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Evaluation of Characteristic Aroma Compounds of *Citrus natsudaidai* Hayata (Natsudaidai) Cold-Pressed Peel Oil

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The characteristic aroma compounds of *Citrus natsudaidai* Hayata essential oil were evaluated by a combination of instrumental and sensory methods. Sixty compounds were identified and quantified, accounting for 94.08% of the total peel oil constituents. Limonene was the most abundant compound (80.68%), followed by γ-terpinene (5.30%), myrcene (2.25%) and α-pinene (1.30%). Nineteen compounds which could not be identified in the original oil were identified in the oxygenated fraction. Myrcene, linalool, α-pinene, β-pinene, limonene, nonanal, γ-terpinene, germacrene D, and perillyl alcohol were the active aroma components (FD-factor > 3^6), whereas β-copaene, *cis*-sabinene hydrate and 1-octanol were suggested as characteristic aroma compounds, having a Natsudaidai-like aroma in the GC effluent. Three other compounds, heptyl acetate, (*E*)-limonene oxide and 2,3-butanediol, which each showed a high RFA value (> 35) were considered to be important in the reconstruction of the original Natsudaidai oil from pure odor chemicals. The results indicate that 1-octanol was the aroma impact compound of *C. natsudaidai* Hayata peel oil.

Key words: citrus essential oil; Natsudaidai; GC/MS; GC/olfactometry; aroma extract dilution analysis

The natural hybrids of the pummelo are known to be abundant in the Orient and exhibit a remarkable diversity of characteristics. Some of them have been found to possess desirable or acceptable qualities as fresh fruit and have come into commercial use. The most important fruits among them are considered to be Natsudaidai (*C. natsudaidai*) in Japan, Attani (*C. rugulosa*) in India and Poorman orange, Wheeny grapefruit and smooth Seville (*C. paradisi*) in New Zealand and Australia.

Citrus natsudaidai Hayata (Natsudaidai) was discovered in the 17th century in Yamaguchi Prefecture of Japan.[1,2] Other names include *natsumikan*, *natsukan*, *daidai mikan*, and Japanese summer orange. It was said to be a natural hybrid between the pummelo (buntan in Japanese) and/or sour orange and/or mandarin. Shaw[3] has reported that *C. natsudaidai* Hayata exhibited the characteristics of pummelo or bitter orange and mandarin. Lota *et al.* have listed *C. natsudaidai* Hayata as a sour orange species.[4] However, according to Iwamasa,[5] Natsudaidai is classified into the sour citrus group of Daidai. This large, rough, and unevenly textured fruit used to be popularly eaten, but nowadays it is mostly used for preparation of jelly and marmalade. The peel is used for the the preparation of liquors, and for aromatizing several drugs in pharmacy.[1,4] Natsudaidai is also used in Japan as an ingredient in Chinese medicines.[1] Its wide consumption is due to the distinct aroma from its peel essential oil and its richness in flavonoids. Citrus essential oils are often isolated from the peel (rind or flavedo) of the fruit, and are industrially produced by distillation, solvent extraction or cold-pressing. Among these methods, cold-pressing has been the preferable method because it is the most simple and natural.

Many researchers have attempted to study the non-volatile components of Natsudaidai peel oil, especially in the pharmaceutical area.[6] Quantitative analyses by high-performance liquid chromatography have shown the occurrence of auraptene in both the peel and sarcocarp of Natsudaidai. Auraptene, a citrus coumarin, is a chemo-preventer of skin tumorigenesis and inhibits tumour promotion in mouse skin.[6] According to several reports on the volatile composition of Natsudaidai peel oil, a total of 55 compounds have so far been reported.[1,7] Among them, limonene, α-terpinene and myrcene were the major components. Sawamura *et al.* have identified 50 volatiles, of which limonene (90.24%), γ-terpinene (4.86%) and myrcene (1.71%)

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were the main components in Natsudaidai essential oil.[7] In another report, the amount of limonene was 93.5%.[5] These studies have focused only on the volatile and non-volatile compounds. There has been no report on the quantification and characterization of the volatiles which constitute the distinctive aroma of Natsudaidai. The oil composition of species of minor commercial importance and of hybrids, on the other hand, is often useful for chemotaxonomic studies. Therefore, this present study was designed to identify the chemical composition of Natsudaidai oil and attempts to uncover the volatile components which contribute to the overall aroma quality of Natsudaidai cold-pressed peel oil.

