Food Chemistry 115 (2009) 1042–1047

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Chemical and aroma profiles of yuzu (*Citrus junos*) peel oils of different cultivars

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ARTICLE INFO

Article history:
Received 16 October 2007
Received in revised form 3 September 2008
Accepted 7 December 2008

Keywords:
Essential oils
Citrus
Aroma
Citrus junos
Yuzu
GC-olfactometry
AEDA

ABSTRACT

The essential oils of six different yuzu cultivars, Kumon (KUM), Nagano (NAG), Yasu (YAS), Jimoto (JIM), Komatsu Sadao (KOS) and Komatsu Koichi (KOK), were extracted by cold-pressing method. A total of 69 compounds of the six samples were identified. Application of GC-olfactometry and aroma extraction dilution analysis technique in three-fold stepwise dilution of the neat oil for all samples indicated eight odourants with the highest flavour dilution (FD) values. Those were limonene, α-pinene, α- and β-phellandrene, myrcene, γ-terpinene, (*E*)-β-farnesene and linalool. 'KOS' was differentiated from the other oil samples by showing the highest number of components having yuzu-like odour notes and also from the PCA analysis of the FD-factor values. This is the first time the aroma characteristics of yuzu essential oils of specified cultivars were investigated.

1. Introduction

Yuzu (*Citrus junos* Sieb. ex Tanaka) originated in China and spread to Japan and Korea around the eighth century. This fruit has an important commercial value as compared to other sour citrus fruit and has become very popular in Japan. Yuzu fruit and its juice have been traditionally used in making vinegar and seasoning. Some products of yuzu own their commercial brand such as "Ponzu" sauces. The peel of yuzu fruit is commonly used in Japanese cuisine and processing ingredient in paste, marmalade and jelly. Yuzu is industrially used in sweet production, beverages, cosmetics and perfumery, and also in aromatherapy (Sawamura, 2005). This fruit has been known for its antioxidant activity that was reported to be higher in peel than in flesh (Yoo, Lee, Park, Lee, & Hwang, 2004) and anti-carcinogenic property (Sawamura, Wu, Fujiwara, & Urushibata, 2005). Yuzu fruit has been used for almost all parts of its peel, juice and seed. Most likely, yuzu is well-known by its pleasant aroma from the outer rind. Recently yuzu essential oil has gained a great interest due to its unique organoleptic properties. The production of yuzu in Japan was estimated to be around 20,000 tons in 2004. Such a current production, however, does not meet the demands of Japanese consumers.

A vast number of studies on the volatile constituents of yuzu cold-pressed oil have been carried out by using gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) equipped with packed column (Kusunose & Sawamura, 1980), glass capillary (Ohta, 1983) and fused-silica capillary column (Njoroge, Ukeda, Kusunose, & Sawamura, 1994; Njoroge, Ukeda, & Sawamura, 1996, Song, Sawamura, Ito, & Ukeda, 1999). However, there is still lack of information on the aroma key compounds of yuzu flavour. GC-olfactometry (GC-O) is a method using human nose as a detector to reveal whether a compound has odour or not and describe the quality of the perceived odour for each separated compound emerging from the GC. This method is usually coupled with other techniques such as aroma extraction dilution analysis (AEDA) (Grosch, 1994), CharmAnalysis (Acree, 1997), and Osme analysis (Miranda-Lopez, Libbey, Watson, & McDaniel, 1992). A study on the aroma characteristics of yuzu cold-pressed oil using GC-O and AEDA technique has been reported. In that study, Song *et al.* claimed that there remained unknown compounds presenting a yuzu-like aroma with high FD-factor (Song, Sawamura, Ito, Kawashimo, & Ukeda, 2000).

From the viewpoint of citrus taxonomy, the cultivar identification of yuzu is still ambiguous. Due to the change of climate, cultivation habit and a long cultivation period, yuzu species includes many different cultivars, which are available in the market with the only name of "yuzu". Each cultivar though closely linked to each other by their appearance, they had somewhat difference in the insect-resistance and/or aroma. Taxonomists identified yuzu cultivars by its morphology (leaf shape, flower colour, fruit size and seed) or using isozyme analysis (Rahman, Nito, & Isshiki, 2001). It is the fact that yuzu is available in a diversity of cultivars and its flavour has been extensively studied. However, the exact cultivar investigated was not mentioned.

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doi:10.1016/j.foodchem.2008.12.024

The aim of this study was, therefore, to obtain adequately the aroma characteristic profile of yuzu essential oil from different cultivars belonging to this species by using GC–MS and GC-O associated with AEDA technique. In this paper, the cold-pressed yuzu oils of the six cultivars harvested in Japan were analysed and the results of the volatiles and their odour characteristics will be presented.

2. Materials and methods

2.1. Materials

The six yuzu cultivars: 'Jimoto' (JIM); 'Komatsu Koichi' (KOK); 'Komatsu Sadao' (KOS); 'Kumon' (KUM); 'Nagano' (NAG) and 'Yasu' (YAS), were collected from the Kochi Fruit Experimental Station, Japan in November, 2005. The peel oil was extracted from the flavedo by the hand-pressing and obtained in a brine solution on ice. The extracts were centrifuged at 4000*g* for 15 min at 4 °C. The supernatants were dehydrated with anhydrous sodium sulphate at 5 °C for 24 h and then filtered. The neat oil was stored at −21 °C until analysed. Authentic chemicals used for identification and characterisation of the oil components were from Wako Pure Chemical Industries (Japan), Aldrich Chemical Co. (USA), Fluka Fine Chemicals (Switzerland), Nacalai Tesque Inc. (Japan) and Tokyo Kasei Kogyo Co. Ltd. (Japan).

