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Essential oil composition of citrus peels in Kikai-jima Island, Japan

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Abstract
Essential oils were extracted from five major citrus cultivars in Kikai-jima Island (Amami archipelago, Japan), namely, 'Shiiku' (*C.* sp.), 'Keraji' (*C. Keraji* hort. ex Tanaka), 'Kunenbo' (*C. nobilis* Lour.), 'Fusuu' (*C. rokugatsu* hort. ex Y. Tanaka), and 'Kuriha' (*C. Keraji* hort. ex Tanaka var. *Kabuchii*), and their volatile components were characterized by gas-chromatography/mass-spectrometry. A total of 54 volatile components were identified in the samples studied. The major components of 'Shiiku' oil were linalool, α-terpinol, geranyl acetate, and geraniol. The other citrus essential oils of Kikai-jima showed a composition similar to that of cultivars in Okinawa.

Keywords: Citrus, Shiiku, essential oil, GC-MS, Kikai-jima

1. Introduction
Citrus fruits are among the most important fruits worldwide. Throughout human history, many parts of *Citrus* plants have been used in various applications such as foods, flavorings, spices, medicines, and perfumes. The volatile components of Japanese citrus fruits [1-10] and common citrus fruits cultivated worldwide [11-16] have been widely investigated. Kikai-jima (28°19'N, 129°58'E) is a raised coral reef in the Amami archipelago in Kagoshima prefecture, Japan [17], where unique citrus species can be found due to the geographical and climatic conditions, significantly different from those of neighboring islands. In this work, the volatile components of five major citrus cultivars in Kikai-jima, namely, 'Shiiku' (*C.* sp.), 'Keraji' (*C. Keraji* hort. ex Tanaka), 'Kunenbo' (*C. nobilis* Lour.), 'Fusuu' (*C. rokugatsu* hort. ex Y. Tanaka), and 'Kuriha' (*C. Keraji* hort. ex Tanaka var. *Kabuchii*), were analyzed. 'Shiiku' is cultivated only in Kikai-jima, and the skin of its fruits has an aroma similar to that of 'Bergamot' (*C. bergamia* Risso et Poiteau) [18-19]; however, its volatile components have not yet been studied. 'Keraji' is a local citrus of Kikai-jima Island [20-22], widely cultivated in the Ryukyu Islands, whereas 'Kunenbo' ('Tokunibu') was introduced into Japan from China or Southeast Asia in the middle of the 15th century [23]. Both 'Keraji' and 'Kunenbo' showed high β-myrcene content [6]. Moreover, the origin of 'Fusuu' ('Rokugatsu-mikan') is still unclear, whereas 'Kuriha' ('Kabuchii') originates from Okinawa. These two citrus fruits grown in Okinawa are characterized by a high γ-terpinene content [5, 6]. In addition, 'Keraji' and 'Kuriha' in Kikai-jima contain high amounts of polymethoxyflavonoids, which are among the major citrus bioactive components [24, 25]. Characterization of the essential oils from Kikai citrus fruits will provide important information for the development of new products by food industries.

2. Materials and Methods
2.1 Plant samples
Mature fruits of Keraji, Fusuu, Kunenbo, Kuriha, and Shiiku were collected from citrus farms in Kikai-jima Island, Japan, in 2012–2016. The fruits were picked from the same tree and then peeled with a peeler or by hand. The fresh peels were stored at -80 °C until extraction of volatile components. Teramoto *et al*. [5] reported minor differences in the volatile components of cold-pressed, distilled, and hexane-extracted citrus peel; thus, the cold-pressed extracts were used in this study. The oils were refrigerated at -20 °C until analysis

2.2 Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the essential oils was carried out using an Agilent Technologies 7890A/5975C GC/MSD system equipped with a DB-WAX capillary column (J&W Scientific Inc., CA, USA; 30 m × 0.25 mm (i.d.), 0.25 □m film thickness). The injection port was heated at 250 °C. The column was maintained at an initial temperature of 50 °C for 5 min and then programmed at 10 °C/min to a final temperature of 240 °C, which was maintained for 20 min. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; mass range 50–800 amu; scan rate 1.0 scan/s. Helium was used as the carrier gas at a flow rate of 1 mL/min. The split ratio was 50:1. Each analysis was performed in triplicate.

2.3 Identification of volatile components

The volatile components were identified on the basis of the retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes under identical experimental conditions. Identification was confirmed by comparison of their mass spectra with those of the NIST (National Institute of Standards and Technology) library[23] and the homemade MS library built from pure substances and components of known essential oils, as well as by comparison of their RI with literature values.

