Multivariate Analyses and Characterization of Volatile Components in Citrus Species

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The composition of volatile components in 18 *Citrus Archicitrus* species, 17 *Citrus Metacitrus* species and two *Fortunella* species, according to the classification system established by Tanaka, was investigated using headspace gas chromatography-mass spectrometry. The composition of 48 volatile compounds in the juice of these species was determined, and principal component analysis was performed. Scores for the 1st and 2nd principal components are plotted as a scatter diagram. In *Citrus Archicitrus*, samples of *Citrophorum*, *Cephacitrus* and *Aurantium* formed individual groups, but others did not. In *Citrus Metacitrus*, samples of *Acrumen* were divided into two different groups. The composition of volatile components in citrus juice was consistent with each grouping of Tanaka's system, in particular, in the case of dividing the *Citrus* genus into the two subgenera *Archicitrus* and *Metacitrus*.

Keywords: Citrus, volatile component, headspace gas, SPME, characterization, classification

Introduction

Citrus fruits are cultivated in many countries the world over, and are widely consumed because of their preferable flavors. The majority of citrus fruits are used for the production of citrus juice. The aroma of citrus juice typically consists of several odor components, including esters, aldehydes, alcohols, ketones and hydrocarbons. Extraction methods such as headspace extraction coupled to gas chromatography-mass spectrometry (GC-MS) have been used for the analysis of volatile components in food (Wu and Wang, 2000; Akiyama *et al.*, 2005), particularly those in citrus (Steffen and Pawliszun, 1996; Jia *et al.*, 1998; Braddock *et al.*, 2004).

Previously, most of the studies on headspace volatile components of citrus juices had been performed on orange juice (Jordán *et al.*, 2005; Mahattanatawee *et al.*, 2005; Berlinet *et al.*, 2007). In contrast to the research conducted on oranges, less information has been reported for other citrus fruits (Tamura *et al.*, 1999; Dharmawan *et al.*, 2007). In addition, few studies comparing volatile components among

several citrus species have been performed. Sawamura *et al.* (1991) classified 37 kinds of citrus fruits into 4 groups using oxygenated components. González-Mas *et al.* (2011) differentiated 4 citrus species from each other using volatile components. Furthermore, a study comparing chemical classification using volatile components and morphological classification, such as Tanaka's classification (Tanaka, 1969), would be useful in resolving *Citrus* taxonomy.

Materials and Methods

Materials The citrus fruit samples used in this study are listed according to Tanaka's classification (Tanaka, 1969) in Table 1. The fruits were purchased from the National Institute of Fruit Tree Science Okitsu Citrus Research Station (Shizuoka, Japan) in early December, 2009. Each juice was obtained by squeezing whole fruits using 260-mm-long hand-press juicers with an 84-mm diameter and 32-mm-deep pressing area.

SPME-GC, GC/MS for volatile components An aliquot (1 mL) of a juice sample was placed in a vial (22 × 38 mm) containing an internal standard solution of 10 µL of 1% cyclohexanol in water. The solution was held at 40°C for 5 min, and a Sigma-Aldrich Carboxen / PDMS SPME

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Table 1. Classification of Citrus Plants Investigated in an Analysis of Volatile Components.

Classification of citrus based on Tanaka's classification				#	Tanaka's NO.	Scientific name	Conventional name
<i>Citrus</i>	<i>Archicitrus</i>	<i>Limonellus</i>	<i>Eulimonellus</i>	1	II-4-14	<i>C. latifolia</i>	Tahiti lime
				2	II-4-15	<i>C. limettoides</i>	Sweet lime
	<i>Citrophorum</i>		<i>Citrioides</i>	3	III-7-31	<i>C. medica</i>	Citron
			<i>Limonioides</i>	4	III-8-36	<i>C. limon</i>	Eureka lemon
				5	III-8-38	<i>C. limetta</i>	Sweet lemon
			<i>Decumanoides</i>	6	III-9-48	<i>C. lumia</i>	Lumie
	<i>Cephalocitrus</i>		<i>Decumana</i>	7	IV-10-56	<i>C. grandis</i>	<i>Hirado buntan</i>
				8	IV-10-61	<i>C. grandis</i>	<i>Shaten yu</i>
		<i>Intermedia</i>	<i>Flavicarpa</i>	9	IV-11-62	<i>C. paradisi</i>	Marsh grapefruit
				10	IV-11-63	<i>C. glaberrima</i>	<i>Kinukawa</i>
				11	IV-12-74	<i>C. hassaku</i>	<i>Hassaku</i>
		<i>Aurantium</i>	<i>Medioglobosa</i>	12	V-13-78	<i>C. natsudaikai</i>	<i>Natsudaikai</i>
			<i>Aurantoides</i>				
			<i>Racemosa</i>	13	V-14-93	<i>C. aurantium</i>	Sour orange
			<i>Sinensoides</i>	14	V-16-100	<i>C. sinensis</i>	Valencia
				15	V-16-100	<i>C. sinensis</i>	Navel
				16	V-16-105	<i>C. iyo</i>	<i>Iyo</i>
			<i>Osmocitroides</i>				
			<i>Tenuicarpa</i>	17	V-17-107	<i>C. tamurana</i>	<i>Hyuganatsu</i>
			<i>Paranobilis</i>	18	V-19-111	<i>C. shunkokan</i>	<i>Shunkokan</i>
	<i>Metacitrus</i>	<i>Osmocitrus</i>	<i>Euosmocitroides</i>	19	VI-21-113	<i>C. junos</i>	Yuzu
				20	VI-21-121	<i>C. sphaerocarpa</i>	Kabosu
	<i>Acrumen</i>		<i>Euacrumen</i>	21	VII-23-123	<i>C. nobilis</i>	King
				22	VII-23-124	<i>C. unshiu</i>	<i>Unshu</i>
				23	VII-23-125	<i>C. yatsushiro</i>	<i>Yatsushiro</i>
		<i>Microacrumen</i>	<i>Anisodora</i>	24	VII-24-126	<i>C. keraji</i>	<i>Keraji</i>
				25	VII-24-127	<i>C. oto</i>	<i>Oto</i>
		<i>Citroidora</i>	<i>Megacarpa</i>	26	VII-25-130	<i>C. reticulata</i>	<i>Ponkan</i>
				27	VII-25-133	<i>C. tangerina</i>	Dancy Tangerine
				28	VII-25-134	<i>C. clementia</i>	Clementine
		<i>Microcarpa</i>		29	VII-25-136	<i>C. succosa</i>	<i>Jimikan</i>
				30	VII-26-143	<i>C. tachibana</i>	<i>Tachibana</i>
				31	VII-26-145	<i>C. kinokuni</i>	<i>Kishu</i>
				32	VII-27-148	<i>C. sunki</i>	Sunki
				33	VII-27-153	<i>C. depressa</i>	<i>Shiikuwasha</i>
				34	VII-27-154	<i>C. leiocarpa</i>	<i>Koji</i>
<i>Fortunella</i>				36		<i>F. margarita</i>	<i>Nagami kumquat</i>
				37		<i>F. crassifolia</i>	<i>Ninpou kumquat</i>