2.2. GC–MS condition

The composition analysis of the oil was carried out by using a gas chromatograph–mass spectrometer (GC–MS QP-5050A, Shimadzu, Kyoto) equipped with two capillary columns, a polar DB-Wax column, 60 m × 0.25 mm i.d., film thickness 0.25 μm (J&W Scientific, Folsom, CA, USA), and a non-polar DB-1 column, 60 m × 0.25 mm i.d., film thickness 0.25 μm (J&W Scientific, Folsom, CA, USA). These two different columns were used alternatively. The column temperature was programmed to rise from 70 (2 min hold) to 230 °C (20 min hold) at 2 °C/min. The injector and detector temperatures were at 250 °C. Nitrogen was the carrier gas at a flow rate of 0.8 ml/min. Mass spectra in the electron impact mode (MS-EI) was generated at 70 eV and the ion source temperature was 250 °C. An oil sample of 0.2 μl was injected in the split mode injection.

2.3. GC-olfactometry (GC-O) and aroma extraction dilution analysis (AEDA)

Samples were prepared for GC-O from the neat oil by making a set of serial dilutions with a three-fold dilution using acetone for each sample. The sample was analysed by two sniffers who had been trained. GC-O was performed by means of a gas chromatograph (GC-17A, Shimadzu) equipped with a DB-Wax wide-bore fused-silica capillary column, 60 m × 0.53 mm i.d., film thickness 1 μm (J & W Scientific, Folsom, CA, USA) connected to a humidifier ODO II (SGE, Japan), and an FID. The GC conditions were as given above for the GC–MS. An oil sample of 0.5 μl was injected. At the end of the column, the effluent was split into the FID and sniffing port at the ratio of 1:5 (by vol.). The flow rate of nitrogen carrier gas was 3.5 ml/min. All dilutions were sniffed in triplicate until no odour was detected in the maximum diluted sample. The highest dilution at which an individual component could be detected was defined as the flavour dilution (FD) factor for that odourant.

2.4. Identification and quantitative determination

The volatile components were identified on the basis of linear retention index (RI) and by the comparison of mass spectra with MS data of reference compounds, by peak enrichment on co-injection with authentic standards if necessary, and also by comparison with previously studied. The linear retention indices were determined for all constituents by using a homologous series of *n*-alkanes (C_9–C_{27}). The two internal standards were used for quantitative analysis: *n*-hexanol being for the peaks up to linalool and methyl myristate for the ones after linalool in the eluted order. The ratio of the neat oil to the two internal standards was 150:1:1.

2.5. Statistical analysis

All data analysis was carried out using SPSS software for Windows (version 13.01; SPSS Inc., USA). Principal component analysis (PCA) was used to resolve the FD-factor data and group the cultivar samples. Pearson's product moment correlation (2-tailed) was used to examine the relationship between odour concentration and odour intensity (FD-factor) of the odourants characterised. One-way ANOVA with Tukey post hoc analysis was applied to test the differences between the means of concentration and FD-factor of the components identified in the oils of the six yuzu cultivars.

3. Results and discussion

3.1. Yuzu samples

All yuzu trees were grown under the same climatic and cultural conditions. The identical extraction method and analytical conditions for all the samples were also carried out. Therefore, it was possible to compare the volatile composition and aroma characteristics of these six yuzu cultivars.

The average weigh of fruit of the six cultivars were from 114.1 to 231.8 g/fruit. The yuzu juices were very sour with pH of 2.37–2.56 and the total soluble solid were ranging from 8.0 to 8.6 °Brix.

3.2. Identification of the volatile components

Sixty-nine compounds were identified, constituting about 98.5–99.6% of the entire volatile concentration as shown in Table 1. The results are expressed as relative weight percentages calculated from the peak areas. The total content of these yuzu oils was mostly summed up by 18 monoterpenes. Amongst them, the most predominant was limonene (63.1–68.1%), followed by γ-terpinene (11.4–12.5%), β-phellandrene (4.6–5.4%), myrcence (3.0–3.2%) and α-pinene (2.3–2.7%). Pseudolimonene, a minor monoterpene compound, was tentatively identified for the first time in yuzu essential oil. Sesquiterpene hydrocarbons occur in a small amount in most citrus essential oils. However, they are important in the characteristic aroma of many kinds of citrus fruits (Shaw, 1979). Bicyclogermacrene was present in the greatest amount (1.5–2.0%) with respect to the other sesquiterpenes in most of the oils analysed. The two isomeric sesquiterpenes (*Z*)- and (*E*)-β-farnesene were also quantified, and the (*E*) isomer was predominant at a higher proportion of 0.9–1.3%. The presence of bicyclogermacrene and (*E*)-β-farnesene in yuzu oil was previously reported at significant amount by Sawamura (2000). α-Ylangene was the sesquiterpene that appeared in only "KUM" oil sample. Germacrenes including germacrene B and D are often identified in citrus oils such as in lime oil (Lan Phi, Minh Tu, Nishiyama, & Sawamura, 2006). Previous study reported that germacrene D was the significant constituent of Japanese yuzu oil (Njoroge et al., 1994). In this study, germacrene B and D accounted for 0.1–0.2% and 0.3–0.4%, respectively. Other sesquiterpenes such as δ-elemene (0.1–0.2%) and β-caryophyllene (0.3%) also commonly existed.

