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Characteristic Odor Components of *Citrus reticulata* Blanco (Ponkan) Cold-pressed Oil

Masayoshi SAWAMURA,[1,†] Nguyen THI MINH TU,[2] Yuji ONISHI,[1]
Eriko OGAWA,[1] and Hyang-Sook CHOI[3]

[1]*Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan*
[2]*Institute of Biological and Food Technology, Hanoi University of Technology, 1 Dai Co Viet Street, Hai Ba Trung, Hanoi, Vietnam*
[3]*Department of Food and Nutrition, Faculty of Natural Science, Duksung Women's University, Seoul 132-714, Korea*

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Citrus reticulata **Blanco (ponkan) cold-pressed oil and its oxygenated fraction were studied by analytical (GC and GC/MS) and sensory analyses. The monoterpene group was predominant, accounting for more than 89.6% (w/w), of which limonene was the most abundant (80.3%). Among the oxygenated compounds, octanal and decanal were the major ones among 12 aldehydes accounting for >1.5%; six alcohols were identified with a total concentration of >0.7%, while oxides, ketones and esters did not quantitatively or qualitatively contribute to the oil. Sniffing the ponkan cold-pressed oil and its oxygenated fraction demonstrated that octanal and decanal were the characteristic odor components of ponkan. Reconstruction of the ponkan aroma model and its sensory evaluation by a hedonic test were performed, showing that, in addition to octanal and decanal which played important roles, (*R*)-(+)-limonene contributed to the aroma model as a background component, making the aroma model very similar to that of the original.**

Key words: *Citrus reticulata* Blanco; ponkan; cold-pressed oil; gas chromatography/olfactometry (GC/O); characteristic odor compound

Ponkan is one of the most important commercial cultivars of mandarin that has been extensively planted in China and Japan since a very early date.[1)] The fruit is round to oblate in shape, with peel of an orange color and 9–12 seedy but very juicy segments. In Japan, ponkan is one of the most popular sweet citrus fruits for consuming fresh. It belongs to *Citrus reticulata* Blanco, a large species with numerous varieties and hybrids, which has been the topic of large-scale investigations. For instance, the peel oil composition of various origins has been reported by many researchers,[2–8)] with limonene and γ-terpinene being the most abundant compounds, amounting to 66.0–74.0% and 9.1–22.7%, respectively in European mandarins;[3)] sesquiterpenes were found in a remarkable quantity in Uruguayan but not in Kenyan mandarin oil,[5–8)] with aliphatic aldehydes being reported to vary in number and have notable concentration in Vietnamese and Kenyan samples.[6,8)] The isolation of terpenes, pigments and flavonoid fractions has also been carried out.[9,10)] The enantiomeric distribution of the volatile components as well as monoterpene hydrocarbons and monoterpene alcohols has been presented mainly for Italian mandarin oil samples.[11,12)] The cold-pressed peel oil of mandarins from Ethiopia, Japan, and Kenya has been analyzed for several species and their hybrids, the results showing that the enantiomeric distribution of linalool is a useful tool for determining the mandarin origin.[13)] Despite this considerable work on *C. reticulata* Blanco, which is accepted as the major source of essential oil mainly for food and beverage flavoring because of its fine aroma quality, there still remains a question about the compounds which induce the specific and attractive odor of Japanese mandarins. The citrus aroma quality according to odor threshold values has been published,[14)] and the key odor compounds have been investigated for a considerable number of citrus samples[15–18)] such as hand-squeezed orange and grapefruit juices, grapefruit and mandarin juices, orange essence oil and aqueous lemon oil. The characteristic odor-active volatile compounds of a wide range of Japanese citrus varieties have been revealed by using gas chromatography/olfactometry (GC/O) and an aroma extract dilution analysis (AEDA): for instance, 2-dodecenal, (*R*)-(+)-citronellal,

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

Abbreviations: AEDA, aroma extract dilution analysis; FD, flavor dilution; FID, flame ionization detector; GC/O, gas chromatography/olfactometry; RFA, relative flavor activity

(*E*)-ocimene, and myrcene and (*Z*)-ocimene were key compounds of *C. grandis* Osbeck forma *Tosa* (Tosa buntan), *C. sphaerocarpa* Tanaka (Kabosu), *C. inflata* Tanaka (Mochiyu) and *C. sp.* (Kiyookadaidai) cold-pressed oils, respectively.[19–22] *C. junos* (Yuzu), *C. aurantium* L. var *Cyathifera* Y. Tanaka (Daidai), *C. flaviculpus* Hort. ex Tanaka (Kimikan) and *C. tamurana* Hort. ex Tanaka (Hyuganatsu) seem to have rather complex aromas, but they are made up by a small group of compounds which are responsible for their characteristic odor.[23–26] The aim of this work is to elucidate the characteristic odor compounds of ponkan cold-pressed oil by using GC/O and AEDA techniques.

