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Novel Character Impact Compounds in Yuzu (*Citrus junos* Sieb. ex Tanaka) Peel Oil

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Yuzu (*Citrus junos* Sieb. ex Tanaka), a tree-grown fruit similar to a kind of sour orange, is widely used in Japanese food/cooking for its pleasant flavor. To clarify the odor-active volatiles that differentiate yuzu from other citrus fruits, sensory evaluations were conducted on yuzu peel oil. The results revealed that the polar part of yuzu peel oil was the source of the characteristic aroma of fresh yuzu fruit. By aroma extract dilution analysis (AEDA) of the polar volatile part of yuzu peel oil, seven odorants were newly identified as odor-active volatiles in yuzu peel oil in the highest flavor dilution (FD) factors of 128 and 32: oct-1-en-3-one, (*E*)-non-4-enal, (*E*)-dec-4-enal, 4-methyl-4-mercaptopentan-2-one, (*E*)-non-6-enal, (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (Yuzunone), and (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (Yuzuol). Among the most odor-active volatiles in yuzu, (*E*)-non-6-enal and Yuzunone were identified for the first time solely in yuzu peel oil and not in the peel of other citrus species, and Yuzuol was identified for the first time in nature. Sensory evaluation of yuzu aroma reconstitutions revealed that the newly identified compound, Yuzunone, contributes greatly to the distinct yuzu aroma.

KEYWORDS: Yuzu (*Citrus junos* Sieb. ex Tanaka); citrus; novel aroma compounds; GC-MS-O; AEDA; sensory evaluation; (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (Yuzunone); (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (Yuzuol)

INTRODUCTION

Yuzu (*Citrus junos* Sieb. ex Tanaka) is a tree-growing fruit similar in appearance to a sour orange. The tree originated in China and now grows mainly wild in Japan and Korea. This citrus fruit is widely used in Japanese and Korean cuisines for its pleasant flavor. Common delicacies with yuzu include slivered yuzu peel to garnish Japanese dishes, *Yuzu-ponzu*, a dressing made from soy sauce and yuzu juice, *Yuzu-kosho*, a seasoning made from salt, milled green chilies, and yuzu peel, and *Yuja-cha*, a hot beverage made from a marmalade-like syrup with sugar, honey, and sliced yuzu peel. Another popular custom in Japan is to add yuzu fruit as is with the fragrance to hot bath water.

The distinct aroma of yuzu, an aroma unlike that of any other citrus fruits, has been studied by a number of researchers (*1*−*6*). These studies revealed that yuzu aroma is composed of hydrocarbons (*5*), aldehydes (*3*, *6*), a lactone (*2*), and a sulfur compound (*4*), in addition to limonene and linalool, two aroma compounds common to other citrus fruits. Our laboratory, meanwhile, has identified ketols (*7*), alcohols (*8*, *9*), and epoxides (*9*, *10*) as potent odorants, and determined the enantio purities of branched aldehydes (*11*) in yuzu peel oil. Many of the components of the total aroma have yet to be found, however, as fresh yuzu aroma is extremely difficult to duplicate using known odor-active volatiles alone. In this research we attempted to analyze yuzu peel oil by sensory analyses to clarify the seemingly missing character impact compounds that differentiate yuzu from other citrus fruits.

MATERIALS AND METHODS

Materials. Fresh yellow yuzu (*C. junos* Sieb. ex Tanaka) fruits cultivated in Kochi, Japan, were purchased at a local market in Tokyo, Japan. The fruit ranged from 140 to 180 g in weight and from 70 to 75 mm in diameter. Orange oil, prepared by the cold-pressed method from *Citrus sinensis* (L.) Osbeck, was purchased from Polarome International (Jersey City, NJ). Grapefruit oil, prepared by the cold-pressed method from *Citrus paradisi* Macfaden, was purchased from Peace River Citrus Products, Inc. (Vero Beach, FL). Lime oil, prepared by steam distillation from *Citrus aurantifolia* (Christmann) Swingle, was purchased from Citrus and Allied Essences Ltd. (Lake Success, NY).

Chemicals. The following compounds were purchased from commercial sources: (*Z*)-hex-3-enal, octanal, oct-1-en-3-one, nonanal, decanal, (*E*)-non-2-enal, linalool, (*Z*)-oct-5-en-1-ol, (2*E*,4*E*)-nona-2,4-dienal, (2*E*,4*E*)-deca-2,4-dienal, and thymol (Sigma-Aldrich Japan, Tokyo, Japan); 4-methyl-4-mercaptopentan-2-one (Oxford Chemicals Ltd., Hartlepool, U.K.); (*E*)-dec-4-enal (Givaudan SA, Vernier, Switzerland). The following compounds were synthesized as reported in the literature: 6-methyloctanal (*11*); (3*E*,5*Z*)-undeca-1,3,5-triene (*12*); (*Z*)-non-4-enal (*13*); (*E*)-non-6-enal (*14*); (3*E*,5*Z*,8*Z*)-undeca-1,3,5,8-tetraene (*15*); 8-methylnonanal (*11*); (*Z*)-dodec-9-eno-12-lactone (*16*); (2*E*)-*cis*-4,5-epoxydec-2-enal (*17*); (2*E*)-*trans*-4,5-epoxydec-2-enal (*18*).

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Table 1. Sensory Evaluation of Citrus Oils

	average score[a]			
attribute	yuzu	orange	grapefruit	lime
fruity	3.8 a	3.8 a	3.4 ab	2.4 b
sweet	3.5 a	3.5 a	3.3 a	1.7 b
floral	3.8 a	3.1 ab	3.3 ab	2.6 b
green	3.0 a	3.0 a	3.5 a	3.1 a
metallic	2.8 b	2.5 b	3.0 b	4.5 a
sour	2.9 a	3.6 a	3.5 a	3.5 a
balsamic	4.4 a	2.8 b	2.3 bc	1.7 c

[a] $n = 11$. Different letters (a−c) cited on the right side of the scores denote significant differences by Tukey's multiple-comparison test ($P < 0.05$). Values correspond to the **Figure 1**.

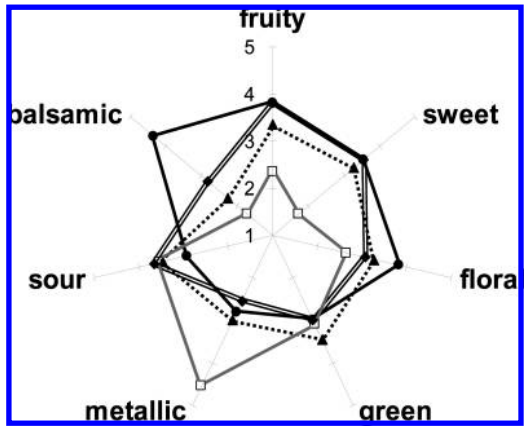

Figure 1. Aroma profile of citrus oils: (●) yuzu peel oil; (◆) orange oil; (▲) grapefruit oil; (□) lime oil.