Monoterpene and sesquiterpene alcohols were the minor component in these yuzu oils. Linalool (1.9–2.9%) and α-terpineol

Table 1
Volatile components of the six cultivars of yuzu cold-pressed peel oils.

Peak No.	Compound	RI		Relative concentration (%)						Identification	Reference
		DB-Wax	DB1	JIM	KOK	KOS	KUM	NAG	YAS		
1	α-Pinene	1040	943	[b]2.7	[a,b]2.6	[a,b]2.6	[a,b]2.5	[a,b]2.4	[a]2.3	RI, MS	1–5
2	Camphene	1085	957	tr	tr	tr	tr	tr	tr	RI, MS	1,2,4,5
3	β-Pinene	1126	980	[b]1.1	[a,b]1.1	[a,b]1.0	[a,b]1.0	[a,b]1.0	[a]0.9	RI, MS	1–5
4	Sabinene	1135	977	0.5	0.5	0.5	0.5	0.5	0.4	RI, MS	1–5
5	δ-3-Carene	1163		tr	tr	tr	tr	nd	tr	RI, CI	
6	Myrcene	1171	991	[a]3.2	[a]3.1	[a]3.0	[a]3.0	[a]3.1	[a]3.0	RI, MS	1–5
7	α-Phellandrene	1179	1006	0.9	0.8	0.9	0.9	0.8	0.7	RI, MS	1, 2, 4, 5
8	Pseudolimonene[m]	1184	1011	tr	tr	tr	tr	tr	tr	MS	
9	α-Terpinene	1193	1018	0.3	0.3	0.3	0.3	0.3	0.3	RI, MS	1–5
10	Limonene	1222	1037	[a]63.1	[a]63.2	[b]67.8	[b]66.6	[b]65.3	[b]68.1	RI, MS	1–3,5
11	β-Phellandrene	1229		5.4	5.1	5.3	5.2	4.8	4.6	RI, MS	1,2
12	(*Z*)-β-Ocimene	1243	1047	tr	tr	tr	tr	tr	tr	RI, MS	1,2,4
13	γ-Terpinene	1262	1061	[b]12.5	[b]12.1	[a]11.4	[a]11.5	[b]12.3	[a]11.6	RI, MS	1–5
14	(*E*)-β-Ocimene	1265		tr	tr	nd	tr	nd	tr	RI, MS	4
15	*p*-Cymene	1282	1021	0.6	0.6	0.4	0.5	0.4	0.4	RI, MS	1–5
16	Terpinolene	1295	1088	0.7	0.7	0.6	0.6	0.7	0.6	RI, MS	1–5
17	Octanal	1297		tr	tr	tr	tr	tr	tr	RI, MS	1,2,4,5
18	Tetradecane	1397		tr	nd	nd	tr	nd	tr	RI	4
19	Nonanal	1399		tr	tr	tr	tr	tr	tr	RI, MS	1,4,5
20	α-*p*-Dimethyl styrene[m]	1445	1082	tr	tr	tr	tr	tr	tr	MS	1,3,4
21	1,3,8-*p*-Menthatriene[m]	1449	1135	tr	tr	tr	tr	tr	tr	MS	1
22	(*Z*)-Limonene oxide	1458		tr	tr	nd	nd	tr	nd	RI, CI	2–5
23	α-Cubebene	1467	1356	tr	tr	tr	tr	tr	tr	RI, MS	1,2,4
24	*trans*-Sabinene hydrate	1475	1064	0.1	0.1	tr	tr	0.1	tr	RI, MS	2,4,5
25	δ-Elemene	1479	1343	0.2	0.2	0.2	0.2	0.2	0.1	RI, MS	1–5
26	(*E*)-Linalool oxide	1481		tr	tr	nd	tr	tr	tr	RI, MS	1,2,4,5
27	Bicycloelemene[m]	1489		tr	tr	tr	tr	tr	tr	MS	5
28	α-Ylangene	1494		nd	nd	nd	tr	nd	nd	RI, MS	4
29	(−)-α-Copaene	1501	1382	0.1	0.1	tr	tr	0.1	tr	RI, MS	2–5
30	Decanal	1504	1192	tr	tr	tr	tr	tr	tr	RI, MS	1,2,4,5
31	β-Cubebene	1548		0.1	tr	tr	tr	tr	tr	RI, MS	1,2
32	Linalool	1555	1093	[d]2.8	[d]2.9	[a]1.9	[b,c]2.4	[c,d]2.6	[a,b]2.1	RI, MS	1–5
33	*cis*-Sabinene hydrate	1560		0.1	0.1	tr	0.1	0.1	0.1	RI, MS	4
34	(*E*)-α-Bergamotene	1564	1408	tr	tr	tr	tr	tr	tr	RI, MS	2,4
35	β-Elemene	1597	1394	0.1	0.1	0.1	0.1	0.1	0.1	RI, MS	1–5