Materials and Methods

Materials. Natsudaidai fruits were collected from Kochi Fruit Experimental Station (Kochi, Japan) in December 2002. The peel oil was extracted from the flavedo by the hand-pressing and obtained in a brine solution on ice. The extract was centrifuged at 4000 *g* for 15 min at 4 °C. The resulting supernatant was dehydrated with anhydrous sodium sulfate at 5 °C for 24 h and then filtered. The neat oil was stored at −25 °C until needed for analysis. Standard chemical compounds purchased from Wako Pure Chemical Industries (Japan), Aldrich Chemical Co. (USA), Fluka Fine Chemicals (Switzerland), Nakalai Tesque (Japan) and Tokyo Kasei Kogyo Co. (Japan) were used for identifying the oil components and for sensory studies of the detected characteristic compounds. Silica gel (Wakogel Q-23, Wako Pure Chemical Industries) was dried at 100 °C for 24 h.

GC and GC–MS. The composition analysis of the oil was carried out by using a GC-17A gas chromatograph (Shimadzu) equipped with a flame ionization detector (FID) and two capillary columns: a DB-Wax column, 60 m × 0.25 mm i.d., 0.25 µm film thickness (J & W Scientific, Folsom, CA, USA), and a DB-1 column, 60 m × 0.25 mm i.d., 0.25 µm film thickness (J & W Scientific, Folsom, CA, USA). The peak areas were integrated with an C-R8A Chromatopack integrator (Shimadzu). The column temperature was programmed to rise from 70 °C (2 min hold) to 230 °C (20 min hold) at 2 °C/min. The injector and detector temperatures were at 250 °C and nitrogen was the carrier gas at a flow rate of 2 ml/min. Authentic compounds of both 1-heptanol and methyl myristate (Wako Pure Chemical Industries) were used as internal standards.[8] The weight percentage of each peak was calculated according to the correlation factor to FID. An oil sample of 0.5 µl was injected, the split ratio of the injector being 1:50.

A GC–MS QP-5050A gas chromatograph mass spectrometer (Shimadzu) was used for the GC–MS analysis. Under the same GC conditions as those just described. The MS conditions run in the electron impact mode (MS-EI) were an ionization voltage of 70 eV and ion source temperature of 250 °C. An oil sample of 0.2 µl was injected. Individual components were identified by comparing both mass spectra and their GC retention times on polar and apolar columns with those of authentic compounds that had previously analysed and stored in the data system, and also by peak enrichment upon co-injection with authentic standards, if necessary. The retention indices were also determined for all constituents by using a homologous series of *n*-alkanes (C_8–C_{27}).

GC-olfactometry (GC-O). Samples were prepared for GC-O from the neat oil by making a set of three-fold serial dilutions for each sample, using acetone. The sample was analysed by two trained sniffers. GC-O was performed by means of a GC-17A gas chromatograph (Shimadzu) equipped with a DB-Wax fused silica capillary column of 60 m × 0.53 mm i.d. and 1 µm film thickness (J & W Scientific, Folsom, CA, USA) connected to an ODO II humidifier (SGE, Japan), and an FID. The conditions were the same as those just given for the GC-17A. An oil sample of 0.5 µl was injected. At the exit of the capillary column, the effluent was split into the FID and sniffing port at the ratio of 1:5. The flow rate of the nitrogen carrier gas was 3.2 ml/min. All dilutions were sniffed in triplicate until no odor could be detected in the maximum-diluted sample.

Aroma extract dilution analysis (AEDA). The cold-pressed Natsudaidai oil was successively diluted three-fold with acetone until the sniffers could not detect any significant odor in a run. The highest dilution at which an individual component could be detected is defined as the flavor dilution (FD) factor for that odorant. According to the Stevens-law ($\psi = k\varphi^n$, where ψ is the intensity of stimulus, k is a constant, φ is the stimulus level and n is the Stevens-exponent (n = 0.3–0.8)) applied to flavor research, we defined the RFA value from the correlation between the concentration (stimulus level) and flavor dilution factor (intensity of stimulus). On the basis of the AEDA results, the relative flavor activity (RFA) was calculated for each detected odorant, using the equation $RFA = \log_{10}(3^n)/S^{0.5}$, where 3^n is the FD factor and S is the weight percentage of a component.