fiber (fiber length of 10 mm, 75 μ m of film thickness) was introduced into the septum-sealed vial and kept in the injection port for 20 min. SPME cryofocusing was carried out as follows. Heating of the GC column-oven was stopped and a column head (10 cm) of the GC was dipped into liquid nitrogen to collect the volatiles in splitless mode. Then, the SPME fiber was introduced into the injector and kept there

for 7 min (Hashimoto *et al.*, 2006). Volatile components of the headspace gas were analyzed using a Shimadzu GC14A gas chromatograph. A 0.25 mm i.d. $\times$ 60 m DB-WAX chemical bond type silica capillary column with 0.25- μ m-thick film (Agilent Technologies, Santa Clara, CA, U.S.A.) was employed, with the FID at 230°C and injection port at 260°C. The temperature was programmed from 40°C to

230°C at 3°C/min, and to be held at that temperature for 10 min. The helium carrier gas flow through the column was 1.0 mL/min. The headspace gas constituents were identified by gas chromatography-mass spectrometry. A Varian 3400 gas chromatograph interfaced to a Finigan MAT Model 800 ion trap detector (Thermo Fisher Scientific, Waltham, MA, USA) was used. Transfer port temperature and ion trap temperature were kept at 230°C. Mass units were monitored from 26–300 amu/s at 1250 eV. Other conditions were the same as those of GC conditions. The components were identified by comparing the mass spectra of eluted compounds with that in a library search system (MAGNUM Library-Search System: NIST MASS SPECTRA DATABASE 62,235 compounds) and their GC retention indices (RI) on a DB-WAX column with those of authentic compounds (Wako Pure Chemical Industries, Osaka, Japan; Tokyo Kasei Kogyo, Tokyo, Japan; Sigma-Aldrich, St. Louis, MO, USA) previously analyzed and stored in our private data file. The GC data was calculated using a Shimadzu GC-6R integrator. All treatments were performed in duplicate and the results are presented as mean values.

Statistical analysis Principal component analysis (PCA) and cluster analysis were performed using the SPSS statistics version 19.0. (IBM Corp., Armonk, NY, USA). The peak area percentages (%) of 48 volatile compounds of the citrus juice were used as variables for principal component analysis. Subsequently, the principal component scores calculated by PCA were used for the cluster analysis. Dendrograms were generated using the Ward method, which uses squared Euclidean distance with z-score normalized variables.

Results and Discussion

Composition of volatile compounds from various citrus juices All the citrus fruits assessed in this study were cultivated under the same weather conditions to reduce factors of variance; samples were collected from the same fields and in the same year. We divided 37 citrus samples into 18 *Archicitrus*, 17 *Metacitrus* and 2 *Fortunella* species based on Tanaka's classification (Tanaka, 1969), as the morphological characteristics differed vastly among *Archicitrus*, *Metacitrus* and *Fortunella*. Because *Pseudofortunella* resembles *Fortunella* in flavor, we decided to compare *Metacitrus* and *Fortunella*. Table 2 shows the volatile compositions in 18 *Archicitrus* citrus juices, and Table 3 summarizes those in 17 *Metacitrus* and 2 *Fortunella* citrus juices. The compounds are listed in a classification that is based on the functional group. Forty-eight compounds from more than 80 peaks on gas chromatograms were identified and quantified.

As shown in Table 2, hydrocarbons were dominant in the headspace gas of *Archicitrus* juice, followed by alcohols,

aldehydes, ketones, esters and acids. Limonene was the most abundant hydrocarbon in the *Archicitrus* juice samples tested in this study, and the second most abundant hydrocarbon was γ -terpinene or myrcene. Limonene was the main constituent of all samples in the *Archicitrus*. *Hirado buntan* (#7) and Marsh grapefruit (#9) contained the highest percentage of limonene in the *Archicitrus*. Sawamura *et al.* (1990) reported that *Hirado buntan* contained the highest percentage of limonene (86.31%), followed by myrcene (2.46%). Attaway *et al.* (1967) showed that in grapefruit the highest percentage was limonene (93%), followed by myrcene (1.9%). Our results generally agree with their data, except for minor compounds. A high percentage of γ -terpinene was present in Tahiti lime (#1) and Citron (#3). Of the alcohols, linalool was the most abundant component, followed by 1-octanol. Lumie (#6) contained exceptionally large amounts of linalool and 1-octanol. *Limonellus* (II) samples contained a low percentage of oxygenated compounds, except for alcohols. Lumie (#6) contained the highest percentage of oxygenated compounds in the *Archicitrus*; however, intermediate percentages of these were detected in other *Citrophorum* (III) citrus. In *Archicitrus* citrus, *Cephalocitrus* (IV) samples, except *Kinukawa* (#10), contained higher percentages of hydrocarbons. *Aurantium* (V) samples contained low percentages of oxygenated compounds, except for aldehydes; however, *Iyo* (#16) contained a small amount of aldehydes.

As shown in Table 3, the most abundant component in the headspace gas of *Metacitrus* citrus juices was hydrocarbons, followed by alcohols, aldehydes, ketones, esters and acids. This composition of volatile components was quite similar to *Archicitrus*. Similarly, dominant components such as limonene, γ -terpinene, myrcene and linalool closely resemble each other. *Yatsushiro* (#23), *Oto* (#25) and *Shiikuwasha* (#33) contained the highest percentage of γ -terpinene. Inafuku *et al.* (2011) revealed *C. depressa* contained the highest percentage of γ -terpinene, except for limonene. *Kabosu* (#20) and *Keraji* (#24) contained a high percentage of myrcene, and *Keraji* contained the highest percentage of citronellol. In the *Osmocitrus* (VI) samples and *Fortunella*, high percentages of hydrocarbons were present; however, only a low percentage of oxygenated compounds was detected. Akakabe *et al.* (2008) compared the Japanese sour citrus in *Osmocitrus*, and detected a high percentage of hydrocarbons. Furthermore, Choi (2005) showed kumquat contained a high percentage of hydrocarbons; our results agree with their data. In the *Acrumen* (VII) samples, it was too difficult to identify distinguishing characteristics, because 1 citrus sample showed several characteristics. Since the 13 *Acrumen* samples exhibited highly variable characteristics, it was difficult to generalize about their characteristics.