Table 2. Sensory Evaluation of Yuzu Peel Oil Fractions

	average score[a]		
attribute	yuzu peel oil	polar part	nonpolar part
fruity	3.5 a	4.0 a	1.8 b
sweet	3.1 a	3.9 a	1.6 b
floral	3.4 a	3.9 a	1.7 b
green	3.5 a	2.7 ab	2.4 b
metallic	3.7 a	1.8 b	4.4 a
sour	3.8 a	2.7 ab	3.2 a
balsamic	4.2 a	4.1 a	1.4 b

[a] $n = 10$. Different letters (a, b) cited on the right side of the scores denote significant differences by Tukey's multiple-comparison test ($P < 0.05$). Values correspond to the **Figure 2**.

(6*E*/*Z*,8*E*)-Undeca-6,8,10-trien-3-one (**1**) (6*E*/6*Z* = 50:50) (*19*, *20*), (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-ol (**2**) (6*E*/6*Z* = 50:50) (*19*, *20*), and (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) (6*E*/6*Z* = 65:35) were synthesized in our laboratory. All other reagents and solvents were of analytical grade.

Nuclear Magnetic Resonance (NMR) Spectra. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL NNM-ECX400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as an internal standard, δ 0.00 (coupling constants J in hertz). The geometric ratios of the synthesized compounds were determined by ^{1}H NMR.

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS analyses were performed with an Agilent 6890 gas chromatograph (GC) combined with a 5973 mass selective detector and a flame ionization detector (FID; 250 °C) equipped with a TC-WAX capillary column (0.25 mm i.d. × 60 m; GL Sciences Co., Tokyo, Japan). The effluent of the column at the end of the capillary was divided into two branches and routed by deactivated fused silica capillaries to the mass detector and FID, respectively. Each sample was injected in 1 μL volumes in a split mode (50:1) at a constant temperature of 250 °C. The oven temperature was kept at 40 °C for the initial 3 min and then increased to 230 °C at a rate of 3 °C/min, with a constant carrier helium gas flow of 1.8 mL/min. Mass spectra (MS) in the electron impact (EI) mode were recorded at 70 eV ionization energy. Linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes. The purity of the synthesized compounds was calculated by integration of the chromatogram obtained by the FID.

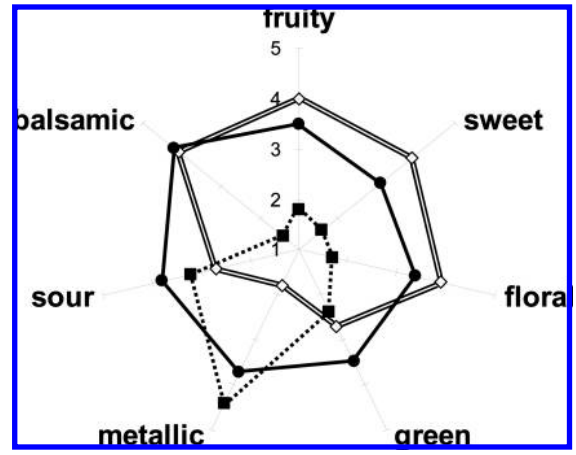

Figure 2. Aroma profile of yuzu peel oil fractions: (●) yuzu peel oil; (◇) polar part; (■) nonpolar part.

Infrared Absorption (IR) Spectra. IR spectra were recorded on a GC-IR, an Agilent 6890 GC connected to a Bio-Rad IRD II equipped with a TC-5 capillary column (0.32 mm i.d. × 60 m; GL Sciences Co.). The sample volume, split rate, injection temperature, oven temperature program, carrier gas, and flow rate were all the same as the conditions of GC-MS described above.

High-Resolution Mass Spectra (HRMS). The HRMS were recorded on a JEOL JMS-700.

Syntheses. (6E/Z,8E)-Undeca-6,8,10-trien-4-ol (3). $NaBH_4$ (0.174 g, 4.60 mmol) was added to a solution of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-one (6*E*/6*Z* = 46:54, 1.50 g, 9.13 mmol) (*19*, *20*) in EtOH (10 mL) at 5 °C. After 1 h of stirring at 5 °C, the reaction mixture was quenched with 1 N aqueous HCl (10 mL) and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. After the addition of tocopherol (0.1 g), the ethyl acetate solution was then concentrated under high vacuum. The residue (1.60 g) was purified by chromatography on silica gel (10 g; silica gel 60N; Kanto Chemical Co., Inc., Tokyo, Japan) using *n*-hexane/ethyl acetate (50:1, v/v) into a colorless oil of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**, 6*E*/6*Z* = 65:35, 1.10 g, yield = 79%, purity = 95%). The (6*Z*)-isomer (**3a**) and (6*E*)-isomer (**3b**) were determined by the coupling constants at δ 5.51 and 5.71, respectively. ^{1}H NMR (both isomers) δ 0.91, 0.92 (each t, $J = 6.8$, $J = 6.8$, total 3H), 1.32−1.50 (m, 5H), 2.15−2.38 (m, 2H), 3.52 (br d, $J = 3.6$, 1H), 5.06, 5.09 (each d, $J = 10.0$, $J = 11.6$, total 1H), 5.18, 5.22 (each d, $J = 12.4$, $J = 12.4$, total 1H), 5.51 (**3a**, dt, $J = 8.0$, 10.4, 0.35H), 5.71 (**3b**, dt, $J = 7.2$, 15.2, 0.65H), 6.10−6.52 (m, 4H); ^{13}C NMR (both isomers) δ 14.1, 18.8, 18.9, 35.9, 39.0, 39.1, 41.0, 70.9, 71.2, 117.0, 117.6, 128.1, 128.3, 131.2, 132.1, 132.9, 133.3, 134.1, 136.9, 137.0; MS-EI [(6*Z*)-isomer **3a**] 166 (M^+, 7), 105 (2), 94 (40), 79 (100), 55 (54), 43 (23), 31 (7), 27 (7); MS-EI [(6*E*)-isomer **3b**] 166 (M^+, 8), 105 (2), 94 (45), 79 (100), 55 (59), 43 (23), 31 (7), 27 (7); GC-IR [(6*Z*)-isomer **3a**] 3652, 3620, 3094, 3023, 2967, 2938, 1000 cm^{-1}; GC-IR [(6*E*)-isomer **3b**] 3652, 3619, 3094, 3016, 2968, 2936, 1001cm^{-1}; HRMS (both isomers) (EI) calcd for $C_{11}H_{18}O$ 166.1358, found 166.1342.