Fractionation of the volatile components. Silica gel was used to separate about 4.5 g of Natsudaidai oil into its hydrocarbon and oxygenated fractions. The column (35 cm × 2 cm i.d.) was prepared by using *n*-hexane. The hydrocarbons were eluted with *n*-hexane until no reaction occurred by the iodine test. Diethyl ether was then employed to obtain the oxygenated fraction. This fraction was concentrated to about 200 µl at room temperature under reduced pressure. The oxygenated fraction was analyzed by GC-O and GC–MS to identify the additional odorants.

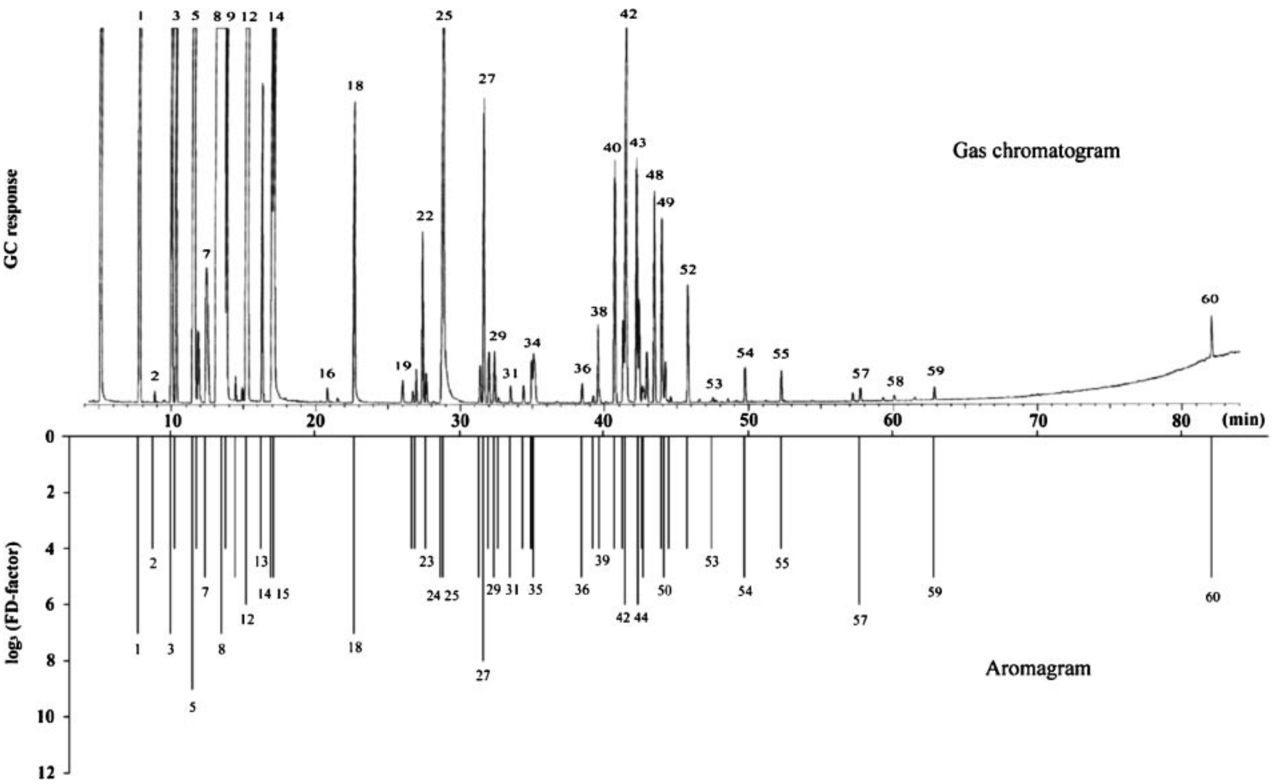

Fig. 1. Gas Chromatogram and Aromagram of the Active Odor Volatiles of Natsudaidai Cold-Pressed Oil.