Table 2-1. Composition of volatile components in the headspace gas of *Citrus Archicitrus* species.

#		1	2	3	4	5	6	7	8	9
Tanaka's No.		II-4-14	II-4-15	III-7-31	III-8-36	III-8-38	III-9-48	IV-10-56	IV-10-61	IV-11-62
Compound	*RI	Tahiti lime	Sweet lime	Citron	Eureka lemon	Sweet lemon	Lumie	Hirado buntan	Shaten yu	Marsh grapefruit
Hydrocarbons										
α -Pinene	1025	1.83	0.10	0.53	1.23	0.05	0.34	0.12	0.10	0.15
α -Thujene	1029	0.38	nd	0.29	0.22	nd	0.10	nd	0.01	0.01
Toluene	1037	tr	0.01	0.01	0.01	0.02	0.01	0.01	0.03	nd
β -Pinene	1114	10.50	nd	0.90	8.36	0.03	5.16	0.59	0.04	0.02
Myrcene	1168	2.10	3.27	2.48	3.10	3.21	3.71	2.37	3.47	1.93
α -Terpinene	1182	0.54	nd	0.78	0.35	0.06	0.02	nd	nd	nd
Limonene	1205	49.27	88.23	49.23	66.69	88.43	64.16	90.16	88.70	90.25
(Z)- β -Ocymene	1240	0.06	0.02	0.36	0.06	0.02	0.31	nd	0.02	0.02
γ -Terpinene	1255	18.90	nd	23.47	9.28	0.34	1.15	nd	0.47	0.07
(E)-b-Ocymene	1256	nd	nd	nd	nd	nd	nd	nd	nd	nd
p-Cymene	1277	1.31	0.38	8.29	1.24	0.92	0.01	0.68	0.56	0.18
Terpinolene	1291	1.04	0.02	1.56	0.68	0.05	0.01	0.13	0.07	0.08
δ -Elemene	1478	nd	0.03	nd	nd	0.02	nd	0.08	0.10	nd
α -Copaene	1502	0.03	nd	0.22	0.02	nd	nd	nd	0.01	0.10
β -Caryophyllene	1616	nd	nd	nd	0.37	nd	0.17	nd	nd	0.75
α -Caryophyllene	1685	0.06	0.05	0.03	0.12	0.02	0.06	nd	0.02	0.10
Germacrene D	1724	nd	0.13	nd	nd	nd	nd	nd	nd	0.09
β -Bisabolene	1748	nd	0.05	0.65	1.33	nd	0.50	nd	0.56	0.20
Acids										
Acetic acid	1449	nd	nd	nd	0.01	nd	nd	0.03	nd	nd
Alcohols										
Ethanol	933	1.05	0.88	0.04	0.19	0.06	0.02	0.09	0.31	nd
1-Penten-3-ol	1159	nd	nd	nd	0.01	nd	0.01	nd	nd	nd
Cineol	1214	0.26	0.04	0.11	nd	nd	0.01	nd	0.14	nd
1-Heptanol	1458	0.04	0.03	0.03	0.04	0.04	0.04	0.01	0.01	0.03
Linalool	1550	0.17	1.20	0.34	0.13	0.80	12.24	0.09	0.57	0.10
1-Octanol	1560	0.04	0.06	0.34	0.04	0.04	5.53	0.04	0.02	0.11
Terpinene-4-ol	1608	1.18	0.02	0.63	0.99	0.02	0.05	0.14	0.02	0.19
p-Mentha-	1633	nd	nd	0.05	nd	0.03	nd	nd	0.02	0.09
(E)-2,8-dien-1-ol										
α -Terpineol	1702	1.10	0.05	1.68	0.31	0.06	0.19	0.11	0.22	0.05
Citronellol	1768	0.88	1.33	0.26	0.52	nd	1.07	0.25	0.21	0.36
p-Cymene-8-ol	1852	0.01	nd	nd	nd	nd	0.14	nd	nd	nd
β -Elemol	2086	nd	nd	nd	0.01	nd	nd	nd	0.01	0.01
τ -Cadinol	2180	nd	nd	nd	nd	nd	0.01	nd	nd	nd
Thymol	2185	0.02	nd	0.16	0.01	0.03	0.01	0.01	0.08	0.03
Iso thymol	2215	nd	nd	0.34	0.01	nd	tr	nd	0.01	nd
Aldehydes										
Hexanal	1081	nd	0.10	nd	0.02	0.18	0.01	nd	0.10	nd
Heptanal	1185	nd	0.01	nd	nd	nd	nd	nd	0.01	nd
Octanal	1290	0.07	0.02	0.16	0.12	0.10	0.05	0.03	0.02	1.30
Nonanal	1395	0.06	0.04	0.45	0.45	nd	nd	nd	0.11	0.20
Decanal	1501	0.29	nd	0.47	0.09	0.31	0.23	0.17	0.47	1.00
Myrtenal	1638	0.05	nd	nd	nd	nd	0.03	0.01	nd	nd
(E)-2-Decenal	1647	0.02	nd	nd	0.04	nd	nd	nd	nd	0.01
Esters										
Ethyl acetate	886	0.01	0.03	0.02	0.01	0.02	0.01	0.01	0.02	nd
Heptyl acetate	1376	nd	nd	nd	nd	nd	nd	nd	nd	nd
Octyl acetate	1477	tr	nd	0.11	0.09	nd	nd	nd	0.02	0.09
Linalyl acetate	1554	nd	0.02	0.06	0.01	0.02	nd	nd	0.03	0.01
Perillyl acetate	1862	0.03	nd	0.02	0.12	nd	0.01	0.05	0.04	0.02
Ketones										
Camphor	1534	nd	nd	nd	0.01	nd	nd	nd	nd	nd
D-Carvone	1742	nd	nd	0.27	0.79	0.06	1.36	nd	0.06	nd
Hydrocarbons		86.01	92.28	88.81	93.05	93.16	75.71	94.14	94.15	93.95
Acids		0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.00	0.00
Alcohols		4.76	3.60	3.98	2.24	1.09	19.32	0.74	1.63	0.96
Aldehydes		0.49	0.18	1.09	0.72	0.59	0.32	0.21	0.72	2.50
Esters		0.04	0.05	0.21	0.23	0.04	0.02	0.06	0.10	0.12
Ketones		0.00	0.00	0.27	0.79	0.06	1.36	0.00	0.06	0.00
Total		91.29	96.11	94.36	97.04	94.92	96.73	95.17	96.67	97.54

mean (n = 2) nd: not detected; tr: trace (< 0.005%) *: RI: Retention indices

Table 2-2. Composition of volatile components in the headspace gas of *Citrus Archicitrus* species (continued).