Gas Chromatography−Mass Spectrometry−Olfactometry (GC-MS-O). The GC-MS-O analyses were performed with an Agilent 6890 GC combined with a 5973 mass selective detector and a sniffing port equipped with a TC-WAX capillary column (0.25 mm i.d. × 60 m; GL Sciences Co.). The effluent of the column at the end of the capillary was divided into two branches and routed by deactivated fused silica capillaries to the mass detector and sniffing port, respectively. The sample volume, split rate, injection temperature, oven temperature program, carrier gas, flow rate, and ionization mode were all the same as the conditions of GC-MS described above.

Table 3. Most Odor-Active (FD ≥ 8) Volatiles in the Polar Part of Yuzu Peel Oil

odorant[a]	odor quality[b]	RI on TC-WAX	FD factor	GC peak area, %[c]	identification mode[d]
(*Z*)-hex-3-enal	green, citrus, apple-like	1149	32	0.090	MS, RI, GC-O
octanal	citrus	1286	8	0.326	MS, RI, GC-O
oct-1-en-3-one	mushroom-like, spicy, earthy	1300	32	0.001	MS, RI, GC-O
6-methyloctanal	citrus, herbal	1358	8	0.012	MS, RI, GC-O
4-methyl-4-mercaptopentan-2-one	sulfury, tropical fruit-like	1381	128	trace	MS, RI, GC-O
(3*E*,5*Z*)-undeca-1,3,5-triene	peely, citrus, fruity	1387	32	0.001	MS, RI, GC-O
nonanal	citrus, peely	1391	32	0.275	MS, RI, GC-O
(*Z*)-non-4-enal	floral, citrus, peely	1435	32	0.002	MS, RI, GC-O
(*E*)-non-6-enal	peely, citrus, albedo-like	1438	128	trace	MS, RI, GC-O
(3*E*,5*Z*,8*Z*)-undeca-1,3,5,8-tetraene	peely, citrus	1449	32	trace	MS, RI, GC-O
8-methylnonanal	peely, citrus	1449	32	0.053	MS, RI, GC-O
decanal	citrus	1497	8	1.117	MS, RI, GC-O
unknown	peely, sweet, floral	1510	32	nd	GC-O
(*E*)-non-2-enal	floral, green, albedo-like	1537	128	0.009	MS, RI, GC-O
(*E*)-dec-4-enal	peely, citrus, oily	1545	128	0.063	MS, RI, GC-O
linalool	citrus, peely, green	1547	128	74.939	MS, RI, GC-O
(*Z*)-oct-5-en-1-ol[e]	fatty, citrus	1616	128	nd	RI, GC-O
(2*E*,4*E*)-nona-2,4-dienal[e]	fatty, citrus	1701	8	nd	RI, GC-O
(2*E*,4*E*)-deca-2,4-dienal	fatty, oily	1812	128	0.110	MS, RI, GC-O
unknown A	peely, balsamic, floral	1899	128	0.010	GC-O
(*Z*)-dodec-9-eno-12-lactone	fatty, spicy, albedo-like	1986	32	0.014	MS, RI, GC-O
(2*E*)-*cis*-4,5-epoxydec-2-enal	peely, citrus, albedo-like	1986	128	trace	MS, RI, GC-O
unknown B	peely, balsamic, albedo-like	1986	128	0.003	GC-O
(2*E*)-*trans*-4,5-epoxydec-2-enal	citrus, peely	2000	128	trace	MS, RI, GC-O
unknown	peely, citrus	2069	8	trace	GC-O
thymol	medicinal, spicy	2177	128	1.434	MS, RI, GC-O

[a] The odorant was identified by matching the mass spectrum, retention index, and odor quality with the reference odorant. [b] Odor quality perceived at the sniffing port. [c] nd, not detected; trace, trace amount (<0.001%). [d] MS, reference mass spectrum; RI, reference retention index; GC-O, gas chromatography coupled with olfactometry. [e] Tentatively identified odrant.

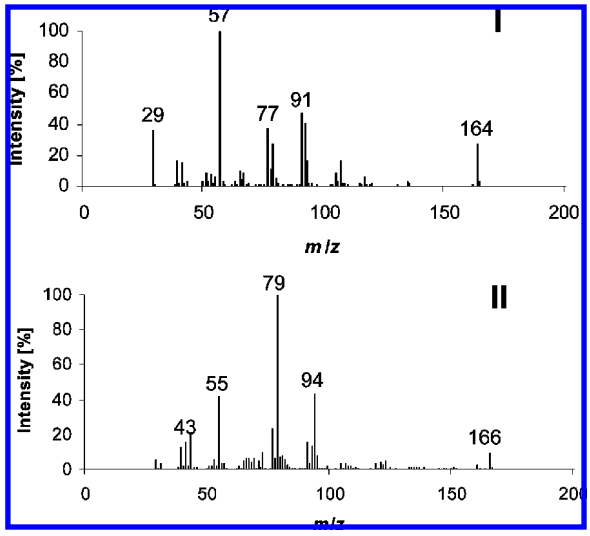

Figure 3. Mass spectra (MS-EI) of (**I**) unknown A and (**II**) unknown B.

Table 4. RI on TC-WAX and TC-1 of Unknowns, (6*E*/*Z*,8*E*)-Undeca-6,8,10-trien-3-ones, and (6*E*/*Z*,8*E*)-Undeca-6,8,10-trienols

		RI on	
no.	compound	TC-WAX	TC-1
	unknown A	1899	1328
	unknown B	1986	1331
1a	(6*Z*,8*E*)-undeca-6,8,10-trien-3-one	1899	1328
1b	(6*E*,8*E*)-undeca-6,8,10-trien-3-one	1924	1339
2a	(6*Z*,8*E*)-undeca-6,8,10-trien-3-ol	2008	1340
2b	(6*E*,8*E*)-undeca-6,8,10-trien-3-ol	2042	1357
3a	(6*Z*,8*E*)-undeca-6,8,10-trien-4-ol	1986	1331
3b	(6*E*,8*E*)-undeca-6,8,10-trien-4-ol	2002	1341

Isolation and Fractionation of Volatiles. *Solvent Extraction.* The flavedo layers of 293 fresh yellow yuzu fruits were carefully peeled off by knives. The peel (5.31 kg) was then cut into pieces and extracted with *n*-pentane (10.6 L) for 30 min. After removal of the fibrous parts of the peel by filtration, the filtrate was dried over Na_2SO_4 and concentrated by distilling off the solvent over a Vigreux column at 43 °C to yield yuzu peel oil (81.5 g).