Table 1. Aroma Volatiles of *C. natsudaidai* Hayata (Natsudaidai) Cold-Pressed Oil

Compound No.	Compound name	Retention index		% (w/w)	Identification	Odor description[d]	$\log_3$(FD-factor)[e]	RFA[f]
		DB-Wax	DB-1					
1	α-Pinene	1031	934	1.30	RI, MS	green, pine-like	7	2.9
2	Camphene	1077		0.01	RI, MS	woody, green	4	24.5
3	β-Pinene	1119	973	0.49	RI, MS	green, resinous	7	4.8
4	Sabinene	1128	968	0.19	RI, MS	grassy	4	4.4
5	Myrcene	1164	982	2.25	RI, MS	grassy, pungent, metallic, herbal	9	2.9
6	α-Phellandrene	1172	925	0.05	RI, MS	cool, grassy	4	8.8
7	α-Terpinene	1187	1010	0.13	RI, MS	green, fresh, floral	5	6.7
8	Limonene	1215	1032	80.68	RI, MS	sour, lemon-like, minty	7	0.4
9	β-Phellandrene	1222	997	0.46	RI, MS	green, herbal	4	2.8
10	(Z)-β-Ocimene	1236	1039	0.01	RI, MS, Co-GC	herbal	5	27.7
11	(E)-β-Ocimene	1247		tr	RI, Co-GC	nutty, fresh	3	33.9
12	γ-Terpinene	1254	1053	5.30	RI, MS	oily, waxy, pungent	6	1.2
13	p-Cymene	1276	1012	0.12	RI, MS	oily, resinous, green	4	5.4
14	Terpinolene	1288	1080	0.22	RI, MS	dry grass-like, smoky, flowery	5	5.1
15	Octanal	1291	980	0.89	RI, MS	grassy, fruity	5	2.5
16	2-Octen-1-ol[a]	1362		tr	MS	green, herbal	3	28.8
17	Heptyl acetate	1374		tr	RI, Co-GC	green, herbal	3	55.5
18	Nonanal	1393	1082	0.05	RI, MS	green, citrusy, fruity	7	14.9

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Results and Discussion

Volatile components of Natsudaidai cold-pressed oil

The integrated FID peaks were observed on a typical chromatogram of Natsudaidai cold-pressed peel oil (Fig. 1). The compounds identified in the order of their elution from the DB-Wax capillary column are listed in Table 1. Sixty volatile components of Natsudaidai oil were identified and quantified, accounting for 94.08% of the total peel oil. They included 14 monoterpene (91.19%) and 10 sesquiterpene (0.58%) hydrocarbons, 9 aldehydes (1.55%), 14 alcohols (0.41%), 9 esters (0.30%), 2 ketones (0.05%) and 2 oxides (0.01%). Limonene was the most abundant compound (80.68%), *Continued*