36	β-Caryophyllene	1607	1425	0.3	0.3	0.3	0.3	0.3	0.3	RI, MS	1–5
37	Terpinen-4-ol	1612	1172	0.1	0.1	tr	0.1	0.1	0.1	RI, MS	1,2,4,5
38	Aromadendrene	1626		tr	nd	nd	nd	nd	nd	RI, MS	1–4
39	Caryophyllene[n]	1644		tr	tr	nd	tr	nd	nd	RI, MS	
40	γ-Elemene	1646	1559	tr	tr	tr	tr	tr	tr	RI, MS	3,4
41	(*E*)-2-Decenal	1651		tr	tr	nd	tr	tr	tr	RI, CI	1,2,4,5
42	(*Z*)-β-Farnesene	1667	1455	tr	tr	tr	tr	tr	tr	RI, MS	
43	(*E*)-β-Farnesene	1671		c1.3	[b,c]1.1	[a]0.9	[a]0.9	[c]1.2	[a,b]0.9	RI, MS	1–4
44	α-Humulene	1680	1459	0.1	0.1	0.1	0.1	0.1	0.1	RI, MS	2–4
45	α-Terpineol	1706	1183	0.3	0.3	0.2	0.2	0.3	0.2	RI, MS	1–5
46	Dodecanal	1716		tr	tr	nd	nd	nd	nd	RI, MS, CI	2,4,5
47	Germacrene D	1719	1487	0.4	0.4	0.3	0.3	0.4	0.3	RI, MS	1–4
48	Guaiene	1724		nd	tr	nd	nd	nd	nd	RI, MS	5
49	α-Muurolene	1734		tr	tr	tr	tr	tr	tr	RI, MS	1–4
50	Piperitone	1740		tr	tr	tr	tr	tr	tr	RI, MS	1,2,5
51	Bicyclogermacrene	1746	1500	[b,c]2.0	[c]2.0	[a]1.5	[b,c]1.8	[c]2.0	[a,b]1.7	RI, MS	1–4
52	α-Farnesene[n]	1753		nd	nd	nd	nd	tr	nd	RI, MS, CI	
53	δ-Cadinene	1766		0.1	0.1	0.1	0.1	0.1	0.1	RI, MS	3–5
54	Citronellol	1770		tr	nd	nd	nd	nd	nd	RI, MS	2,4,5
55	β-Sesquiphellandrene	1777		0.1	0.1	0.1	0.1	0.1	0.1	RI, MS	1,2,4
56	Perillaldehyde	1790		tr	tr	tr	tr	tr	tr	RI, MS	1,2,4,5
57	Nerol	1794		tr	tr	nd	nd	tr	tr	RI, MS	1–3,5
58	Germacrene B	1841		0.2	0.2	0.1	0.1	0.2	0.1	RI, MS	1,2
59	β-Ionone	1953		tr	tr	nd	tr	tr	nd	RI, CI	
60	Perillyl alcohol	2007		tr	tr	tr	tr	tr	tr	RI, MS, CI	1–5
61	(*E*)-Nerolidol	2048		tr	tr	tr	tr	tr	tr	RI, MS, CI	1,2
62	Germacrene D-4-ol[m]	2063		0.4	0.4	0.3	0.4	0.4	0.4	MS	
63	Elemol	2091		tr	tr	tr	tr	tr	tr	RI, MS	1–5
64	Spathulenol	2119		tr	tr	nd	nd	nd	nd	RI, MS	4
65	Eugenol	2166		tr	nd	nd	nd	nd	nd	RI	2,4
66	Thymol	2193	1280	0.3	0.2	0.2	0.2	0.2	0.2	RI, MS	1,3–5
67	α-Cadinol	2213		tr	nd	nd	nd	tr	nd	RI	2
68	(*E,E*)-Farnesyl acetate	2236		nd	tr	nd	nd	nd	nd	RI, CI	1,2
69	Unknown	2241		nd	tr	tr	nd	nd	tr		
70	β-Eudesmol	2248		tr	tr	tr	tr	tr	tr	RI, MS	2
	Aliphatics (1)			tr	nd	nd	tr	nd	tr		
	Monoterpenes (18)			[a,b]91. 1	[a]90.0	[b]93.6	[a,b]92.6	[a,b]91.5	[b]93.0		
	Sesquiterpenes (23)			[a]4.5	[a]4.3	[a]3.3	[a]3.6	[a]4.3	[a]3.6		
	Aldehydes (6)			[b]0.1	[c]0.1	[a]tr	[a,b]0.1	[a,b]0.1	[a]tr		
	Alcohols (16)			[c,d]3.9	[d]4.0	[a]2.7	[b]3.4	[b,c]3.7	[a]3.0		
	Esters and ketones (3)			tr	nd	tr	tr	nd	tr		
	Oxides (2)			tr	tr	nd	tr	tr	tr		
	Total (69)			[b]99.5	[a]98.5	[b]99.6	[b]99.6	[b]99.5	[b]99.6		