Silica Gel Chromatography. The yuzu peel oil (80.5 g) was pipetted into the top of a water-cooled (10−12 °C) glass column filled with silica gel slurry (400 g; Wakogel C-100; Wako Pure Chemical Industries, Ltd., Osaka, Japan) in *n*-pentane. Chromatography was performed using *n*-pentane, followed by *n*-pentane/diethyl ether (200:1, v/v) to give a nonpolar fraction, followed by *n*-pentane/diethyl ether (1:1, v/v) and diethyl ether to give a polar fraction. Each of the fractions was dried over Na_2SO_4, filtered, and freed from solvent under high vacuum to yield 76.0 g of nonpolar part and 4.4 g of polar part. Next, the volatiles of the polar part were isolated by the solvent-assisted flavor evaporation (SAFE) method (*21*) at 40 °C. The distillate was dried over Na_2SO_4 and concentrated to yield 3.2 g of polar volatiles of yuzu peel oil.

Aroma Extract Dilution Analysis (AEDA). The flavor dilution (FD) factors of the odor-active compounds were determined by the AEDA approach (*22*). The polar volatile part of yuzu peel was diluted stepwise with diethyl ether to obtain dilutions at 1:8, 1:32, and 1:128 ratios, and each dilution was analyzed by GC-MS-O equipped with the TC-WAX capillary. AEDA was performed by two experienced assessors.

Multidimensional Gas Chromatography−Mass Spectrometry−Olfactometry (MD-GC-MS-O). MD-GC-MS-O analyses were performed with an Agilent 6890 GC combined with an FID (250 °C), connected to an Agilent 6890 GC combined with a 5973 mass selective detector and a sniffing port equipped with a Gerstel multicolumn switching system. The effluent of the first column (TC-WAX capillary column; 0.25 mm i.d. × 60 m; GL Sciences Co.) at the end of the capillary was divided into two branches and routed by deactivated fused silica capillaries to the FID and to the column switching device, where the compounds eluted from the first column could be eliminated or transferred directly into the second column (TC-1 capillary column; 0.25 mm i.d. × 60 m; GL Sciences Co.). The effluent of the second column at the end of the capillary was divided into two branches and routed by deactivated fused silica capillaries to the mass detector and sniffing port, respectively. Each sample was injected in 1 μL volumes in a splitless mode. The injection temperature, oven temperature

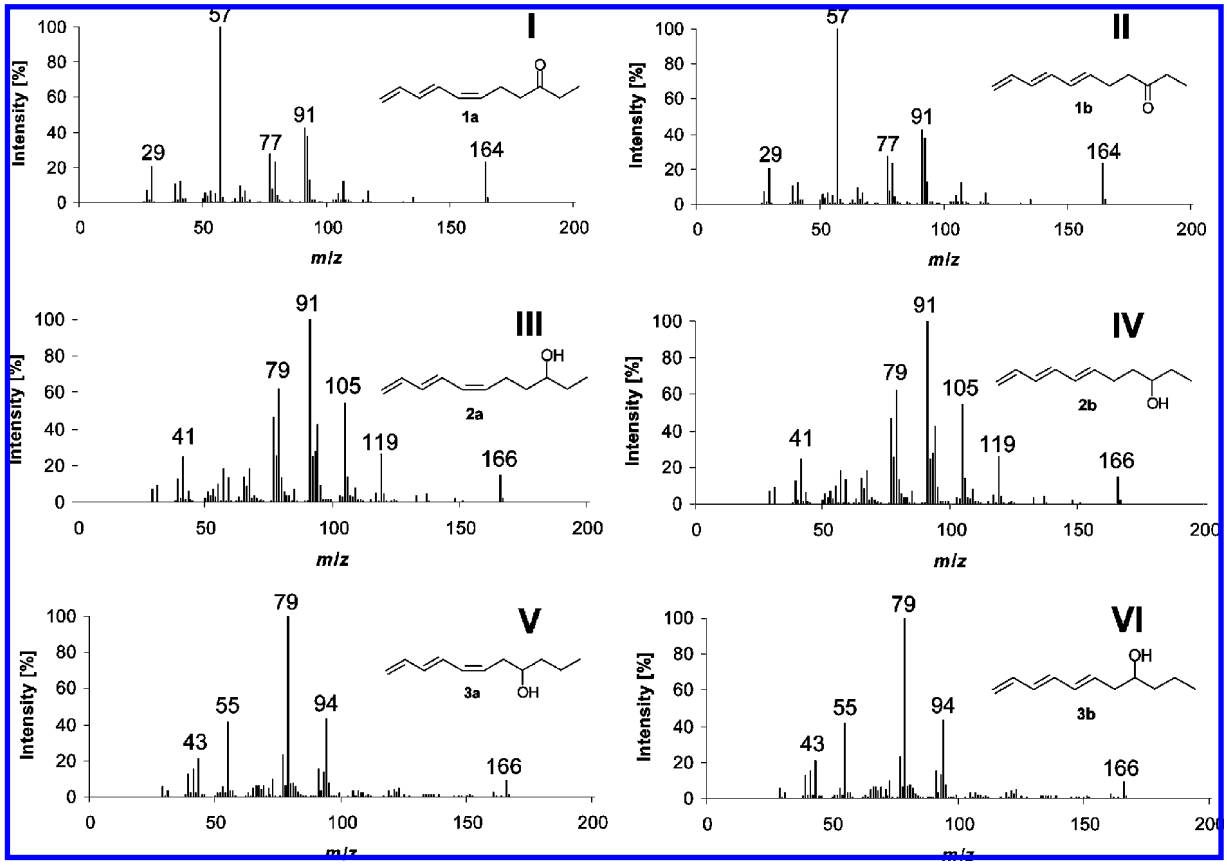

Figure 4. Chemical structures and mass spectra (MS-EI) of candidate compounds for odor-active unknowns in yuzu peel oil: (**I**) (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1a**); (**II**) (6*E*,8*E*)-undeca-6,8,10-trien-3-one (**1b**); (**III**) (6*Z*,8*E*)-undeca-6,8,10-trien-3-ol (**2a**); (**IV**) (6*E*,8*E*)-undeca-6,8,10-trien-3-ol (**2b**); (**V**) (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3a**); (**VI**) (6*E*,8*E*)-undeca-6,8,10-trien-4-ol (**3b**).