19	*cis*-Limonene oxide	1452		0.01	RI, Co-GC	sour, herbal	3	17.5
20	*trans*-Limonene oxide	1463		tr	RI, MS	grassy	4	41.6
21	*trans*-Sabinene hydrate	1467	1056	0.01	RI, MS	floral, fruity	4	17.2
22	Octyl acetate	1475	1191	0.02	RI, MS	green, herbal	3	10.4
23	Citronellal	1479	1131	0.01	RI, MS	floral, fresh	4	22.6
24	α-Copaene	1482	1375	0.01	RI, MS	herbal	5	20.1
25	Decanal	1497	1183	0.28	RI, MS	grassy, herbal, sour	5	4.5
26	2,3-Butanediol[b]	1541		0.01	RI, MS, Co-GC	grassy, powdery	5	21.7
27	Linalool	1546	1083	0.11	RI, MS	citrusy, fruity, sweet	8	11.7
28	*cis*-Sabinene hydrate	1552		0.04	RI, MS	natsudaidai-like, flowery	4	10.1
29	1-Octanol	1558		0.02	RI, MS	natsudaidai-like, sour	5	18.2
30	β-Copaene	1562		0.01	RI, MS	natsudaidai-like, sweet	4	23.8
31	2,3-Butanediol[b]	1576		tr	RI, MS	bitter, citrusy, sour	5	38.3
32	β-Elemene	1590	1416	0.02	RI, MS	citrusy, sour	4	14.8
33	β-Caryophyllene	1599		0.03	RI, MS	green, floral	4	11.7
34	Undecanal	1601	1284	0.03	RI, MS	citrusy, sour	4	12.1
35	Terpinen-4-ol	1603	1162	0.02	RI, MS	bitter, green, smoky	5	16.9
36	Citronellyl acetate	1660		0.01	RI, MS	grassy, burnt	5	30.0
37	α-Humulene	1672		0.01	RI, MS	citrusy, cool	4	22.3
38	Decyl acetate	1678	1389	0.02	RI, MS	bitter, green, fruity	3	9.8
39	Neral	1680		0.01	RI, Co-GC	dry grassy, sour	4	21.1
40	α-Terpineol	1696	1172	0.13	RI, MS	green, herbal	4	5.3
41	Dodecanal	1707	1386	0.04	RI, MS, Co-GC	nutty, dry grassy	4	9.1
42	Germacrene D	1710	1474	0.27	RI, MS	herbal, bitter, smoky	6	5.5
43	Neryl acetate	1723	1341	0.13	RI, MS	smoky, nutty	3	4.0
44	Valencene	1726		0.06	RI, MS	burnt, smoky	6	11.9
45	Piperitone[c]	1730		0.03	RI, Co-GC	green, smoky	4	nf
46	Geranial[c]	1732			RI, Co-GC	bitter, dry grassy	5	
47	Bicyclogermacrene	1736		0.03	RI, MS	green, minty	3	8.5
48	α-Farnesene[b]	1745	1493	0.11	RI, MS	floral, smoky	3	4.3
49	Geranyl acetate	1754	1358	0.08	RI, MS	green, herbal	4	6.6
50	δ-Cadinene	1758	1512	0.04	RI, MS	green, grassy	5	12.0
51	β-Citronellol	1764		tr	RI, Co-GC	herbal, woody	4	33.9
52	Perrilaldehyde	1784	1244	0.23	RI, MS, Co-GC	green, herbal	4	4.0
53	Geranyl propionate	1815		tr	RI, Co-GC	herbal	4	33.6
54	Limonen-10-yl acetate[a]	1856		0.02	MS	herbal, smoky	5	16.4
55	Perillyl acetate	1901	1405	0.02	RI, MS	citrusy, dry grassy	4	13.6
56	*cis*-Nerolidol	1994		0.01	RI, Co-GC	dry grass-like, burnt	3	15.8
57	Perillyl alcohol	2004	1275	0.04	RI, MS, Co-GC	green, citrusy	6	14.1
58	*trans*-Nerolidol	2051	1512	0.01	RI, Co-GC	green, burnt	3	19.7
59	Viridiflorol[a]	2105		tr	MS	dry grassy	5	33.9
60	Nootkatone	2526		0.03	RI, MS	bitter, grassy, herbal	5	13.2

tr, Detected weight percent less than 0.005%.
RI, Identification based on retention index.
MS, Identification based on comparison of mass spectra.
Co-GC, Identification based on co-injection with authentic standards.
nf, Not calculated.
[a], Tentatively identified.
[b], Correct isomer not identified.
[c], Co-eluted peaks.
[d], Odor description at the GC-sniffing port during GC-O analysis.
[e], FD-factor in DB-Wax column.
[f], RFA $= \log_{10}(3^n)/S^{0.5}$, where S is the weight percentage.

followed by γ-terpinene (5.30%), myrcene (2.25%) and α-pinene (1.30%). Among the sesquiterpene hydrocarbons, germacrene D (0.27%) was the major component. Aldehydes are the major and important oxygenated components in citrus essential oils. They comprised about 67% of the total oxygenated components in the Natsudaidai oil. The Natsudaidai oil composition studied by Maekawa *et al.* showed high levels of 1,8-cineole and octanal, which also occurred in bitter orange oils examined by the same workers.[3)] The amount of octanal in this present study was 0.89%, but no 1,8-cineole was detected in this oil sample. The detailed composition may vary according to maturity and growing conditions. However, as long as we use an oil sample with the characteristic aroma of Natsudaidai, the characteristic aroma compound of the flavor is not variable. Saturated straight-chain aldehydes from C_8 to C_{12} were quantified, octanal and decanal being present in greater quantities than the others. As has been reported, octanal and decanal are the two main aldehydes in citrus oils, the level of octanal being higher in orange and grapefruit oil but lower in mandarin oil than that of decanal.[3,9)]