#		10	11	12	13	14	15	16	17	18
Tanaka's No.		IV-11-63	IV-12-74	V13-78	V-14-93	V-16-100	V-16-100	V-15-105	V-17-107	V-19-111
Compound	*RI	<i>Kinukawa</i>	<i>Hassaku</i>	<i>Natsudaiddai</i>	Sour orange	Valencia	Navel	<i>Iyo</i>	<i>Hyuganatsu</i>	<i>Shunkokan</i>
Hydrocarbons										
α -Pinene	1025	0.11	0.16	0.23	0.30	0.25	0.10	0.31	0.27	0.14
α -Thujene	1029	0.04	0.04	0.05	0.04	0.04	nd	0.09	0.08	nd
Toluene	1037	nd	nd	0.01	nd	0.01	0.22	0.01	0.01	0.02
β -Pinene	1114	0.17	0.20	0.18	0.97	0.03	0.02	0.30	0.35	0.61
Myrcene	1168	2.96	3.39	2.64	2.79	3.16	2.34	2.49	1.58	3.09
α -Terpinene	1182	0.22	0.17	0.06	nd	0.14	0.05	0.05	0.16	0.09
Limonene	1205	73.17	81.14	84.23	88.84	89.45	86.85	82.60	77.48	85.71
(Z)- β -Ocymene	1240	0.05	0.02	0.02	nd	nd	0.02	tr	0.05	0.14
γ -Terpinene	1255	4.65	6.61	5.06	1.09	nd	0.26	0.79	8.36	0.39
(E)- β -Ocymene	1256	0.73	nd	nd	nd	nd	nd	6.65	nd	0.19
<i>p</i> -Cymene	1277	2.17	2.20	0.92	0.17	nd	0.40	0.64	1.12	0.50
Terpinolene	1291	0.47	0.58	0.38	0.08	0.09	0.14	0.48	0.57	0.14
δ -Elemene	1478	0.09	0.11	0.02	0.20	nd	nd	nd	nd	nd
α -Copaene	1502	0.03	nd	0.04	tr	nd	0.01	tr	0.01	0.04
β -Caryophyllene	1616	nd	nd	nd	0.04	nd	0.01	0.01	nd	0.04
α -Caryophyllene	1685	0.07	0.05	0.05	0.07	nd	0.01	0.02	0.04	0.02
Germacrene D	1724	nd	nd	0.04	0.09	0.05	0.21	0.03	nd	nd
β -Bisabolene	1748	0.04	0.03	0.06	0.12	0.32	0.32	nd	0.13	0.18
Acids										
Acetic acid	1449	nd	nd	nd	nd	0.01	nd	nd	nd	nd
Alcohols										
Ethanol	933	1.25	0.02	0.06	0.01	0.30	1.05	0.01	0.25	0.05
1-Penten-3-ol	1159	nd	nd	nd	nd	nd	nd	nd	nd	0.02
Cineol	1214	0.06	0.10	0.05	nd	nd	0.04	nd	0.06	0.05
1-Heptanol	1458	0.04	0.02	0.04	0.03	nd	nd	0.02	0.02	nd
Linalool	1550	4.86	0.18	0.13	0.25	0.15	0.48	1.48	3.19	0.91
1-Octanol	1560	1.34	0.63	0.42	0.04	0.12	1.09	0.07	0.03	0.26
Terpinene-4-ol	1608	0.15	0.08	0.12	0.13	0.18	0.58	0.13	0.38	0.38
<i>p</i> -Mentha-	1633	nd	nd	0.02	0.01	nd	nd	0.01	0.04	nd
(E)-2,8-dien-1-ol										
α -Terpineol	1702	0.19	0.19	0.12	0.12	0.09	0.11	0.10	0.32	0.54
Citronellol	1768	0.49	0.27	0.29	0.45	nd	0.22	0.09	0.05	0.03
<i>p</i> -Cymene-8-ol	1852	0.02	nd	nd	nd	nd	nd	0.01	0.02	0.03
β -Elemol	2086	0.04	nd	0.02	tr	nd	nd	nd	nd	nd
τ -Cadinol	2180	nd	nd	nd	nd	nd	nd	0.01	nd	nd
Thymol	2185	0.03	nd	0.01	0.01	0.01	0.01	0.01	0.01	0.04
Iso thymol	2215	nd	nd	nd	nd	0.01	0.01	tr	nd	nd
Aldehydes										
Hexanal	1081	0.39	0.21	0.02	nd	0.01	0.02	0.02	0.23	0.12
Heptanal	1185	nd	0.09	0.01	nd	nd	0.03	nd	0.04	nd
Octanal	1290	0.04	0.05	0.72	1.09	nd	1.27	0.16	0.37	0.02
Nonanal	1395	0.19	0.13	0.23	0.11	nd	0.13	0.09	0.15	0.73
Decanal	1501	0.20	0.19	0.81	0.53	0.90	0.85	0.25	0.15	0.29
Myrtenal	1638	nd	nd	nd	nd	nd	nd	nd	nd	nd
(E)-2-Decenal	1647	nd	nd	nd	0.04	nd	0.01	0.02	nd	nd
Esters										
Ethyl acetate	886	nd	nd	nd	tr	tr	0.01	tr	0.03	0.19
Heptyl acetate	1376	nd	nd	0.01	0.01	nd	nd	nd	nd	0.03
Octyl acetate	1477	0.15	0.08	0.24	0.13	nd	0.03	0.03	0.04	0.02
Linalyl acetate	1554	0.05	0.02	0.04	0.04	nd	0.02	0.03	0.04	0.02
Perillyl acetate	1862	0.13	0.03	0.08	tr	0.01	0.02	0.04	0.05	0.10
Ketones										
Camphor	1534	nd	nd	nd	nd	nd	nd	nd	nd	0.09
D-Carvone	1742	0.05	0.03	0.02	0.04	nd	0.02	0.03	0.02	0.06
Hydrocarbons		84.96	94.71	93.97	94.81	93.55	90.97	94.48	90.21	91.29
Acids		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Alcohols		8.46	1.48	1.28	1.04	0.86	3.59	1.95	4.36	2.31
Aldehydes		0.82	0.66	1.78	1.78	0.91	2.31	0.54	0.95	1.16
Esters		0.33	0.14	0.37	0.18	0.01	0.07	0.09	0.16	0.36
Ketones		0.05	0.03	0.02	0.04	0.00	0.02	0.03	0.02	0.16
Total		94.63	97.01	97.42	97.84	95.34	96.95	97.09	95.71	95.28

mean (n = 2) nd: not detected; tr: trace (< 0.005%) * RI: Retention indices

Table 3-1. Composition of volatile components in the headspace gas of *Citrus Metacitrus* and *Fortunella* species.