Table 5. Composition of Aroma Reconstitutions for Sensory Evaluation

				weight (mg)		
odorant	RI on TC-WAX	FD factor	GC peak area, %[a]	i[b]	ii[c]	iii[d]
oct-1-en-3-one	1300	32	0.001	1.00	1.00	1.00
4-methyl-4-mercaptopentan-2-one	1381	128	trace	0.05	0.05	0.05
(3*E*,5*Z*)-undeca-1,3,5-triene	1387	32	0.001	1.00	1.00	1.00
nonanal	1391	32	0.275	280.00	280.00	280.00
(*Z*)-non-4-enal	1435	32	0.002	2.00	2.00	2.00
(*E*)-non-6-enal	1438	128	trace	0.05	0.05	0.05
(3*E*,5*Z*,8*Z*)-undeca-1,3,5,8-tetraene	1449	32	trace	0.05	0.05	0.05
8-methylnonanal	1449	32	0.053	50.00	50.00	50.00
(*E*)-non-2-enal	1537	128	0.009	10.00	10.00	10.00
(*E*)-dec-4-enal	1545	128	0.063	60.00	60.00	60.00
linalool	1547	128	74.939	74900.00	74900.00	74900.00
(*Z*)-oct-5-en-1-ol	1616	128	nd	0.05	0.05	0.05
(2*E*,4*E*)-deca-2,4-dienal	1812	128	0.110	110.00	110.00	110.00
(*Z*)-dodec-9-eno-12-lactone	1986	32	0.014	20.00	20.00	20.00
(2*E*)-*trans*-4,5-epoxydec-2-enal	2000	128	trace	0.05	0.05	0.05
thymol	2177	128	1.434	1400.00	1400.00	1400.00
(6*Z*,8*E*)-undeca-6,8,10-trien-3-one	1899	128	0.010			
(6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one[e]					20.00	
(6*Z*,8*E*)-undeca-6,8,10-trien-4-ol	1986	128	0.003			
(6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol[f]						9.00
medium-chain triglyceride				23165.75	23145.75	23156.75

[a] nd, not detected; trace, trace amount (<0.001%). [b] Yuzu aroma model mixture. [c] Yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one. [d] Yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol. [e] 6*E*/6*Z* = 50:50. [f] 6*E*/6*Z* = 65:35.

program, carrier gas, and flow rate were all the same as the conditions of GC-MS described above. At a target retention time, the effluent was eliminated again, whereupon the trapped material was heated to 250 °C and then directed to the second column. The oven temperature of the second GC was kept at 40 °C for the initial 3 min and then increased to 300 °C at a rate of 3 °C/min. The carrier gas, flow rate, and ionization mode were the same as the conditions of GC-MS described above.

Sensory Evaluation. A 0.5 mL portion of each sample was put into a closed sensory vial (total volume = 30 mL) and coded by a random three-digit number. Each panelist was presented with a set of test samples with instructions to sniff each sample and rate the intensity in seven attributes using a five-point linear scale from 1 (none) to 5 (very strong). The results were averaged for each attribute and plotted on a spider web diagram. The evaluation was conducted in a quiet room

Table 6. Sensory Evaluation of Aroma Reconstitutions

attribute	average score[a] i[b]	ii[c]	iii[d]
fruity	3.0 a	3.6 a	3.1 a
sweet	2.6 b	3.5 a	3.2 ab
floral	3.1 b	3.9 a	3.1 ab
green	3.2 a	3.6 a	3.1 a
metallic	2.7 a	3.3 a	3.4 a
sour	2.9 a	3.3 a	3.2 a
balsamic	3.0 b	4.2 a	3.5 ab

[a] $n = 10$. Different letters (a, b) cited on the right side of the scores denote significant differences by Tukey's multiple-comparison test ($P < 0.05$). Values correspond to **Figure 5**. [b] Yuzu aroma model mixture. [c] Yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one. [d] Yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol.

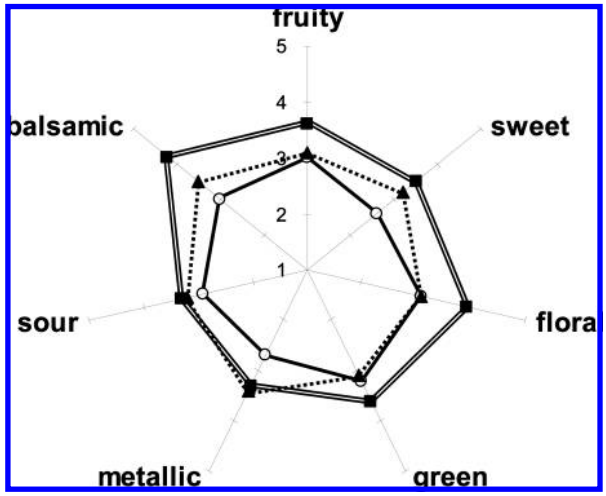

Figure 5. Aroma profiles of aroma reconstitutions: (○) yuzu aroma model mixture; (■) yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**); (▲) yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**).

kept at 23 °C. Two booths were set up in the room, and each panelist performed the assessment alone in one booth, to ensure that the panelists would not influence each other.

Panelists. The panel consisted of seven males and four females, all of whom were employees of the Technical Research Center of T. Hasegawa Co., Ltd., Kawasaki, Japan. They were trained to recognize and quantify aromas with about 100 odorous chemicals and raw materials.

Attributes. To develop a vocabulary suitable for describing the aroma of yuzu peel oil before the sensory evaluation, the panelists were presented with yuzu peel oil, orange oil, grapefruit oil, and lime oil, together with a list of 44 descriptors (*23*, *24*). After the session, they collaboratively discussed, generated, and modified descriptive terms using yuzu peel oil and other citrus oils. As a result of several consultations, they reached a consensus on seven attributes to be used for their descriptive evaluations of the yuzu aroma: fruity, sweet, floral, green, metallic, sour, and balsamic.

Citrus Oils. Four test samples were evaluated as is by the 11 panelists: yuzu peel oil, orange oil, grapefruit oil, and lime oil.

Yuzu Peel Oils. Three test samples were evaluated by 10 panelists: yuzu peel oil, the polar part of yuzu peel oil, and the nonpolar part of yuzu peel oil. To adjust the concentrations of the fractionated samples with yuzu peel oil, medium-chain triglycerides (MCTs) were added to both the polar part and the nonpolar part. The test samples were prepared by adding 17.3 g of MCTs to 1.0 g of the polar part and 0.58 g of MCTs to 10 g of the nonpolar part and then subjected to the sensory evaluation.