Materials and Methods

Materials. Mature ponkan fruits were obtained from Kochi Fruit Experimental Station (Kochi, Japan) in December 2002. Twenty kilograms of ponkan fruits were used. Ponkan cold-pressed oil was collected by the hand-pressing method as previously described.[20] Each ponkan fruit weighed about 160 g which respectively yielded 0.12% and 0.53% cold-pressed oil (w/w) from the whole fruit and from the separated flavedo (36.2 g). The oil was kept at $-25\,^{\circ}$C until needed for analysis. Authentic standard chemicals were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Nacalai Tesque Inc. (Kyoto, Japan), Aldrich Chemical Co. (USA) and Fluka Fine Chemicals (Switzerland) for identification of the oil components and sensory studies on the detected characteristic compounds, as well as for sensory analysis of the characteristic ponkan compounds.

Silica gel column chromatography. The ponkan essential oil (about 3 g) was fractionated into hydrocarbon and oxygenated fractions by silica gel column chromatography (25 cm × 2 cm i.d.) on Wako Q-23 gel (about 60 g) which had been preliminarily activated at 100 °C for 24 hr. The hydrocarbon and oxygenated compounds were respectively eluted with *n*-hexane (about 960 ml) and diethyl ether (about 240 ml). Each fraction was carefully concentrated under reduced pressure at room temperature. This method has been described and applied to the fractionation of citrus essential oil in a previous study.[21]

GC and GC/MS. The composition of the oil was analyzed by a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (FID) and two capillary columns. The first was a DB-Wax column of 60 m × 0.25 mm i.d. with a film thickness of 0.25 μm, and the second was a DB-1 column of 60 m × 0.25 mm i.d. with a film thickness of 0.25 μm (both from J & W Scientific, Folsom, CA, USA). The peak areas were integrated with a Shimadzu C-R6A Chromatopack integrator. 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 both 250 °C. 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.[19,24] The percentage weight of each peak was calculated according to the correlation factor to FID.[19,24] An oil sample of 1 μl was injected, the split ratio of the injector being 1:50.

A Shimadzu GC-17A coupled with a Shimadzu QP-5000 instrument used for the GC-MS analysis under the same conditions as those just described. The MS conditions were as follows: ionization voltage, 70 eV; ion source temperature, 250 °C.

Individual components were identified by comparing both mass spectra and their GC retention times in polar and apolar columns with those of authentic compounds that had previously been analyzed and stored in the data system, and also by peak enrichment up on co-injection with authentic standards wherever possible. The retention indices were also determined for all constituents by using a homologous series of *n*-alkanes (C_7-C_{27}).

GC/Olfactometry (GC/O). A Shimadzu GC-14A gas chromatograph equipped with a DB-Wax fused silica capillary column (60 m × 0.53 mm i.d.; film thickness of 1 μm (J & W Scientific) and FID was used.[20] The column oven and other operating conditions were the same as those already described for the GC-14A instrument. An oil sample of 0.5 μl was injected. At the exit from the capillary column, the effluent was split into channels to the FID and a sniffing port. The flow rate of nitrogen carrier gas was 9 ml/min, and the split ratio was 1:5. Humid air was constantly added to the effluent at the sniffing port.