Fourteen alcohols in Natsudaidai oil were identified and quantified. Most of them were detected in a quantity of less than 0.04%, except for linalool (0.11%) and α-terpineol (0.13%). It is considered that linalool, α-terpineol and terpinen-4-ol are very important to the flavor of orange oil.[3] Although octanol is the major component in grapefruit oil,[3,10] its concentration was low in Natsudaidai oil (0.02%). However, 1-octanol was conspicuously detected as having a strong natsudaidai-like aroma in both the neat oil and the oxygenated fraction in the GC effluent from the sniffing test and from having the highest FD factor among three identified characteristic aroma compounds of Natsudaidai. We consider that the characteristic aroma compounds (showing a strong natsudaidai-like aroma at the sniffing port) were the most contributive to the overall aroma of this oil, especially those compounds with higher FD factor. Therefore, 1-octanol is considered to have been the aroma impact compound in Natsudaidai oil, although its concentration was low. Other minor compounds such as esters, ketones and oxides were present in trace amounts in Natsudaidai oil. Nine esters were quantified at a low level, but the level of neryl acetate was as much as 0.13%. The two ketones detected in this oil were piperitone and nootkatone. The level of nootkatone is regarded as an indicator of the quality of the essential oil from grapefruit and pumello.[3] This compound was also present in Natsudaidai oil at the level of 0.03%. In conclusion, the chemical composition of the oil was quite different from that of pummelo, orange and mandarin oils. It would be interesting to determine each odorant characterizing the Natsudaidai aroma.

Characterization of the detected aroma volatiles

The results of AEDA and the descriptive aroma analysis are summarized in Table 1. An aromagram of the FD factor value *versus* retention time from the gas chromatogram of Natsudaidai peel oil is shown in Fig. 1. The Natsudaidai peel oil had green and sour-citrusy top notes and a somewhat mandarin-like aroma.

As shown in Table 1 and Fig. 1, myrcene (grassy, pungent, metallic and herbal) had the highest FD factor (3^9), followed by linalool (citrusy, fruity and honey) with an FD factor of 3^8. Other components with an FD factor of 3^7 were α-pinene (green and pine-like), β-pinene (green and resinous), limonene (sour, lemon-like and minty) and nonanal (green, citrusy and fruity), and others with an FD factor of 3^6 were γ-terpinene (oily, waxy and pungent), germacrene D (herbal, bitter and smoky) and perillyl alcohol (green and citrusy). The results show that the aroma components with higher FD factor were the most active.[11,12] It is therefore suggested that these nine compounds identified by GC-sniffing were the active odor compounds in Natsudaidai peel oil. In our previous study, myrcene, (*E*)-ocimene, β-copaene, perillaldehyde and perillyl alcohol were the characteristic odor compounds of Mochiyu (*C. inflata* Hort. ex Tanaka) peel oil, showing a mochiyu-like odor.[13] Interestingly, β-copaene was also described as a Natsudaidai-like odor in this peel oil. Two other compounds, perillaldehyde and perillyl alcohol, had a green and herbal/citrusy aroma. Limonene, in general, is not the most significant odor-character component in citrus oils, although it is still an odor contributor.[11] Limonene has been as lemon-like and minty in Natsudaidai oil. Although this is the predominant constituent of citrus oils, limonene is considered less important in contributing to the overall aroma of citrus essential oils. It only forms a background in an aroma model of citrus flavor.[14] The minor components such as sequiterpenes and oxygenated compounds are considered to be important to the characteristic aroma of citrus peel oils. The GC-sniffing result as described the characteristic aroma compounds of Natsudaidai oil as β-copaene (sesquiterpene), *cis*-sabinene hydrate (monoterpene alcohol) and 1-octanol (aliphatic alcohol). They were described as having a natsudaidai-like aroma in the GC effluent.