Aroma Reconstitutions. Three test samples were evaluated by 10 panelists: a yuzu aroma model mixture, a yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**), and a yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**). The yuzu aroma model mixture was prepared by mixing the following 16 reference compounds at concentrations based on the GC peak area percentage of the polar volatile part of the yuzu peel oil, then adding MCTs until the gross quantity became 100 g: oct-1-en-3-one (1 mg), 4-methyl-4-mercaptopentan-2-one (50 μg), (3*E*,5*Z*)-undeca-1,3,5-triene (1 mg), nonanal (280 mg), (*Z*)-non-4-enal (2 mg), (*E*)-non-6-enal (50 μg), (3*E*,5*Z*,8*Z*)-undeca-1,3,5,8-tetraene (50 μg), 8-methylnonanal (50 mg), (*E*)-non-2-enal (10 mg), (*E*)-dec-4-enal (60 mg), linalool (74.9 g), (*Z*)-oct-5-en-1-ol (50 μg), (2*E*,4*E*)-deca-2,4-dienal (110 mg), (*Z*)-dodec-9-eno-12-lactone (20 mg), (2*E*)-*trans*-4,5-epoxy-dec-2-enal (50 μg), and thymol (1.4 g). To evaluate the effect of the newly identified odor-active compounds, synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**) (20 mg; 6*E*/6*Z* = 50:50) and (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) (9 mg; 6*E*/6*Z* = 65:35) were added to the aroma model mixture, respectively, on the basis of their GC peak area percentage of the polar volatile part of the yuzu peel oil: (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1a**) (0.010%) and (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3a**) (0.003%). To adjust the concentrations of the aroma reconstitutions with yuzu peel oil, the test samples were prepared by adding 17.3 g of MCTs to 1.0 g of each aroma reconstitution before the sensory evaluation.

Statistical Analyses. The differences among the average scores of the evaluated samples were compared by the analyses of variance (ANOVA) and Tukey's multiple-comparison tests (*25*−*32*). On the ANOVAs, the correction factor (CF), sum of square (SS), degree of freedom (df), mean square (ms), and *F* value for each attribute were calculated. On the Tukey's multiple-comparison tests, the standard error measurement (SEM) and least significant differences (LSD) for each attributes were calculated ($P < 0.05$) and compared with the subtracted values between the evaluated samples.

RESULTS AND DISCUSSIONS

Sensory Evaluation of Citrus Oils. Our first step in studying the aroma of yuzu fruit, a popular citrus in East Asia, was to conduct a sensory evaluation to evaluate differences of the aroma compared with other citrus fruits. The evaluation of four citrus oils was performed by panelists: yuzu peel oil prepared by solvent extraction from fresh yellow yuzu fruit purchased in the Japanese local market, and orange, grapefruit, and lime citrus oils purchased from U.S. companies. Among the attributes fruity, sweet, floral, green, metallic, sour, and balsamic, the balsamic note of yuzu received a significantly different score from those of the other citruses by Tukey's multiple-comparison test ($P < 0.05$) (*25*−*32*). The balsamic note was thus found to be the most characteristic factor in yuzu aroma, whereas the other attributes of yuzu, namely, fruity, sweet, floral, green, metallic, and sour notes, were scored similar to those of orange and grapefruit (**Table 1** and **Figure 1**).

Sensory Evaluation of Yuzu Peel Oil Fractions. Yuzu peel oil was fractionated by silica gel chromatography into two parts, namely, a nonpolar part, in which hydrocarbons such as limonene and γ-terpinene were abundant, and a polar part, in which oxygenated compounds such as linalool and α-terpineol were abundant. Another sensory evaluation was performed on the same seven attributes in three samples, that is, yuzu peel oil, the nonpolar part, and the polar part. The results revealed that components of the polar part contributed the balsamic, fruity, sweet, and floral notes, whereas those of the nonpolar part contributed the sour and metallic notes (**Table 2** and **Figure 2**). We noted with interest that the balsamic note, the note evaluated as the most characteristic factor in yuzu aroma in the first sensory session, was detected exclusively in the polar part and not in the nonpolar part. On the basis of this finding, we presumed that we would be able to clarify the distinct odor volatiles of yuzu by focusing our analysis on the polar part in detail using GC-MS-O.

Identification of Odor-Active Compounds of the Polar Part. To rank the odorants of the polar part of yuzu peel oil according to their odor potencies, AEDA was applied on polar volatiles that had been prepared by SAFE distillation. As a result, 26 odor-active regions were detected within the FD factor range of 8−128 (**Table 3**). Within the FD factor range of 32−128 of 21 odor-active regions, we newly identified five odorants in yuzu peel oil from among 17 odorants identified by matching the MS, RI, and odor qualities with the reference odorants in our laboratory, namely, oct-1-en-3-one (FD 32), (*Z*)-non-4-enal (FD 32), (*E*)-dec-4-enal (FD 128), 4-methyl-4-mercaptopentan-2-one (FD 128), and (*E*)-non-6-enal (FD 128). Although it was a tentative identification, (*Z*)-oct-5-en-1-ol (FD 128) was also newly identified in yuzu peel oil by matching the RI and odor quality with the reference odorant. Moreover, we found that the distinct yuzu peel aroma was partly composed of two more odorants, each of which received the highest FD factor of 128 in the AEDA. We hereinafter refer to these odorants as "unknown A" and "unknown B."

Identification of the Candidate Compounds for Characteristic Odor. *Unknown A.* The MS of unknown A was obtained by MD-GC-MS-O of the polar volatile part of yuzu peel oil. Upon study, the MS data of unknown A (**Figure 3**) proved to be exactly the same as those of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**), a compound recently identified by our group as one of the characteristic odorants in galbanum oil (*19*, *20*). For confirmation, a synthesized authentic sample of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**) was analyzed according to the same method as that used for unknown A in yuzu peel oil. We were thereby able to identify unknown A as (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1a**) by matching the MS, RI, and odor qualities (**Table 4** and **Figure 4**). Moreover (6*E*,8*E*)-undeca-6,8,10-trien-3-one (**1b**) was also identified in yuzu peel oil by matching the respective MS and RI, although the compound was not detected as an odor-active volatile by AEDA. As we reported recently (*19*, *20*), the odor of the (6*Z*)-isomer **1a** noted at the sniffing port was stronger than that of the (6*E*)-isomer **1b**. Both the (6*Z*) and (6*E*)-isomers **1** were identified for the first time in citrus oils.