#		19	20	21	22	23	24	25	26	27	28
Tanaka's No.		VI-21-113	VI-21-121	VII-23-123	VII-23-124	VII-23-125	VII-24-126	VII-24-127	VII-25-130	VII-25-133	VII-25-134
Compound	*RI	<i>Yuzu</i>	<i>Kabosu</i>	King	<i>Unshu</i>	<i>Yatsushiro</i>	<i>Keraji</i>	<i>Oto</i>	<i>Ponkan</i>	Dancy tangerine	Clementine
Hydrocarbons											
α-Pinene	1025	1.04	0.11	0.32	0.14	0.40	0.20	0.59	0.24	0.19	0.07
α-Thujene	1029	0.28	nd	nd	0.03	0.08	0.07	0.19	0.08	0.03	0.06
Toluene	1037	0.03	0.06	tr	nd	0.01	0.01	0.01	0.01	nd	0.02
β-Pinene	1114	0.66	0.06	0.10	0.12	0.52	0.24	0.69	0.18	0.15	nd
Myrcene	1168	2.04	19.60	1.83	3.45	3.02	28.07	2.33	1.95	3.11	3.25
α-Terpinene	1182	0.03	0.07	1.25	nd	0.50	nd	0.30	0.09	nd	0.26
Limonene	1205	76.12	71.50	92.47	83.16	63.00	40.60	64.02	84.55	75.53	80.75
(Z)-β-Ocymene	1240	0.02	0.02	nd	nd	0.03	nd	0.03	0.01	nd	0.03
γ-Terpinene	1255	8.83	2.38	0.21	3.14	15.68	6.03	18.09	4.25	4.24	0.48
(E)-β-Ocymene	1256	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.38
p-Cymene	1277	1.44	0.93	0.12	1.65	3.90	0.77	1.94	0.96	1.21	0.75
Terpinolene	1291	0.08	0.24	1.23	0.36	1.12	0.41	1.16	0.37	0.43	0.22
δ-Elementene	1478	0.23	nd	nd	0.06	0.02	0.03	0.02	0.06	0.04	nd
α-Copaene	1502	0.04	nd	0.01	nd	0.06	0.08	0.10	0.01	nd	0.04
β-Caryophyllene	1616	nd	nd	tr	nd	0.15	nd	0.22	0.01	nd	nd
α-Caryophyllene	1685	nd	0.08	0.02	0.02	0.02	0.14	0.03	0.01	0.01	nd
Germacrene D	1724	0.21	nd	nd	nd	nd	0.13	nd	nd	nd	0.03
β-Bisabolene	1748	0.38	nd	0.04	nd	0.19	1.85	0.48	0.10	0.10	0.05
Acids											
Acetic acid	1449	nd	0.06	nd	0.01	nd	nd	nd	nd	nd	nd
Alcohols											
Ethanol	933	0.15	0.75	0.62	0.46	0.16	0.48	0.41	0.04	1.51	2.56
1-Penten-3-ol	1159	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cineol	1214	nd	0.02	nd	0.09	0.06	0.01	0.05	0.01	0.05	0.14
1-Heptanol	1458	0.06	0.06	tr	0.02	0.02	0.12	0.04	nd	nd	nd
Linalool	1550	0.80	0.04	0.12	0.32	2.29	1.29	1.55	0.76	4.75	3.11
1-Octanol	1560	0.01	nd	0.02	0.03	0.07	0.05	0.03	0.04	0.29	1.50
Terpinene-4-ol	1608	0.41	0.15	0.06	0.09	0.58	0.22	0.45	0.02	0.24	1.30
p-Mentha-(E)-2,8-dien-1-ol	1633	0.22	nd	tr	nd	nd	0.82	nd	nd	nd	nd
α-Terpineol	1702	nd	nd	0.04	0.07	0.48	0.32	0.52	0.13	0.25	0.19
Citronellol	1768	0.06	0.02	0.06	0.10	nd	8.19	0.25	0.04	0.05	0.27
p-Cymene-8-ol	1852	nd	nd	nd	nd	0.03	nd	nd	nd	0.03	nd
b-Elemol	2086	0.01	nd	nd	nd	0.02	0.01	0.02	nd	nd	nd
τ-Cadinol	2180	nd	nd	nd	nd	nd	nd	nd	nd	0.03	nd
Thymol	2185	0.20	nd	0.01	0.02	2.16	0.05	1.48	0.07	0.31	nd
Iso thymol	2215	0.01	nd	nd	nd	0.02	0.01	0.01	nd	nd	nd
Aldehydes											
Hexanal	1081	0.03	0.05	0.02	0.33	0.07	0.04	0.02	0.03	0.03	0.25
Heptanal	1185	0.01	nd	0.01	nd	0.03	0.18	0.19	0.04	0.21	nd
Octanal	1290	0.51	nd	tr	0.04	0.42	0.79	0.48	1.67	0.03	0.03
Nonanal	1395	0.10	nd	tr	nd	0.18	0.32	0.12	0.04	0.11	0.11
Decanal	1501	0.12	0.68	0.32	0.16	0.29	0.82	0.32	0.94	0.62	0.27
Myrtenal	1638	0.03	nd	nd	nd	0.02	nd	0.01	0.02	0.01	nd
(E)-2-Decenal	1647	nd	nd	0.01	nd	nd	0.07	nd	nd	0.02	nd
Esters											
Ethyl acetate	886	0.01	0.01	nd	0.01	nd	nd	nd	0.01	0.02	0.05
Heptyl acetate	1376	0.05	nd	0.03	nd	nd	nd	nd	nd	nd	nd
Octyl acetate	1477	nd	nd	0.04	nd	0.09	0.15	nd	0.02	nd	0.04
Linalyl acetate	1554	0.02	nd	0.01	0.01	0.03	0.03	0.02	0.01	0.03	0.03
Perillyl acetate	1862	0.01	nd	0.02	0.02	0.03	0.02	0.04	0.01	0.03	0.03
Ketones											
Camphor	1534	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
D-Carvone	1742	0.05	nd	0.03	nd	0.27	nd	0.47	0.02	0.03	nd
Hydrocarbons		91.42	95.04	97.59	92.12	88.70	78.61	90.20	92.87	85.05	86.38
Acids		0.00	0.06	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Alcohols		1.92	1.04	0.92	1.19	5.89	11.57	4.79	1.12	7.50	9.07
Aldehydes		0.81	0.73	0.35	0.53	1.01	2.22	1.14	2.74	1.04	0.66
Esters		0.10	0.01	0.10	0.05	0.14	0.20	0.05	0.04	0.07	0.16
Ketones		0.05	0.00	0.03	0.00	0.27	0.00	0.47	0.02	0.03	0.00
Total		94.30	96.88	98.99	93.91	96.01	92.60	96.65	96.79	93.69	96.27

