	0.01	0.01	RI, MS, Co-GC	7−9
29	(−)-α-cubebene	1466	tr	tr	tr	tr	-	-	RI, MS, Co-GC	7, 9, 10
30	(+)-*trans*-limonene oxide	1470	tr	tr	tr	tr	tr	tr	RI, MS, Co-GC	7−9
31	menthone	1476	0.03	0.03	0.03	0.03	0.03	0.03	RI, Co-GC	8
32	*trans*-linalol furanoxide	1482	tr	tr	tr	tr	tr	tr	RI, Co-GC	8, 9
33	citronellal	1486	0.27	0.26	0.26	0.27	0.25	0.26	RI, MS, Co-GC	7−10
34	α-ylangene	1494	0.01	tr	0.01	0.01	tr	0.02	RI	5
35	(−)-α-copaene	1500	0.03	0.03	0.03	0.03	tr	tr	RI, MS, Co-GC	7−10
36	pentadecane	1504	0.04	0.04	0.04	0.05	0.06	0.05	RI, Co-GC	7, 8, 10
37	decanal	1509	0.01	0.01	0.01	0.01	0.01	tr	RI, MS, Co-GC	7−10
38	*d*-camphor	1529	tr	0.01	tr	tr	tr	tr	RI, Co-GC	7, 8
39	borneol	1548	0.02	0.02	0.02	0.02	tr	tr	RI, MS, Co-GC	7−10
40	*β*-cubebene	1552	tr	tr	tr	tr	0.02	0.02	RI, MS, Co-GC	7−10
41	linalol	1557	1.37	1.64	1.54	2.01	1.69	1.49	RI, MS, Co-GC	7−10
42	octanol	1562	0.06	0.08	0.09	0.07	0.07	0.05	RI, MS, Co-GC	7, 8, 10
43	linalyl acetate	1570	0.01	0.01	0.01	0.01	0.03	0.02	RI, MS, Co-GC	7−10
44	(−)-α-cedrene	1574	0	tr	tr	tr	0.01	0.01	RI, Co-GC	
45	nonyl acetate	1581	tr	0.01	0.01	0.01	0.01	0.01	RI, Co-GC	7, 8, 10
46	bornyl acetate	1592	0.05	0.07	0.08	0.07	0.10	0.07	RI, Co-GC	7−10
47	*β*-elemene	1596	0.01	0.01	0.01	tr	0.01	tr	RI, MS, Co-GC	7−10
48	*β*-caryophyllene	1606	0.04	0.05	0.05	0.04	0.05	0.07	RI, MS, Co-GC	7−10
49	terpinen-4-ol	1613	0.01	0.02	0.02	0.01	0.02	0.01	RI, MS, Co-GC	7, 8, 10
50	myrcenol	1625	tr	tr	tr	tr	0.01	tr	RI, Co-GC	
51	citronellyl formate	1628	0.01	0.01	0.01	0.01	0.01	0.01	RI, MS, Co-GC	
52	*γ*-elemene	1633	tr	-	tr	-	-	-	RI	7, 9, 10
53	*trans*-*p*-mentha-2,8-diene-1-ol	1641	tr	tr	tr	tr	-	tr	RI	7, 10
54	trans−2-decenal	1648	tr	tr	tr	tr	-	tr	RI, MS, Co-GC	7
55	*l*-menthol	1652	tr	tr	tr	tr	tr	tr	RI, Co-GC	
56	*cis*-*β*-farnesene	1657	tr	tr	tr	tr	tr	tr	RI, Co-GC	9, 10
57	citronellyl acetate	1668	0.03	0.04	0.04	0.04	0.02	-	RI, MS, Co-GC	7, 8, 10
58	trans-*β*-farnesene	1673	0.60	0.82	0.93	0.80	1.04	0.77	RI, MS, Co-GC	7, 9, 10
59	α-humulene	1678	0.02	0.02	0.02	0.02	0.02	0.02	RI, MS, Co-GC	7−10
60	*δ*-muurolene	1683	tr	tr	tr	tr	tr	tr	RI	7, 8
61	decyl acetate	1690	0.02	0.02	0.03	0.03	0.01	0.03	RI, MS, Co-GC	8, 10
62	*trans*-piperitol	1691	-	tr	-	-	0.01	0.01	RI	
63	neral	1697	0.01	0.01	0.01	0.01	0.01	tr	RI, MS, Co-GC	7−9
64	terpinyl acetate	1701	0.01	0.01	0.01	tr	0.01	0.01	RI, Co-GC	8
65	α-terpineol	1708	0.09	0.11	0.11	0.09	0.13	0.11	RI, MS, Co-GC	7−10
66	dodecanal	1718	0.11	0.13	0.15	0.11	0.03	0.02	RI, MS, Co-GC	7−10
67	germacrene-D	1724	tr	0.01	0.01	tr	0.07	0.07	RI, MS	7−10
68	valencene	1728	tr	0.01	0.01	tr	tr	tr	RI, Co-GC	8
69	neryl acetate	1734	0.05	0.07	0.07	0.05	0.07	0.06	RI, MS, Co-GC	8−10
70	*l*-carvone	1744	0.38	0.43	0.57	0.41	0.56	0.37	RI, MS, Co-GC	
71	*cis*-linalol pyranoxide	1755	0.01	0.01	0.01	0.01	0.02	0.02	RI, Co-GC	
72	trans−2-undecenal	1761	0.01	0.01	0.01	0.01	0.01	-	RI, Co-GC	
73	geranyl acetate	1766	0.05	0.06	0.07	0.05	0.05	0.05	RI, MS, Co-GC	7, 8, 10