Unknown B. The MS of unknown B (**Figure 3**) was obtained by MD-GC-MS-O of concentrated effluent obtained by repeated silica gel chromatography of the polar volatile part of the yuzu peel oil. We assumed that the chemical structure might be (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-ol (**2**) (*19*, *20*), the reduced derivative of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**), as the MS data showed that the molecular weight was 166 and as the odor quality of unknown B was similar to that of unknown A. Although the odor of the alcohol **2** was similar to that of unknown A, the MS and RI of synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-ol (**2**) did not match with those of unknown B (**Table 4** and **Figure 4**). Again, we assumed that the chemical structure might be (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**), the reduced derivative of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-one, another characteristic odorant identified in galbanum oil (*19*, *20*). The synthesis of the alcohol **3** was accomplished by the reduction of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-one with $NaBH_4$. For confirmation, a synthesized authentic sample of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) was analyzed according to the same method as that used for unknown B in yuzu peel oil. We were thereby able to identify unknown B as (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3a**) by matching the MS, RI, and odor qualities (**Table 4** and **Figure 4**). Although the MS data on (6*Z*) and (6*E*)-isomers were almost the same, differences of their RI allowed us to identify unknown B as (6*Z*)-isomer **3a**. In addition, (6*Z*,8*E*)-undeca-6,8,10-trien-3-ol (**2a**) was also identified in yuzu peel oil by matching the respective MS and RI data, although it was not identified as unknown B and not detected as an odor-active volatile by AEDA. Both (6*E*,8*E*)-isomers, (6*E*,8*E*)-undeca-6,8,10-trien-4-ol (**3b**) and (6*E*,8*E*)-undeca-6,8,10-trien-3-ol (**2b**), were also identified in yuzu peel oil by matching the respective MS and RI data, although the odors of the (6*E*)-isomers **3b** and **2b** noted at the sniffing port were weaker than those of the corresponding (6*Z*)-isomers **3a** and **2a**. In yuzu peel oil, however, we were not able to identify (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-one despite the presence of the alcoholic derivatives **3**. (6*E*/*Z*,8*E*)-Undeca-6,8,10-trien-3-ol (**2**) and the newly synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) were nature-identified for the first time. Although previous investigators have proposed that aldehydes such as nonanal, undecanal, and 6-methyloctanal contributed to the characteristic odor of yuzu aroma (*3*), we were able to identify the ketone **1a** and alcohol **3a** as individually having the characteristic yuzu aroma of peely and balsamic notes.

Effect of Newly Identified Odor-Active Compounds on Yuzu Aroma. We here name two characteristic volatiles of yuzu peel oil, both of which have been discovered in our laboratory, as Yuzunone for (6*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1a**) and Yuzuol for (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3a**). To investigate the effect of two characteristic volatiles, Yuzunone (**1a**) and Yuzuol (**3a**), on yuzu aroma, we prepared three yuzu aroma reconstitutions: a yuzu aroma model mixture composed of 16 odor-active volatiles identified by AEDA analysis of the polar volatile part of yuzu peel oil, a yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**) including Yuzunone (**1a**), and a yuzu aroma model mixture with synthesized (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) including Yuzuol (**3a**) (**Table 5**). We mixed the compounds at concentrations based on the GC peak area percentage of the polar volatile part of the yuzu peel oil, although they were not precise quantities existing in yuzu peel oil.

Yuzunone. Compared to the yuzu aroma model mixture, all attribute scores were increased by the addition of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**) including Yuzunone (**1a**). The scores for balsamic, sweet, and floral notes of the yuzu aroma model mixture with (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-3-one (**1**) were significantly higher by Tukey's multiple-comparison test ($P < 0.05$) than those of the yuzu aroma model mixture. Only through the addition of 10 ppm of Yuzunone (**1a**) to the yuzu aroma model mixture did the compound significantly improve the balsamic note, the most characteristic factor in yuzu aroma. This result indicates that Yuzunone (**1a**) is highly contributive to the yuzu aroma (**Table 6** and **Figure 5**).

Yuzuol. Compared to the yuzu aroma model mixture, four of the seven attribute scores were increased by the addition of (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) including Yuzuol (**3a**). Although no significant differences were observed in any of the attributes by Tukey's multiple-comparison test ($P < 0.05$), the scores for balsamic, sweet, metallic, and sour notes of the yuzu aroma model mixture with (6*E*/*Z*,8*E*)-undeca-6,8,10-trien-4-ol (**3**) tended to be higher than those of the yuzu aroma model mixture (**Table 6** and **Figure 5**).

In conclusion, we identified seven odorants as novel character impact compounds in yuzu peel oil. We point out two results as the most significant: first, (*E*)-non-6-enal (*33*−*35*) and Yuzunone (**1a**) were identified for the first time solely in yuzu peel oil and not in the peel of other citrus species; second, Yuzuol (**3a**) was identified for the first time in nature. Sensory evaluation of yuzu aroma reconstitutions revealed that Yuzunone (**1a**) is the aroma compound that differentiates yuzu from other citrus fruits.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; ANOVA, analysis of variance; CF, correction factor; df, degree of freedom; EI, electron impact; FD, flavor dilution; FID, flame ionization detector; GC, gas chromatograph; GC-MS, gas chromatography−mass spectrometry; GC-MS-O, gas chromatography−mass spectrometry−olfactometry; HRMS, high-resolution mass spectrum; IR, infrared absorption; LSD, least significant differences; MCT, medium-chain triglyceride; MD-GC-MS-O, multidimensional gas chromatography−mass spectrometry−olfactometry; MS, mass spectrum; ms, mean square; NMR, nuclear magnetic resonance; RI, retention index; SAFE, solvent-assisted flavor evaporation; SEM, standard error measurement; SS, sum of square.

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LITERATURE CITED

(1) Shinoda, N.; Shiga, M.; Nishimura, K. Constituents of yuzu (*Citrus junos*) oil. *Agric. Biol. Chem*. **1970**, *34*, 234–242.

(2) Matsuura, Y.; Hata, G.; Abe, S.; Sakai, I.; Abe, A. A novel 13-membered macrolide. A flavour component of yuzu (*Citrus junos*) oil. In *Proceedings of the 8th International Congress of Essential Oils*, Cannes-Grasse, France, Oct 12−17; Fedarom: Grasse, France, 1980; pp 497−499.

(3) Tajima, K.; Tanaka, S.; Yamaguchi, T.; Fujita, M. Analysis of green and yellow yuzu peel oils (*Citrus junos* Tanaka). Novel aldehyde components with remarkably low odor thresholds. *J. Agric. Food Chem*. **1990**, *38*, 1544–1548.

(4) Tajima, K.; Fukuda, K.; Hasegawa, Y.; Toi, N. Research on new key odorants in yuzu peel oil (*Citrus junos* Tanaka). In *Proceedings of the 36th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics*, Nishinomiya, Japan, Nov 14−16, 1992; pp 28−30.