AEDA. The cold-pressed ponkan oil was stepwise diluted 3-fold with acetone until the sniffers could not detect any significant odor in a run.[27,28] The odor potency and odor description of volatiles in the cold-pressed oil were evaluated in duplicate by three trained panelists. The highest dilution at which an individual component could be detected is defined as the flavor dilution (FD) factor for that odorant. An FD factor was assigned only if the odorant was detected in at least 4 out of 6 GC/O evaluations for a particular dilution test. On the basis of the AEDA results, the relative flavor activity (RFA) was calculated for each detected odorant by using the following equation: $\mathrm{RFA} = \log 3^{n}/S^{0.5}$, where 3^{n} is the FD factor and S is the percentage weight of a component.[23]

Sensory evaluation. The panelists were trained to use descriptions of odors that have commonly been found in citrus peel oil.[21–23,29] During training, the panelists discussed terms and attributes and learned to consistently use all the descriptors. In respect of "ponkan-like", the panelists were asked to remember the

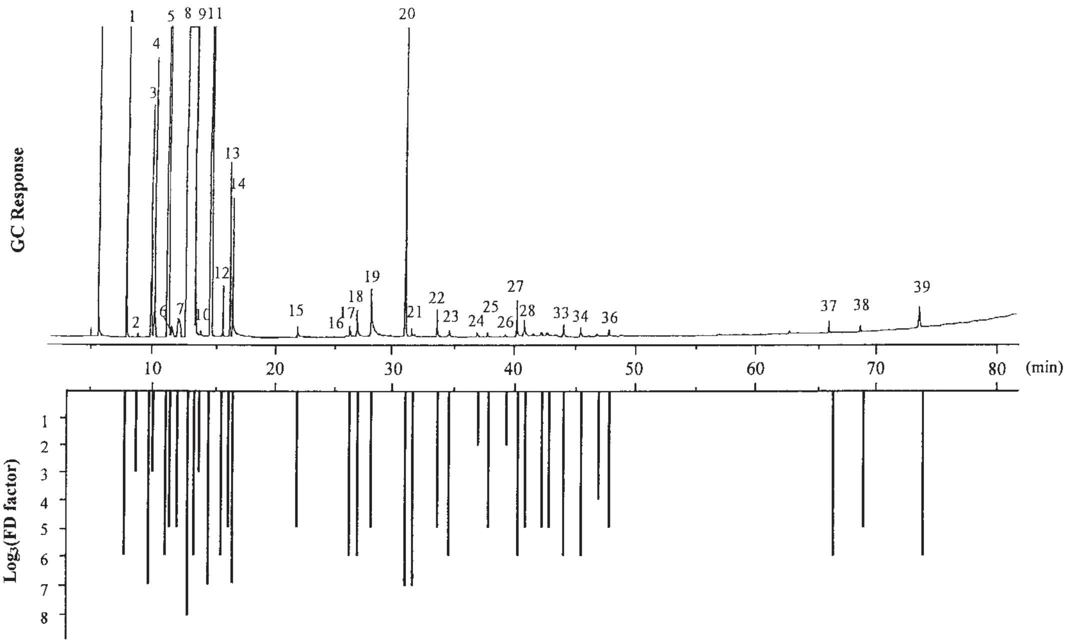

Fig. 1. Gas Chromatogram (top) and Aromagram (bottom) of the Odor-active Volatiles of Ponkan Cold-pressed Oil.

characteristic odor of the cold-pressed oil. A sensory evaluation of the similarity between ponkan peel oil and its aroma model was performed with a hedonic scale by 14 panelists who already had experience in sensory analyses.[30] Assessors were asked to give the degree of similarity to or difference from the ponkan oil and the prepared model in agreement with the nine-point hedonic scale statements. The sensory test was triplicated.

Results and Discussion

Volatile composition of ponkan cold-pressed oil

Thirty-nine compounds, accounting for more than 92% (w/w), were detected and identified by injecting the ponkan cold-pressed peel oil into the polar DB-Wax and apolar DB-1 colums. A gas chromatogram from the DB-Wax column is shown in Fig. 1. The components are listed in order of their elution from the DB-Wax column, and their percentage weights are given in Table 1. All the compounds matched those from a previous study, which was conducted on the same variety.[31] The monoterpene fraction was predominant, accounting for more than 89.6%, where limonene was the most abundant component (80.3%), followed by γ-terpinene (4.7%), myrcene (2.1%) and α-pinene (1.2%). This proportion of monoterpenes is similar to that in previous studies,[7,29] except for limonene, whose concentration amounted to 89.9%. α-Thujene, a monoterpene hydrocarbon, was at a level of 0.2% in ponkan peel oil, according to Lota *et al.*,[7] although it was not detected in the present study. β-Elemene (0.1%) was the major of five sesquiterpenes identified.