mean (n = 2) nd: not detected; tr: trace (< 0.005%) *: RI: Retention indices

Table 3-2. Composition of volatile components in the headspace gas of *Citrus Metacitrus* and *Fortunella* species (continued).

#	29	30	31	32	33	34	35	36	37	
Tanaka's No.	VII-25-136	VII-26-143	VII-26-145	VII-26-148	VII-27-153	VII-27-154	VIII-28-159			
Compound	*RI	<i>Jimikan</i>	<i>Tachibana</i>	<i>Kishu</i>	<i>Sunki</i>	<i>Shiikuwasha</i>	<i>Koji</i>	<i>Shikikitsu</i>	<i>Nagami kumquat</i>	<i>Ninpo kinquat</i>
Hydrocarbons										
α -Pinene	1025	0.26	0.51	0.34	0.46	0.61	0.26	0.15	0.20	0.09
α -Thujene	1029	0.06	0.13	0.09	0.16	0.19	0.02	nd	nd	0.04
Toluene	1037	0.01	tr	0.01	nd	0.01	0.06	0.05	nd	0.01
β -Pinene	1114	0.22	2.00	1.19	0.42	0.77	nd	0.39	0.01	nd
Myrcene	1168	1.06	2.19	1.65	1.89	1.18	3.01	2.75	3.04	3.23
α -Terpinene	1182	0.02	0.29	0.18	0.04	0.13	nd	0.18	nd	nd
Limonene	1205	80.40	65.24	79.86	78.79	57.46	62.70	78.47	92.94	86.78
(Z)- β -Ocymene	1240	0.02	nd	0.02	tr	nd	nd	nd	0.01	nd
γ -Terpinene	1255	6.20	14.46	6.15	8.96	18.58	13.05	0.65	0.05	0.39
(E)- β -Ocymene	1256	nd	nd	nd	nd	nd	nd	nd	0.06	nd
<i>p</i> -Cymene	1277	1.01	1.11	1.73	3.14	5.13	7.78	3.84	0.10	0.24
Terpinolene	1291	0.55	0.76	0.52	0.58	1.23	1.09	0.29	0.07	0.11
δ -Elemene	1478	nd	0.83	0.07	nd	nd	0.60	0.22	0.11	0.25
α -Copaene	1502	nd	0.11	0.01	0.04	0.04	nd	nd	nd	0.01
β -Caryophyllene	1616	nd	nd	0.03	0.02	nd	nd	nd	0.02	0.03
α -Caryophyllene	1685	0.01	0.26	0.01	0.03	0.03	nd	0.14	nd	0.09
Germacrene D	1724	nd	0.25	nd	nd	0.11	nd	0.36	0.05	0.06
β -Bisabolene	1748	0.14	0.36	0.41	0.39	2.04	nd	0.09	0.05	0.26
Acids										
Acetic acid	1449	nd	nd	0.01	nd	nd	0.04	0.11	nd	nd
Alcohols										
Ethanol	933	1.65	0.24	0.56	0.03	0.14	0.30	0.45	0.08	0.03
1-Penten-3-ol	1159	nd	nd	nd	0.01	nd	nd	0.07	nd	nd
Cineol	1214	0.03	0.05	0.03	tr	nd	nd	0.14	0.02	0.06
1-Heptanol	1458	0.01	0.12	0.02	0.01	0.02	0.05	0.10	0.05	0.04
Linalool	1550	0.76	2.30	0.54	0.78	1.70	1.91	1.35	0.14	0.93
1-Octanol	1560	0.12	0.08	0.32	0.05	0.12	0.94	0.41	0.01	0.04
Terpinene-4-ol	1608	0.24	0.90	0.26	0.22	0.62	0.66	0.31	0.07	0.15
<i>p</i> -Mentha-	1633	nd	nd	0.04	0.02	0.09	nd	0.13	nd	nd
(E)-2,8-dien-1-ol										
α -Terpineol	1702	0.22	0.69	0.17	0.16	0.47	0.40	0.34	0.26	0.17
Citronellol	1768	0.05	0.56	0.49	0.01	nd	0.08	0.51	0.40	0.05
<i>p</i> -Cymene-8-ol	1852	nd	0.04	0.01	0.01	0.01	nd	nd	tr	0.19
β -Elemol	2086	nd	0.05	nd	tr	0.01	nd	0.05	tr	nd
τ -Cadinol	2180	nd	nd	nd	0.01	0.01	nd	nd	tr	0.03
Thymol	2185	0.01	0.03	0.26	0.02	0.12	0.03	nd	0.01	0.01
Iso thymol	2215	nd	0.02	0.01	tr	0.03	0.02	0.07	0.01	0.01
Aldehydes										
Hexanal	1081	0.01	0.03	0.02	tr	0.03	nd	0.29	tr	0.40
Heptanal	1185	0.02	nd	0.01	0.05	0.20	nd	0.04	0.05	nd
Octanal	1290	2.74	0.06	0.05	0.52	1.39	0.11	1.31	0.03	0.08
Nonanal	1395	nd	0.22	0.09	0.04	0.24	nd	0.63	0.02	nd
Decanal	1501	0.89	0.07	0.32	0.63	1.01	nd	0.68	0.05	0.08
Myrtenal	1638	nd	nd	nd	0.04	0.07	nd	0.10	0.01	nd
(E)-2-Decenal	1647	0.01	0.07	0.01	0.01	nd	nd	0.09	0.02	nd
Esters										
Ethyl acetate	886	0.01	0.03	0.02	0.01	0.01	0.06	0.06	tr	0.06
Heptyl acetate	1376	nd	0.01	nd	0.01	nd	nd	nd	0.01	0.03
Octyl acetate	1477	0.02	0.48	0.05	0.06	0.09	nd	0.18	nd	0.20
Linalyl acetate	1554	0.02	0.06	0.02	0.02	0.02	nd	nd	0.01	0.02
Perillyl acetate	1862	0.01	0.04	0.02	nd	0.02	0.05	nd	0.04	nd
Ketones										
Camphor	1534	nd	nd	nd	nd	nd	nd	0.15	0.01	nd
D-Carvone	1742	0.04	nd	0.12	nd	0.10	0.09	0.06	0.03	0.08
Hydrocarbons		89.95	88.49	92.27	94.94	87.50	88.57	87.57	96.71	91.58
Acids		0.00	0.00	0.01	0.00	0.00	0.04	0.11	0.00	0.00
Alcohols		3.08	5.07	2.72	1.34	3.34	4.40	3.93	1.03	1.72
Aldehydes		3.67	0.43	0.51	1.29	2.93	0.11	3.14	0.18	0.55
Esters		0.05	0.61	0.12	0.10	0.14	0.11	0.25	0.05	0.31
Ketones		0.04	0.00	0.12	0.00	0.10	0.09	0.21	0.04	0.08
Total		96.80	94.60	95.74	97.67	94.01	93.33	95.22	98.02	94.23