Table 1 (Continued)

no.	component	retention index (DB-Wax)	% (w/w)[a,b] group I L	group I S	group II L	group II S	group III L	group III S	identification[c,d,e]	references[f]
74	citronellol	1772	0.01	0.01	0.01	0.01	0.04	0.03	RI, MS, Co-GC	7−9
75	sesquiphellandrene	1777	0.06	0.07	0.08	0.07	0.06	0.04	RI, MS	7−10
76	cumin aldehyde	1785	tr	-	-	-	-	-	RI, Co-GC	7−9
77	perillaldehyde	1793	0.03	0.03	0.03	0.02	0.03	0.02	RI, MS, Co-GC	9, 10
78	octadecane	1809	tr	0.01	0.01	0.01	0.01	0.01	RI, Co-GC	8
79	methyl laurate	1812	tr	-	tr	tr	-	tr	RI, Co-GC	
80	carvone oxide	1817	tr	tr	tr	tr	tr	tr	RI	9
81	tridecanal	1822	tr	0.01	tr	tr	tr	tr	RI, MS, Co-GC	9, 10
82	geranyl propionate	1830	tr	tr	tr	tr	tr	tr	RI, Co-GC	7, 8
83	*p*-mentha-1-en-9-yl acetate	1832	tr	tr	tr	tr	-	-	RI	8
84	isopiperitone	1838	tr	tr	tr	tr	0.01	0.01	RI	10
85	*cis*-carveol	1845	tr	tr	0.01	tr	-	-	RI, MS, Co-GC	7−9
86	nerol	1851	0.03	0.05	0.05	0.04	0.06	0.03	RI, MS, Co-GC	7−10
87	geraniol	1859	tr	0.01	tr	tr	-	-	RI, MS, Co-GC	7−10
88	trans−2-dodecenal	1867	0.03	0.04	0.04	0.02	0.04	0.03	RI, Co-GC	
89	*trans*-carveol	1877	0.01	0.01	0.01	0.01	tr	tr	RI, MS, Co-GC	7, 9
90	perillyl acetate	1912	tr	tr	tr	tr	-	-	RI, Co-GC	8−10
91	tetradecanal	1929	0.01	0.01	0.01	0.01	0.01	tr	RI, Co-GC	7−10
92	dehydrocarveol	1943	tr	tr	tr	tr	-	-	RI	
93	*p*-mentha-1-en-9-ol	1948	tr	tr	tr	tr	tr	tr	RI, Co-GC	
94	*β*-ionone	1953	tr	0.01	tr	tr	tr	tr	RI, Co-GC	
95	tetradecenal	1964	tr	tr	tr	tr	-	tr	RI	7
96	caryophyllene oxide	2001	0.01	0.01	0.01	0.01	0.01	tr	RI, Co-GC	
97	*cis*-nerolidol	2008	0.03	0.03	0.03	0.02	0.03	0.02	RI, MS, Co-GC	9, 10
98	fugenol methyl ether	2031	tr	tr	tr	tr	-	-	RI, Co-GC	
99	*trans*-dodec-2-enol	2036	tr	0.01	0.01	0.01	0.01	0.01	RI	
100	*trans*-nerolidol	2050	0.05	0.07	0.08	0.07	0.09	0.05	RI, MS, CO-GC	9, 10
101	globulol	2062	0.05	0.06	0.08	0.06	0.07	0.06	RI	7, 8
102	octanoic acid	2084	tr	0.01	tr	tr	tr	tr	RI, Co-GC	9
103	elemol	2090	0.01	0.01	0.01	tr	0.01	0.01	RI, Co-GC	7
104	viridiflorol	2108	tr	tr	tr	tr	tr	-	RI	7, 9, 10
105	cedrol	2112	0.01	0.01	0.01	0.01	0.01	0.01	RI, Co-GC	8, 9
106	spathulenol	2121	0.01	0.02	0.02	0.02	0.02	0.01	RI	7, 9
107	cedrenol	2142	0.01	0.01	0.02	0.01	0.02	0.01	RI, Co-GC	7, 9, 10