The aldehyde group was notable for its total concentration and constituted >1.5% of the oil. Octanal and decanal were the most predominant among 12 aldehyde compounds detected, constituting 1.0% and 0.3%, respectively. Six alcohols were identified among which linalool (0.6%) and α-terpineol (0.1%) were in relatively high concentrations. Oxides, ketones and esters did not contribute quantitatively or qualitatively to the ponkan cold-pressed oil. No oxide or ketone was found in this study, only one or two esters having been identified in other two studies.[7,31]

The oxygenated fraction of ponkan was composed of 40 compounds: 13 aldehydes, 17 alcohols, 4 esters, 2 ketones, 2 oxides and 2 miscellaneous ones (Table 2). Many compounds which were present in such small quantity that they could not be found in ponkan cold-pressed oil were detectable in the oxygenated fraction: hexadecanal as an aldehyde; β-terpineol, 2-decyn-1-ol, (*E*)-piperitol, geraniol, *p*-menth-1-en-9-ol, perillyl alcohol, (*Z*)-nerolidol, viridiflorol, β-eudesmol and α-bisabolol as alcohols; bornyl acetate, neryl acetate and citronellyl acetate as esters; carvone and *p*-mentha-1,8-dien-3-one as ketones; and (*Z*)- and (*E*)-limonene oxides as oxides were each identified.

GC/O and AEDA

The results of GC/O and AEDA studies are given in Table 3. The chromatogram for ponkan cold-pressed oil is shown in Fig. 1. The highest dilution of an individual component detected by the assessors is defined as its FD factor and expressed as a power of 3. As is summarized in Table 3, 37 of the 39 compounds identified in ponkan oil were evaluated by the sensory analyses. The first 13 peaks were monoterpenes and possessed green or herbal odor properties. Among them, limonene was determined as having the highest FD factor (8), being followed by β-pinene, γ-terpinene, octanal, linalool and octanol, whose

Table 1. Volatile Components of *Citrus reticulata* Blanco (Ponkan) Cold-pressed Peel Oil

Peak no.	Component	Retention index		w/w (%)	Identification
		DB-Wax	DB-1		
1	α-Pinene	1022	942	1.2	RI, MS
2	Camphene	1067	955	*	RI, MS
3	β-Pinene	1109	975	0.5	RI, MS
4	Sabinene	1118	981	0.5	RI, MS
5	Myrcene	1151	988	2.1	RI, MS
6	α-Phellandrene	1161	1005	**	RI, MS
7	α-Terpinene	1175	1020	**	RI, MS
8	Limonene	1213	1044	80.3	RI, MS
9	β-Phellandrene	1216		nq	RI, MS
10	(*Z*)-β-Ocimene	1224	1234	**	RI, MS
11	γ-Terpinene	1245	1062	4.7	RI, MS
12	*p*-Cymene	1264	1021	0.1	RI, MS
13	Terpinolene	1277	1086	0.2	RI, MS
14	Octanal	1281	1175	1.0	RI, MS
15	Nonanal	1382	1092	**	RI, Co-GC
16	(*E*)-Sabinene hydrate	1452		*	RI, MS
17	δ-Elemene	1457		**	RI, MS
18	Citronellal	1467	1138	0.1	RI, MS
19	Decanal	1485	1193	0.3	RI, MS
20	Linalool	1532	1090	0.6	RI, MS
21	Octanol	1541		**	RI, MS
22	β-Elemene	1575		0.1	RI, Co-GC
23	Terpinen-4-ol	1591	1170	**	RI, MS
24	(*E*)-2-Decenal	1629		**	RI
25	(*E*)-β-Farnesene	1643	1679	**	RI, MS
26	Neral	1667	1688	*	RI, Co-GC
27	α-Terpineol	1682	1179	0.1	RI, MS
28	Dodecanal	1691	1392	**	RI, Co-GC
29	Germacrene D	1703		**	RI, MS
30	Geranial	1714	1738	**	RI, Co-GC
31	α-Farnesene[a]	1723		**	RI, MS
32	Geranyl acetate	1735	1343	**	RI, Co-GC
33	Citronellol	1747	1220	**	RI, MS
34	Perilaldehyde	1771	1253	0.1	RI, MS
35	2,4-Decadienal	1781		*	RI
36	Germacrene B	1810		**	RI, MS
37	Thymol	2152	1271	**	RI, MS
38	β-Sinensal	2203		**	RI, MS
39	α-Sinensal	2304		0.1	RI, MS

*, Peak area detected less than 0.005%.
**, Peak area detected between 0.005% and 0.05%.
RI, Identification based on retention index.
MS, Identification based on comparision of mass spectra.
Co-GC, Identification based on co-injection with authentic standards.
[a], Correct isomer not identified.
nq, Not quantified.