[m]Tentatively identified; [n]correct isomer not identified; tr: trace, peak area quantified less than 0.05%; a,b,c,d,e,f: means with different superscript are significant different ($p < 0.05$); nd: not detected. RI: identification based on retention index; MS: identification based on mass spectra; CI: identification based on co-injection with authentic chemicals; the values are means of triplicated analyses for each sample. MS-EI, *m/z* (rel. int.) of peak 8: 93 (100), 136 (32), 79 (25), 91 (20), 94 (15), 121 (11); peak 20: 132 (100), 117 (92), 115 (50), 91 (30), 131 (16), 92 (15); peak21: 119 (100), 91 (85), 134 (76), 105 (36), 92 (27), 93 (25), 117 (20); peak 27: 121 (100), 93 (54), 107 (42), 91 (23), 136 (23), 105 (20); peak 62: 81 (100), 123 (31), 105 (24), 161 (21), 95 (18), 109 (18), 93 (17); References: [1]Song et al., 1999; [2]Song et al., 2000; [3]Njoroge et al., 1996; [4]Njoroge et al., 1994; [5]Yang, Sugisawa, Nakatani, Tamura, & Takagi, 1992.

(0.2–0.3%) were predominant as the former, whilst germacrene D-4-ol (0.3–0.4%) as the latter. Thymol, a monoterpene phenol commonly found in thyme oil, presented at the amount of 0.2–0.3%.

Table 2
FD-factor values of the aroma active compounds of yuzu cold-pressed peel oils.

Peak	Compound	$\log_3$(FD-factorA)[A]					
		JIM	KOK	KOS	KUM	NAG	YAS
1	α-Pinene	7^{b}	8^{b}	5^{a}	8^{b}	8^{b}	8^{b}
2	Camphene	6^{b}	6^{b}	*	7^{b}	7^{b}	5^{b}
3	β-Pinene	$7^{a,b,c}$	8^{c}	$6^{a,b}$	$7^{b,c}$	8^{c}	5^{a}
4	Sabinene	$7^{b,c}$	$7^{b,c}$	6^{b}	8^{c}	$7^{b,c}$	4^{a}
6	myrcene	7^{b}	8^{b}	5^{a}	8^{b}	7^{b}	8^{b}
7	α-Phellandrene	7^{a}	7^{a}	6^{a}	7^{a}	$8^{a,b}$	9^{b}
8	Pseudolimonene	*	5	*	4	4	*
9	α-Terpinene[N]	6^{a}	6^{a}	5^{a}	7^{a}	7^{a}	5^{a}
10	Limonene	$8^{a,b}$	7^{a}	7^{a}	9^{b}	$8^{a,b}$	$7^{a,b}$
11	β-Phellandrene	7^{a}	7^{a}	9^{b}	$8^{a,b}$	$7^{a,b}$	6^{a}
12	(*Z*)-β-Ocimene	6	4	*	9	5	*
13	γ-Terpinene	7^{a}	7^{a}	8^{a}	6^{a}	7^{a}	7^{a}
14	(*E*)-β-Ocimene	5	4	–	*	–	*
15	*p*-Cymene	6^{a}	4^{a}	4^{a}	8^{b}	4^{a}	5^{a}
16	Terpinolene	6^{a}	$5^{a,b,c}$	4^{a}	9^{d}	$5^{a,b}$	$6^{b,c}$
17	Octanal	4^{a}	5^{a}	4^{a}	7^{b}	4^{a}	4^{a}
19	Nonanal	*	4	*	4	4	*
20	α-*p*-Dimethyl styrene	6	4	*	7	5	5
21	1,3,8-*p*-Menthatriene[N]	4	5	*	5	4	4
22	*cis*-Limonene oxide	*	4	–	–	*	–
23	α-Cubebene	*	4	*	*	4	4
24	*trans*-Sabinene hydrate	4	5	4	*	4	4
25	5-Elemene[a]	7^{b}	4^{a}	4^{a}	7^{b}	6^{b}	4^{a}
26	*trans*-Linalool furanoxide	4	*	–	*	*	*
27	Bicycloelemene[a]	5	*	6	5	*	4
28	α-Ylangene	–	–	–	5	–	–
29	(−)-a-Copaene	5	4	7	6	5	*
30	Decanal	4^{a}	5^{a}	7^{b}	4^{a}	5^{a}	4^{a}
31	β-Cubebene[N]	5^{a}	6^{a}	6^{a}	5^{a}	6^{a}	5^{a}
32	Linalool	7^{a}	8^{a}	6^{a}	7^{a}	8^{a}	7^{a}
33	*cis*-Sabinene hydrate	4	5	*	*	4	4
34	*trans*-α-Bergamotene	*	*	*	5	*	4
35	β-Elemene	$4^{a,b}$	$4^{a,b}$	7^{c}	$5^{b,c}$	$4^{a,b}$	4^{a}
36	β-Caryophyllene	5^{a}	$6^{a,b}$	$6^{a,b}$	8^{c}	$6^{b,c}$	$6^{a,b}$
37	4-Terpineol	4^{a}	5^{a}	6^{a}	6^{a}	5^{a}	4^{a}
38	Aromadendrene	4	–	–	–	–	–
39	Caryophyllene	4	4	–	*	–	–
40	γ-Elemene	4^{a}	4^{a}	7^{b}	4^{a}	4^{a}	5^{a}
41	(*E*)-2-Decenal	4	5	–	*	4	5
42	*cis*-β-Farnesene	4	4	5	*	*	7
43	*trans*-β-Farnesene	7^{a}	6^{a}	6^{a}	9^{b}	6^{a}	9^{b}
44	α-Humulene	4	5	*	5	5	6
45	α-Terpineol[N]	$6^{a,b}$	5^{a}	$6^{a,b}$	$6^{a,b}$	$6^{a,b}$	7^{b}
46	Dodecanal	*	4	–	–	–	–
47	Germacrene D	6^{a}		$6^{a,b}$	6^{a}	$6^{a,b}$	8^{b}
48	Guaiene	*	4	–	–	–	–
49	α-Muurolene	7	6	4	*	4	4
50	Piperitone	*	4	4	*	*	*
51	Bicyclogermacrene	6^{a}	$7^{a,b}$	5^{a}	$8^{b,c}$	$6^{a,b}$	9^{c}
52	α-Farnesene	5	–	–	–	*	–
53	δ-Cadinene	*	5	4	6	4	6
54	Citronellol	7	–	–	–	–	–
55	β-Sesquiphellandrene[N]	4^{a}	5^{a}	4^{a}	4^{a}	4^{a}	5^{a}
56	Perillaldehyde	4	4	*	*	*	*
57	Nerol	4	4	–	–	4	6
58	Germacrene B	*	5	*	5	4	4
59	β-Ionone	4	*	–	5	4	4
60	Perillyl alcohol	4	*	5	4	5	5
61	(*E*)-nerolidol[N]	4^{a}	4^{a}	5^{a}	5^{a}	4^{a}	4^{a}
62	Germacrene D-4-ol	*	*	6	6	4	5
63	Elemol	4	4	*	4	5	*
65	Thymol	4	4	5	6	6	*
70	β-Eudesmol	4	*	*	6	4	*