The sniffing test and AEDA report both the active aroma and aroma character of a component. They are valuable criteria in determining the role of a component in the total aroma of an essential oil. However, the organoleptic response to a compound generally depends on its concentration.[12] Therefore, the RFA value from the correlation between the concentration (w/w) and flavor dilution factor was established as an additional criterion. Three compounds, heptyl acetate (peak no. 17), *trans*-limonene oxide (peak no. 20) and 2,3-butanediol (peak no. 31) showed high RFA values (>35) of 55.5, 41.6 and 38.3, respectively. It is considered that compounds with the highest RFA values are important in the reconstruction of an original oil from pure odor chemicals. Hence, heptyl acetate (green and herbal), *trans*-limonene oxide (grassy) and 2,3-butanediol (bitter, citrusy and sour) would play important roles in reconstructing the Natsudaidai aroma model.

The results obtained by GC-O depend directly on the sample preparation and analytical conditions. The analysis of an aroma extract by the dilution technique combined with static headspace GC-O provides complementary data of the aroma composition of a food. Therefore, we tried to identify the volatile composition of Natsudaidai oil using GC–MS together with the static headspace technique. The neat oil was put into a 2-ml headspace vial regulated at 30 °C, and then allowed to stand for 30 min to establish equilibrium at that temperature. Once the volatiles had equilibriated, 500 µl of the headspace gas was withdrawn with a gas tight syringe (Hamilton, USA) and injected into the gas chromatograph injection port of the QP-5050A GC–MS instrument (Shimadzu, Japan). The analysis was conducted using the same analytical conditions as those for the directly cold-pressed oil analysis. The result shows that the 11 constituents with the largest peaks were limonene, α-pinene, γ-terpinene, myrcene, β-pinene, *p*-

Table 2. Aroma Volatiles in the Oxygenated Fraction of Natsudaidai Cold-Pressed Oil

Peak	Compound	Retention index		Odour discription[a]	Identification[c]
		DB-Wax	DB-1		
1	Octanal	1289	979	floral, grassy	RI, MS
2	3-Methyl-cyclopentanol[b]	1342		smoky, bitter	MS
3	1-Hexanol	1352	866	grassy	RI, MS
4	2-Octen-1-ol[b]	1360		floral	MS
5	Nonanal	1392	1082	floral, fruity	RI, MS
6	*cis*-Linalool oxide (furanoid)	1445	1059	floral, green, dry grassy	RI, MS
7	Acetic acid[b]	1449		straw-like	MS
8	*cis*-Limonene oxide	1454		rubbery	RI
9	*trans*-Limonene oxide	1462	1121	resinous, citrusy	RI, MS
10	*trans*-Linalool oxide (furanoid)	1473	1073	citrusy	RI, MS
11	Decanal	1496	1183	fruity, sweet, citrusy, warm	RI, MS
12	Linalool	1545	1083	herbal, pungent, spicy	RI, MS
13	1-Octanol	1557	1255	hot, spicy, natsudaidai-like	RI, MS
14	Neoisopulegol	1574	1130	pungent, spicy	RI, MS
15	Undecanal	1600	1285	herbal	RI, MS
16	Terpinen-4-ol	1601	1162	grassy, citrusy	RI, MS
17	Isopulegol	1628	1148	citrusy, cool, herbal	RI, MS
18	Nonanol	1658	1154	spicy, herbal	RI, MS
19	Decyl acetate	1677	1390	spicy, herbal, floral	RI, MS
20	Neral	1679	1242	metallic, green	RI, MS
21	β-Terpineol	1681		herbal	RI, MS
22	α-Terpineol	1696	1173	citrusy, metallic, bitter	RI, MS
23	Dodecanal	1705	1386	spicy	RI, MS
24	Neryl acetate	1722	1341	herbal, citrusy	RI, MS
25	Piperitone	1729		green	RI, MS
26	Geranial	1731	1242	green	RI, MS
27	*cis*-Linalool oxide (pyranoid)	1737		green, smoky	RI, MS
28	Geranyl acetate	1752	1359	oily, green	RI, MS
29	*trans*-Linalool oxide (pyranoid)	1760		green	RI, MS
30	β-Citronellol	1763	1207	spicy, hot	RI, MS
31	Perillaldehyde	1783	1245	flowery	RI, MS
32	Nerol	1796		dry grass-like, smoky	RI, MS
33	*trans*-Carveol	1832	1195	citrusy, green	RI, MS
34	Geraniol	1844		citrusy	RI, MS
35	Limonen-10-yl acetate[b]	1854	1297	minty	MS