FD factors were each 7. Since the percentage weight of volatile components is concerned with its odor detection during serial dilution, the relative flavor activity (RFA) was calculated in which both the FD factor and percentage weight of each compound are involved.[23–26,32,33] Thus, high RFA values were found for camphene (20.2), δ-elemene (17.6), octanol (22.5), terpinen-4-ol (22.0), (*E*)-β-farnesene (17.1), dodecanal (18.5), geranial (17.3), 2,4-decadienal (19.7), thymol (17.4) and β-sinensal (17.3), while their FD factors ranged from 3 to 7.

As shown in Table 3, octanal (peak **14**), decanal (peak **19**) and β-elemene (peak **22**), whose FD factors and RFA values were 7, 3.3; 5, 4.4; and 5, 7.5, respectively, gave a ponkan-odor note when being eluted at the sniffing port. The ponkan aroma was a complex one involving, fresh, floral, fruity, and citrusy, and each of these can be found in many descriptions of aldehyde and alcohol compounds. This led us to focus on an analysis of the oxygenated fraction obtained by silica gel fractionation. The result is shown in Table 2, where the aldehyde and alcohol groups were also more predominant in volatile number and more persistent in FD factor than the other oxygenated functional groups. Octanal and decanal were evaluated as having a ponkan-like aroma among the 40 oxygenated volatiles. In previous reviews reported by Shaw and Boelenes,[2,3] octanal and decanal were the main aldehydes in orange and mandarin oils. Octanal has been described as citrusy in sensory analyses of orange and grapefruit hand-squeezed juices,[15,16] as well as of clementine peel oil prepared by solvent extraction.[34] It has been used in an aroma model of orange and grapefruit odors at different concentrations.[15,16] Thus, not only the molecular structure, but also the concentration of a chemical compound one related to its odor quality. Moreover, descriptors for each odorant generally depend on the concentration.[35,36] The odor quality of oxygenated aliphatic molecules, as reported by Laing *et al.*, was generally pleasant at lower concentrations.[35] Octanal and decanal which are very common aliphatic aldehydes in citrus essential oils, may also play an important role to the specific ponkan aroma at a certain amount in respect of the relationship between the odor quality and concentration.

Aroma model

In order to substantiate the results of the sensory evaluation, an aroma model of ponkan was constructed. The FD factor and RFA value may be useful criteria for constructing a model of the original odor. However, neither has any relationship to the aroma character of each compound.[19] β-Elemene, octanal and decanal were detected as having a ponkan-like odor, but they do not have a high RFA value. In this experiment, we decided to consider the odor eluted from the sniffing port, rather than the FD factor or RFA value. β-Elemene, octanal and decanal were used in the ponkan aroma model in different combinations and concentrations. A solution of β-elemene alone showed a citrusy and ponkan-like odor; however, it was not stable in an aqueous medium which made the aroma keep changing. We were therefore not successful with β-elemene in any combination examined. Considering that the ponkan aroma has the nuance of an aldehyde odor, decanal and octanal may make an important contribution. These were major aldehydes, decanal accounting for 18.8% of total aldehydes, followed by octanal with a level of 62.5%, since the total amount of aldehydes was 1.6% (Table 1). Decanal and octanal played key roles, while limonene was in the background. The importance in the model was the ratio between octanal and decanal, whose RFA values were

Table 2. Aroma Components of the Oxygenated Fraction of Ponkan Cold-pressed Peel Oil