The values are means of triplicates for each sample.
[a,b,c,d]Means with different superscript are significantly different ($p < 0.05$); [N]not significant difference amongst samples.
*FD-factor values less than 3^4.
[A] The base-3 logarithm of flavour dilution factor value on DB-Wax column.

Germacrene D-4-ol was found in tangerine oil (Dugo et al., 2005), but it is tentatively identified for the first time in yuzu oil. Alcohols (2.7–4.0%) were summed up most of the oxygenated content.

The concentration of aldehydes in these samples was low compared to that of other groups identified. Six aldehydes were detected and their content ranged from trace to 0.1%. Octanal and decanal, which play a remarkable role in some citrus fruits, were also determined at very low quantity. Other minor components including one ester, two ketones and two oxides were presented in a trace amount. Statistical analysis showed that there were significant differences between the concentration of major components and of functional groups in the six samples, except for myrcene and sesquiterpene group as seen in Table 1.

3.3. Characterisation of the odourants by olfactory analyses

The samples presented green and sweet as the top notes with a background of citrusy, sour and sharp notes. The odour-active components of yuzu oils were determined on the basis of flavour dilution (FD) factor value resulted from GC-sniffing and AEDA. The odour-active volatiles were defined if their FD-factor value was $\geqslant 3^4$. The data in Table 2 showed that limonene (peak 10), α-pinene (peak 1), and α- and β-phellandrene (peaks 7 and 11, respectively) had the highest FD value. The other odourant with high FD values were myrcene (peak 6), linalool (peak 32), (*E*)-β-farnesene (peak 43) and γ-terpinene (peak 13). Amongst sesquiterpene hydrocarbons, *trans*-β-farnesene, germacrene D (peak 47) and bicyclogermacrene (peak 51) were having the highest FD-factors. Two alcohols, linalool and α-terpineol (peak 45) were the odour-active compounds amongst the alcohols found in these yuzu oils. Octanal (peak 17) and decanal (peak 30) were representative odour-active components of aldehydes. There were some significant differences between the FD-factor values of the odour-active compounds amongst the samples. However, those of γ-terpinene, 1,3,8-*p*-menthatriene, β-cubebene, α-terpineol, β-sesquiphellandrene and (*E*)-nerolidol were not significantly different amongst the six samples. The scatter plot of scores for the PCA analysis of FD-factors is shown in Fig. 1. PC1 and PC2 explain 60.2% of the total variances. The plot illustrated a clear separation between "KOS" and the other five yuzu oil samples.

Results from the Pearson's product moment correlation test revealed that there was a slightly positive correlation ($r = 0.286$, $P < 0.01$, $N = 338$) between weights of detected compounds and their FD values, in which the correlation coefficient of the "JIM"

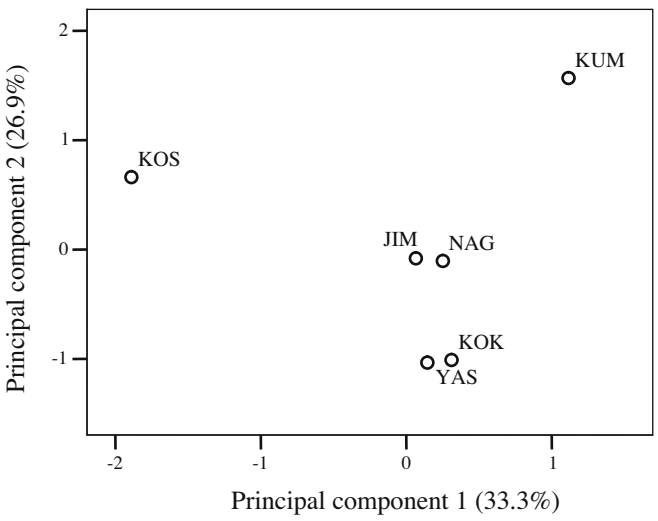

Fig. 1. Scatter plot of scores on principal components 1 and 2 of the FD-factors.