(5) Song, H. S.; Sawamura, M.; Ito, T.; Kawashimo, K.; Ukeda, H. Quantitative determination and characteristic flavor of *Citrus junos* (yuzu) peel oil. *Flavour Fragrance J*. **2000**, *15*, 245–250.

(6) Miyazato, H.; Hashimoto, S.; Hayashi, S. Research on new key odorants in yuzu peel oil (*Citrus junos* Tanaka). In *Proceedings of the 51st Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics*, Nagahama, Japan, Nov 10−12, 2007; pp 79−81.

(7) Kitahara, T.; Takagi, Y.; Matsui, M. Structure and the synthesis of novel constituents of yudzu peel oil and their conversion to related monoterpenes. *Agric. Biol. Chem*. **1980**, *44*, 897–901.

(8) Watanabe, I.; Yanai, T.; Furuhata, A.; Awano, K.; Kogami, K.; Hayashi, K. Volatile components of yuzu (*Citrus junos*). In *Proceedings of the 9th International Congress of Essential Oils*, Singapore, March 13−17; Essential Oil Technical Paper Book 4; 1983; pp 78−82.

(9) Watanabe, I.; Yanai, T.; Awano, K.; Kogami, K. Volatile components of yuzu (*Citrus Junos*). In *Proceedings of the 28th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics*, Kanazawa, Japan, Oct 13−15, 1984; pp 215−217.

(10) Watanabe, I.; Yanai, T.; Awano, K.; Habu, T.; Kogami, K. Volatile components of yuzu (*Citrus junos*) peel oil. In *Proceedings of the 29th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics*, Mie, Japan, Oct 12−14, 1985; pp 112−114.

(11) Ohkubo, Y.; Tamura, H.; Tachihara, T.; Kurobayashi, Y.; Tomita, N.; Hirasawa, H. Syntheses and odor evaluation of both enantiomers of the aldehydes in yuzu oil. In *Proceedings of the 50th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics*, Yokohama, Japan, Nov 10−12, 2006; pp 61−63.

(12) Näf, F.; Decorzant, R.; Thommen, W.; Willhalm, B.; Ohloff, G. The four isomeric 1,3,5-undecatrienes. *Helv. Chim. Acta* **1975**, *58* (4), 1016–1037.

(13) Czerny, M.; Schieberle, P. Influence of the polyethylene packaging on the adsorption of odor-active compounds from UHT-milk. *Eur. Food Res. Technol*. **2007**, *225*, 215–223.

(14) Iwamoto, M.; Takagi, Y.; Kubota, N.; Kogami, K. Preparation of aliphatic aldehyde. Jpn. Kokai Tokkyo Koho JP 19790170297 A, 1979.

(15) Marner, F.-J.; Boland, W.; Jaenicke, L. Synthese isomerer 1,3,5,8-undecatetraene. *Liebigs Ann. Chem.* **1982**, 579–584.

(16) Matsuura, Y.; Sakai, I.; Abe, A. Novel unsaturated macrocyclic lactone and perfumery composition containing said compound as effective component. Jpn. Kokai Tokkyo Koho JP 19790165531 A, 1979.

(17) Kumazawa, K.; Wada, Y.; Masuda, H. Characterization of epoxydecenal isomers as potent odorants in black tea (dimbula) infusion. *J. Agric. Food Chem*. **2006**, *54*, 4795–4801.

(18) Lin, J.; Fay, L. B.; Welti, D. H.; Blank, I. Synthesis of trans-4,5-epoxy-(*E*)-2-decenal and its deuterated analog used for the development of a sensitive and selective quantification method based on isotope dilution assay with negative chemical ionization. *Lipids* **1999**, *34*, 1117–1126.

(19) Miyazawa, N.; Nakanishi, A.; Tomita, N.; Ohkubo, Y.; Maeda, T.; Fujita, A. Novel key aroma components of galbanum oil. *J. Agric. Food Chem*. **2009**, *57*, 1433−1439.

(20) Miyazawa, N.; Nakanishi, A.; Tomita, N.; Ohkubo, Y.; Ishizaki, S.; Maeda, T. Novel key aroma components of galbanum oil. In *Abstracts of the 12th Weurman Flavour Research Symposium*, Interlaken, Switzerland, July 1−4, 2008; pp 272.

(21) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol*. **1999**, *209*, 237–241.

(22) Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier: Amsterdam, The Netherlands, 1995; pp 403−431.

(23) Shimoda, M.; Sasaki, H.; Doi, Y.; Kameda, W.; Osajima, Y. Characterization of abstract terms for odor-description of food products. *J. Jpn. Soc. Food Sci. Technol.* **1989**, *36*, 7–16.

(24) Shimoda, M.; Sasaki, H.; Doi, Y.; Kameda, W.; Osajima, Y. Characterization of concrete terms for odor-description of food products. *J. Jpn. Soc. Food Sci. Technol.* **1989**, *36*, 17–25.

(25) Snedecor, G. W.; Cochran, W. G. In *Statistical Methods*, 8th ed.; Iowa State University Press: Ames, IA, 1989.

(26) Poste, L. M.; Mackie, D. A.; Butler, G.; Larmond, E. In *Laboratory Methods for Sensory Analysis of Food*; Agriculture Canada: Ottawa, Canada, 1991.

(27) Aishima, T. Laboratory methods for sensory analysis of food: part 1. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 311–320.

(28) Aishima, T. Laboratory methods for sensory analysis of food: part 2. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 378–392.

(29) Aishima, T. Laboratory methods for sensory analysis of food: part 3. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 453–466.

(30) Aishima, T. Laboratory methods for sensory analysis of food: part 4. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 539–548.

(31) Aishima, T. Laboratory methods for sensory analysis of food: part 5. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 637–642.

(32) Aishima, T. Laboratory methods for sensory analysis of food: part 6. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 697–703.

(33) Kawakami, M.; Kobayashi, A.; Kator, K. Volatile constituents of rooibos tea (*Aspalathus linearis*) as affected by extraction process. *J. Agric. Food Chem*. **1993**, *41*, 633–636.

(34) Smith, P. W.; Parks, O. W.; Schwartz, D. P. Characterization of male goat odors: 6-*trans*-nonenal. *J. Dairy Sci*. **1984**, *67*, 794–801.

(35) Parks, O. W.; Wong, N. P.; Allen, C. A.; Schwartz, D. P. 6-*trans*-Nonenal: an off-flavor component of foam-spray-dried milks. *J. Dairy Sci*. **1969**, *52*, 953–956.

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