Peak No.	Compound	Retention index		Odor description[a]	FD factor	Identification
		DB-Wax	DB-1			
Aldehydes						
1	Octanal	1278	1175	sweet, ponkan-like	11	RI, MS
2	Nonanal	1379	1093	fresh, citrusy	6	RI, MS
6	Citronellal	1464	1141	geranium-like	6	RI, Co-GC
7	Decanal	1483	1192	sour, acidic, ponkan-like,	5	RI, MS
16	(*E*)-2-Decenal	1627		citrusy, green	6	RI, MS
18	Neral	1664	1689	green, citrusy	6	RI, MS
20	Dodecanal	1687	1395	green, grassy	5	RI, MS
22	Geranial	1711	1251	sour, green	6	RI, MS
27	Perillaldehyde	1768	1256	oily	8	RI, MS
29	2,4-Decadienal	1789		oily	8	RI, MS
31	Tetradecanal	1896		fishy	6	RI, MS
37	β-Sinensal	2200		woody	7	RI, MS
40	α-Sinensal	2301		woody	8	RI, MS
Peak total: 13						
Alcohols						
8	Linalool	1531	1093	floral, rose-like	10	RI, MS
9	β-Terpineol[c]	1593		floral, rose-like	12	MS
10	Octanol	1543		floral	10	RI, MS
11	Terpineol[c]	1552		sweet, floral	8	MS
12	2-Decyn-1-ol[c]	1562		sweet, floral	8	MS
14	Terpinen-4-ol	1589	1179	earthy, green	6	RI, MS
19	α-Terpineol	1680	1182	green	5	RI, MS
24	(*E*)-Piperitol	1727		green	6	RI, MS
26	Citronellol	1744	1220	green, minty	5	RI, MS
28	Geraniol	1779	1246	oily, nutty	8	RI, MS
32	*p*-Menth-1-en-9-ol	1912		fishy	6	RI, MS
33	Perillyl alcohol	1977	1282	fishy	6	RI, MS
34	(*Z*)-Nerolidol	2009		pungent	8	RI, MS
35	Viridiflorol	2127		woody	7	RI, MS
36	Thymol	2150		woody	7	RI, MS
38	β-Eudesmol	2207		woody	5	RI, MS
39	α-Bisabolool	2275		woody	7	RI, MS
Peak total: 17						
Esters						
15	Bornyl acetate	1614	1285	grassy	6	RI, MS
17	Citronellyl acetate	1640	1341	coriander-like	6	RI, MS
21	Neryl acetate	1700	1352	sour	7	RI, MS
25	Geranyl acetate	1732	1339	minty	7	RI, MS
Peak total: 4						
Peak No.	Compound	Retention time		Aroma description[a]	FD factor	Identification
		DB-Wax	DB-1			
Ketones						
23	Carvone[c]	1718		green	5	RI, MS
30	*p*-Mentha-1,8-dien-3-on	1814		oily	8	MS
Peak total: 2						
Oxides						
3	*cis*-Limonene oxide	1437	1126	sweet, floral	5	RI, MS
4	*trans*-Limonene oxide	1449	1131	fresh	4	RI, MS
Peak total: 2						
Miscellaneous						
5	(*E*)-Sabinene hydrate	1459		fruity	4	RI
13	Methyl thymyl ether[b]	1573		minty	6	MS
Peak total: 2						

[a], Odor description at the GC-sniffing port during GC/O.
[b], Tentatively identified.
[c], Correct isomer not identified.
RI, MS and Co-GC as seen in Table 1.

very close at 3.3 and 4.4, respectively (Table 3). We thought that the appropriate ratio of octanal and decanal would be similar. In attempting to construct a similar aroma to that of ponkan, limonene, which was essential as a background to the citrus aroma in previous studies,[22–24)] was also expected to play a part in the

Table 3. Odor Description of the Volatile Components of Ponkan Cold-pressed Peel Oil