Table 3
Odour description of peaks having yuzu-like odour in the six cultivars of yuzu peel oils.

Peak no.	Odour description					
	JIM	KOK	KOS	KUM	NAG	YAS
11	Cool, minty	Minty, yuzu-like, sour	Minty, pungent, sweet	Minty (strongly), sweet	Minty, sweet	Sweet, minty
23	Floral	Green, grassy	Yuzu-like	Citrusy, herbal	Herbal, sour	Dry grassy, herbal
25	Green, grassy	Floral, green, sweet	Yuzu-like, leafy	Sour, herbal, cool	Dry grassy, herbal	Floral, citrusy
30	Herbal, grassy	Floral, sweet, waxy	Sweet, floral	Yuzu-like, floral	Grassy	Citrusy, floral
32	Citrusy, floral, fresh	Green, grassy	Yuzu-like, fruity, floral	Floral, herbal, sweet	Dry grassy, floral, herbal	Leafy, floral, herbal
35	Yuzu-like, floral	Grassy, floral	Floral, herbal	Citrusy, floral, cool	Floral, citrusy	Citrusy, cool
36	Fresh, citrusy	Grassy, citrusy	Yuzu-like, pungent	Citrusy, grassy	Citrusy, grassy	Citrusy, floral
40	Green, citrusy	Grassy, cool	Floral, yuzu-like	Citrusy, cool	Floral, citrusy	Metallic, herbal, citrusy
43	Green, herbal, yuzu-like	Cool, floral, citrusy	Yuzu-like, leafy	Citrusy, cool, floral	Green, grassy	Citrusy, floral
44	Yuzu-like	Floral, citrusy	Citrusy, floral	Cool, citrusy, floral	Citrusy, floral	Yuzu-like, grassy
45	Grassy, citrusy	Floral, citrusy	Yuzu-like, pungent	Citrusy	Yuzu-like, grassy	Yuzu-like, floral
49	Grassy	Citrusy, floral	Yuzu-like, floral	Floral, herbal	Citrusy, herbal	Herbal, citrusy
51	Herbal, leafy	Herbal, citrusy, grassy	Floral, citrusy, sweet	Floral, cool, citrusy	Citrusy, cool	Yuzu-like, herbal
53	Floral	Grassy, citrusy	Yuzu-like	Cool, citrusy, herbal	Sweet, citrusy	Citrusy, floral
55	Green, fruity, herbal	Citrusy, grassy	Citrusy, bitter	Grassy, cool, yuzu-like	Sweet, citrusy, green	Yuzu-like
57	Yuzu-like, fresh	Floral, citrusy	–	–	Grassy	Herbal, citrusy
58	Citrusy, sweet	Citrusy, herbal	Grassy, bitter	Citrusy, cool	Citrusy	Yuzu-like, grassy

sample was the highest ($r = 0.345$, $P < 0.01$, $N = 62$), followed by those of the "NAG" ($r = 0.341$, $P < 0.05$, $N = 56$), "KOK" ($r = 0.287$, $P < 0.05$, $N = 59$) and "KUM" ($r = 0.281$, $P < 0.05$, $N = 56$). Although, there was no significant correlation between the two values of the "KOS" ($r = 0.269$, $N = 50$) and "YAS" ($r = 0.229$, $N = 55$) samples at the 0.05 level. There is sufficient evidence from this study to conclude that there was a positive correlation between the odourant concentration and the odour intensity, and the higher odourant concentration usually have the higher FD-factor value. The low value of correlation coefficient may be caused by some components that existed in a trace amount, having rather high FD values. In these oil samples, such components were as camphene, α-*p*-dimethyl styrene, (*Z*)-β-ocimene, β-cubebene and nerol.

The term of sensory properties usually includes odour activity and odour quality. Odour quality was obtained by means of olfactory evaluation and description perceived by sniffing the effluent of GC for all compounds identified. The odour profiles of the six yuzu samples could be described by the same descriptors. Amongst characterised odourants, the compounds possesses yuzu-like note are often considered for its remarkable contribution in reconstruction of yuzu aroma model. In this study, those compounds representing yuzu-like odour note during GC-sniffing analysis are shown in Table 3. A total of 17 compounds of the six samples were described as having yuzu-like odour note. "KOS" owned the highest number of components having yuzu-like odour. β-Elemene, β-caryophyllene, γ-elemene, α-muurolene, bicyclogermacrene, δ-cadinene and germacrene B indicated yuzu-like and/or citrusy note in at least four out of the six samples. Bicyclogermacrene, however, little contributed to yuzu flavour as previously reported (Song et al., 2000). There were no compounds showing yuzu-like odour in all samples. Although, the six cultivars investigated had essentially yuzu characteristic odour, some differences in the aroma profiles that were recognised each other.