Continued on next page

cymene, α-phellandrene, sabinene, terpinolene, α-terpinene and copaene. Most oxygenated compounds and important minor compounds like 1-octanol were not detectable in the gas chromatogram. Furthermore, some artifacts may have been formed during equilibrium at a temperature higher than ambient. It is suggested, in summary, that directly using the cold-pressed oil analysis provides a sufficiently reliable basis for quantitatively characterizing aroma impact compounds which are very similar to their natural counterparts. This information will be useful in creating the aroma of Natsudaidai.

As already mentioned, a small quantity of compounds in the range from trace to 0.05% such as heptyl acetate, nonanal and *cis*-limonene oxide were important to the characteristic aroma of Natsudaidai. On the other hand, an aliphatic alcohol like 1-octanol showed a very strong natsudaidai-like odor from the sniffing test. Thus, a further study aimed at determining other minor compounds important to the characteristic aroma of Natsudaidai oil will be necessary.

Identification and characterization of the oxygenated compounds

Forty-eight compounds in the oxygenated fraction from Natsudaidai cold-pressed peel oil were identified. Their odor descriptions are shown in Table 2. Eight aldehydes, twenty-four alcohols, five esters, two acids, two ketones, six oxides and one ether were detected. Nineteen compounds which could not be identified in the original oil were identified in the oxygenated fraction. Those were 1-hexanol, 3-methyl-cyclopentanol, *cis*- and *trans*-linalool oxides (pyranoid form), *cis*- and *trans*-linalool oxides (furanoid form), isopulegol, nonanol, β-terpineol, nerol, geraniol, *cis*- and *trans*-carveols, exo-2-hydroxycineole, *p*-menth-1-en-9-ol, α- and β-eudesmols, isothymol, acetic acid and decanoic acid. Only one of these compounds, 1-octanol, was described as hot, spicy and natsudaidai-like in odor.

Conclusion

The characteristic aroma compounds of *Citrus natsu-*

Continued

36	exo-2-Hydroxycineole[b]	1859	1304	bitter, spicy, earthy	MS
37	*cis*-Carveol	1863		herbal	RI, MS
38	Perillyl acetate	1900	1406	herbal	RI, MS
39	*p*-Menth-1-en-9-ol	1933		herbal	RI, MS
40	(*Z*)-Nerolidol	1992	1493	citrusy, green, cool	RI, Co-GC
41	Perillyl alcohol	2003	1274	herbal, cool, dry grassy	RI, MS
42	(*E*)-Nerolidol	2052		straw-like	RI, Co-GC
43	Viridiflorol[b]	2103	1636	citrusy, warm	MS
44	α-Eudesmol[b]	2223	1638	floral, flowery	MS
45	β-Eudesmol	2233	1633	green, bitter, dry grassy	RI, MS
46	Isothymol	2256		herbal, spicy	RI
47	Decanoic acid	2289		citrusy, flowery, floral	RI, MS
48	Nootkatone	2525	1774	herbal, bitter	RI, MS
	Aldehydes				
	Aliphatics	5			
	Terpenes	3			
	Alcohols	24			
	Esters	5			
	Acids	2			
	Ketones	2			
	Ether	1			
	Oxides	6			
	Total	48			

[a], Odour description at the sniffing port during the GC-O analysis.
[b], Tentatively identified.
[c], RI, MS, Co-GC: Identification based on RI, MS and Co-injection (see Table 1).

daidai Hayata essential oil were investigated. Sixty compounds were identified and quantified, accounting for 94.08% of the total peel oil. In addition, nineteen compounds which could not be identified in the original oil were identified in the oxygenated fraction. β-copaene, *cis*-sabinene hydrate and 1-octanol were suggested as the characteristic aroma compounds, having a Natsudaidai-like aroma in the GC effluent. Three other compounds, heptyl acetate, *trans*-limonene oxide and 2,3-butanediol, which each showed a high RFA value (> 35) are considered to have been important in the reconstruction of the Natsudaidai original oil from pure odor chemicals. This study identified 1-octanol as an aroma impact compound of *C. natsudaidai* Hayata peel oil.

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