108	cedryl acetate	2150	tr	tr	0.01	tr	0.01	tr	RI	7, 9
109	eugenol	2172	tr	tr	tr	tr	-	tr	RI, Co-GC	7, 8
110	nonanoic acid	2194	0.01	0.01	0.01	0.01	0.01	tr	RI, Co-GC	9
111	*γ*-eudesmol	2210	tr	-	tr	tr	-	-	RI	10
112	α-cadinol	2219	tr	tr	tr	tr	0.01	tr	RI, MS	7, 10
113	isothymol	2225	tr	0.01	0.01	0.01	-	-	RI, Co-GC	7, 8
114	(−)-α-bisabolol	2229	tr	tr	-	-	-	-	RI, Co-GC	7−9
115	*β*-sinensal	2239	tr	tr	tr	tr	tr	tr	RI, MS, Co-GC	4
116	*β*-eudesmol	2246	0.01	0.01	0.01	0.01	0.01	0.01	RI, Co-GC	7−9
117	isoeugenol	2271	0.01	0.01	0.02	0.02	0.01	tr	RI, Co-GC	8, 9
118	trans, trans-farnesyl acetate	2276	0.02	0.03	0.04	0.05	0.05	0.02	RI, Co-GC	7, 9, 10
119	cinnamyl alcohol	2303	0.01	0.01	0.01	0.01	tr	tr	RI, Co-GC	7, 9, 10
120	*p*-mentha-1,8-dien-10-ol	2314	tr	tr	tr	tr	tr	0.01	RI	8
121	limonene-diol	2325	0.01	0.01	0.01	0.01	0.02	0.01	RI	7
122	cis, trans-farnesol	2352	0.01	0.01	0.01	0.01	tr	tr	RI	7
123	trans, trans-farnesol	2368	0.02	0.03	0.03	0.04	0.02	0.01	RI, Co-GC	7, 9
124	nerol oxide	2381	0.01	0.01	0.01	0.01	0.01	tr	RI, Co-GC	
125	octadecanal	2390	tr	0.01	0.01	tr	0.01	tr	RI	10
126	undecanoic acid	2417	tr	tr	tr	tr	tr	tr	RI, Co-GC	10

[a] tr, trace, less than 0.005% (weight percent). [b] nq, not quantified. [c] RI, identification based on retention index. [d] Co-GC, identification based on co-injection with authentic compounds. [e] MS, identification based on comparison of mass spectra. [f] References: (1) Bagci et al., 1999; (2, 3) Brophy et al., 1999a,b; (4) Chagonda et al., 1999; (5) Kaya et al., 1999; (6) Ngassoum et al., 1999; (7−10) Njoroge et al., 1994a,b, 1995, 1996.

Ketones. Ketones occurred in low levels (0.40−0.62%), with *l*-carvone being the main component (0.37−0.57%). Nevertheless, the number and the content of ketones in Hyuganatsu oils were higher than in other citrus fruit oils (Njoroge et al., 1994b, 1995, 1996). The content of *l*-carvone in Hyuganatsu oils was higher than that found in other citrus fruits (Njoroge et al., 1994a,b, 1995). Isopiperitone, a rarely reported component in *Citrus* oils, was detected in this study at a level of less than 0.01%. In Hyuganatsu oils, α- and *β*-thujones and *β*-ionone, which have rarely been found in other citrus oils, were detected at low levels.