In conclusion, the instrumental and sensory analyses provided the chemical and aroma profiles of different commercial yuzu cultivars. Amongst them Komatsu sadao were discriminated from the other cultivars by having nine out of seventeen yuzu-like odourants and was classified into different group from the PCA analysis of FD-factor values. The difference of yuzu aroma amongst the investigated cultivars, in other words, would be relative to the odour quality (odour description) and odour intensity (FD-factor) resulted from the olfactory evaluation. This is the first time the specified cultivars of yuzu have been investigated. Though the major components were identified and characterised in these yuzu essential oils, there were also unidentified compounds presented in trace amount. Some of them exhibited yuzu-like odour from the GC-sniffing analysis. Further experiments would be carried out to identify these trace aroma compounds in yuzu oils in addition to the omission test and the reconstruction of the aroma model.

Acknowledgements

We are grateful to Sakamoto Koryo Co. Ltd., Tokyo and Mizkan Co. Ltd., Handa, Japan, for technical support.

References

Acree, T.E. (1997). GC/Olfactometry. Analytical chemistry: News and features (pp. 170–175A).

Dugo, P., Mondello, L., Favoino, O., Cicero, L., Zenteno, N. A. R., & Dugo, G. (2005). Characterization of cold-pressed Mexican dancy tangerine oils. *Flavour Fragrance Journal, 20*, 60–66.

Grosch, W. (1994). Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour and Fragrance Journal, 9*(4), 147–158.

Kusunose, H., & Sawamura, M. (1980). Aroma constituents of some sour citrus oils. *Nippon Shokuhin Kogyo Gakkaishi, 27*, 517–521.

Lan Phi, N. T., Minh Tu, N. T., Nishiyama, C., & Sawamura, M. (2006). Characterisation of the odour volatiles in *Citrus aurantifolia* Persa lime oil from Vietnam. In W. L. P. Bredie & M. A. Petersen (Eds.), *Flavor science: Recent advances and trends* (pp. 193–196). Amsterdam, The Netherlands: Elsevier.

Miranda-Lopez, R., Libbey, L. M., Watson, B. T., & McDaniel, M. R. (1992). Odour analysis of Pinot Noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). *Journal of Food Science, 57*, 985–993.

Njoroge, S. M., Ukeda, H., Kusunose, H., & Sawamura, M. (1994). Volatile components of Japanese yuzu and lemon oils. *Flavour Fragrance Journal, 9*, 159–166.

Njoroge, S. M., Ukeda, H., & Sawamura, M. (1996). Change in volatile composition of yuzu (*Citrus junos* Tanaka) cold-pressed oil during storage. *Journal of Agriculture and Food Chemistry, 4*, 550–556.

Ohta, H. (1983). Glass capillary gas chromatographic analysis of oil components extracted from yuzu (*Citrus junos*) juice. *Journal of Chromatography, 268*, 336–340.

Rahman, M. M., Nito, N., & Isshiki, S. (2001). Cultivar identification of 'yuzu' (*Citrus junos* Sieb. ex Tanaka) and related acid citrus by leaf isozymes. *Scientia Horticulturae, 87*(3), 191–198.

Sawamura, M. (2000). Volatile components of essential oils of the *Citrus* genus. In S. G. Pandalai (Ed.). *Recent research development agricultural and food chemistry* (Vol. 4, pp. 131–164). Trivandrum, India: Research Signpost.

Sawamura, M. (2005). *Citrus junos* Sieb. ex Tanaka (yuzu) fruit. In R. Dris (Ed.), *Fruits. Growth, nutrition, and quality* (pp. 1–24). Helsinki, Finland: WFL Publisher.

Sawamura, M., Wu, Y., Fujiwara, C., & Urushibata, M. (2005). Inhibitory effect of yuzu essential oil on the formation of *N*-nitrosodimethylamine in vegetables. *Journal of Agriculture and Food Chemistry, 53*, 4281–4287.

Shaw, P. E. (1979). Review of quantitative analyses of citrus essential oils. *Journal of Agriculture and Food Chemistry, 27*, 246–257.

Song, H. S., Sawamura, M., Ito, T., Kawashimo, K., & Ukeda, H. (2000). Quantitative and characteristic flavour of *Citrus junos* (yuzu) peel oil. *Flavour Fragrance Journal, 15*, 245–250.

Song, H. S., Sawamura, M., Ito, T., & Ukeda, H. (1999). Chemical compositions of the volatile part of yuzu (*Citrus junos* Tanaka) peel cold-pressed oils from Japan and Korea. *Flavour Fragrance Journal, 14*, 383–389.

Yang, R., Sugisawa, H., Nakatani, H., Tamura, H., & Takagi, N. (1992). Comparison of odour quality in peel oils of acid citrus. *Nippon Shokuhin Kagaku Kogaku Kaishi, 39*, 16–24.

Yoo, K. M., Lee, K. W., Park, J. B., Lee, H. J., & Hwang, I. K. (2004). Variation in major antioxidants and total antioxidant activity of yuzu (*Citrus junos* Sieb. ex Tanaka) during maturation and between cultivars. *Journal of Agriculture and Food Chemistry, 52*, 5907–5913.