mean (n = 2) nd: not detected; tr: trace (< 0.005%) *: RI: Retention indices

Principal component analysis Principal component analysis (PCA) was performed in order to differentiate *Citrus* species based on the composition of volatile components. The data for the 48 volatile components was subjected to PCA.

Principal components and factor loadings for the composition of volatile components are listed in Table 4. Factor loadings indicate the relative extent of the contribution that each original variable contributes to the variance contained in each principal component. The cumulative contribution ratio for first principal component (PC1) and second principal component (PC2) were 36.76% in *Archicitrus*, 37.39% in *Metacitrus*. Previous studies showed that cumulative contribution ratios for PC1 and PC2 ranged from 33.5% to 41.05%, in an analysis of flavonoids in various citrus samples (Kawaii *et al.*, 1999; Kawaii *et al.*, 2000; Nogata *et al.*, 2006), which is in keeping with the present results.

Principal components for the volatile components in *Archicitrus* are listed in the left column of Table 4. The PC1

positively correlates with γ -terpinene most closely, followed by α -terpinene, α -thujene, α -terpineol and terpinolene, and negatively correlates with limonene most closely. PC2 positively correlates with *p*-cymene-8-ol most closely, followed by D-carvone, linalool and 1-octanol, and negatively correlates with linalyl acetate and *p*-metha-(*E*)-2, 8-dien-1-ol most closely. It was assumed that PC1 represented the hydrocarbons, and PC2 represented the oxygenated compounds.

The scores for the two PCs are plotted as a scatter diagram in Fig. 1 (a) and (b). This figure shows that the distribution of *Archicitrus* species belonging to different classes can be observed. This figure shows that three groups according to Tanaka's classification appear to be separated, although some samples were not consistent with the classification. In the *Limonellus* (II) samples, the plots for Tahiti lime (#1) and Sweet lime (#2) were separated from each other. In the *Citrophorum* (III) samples, the plots for Citron (#3) and Sweet lemon (#5) are located in the fourth quadrant and in the *Aurantium* (V) group, respectively. In addition, they were

Table 4. Principal components and factor loadings for the composition of volatile components.

	<i>Archicitrus</i>		<i>Metacitrus</i> and <i>Fortunella</i>			<i>Archicitrus</i>		<i>Metacitrus</i> and <i>Fortunella</i>	
	1	2	1	2		1	2	1	2
Eigenvalue	10.96	6.68	9.87	8.08	Factor loadings				
Contribution %	22.84	13.92	20.56	16.83	Alcohols				
Cumulative %	22.84	36.76	20.56	37.39	Linalool	0.03	0.74	0.23	0.20
Factor loadings					1-Octanol	0.04	0.73	0.01	-0.26
Hydrocarbons					Terpinene-4-ol	0.73	0.05	0.33	0.25
α -Pinene	0.72	0.31	0.17	0.63	<i>p</i> -Mentha-(<i>E</i>)-2,8-dien-1-ol	0.20	-0.50	0.40	0.09
α -Thujene	0.92	0.16	0.16	0.64	α -Terpineol	0.92	-0.19	0.67	0.50
Toluene	-0.18	-0.15	0.20	-0.42	Citronellol	0.21	0.59	0.35	0.10
β -Pinene	0.61	0.59	0.55	0.48	<i>p</i> -Cymene-8-ol	0.09	0.81	0.01	-0.12
Myrcene	-0.20	0.42	0.16	-0.05	β -Elemol	-0.03	-0.15	0.89	0.02
α -Terpinene	0.94	-0.19	-0.06	0.17	τ -Cadinol	-0.06	0.53	-0.16	-0.16
Limonene	-0.94	-0.26	-0.55	-0.52	Thymol	0.60	-0.43	0.05	0.62
(<i>Z</i>)- β -Ocymene	0.67	0.24	-0.27	0.38	Iso thymol	0.70	-0.41	0.83	-0.26
γ -Terpinene	0.95	-0.17	0.34	0.82	Aldehydes				
(<i>E</i>)- β -Ocymene	-0.11	0.05	-0.17	-0.18	Hexanal	-0.15	-0.11	0.10	-0.59
<i>p</i> -Cymene	0.76	-0.44	0.32	0.13	Heptanal	-0.15	-0.17	0.18	0.44
Terpinolene	0.90	-0.26	0.12	0.62	Octanal	-0.23	-0.36	0.14	-0.02
δ -Elemene	-0.30	-0.17	0.51	0.00	Nonanal	0.41	-0.27	0.88	-0.16
α -Copaene	0.69	-0.49	0.51	0.72	Decanal	-0.18	-0.42	0.09	-0.03
β -Caryophyllene	0.03	0.07	0.01	0.61	Myrtenal	0.44	0.61	0.56	-0.23
α -Caryophyllene	0.33	0.20	0.79	-0.08	(<i>E</i>)-2-Decenal	0.16	0.12	0.83	-0.26
Germacrene D	-0.41	-0.21	0.85	-0.26	Esters				
β -Bisabolene	0.43	0.16	0.39	0.43	Ethyl acetate	-0.02	0.00	0.35	-0.57
Acids					Heptyl acetate	-0.09	-0.07	-0.15	-0.02
Acetic acid	-0.19	0.04	0.45	-0.66	Octyl acetate	0.18	-0.42	0.73	0.03
Alcohols					Linalyl acetate	0.28	-0.58	0.29	0.49
Ethanol	0.08	0.05	-0.24	-0.21	Perillyl acetate	0.17	-0.01	0.02	0.46
1-Penten-3-ol	0.09	0.44	0.61	-0.59	Ketones				
Cineol	0.58	-0.05	0.31	-0.45	Camphor	-0.02	0.02	0.61	-0.61
1-Heptanol	0.36	0.26	0.81	-0.07	D-Carvone	0.30	0.75	0.09	0.59

Fig. 1. Scatter diagram of principal component analysis based on the composition of volatile components in citrus juice (a) and a detail of the plot (b).