Esters. The total ester content (0.28−0.39%) of Hyuganatsu oils was higher than that found in other citrus oils such as yuzu, kabosu, yuko, naoshichi, sudachi, and mochiyuzu, while it was lower than that in the oils of Tahiti lime and daidai (Njoroge et al., 1994a,b, 1995, 1996). The content of linalyl acetate was high in stored Hyuganatsu. Neryl acetate (0.05−0.07%), geranyl acetate (0.05−0.07%), and bornyl acetate (0.05−0.10%), which have been used in perfume products, as sweeteners, and as modifiers presenting floral or fruity flavors, were identified in Hyuganatsu oils. Citronellyl acetate (0−0.04%) and trans, trans-farnesyl acetate were also

Table 2. Constitution of Functional Groups in the Essential Oils of *Citrus tamurana* Hort. ex Tanaka

	group I				group II				group III			
	L		S		L		S		L		S	
functional group	total no.	%[a]	total no.	%	total no.	%	total no.	%	total no.	%	total no.	%
hydrocarbons												
aliphatics	6	0.05	6	0.06	6	0.06	6	0.07	6	0.08	5	0.07
monoterpenes	15	95.97	15	95.19	15	94.91	15	94.96	15	94.80	15	95.84
sesquiterpenes	13	0.72	13	0.94	14	1.06	13	0.92	13	1.29	13	1.04
aldehydes												
aliphatics	10	0.28	10	0.31	10	0.30	10	0.26	8	0.12	8	0.04
terpenes	5	0.31	4	0.31	4	0.31	4	0.31	4	0.29	4	0.29
alcohols												
aliphatics	2	0.07	2	0.09	2	0.09	2	0.08	2	0.08	2	0.08
monoterpenes	20	1.63	21	1.96	20	1.89	20	2.32	15	2.00	17	1.71
sesquiterpenes	14	0.21	13	0.25	13	0.30	13	0.24	12	0.29	11	0.19
ketones	8	0.43	7	0.48	8	0.62	8	0.45	7	0.60	7	0.40
esters	18	0.28	18	0.34	18	0.39	18	0.33	14	0.37	14	0.28
oxides	8	0.04	8	0.05	8	0.04	8	0.04	8	0.06	8	0.04
acids	3	0.01	3	0.01	3	0.01	3	0.01	3	0.01	3	0.01
miscellaneous	1	tr	1	tr	1	tr	1	tr	0	0	0	0
total	123	100	121	99.99	122	99.98	121	99.99	107	99.99	107	99.99

[a] Weight percent.

identified in Hyuganatsu oils in low amounts (0.02−0.05%).

Oxides and Acids. Oxides (0.04−0.06%) and acids (0.01%) occurred at low levels. Eight kinds of oxides and 3 kinds of acids were found in this study. According to Arctander (1969), linalol oxide has a powerful sweet odor and is a component of importance in the perfume industry. In this study, cis- and trans-linalol furanoxides, and cis-linalol pyranoxide were identified.

In conclusion, Hyuganatsu peel oils were found to be a rich source of monoterpene hydrocarbons. Limonene, γ-terpinene, myrcene, linalol, and α-pinene were the most abundant components in Hyuganatsu oils. The content of sesquiterpene hydrocarbons was high in stored Hyuganatsu. trans-β-Farnesene was a principal sesquiterpene hydrocarbon, and its content in Hyuganatsu oils was higher than in the oils of other citrus fruits. The number of ketones and the content of *l*-carvone in Hyuganatsu oils were higher than in other citrus oils. A slight decrease of α-pinene, δ-3-carene, and α-phellandrene contents was observed in the Hyuganatsu CPO when these fruits were harvested at a late stage of ripeness, whereas γ-terpinene, linalol, trans-β-farnesene, and *l*-carvone were observed to have increased. The contents of germacrene-D, linalyl acetate, β-phellandrene, and α-terpineol were higher in stored Hyuganatsu. However, the contents of octanal and dodecanal were lower. Furthermore, geraniol was not detected in stored Hyuganatsu. Although it seems to be difficult to find a consistent relationship between fruit size and volatile constituent content, it is clear that fruit size does affect the flavor of *Citrus* Hyuganatsu. As oxygenated compounds such as aldehydes, alcohols, ketones, esters, and acids, in general, have low odor threshold values, they seem to be mainly responsible for the characteristic flavor of some *Citrus* oils (Merory, 1968). Further study will be carried out to determine the odor-active compounds in Hyuganatsu oils.

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