Peak no.	Compound	Odor description[a]	$\log_3$(FD factor)[b]	Relative flavour activity[c]
1	α-Pinene	pine-like	6	2.6
2	Camphene	herbal	3	20.2
3	β-Pinene	green, fresh	7	4.7
4	Sabinene	woody	3	2.0
5	Myrcene	sour, pungent, metallic	6	2.0
6	α-Phellandrene	herbal, chalky	5	13.3
7	α-Terpinene	herbal, green	5	8.9
8	Limonene	pungent, lemon-like	8	0.4
9	β-Phellandrene	pine-like	6	nq
10	(*Z*)-β-Ocimene	citrusy	3	15.4
11	γ-Terpinene	gasoline-like	7	1.5
12	*p*-Cymene	green, fresh	6	9.1
13	Terpinolene	fresh, sweet	5	5.3
14	Octanal	sweet, ponkan-like	7	3.3
15	Nonanal	sweet, citrusy	5	15.7
17	δ-Elemene	sweet, fruity, citrusy	6	17.6
18	Citronellal	sweet, geranium-like	6	9.1
19	Decanal	sour, acidic, ponkan-like	5	4.4
20	Linalool	floral, sweet	7	4.3
21	Octanol	sweet, floral	7	22.5
22	β-Elemene	floral, ponkan-like	5	7.5
23	Terpinen-4-ol	sweet	6	22.0
24	(*E*)-2-Decenal	fragrant	2	9.5
25	(*E*)-β-Farnesene	citrusy, floral	5	17.1
26	Neral	fresh, fragrant	2	13.5
27	α-Terpineol	grassy, tea-like	6	9.1
28	Dodecanal	sour, grassy	5	18.5
29	Germacrene D	cut-grass-like	6	12.8
30	Geranial	grassy	5	17.3
31	α-Farnesene	herbal	5	15.8
33	Citronellol	herbal, fruity	6	15.8
34	Perilaldehyde	oily	6	9.1
35	2,4-Decadienal	nutty	4	19.7
36	Germacrene B	oily	5	16.7
37	Thymol	green, grassy	6	17.4
38	β-Sinensal	grassy	5	17.3
39	α-Sinensal	green, grassy	6	9.1

[a], Odor description at the GC-sniffing port during GC/O.
[b]: FD factor: flavor dilution factor in the DB-Wax column.
[c], Relative flavour activity = log (FD factor)/$S^{0.5}$, where S is the percentage weight.
nq, Not quantified.

Table 4. Composition of the Odorants in the Aroma Model of Ponkan Cold-pressed Peel Oil

Compound	Concentration in ponkan oil (% w/w)	Stock solution (% in Milli-Q water)	Volume of stock solution in aroma model (μl)
Octanal	1.0	0.01	50
Decanal	0.3	0.01	50
Limonene	80.3	0.08*	2400

*, Stock solution was prepared from (*R*)-(+)-limonene.

aroma model. It has been reported that (*R*)-(+)-limonene (99.3%) was superior to (*S*)-(−)-limonene (0.7%) in ponkan cold-pressed oil.[13] This interesting result was applied to the ponkan aroma model preparation. A variety of aroma models were constructed and tested until the aroma had been reproduced. Stock solutions of (*R*)-(+)-limonene, octanal and decanal were respectively prepared at concentrations of 0.08%, 0.01% and 0.01% (w/w) in Milli-Q water. A 2500 μl aroma model (Table 4) consisting of 2400 μl of limonene, 50 μl of octanal and 50 μl of decanal was presented for evaluation as aliquots in 30-ml brown vials. It was demonstrated, as shown in Table 5, that the aroma model was similar to the ponkan cold-pressed oil aroma, for which the hedonic test score was 6.9 (S.D. = 1.27), meaning that the odor of the aroma model was between close and moderately similar to that of ponkan cold-pressed oil.[30] The highest score for similarity in a hedonic test is 9,

Table 5. Sensory Test for the Similarity of the Model to the Ponkan Aroma

Panelist	Run			S.D.[a]
	1	2	3	
a	8	8	8	0.00
b	6	6	7	0.58
c	5	7	4	1.53
d	6	8	8	1.15
e	8	7	7	0.58
f	8	7	9	1.00
g	6	7	7	0.58
h	6	8	8	1.15
i	7	7	6	0.58
j	7	7	8	0.58
k	4	4	6	1.15
l	8	7	7	0.58
m	7	6	7	0.58
n	8	6	7	1.00
Average		6.86		1.27[b]

Similarity is expressed by score from 1 to 9: 1, extremely different; 9, extremely similar.
[a], Standard deviation.
[b], S.D. of all the scores.

and the lowest is 1. The reliability of this result is good, because the standard deviation of all the scores was as low as 1.3. The omission test[24)] also supported the result of the hedonic test in that both compounds, octanal and decanal, were found essential to produce the characteristic aroma of ponkan.

This study on the characteristic odor components of ponkan was carried out by a comprehensive analytical procedure, including analyses of ponkan cold-pressed oil and its oxygenated fraction, GC/O and AEDA analyses and then sensory analyses of the constructed aroma model. The results reveal the important roles of octanal and decanal in the characteristic ponkan odor, with (*R*)-(+)-limonene as a background characteristic of the whole aroma.

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