Symbols: ■, *Limonellus* (II); ◆, *Citrophorum* (III); □, *Cephacitrus* (IV); ◇, *Aurantium* (V)

Roman numerals indicate Tanaka's category number. See Table 1 for the identification of numbers.

Fig. 2. Scatter diagram of principal component analysis based on the composition of volatile components in citrus juice (a) and a detail of the plot (b).

Symbols: ▲, *Osmocitrus* (VI); △, *Acrumen* (VII); ●, *Pseudofortunella* (VIII); ○, *Fortunella*.

Roman numerals indicate Tanaka's category number. See Table 1 for the identification of numbers.

separated from those of Eureka lemon (#4) and Lumie (#6). Lota *et al.* (2002) performed PCA on volatile components in the peels of lemons and limes, and showed three major chemotypes in lemon peel oils and four chemotypes in lime peel oils. It was assumed that the *Limonellus* (II) and the *Citrophorum* (III) did not separate cleanly. Species of the *Cephacitrus* (IV) formed a group separate from the others, except for *Hirado buntan* (#7). In the *Aurantium* (V) samples, the plots for *Natsudaidai* (#12) and *Hyuganatsu* (#17) are close to each other, and those of others in this section formed a group within Sweet lemon (#5).

Principal components for the volatile components in *Metacitrus* and *Fortunella* are listed in the right column of Table 4. The PC1 positively correlates with β -elemol most closely, followed by nonanal, germacrene D, isothymol, (*E*)-2-decanal and 1-heptanol, and negatively correlates with limonene most closely. The PC2 positively correlates with γ -terpinene and α -copaene closely, and negatively correlates with acetic acid and camphor closely. It was assumed that PC1 represents the oxygenated compounds, and PC2 represents the hydrocarbons.

The scores for the two PCs are plotted as a scatter dia-

gram in Fig. 2 (a) and (b). The plot for *Shikikitsu* (#35) is apart from other groups. In the *Osmocitrus* (VI) samples, the plots for *Yuzu* (#19) and *Kabosu* (#20) are located in the second quadrant and the third quadrant, respectively. Akakabe *et al.* (2008) compared the Japanese sour citruses in *Osmocitrus*, and showed that the volatile composition of *Yuzu* differed from that of *Kabosu*. Interestingly, samples belonging to group VII could be divided into 2 different subgroups: VIIa and VIIb. Subgroup VIIa consists of 5 species of the VII group (i.e., *Yatsushiro* (#23), *Keraji* (#24), *Oto* (#25), *Tachibana* (#30) and *Shiikuwasha* (#33)), whereas subgroup VIIb contained 8 species (i.e., *Unshu* (#22), *Ponkan* (#26), *Dancy tangerine* (#27), *Clementine* (#28), *Jimikan* (#29), *Kishu* (#31), *Sunki* (#32) and *Koji* (#34)). *Shikikitsu* (#35) which belongs to the *Pseudofortunella* (VIII) section has a characteristic kumquat-like flavor. However, the plots of *Nagami* kumquat (#36) and *Ninpo* kumquat (#37) belong to the *Fortunella* genus, and that of *Shikikitsu* (#35) were located far apart.

Sawamura *et al.* (1991) classified 37 kinds of citrus fruits into 4 groups using oxygenated components. The classification data differed in general from our result because we analyzed by PCA using different variables from those

of Sawamura *et al.* (1991). Nogata *et al.* (2006) separated 45 kinds of citrus fruits by PCA of flavonoids. Our results showed some similarity to their data, and it appears that the volatile components were associated in some way with flavonoids.

Cluster analysis The dendrogram generated by cluster analysis based on PC1 and PC2 in *Archicitrus* is shown in Fig. 3. Citron (#3), Lumie (#6), Tahiti lime (#1) and Eureka lemon (#4) are separated from other species. Species of *Cephalocitrus*, except *Hirado buntan* (#7), and those of *Aurantium*, except Valencia (#14) and *Iyo* (#16), are close to each other.

The dendrogram generated by cluster analysis based on PC1 and PC2 in *Metacitrus* and *Fortunella* is shown in Fig. 4. The species in these sections are roughly classified into three groups (C-1, C-2 and C-3). The C-1 includes *Yuzu* (#19) of *Osmocitrus* and the *Acumen Microacumen Citroidora Microcarpa* samples, except for *Tachibana* (#30) and *Shiikuwasha* (#33). C-2 includes *Kabosu* (#20) of *Osmocitrus*, the *Fortunella*, the *Acumen Euacrumen* samples and the *Acumen Microacumen Citroidora Megacarpa* samples. C-3 includes *Yatsushiro* (#23), *Oto* (#25), *Keraji* (#24), *Shiikuwasha* (#33) and *Tachibana* (#30), and is separated from C-1 and C-2

Fig. 3. Dendrogram generated from cluster analysis based on the PC1 and PC2 in *Archicitrus*.

Roman numbers in parentheses indicate Tanaka's category number. See Table 1 for the identification of numbers.

Fig. 4. Dendrogram generated from cluster analysis based on the PC1 and PC2 in *Metacitrus* and *Fortunella*.

Roman numbers in parentheses indicate Tanaka's category number. See Table 1 for the identification of numbers.

groups. *Shikikitsu* (#35) is distinguished from other species.

In conclusion, the composition of volatile components in citrus species revealed their characteristics and the correlation among samples. The composition of volatile components in citrus juices classified by Tanaka's system was consistent with each section, in particular, in the case of dividing the *Citrus* genus into the two subgenera *Archicitrus* and *Metacitrus*.

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