3. Results and Discussion

Table 1 shows the essential oil composition of the five studied *Citrus* species. A total of 54 volatile compounds were identified in the citrus peel essential oils, and each species exhibited a characteristic composition. The oils distilled from the five *Citrus* species contained 32–47% of limonene (peak No.7 in Table 1), the most well-known and abundant compound in *Citrus*, which has been reported to have antibacterial [26] and antitumor activities [27]. This value was much lower than the limonene content of orange, grapefruit (>95%), and mandarin oils (70–75%) [28], and slightly lower than that of 'Lime' (*Citrus aurantifolia*) (55%) [29]. The most distinctive citrus in this study was 'Shiiku', native of Kikai-jima Island [30], because of its high levels of linalool (peak No.21; 22%), α-terpinol (peak No.31; 12%), geranyl acetate (peak No.37; 5.9%), and geraniol (peak No.44; 5.4%). This citrus showed some similarities to bergamot [11, 31], and could find application as a new essential oil in aromatherapy. Moreover, 'Shiiku' can be easily grown in Kikai-jima, where it can be exploited by the food industry. The volatile components of the essential oils from the other four *Citrus* species, namely, 'Keraji', 'Kunenbo' ('Tokunibu'), 'Fusuu' ('Rokugatsu-mikan'), and 'Kuriha' ('Kabuchii'), in Kikai-jima were almost the same as those in Okinawa, previously characterized by GC-MS [6], although in this study α-pinene and α-thujene could be distinguished on the basis of their GC retention times. In 'Keraji' and 'Kunenbo', the second most abundant component was β-myrcene (peak No.5; 22.7 and 25.4%, respectively), and other minor aromatic compounds, namely, γ-terpinene (peak No.10; 6.37 and 7.28%, respectively) and *p*-cymene (peak No.12; 5.29 and 8.56%, respectively), were detected. On the other hand, in 'Fusuu' and 'Kuriha', low levels of β-myrcene (3.51 and 3.50%, respectively) and higher levels of γ-terpinene (11.6 and 24.2%, respectively) and *p*-cymene (11.1 and 6.97%, respectively) were found.

Recently, in Japan, the oils extracted from 'Shiiku' and 'Keraji' have been used as aromatic and flavoring compounds for tea. Moreover, treatments such as drying, fermentation, and freeze-drying may be applied to obtain forms suitable for the manufacture of other products. Further studies should be carried out to evaluate the effects of citrus essential oils of Kikai-jima on human health. Our study of the composition of essential oils from Kikai citrus peels could contribute to the development of new products by Kikai citrus industries.

Table 1: The volatile constituents of five mature citrus peel grown on Kikai-jima[a]

No.	RI	compounds	*C.* sp. Shiiku'	*C. keraji* Keraji'	*C. rokugatsu* Fusuu'	*C. nobilis* Kunenbo'	*C. keraji* Kuriha'	Identification	RT (min.)
1	1015	α-Pinene	0.62	1.46	2.50	1.78	5.09	RI[b], MS[c], CO[d]	4.08
2	1018	α-Thujene	t[e]	0.38	0.71	0.46	2.01	RI, MS	4.17
3	1099	β-Pinene	3.04	0.86	1.66	1.09	3.56	RI, MS, CO	6.09
4	1114	Sabinene	0.20	0.24	0.27	0.26	0.55	RI, MS	6.44
5	1160	β-Myrcene	3.45	22.68	3.51	25.41	3.50	RI, MS, CO	7.54
6	1173	α-Terpinene	-[f]	t	0.26	t	0.95	RI, MS	7.79
7	1196	Limonene	32.51	35.67	46.70	44.65	40.87	RI, MS, CO	8.31
8	1200	β-Phellandrene	0.24	0.49	0.49	0.59	0.56	RI, MS	8.42
9	1231	(*Z*)-β-Ocimene	0.94	-	t	-	-	RI, MS	8.97
10	1243	γ-Terpinene	t	6.37	11.56	7.28	24.22	RI, MS, CO	9.18
11	1250	(*E*)-β-Ocimene	1.65	0.31	1.86	t	0.76	RI, MS	9.29
12	1268	*p*-Cymene	0.31	5.29	11.07	8.56	6.97	RI, MS	9.62
13	1282	α-Terpinolene	0.48	0.46	0.86	0.53	1.92	RI, MS	9.86
14	1289	Octanal	-	0.18	-	-	0.49	RI, MS	9.98
15	1388	Nonanal	-	t	-	-	t	RI, MS	11.71
16	1444	*cis*-Limonene oxide	t	t	0.44	0.36	0.27	RI, MS	12.53
17	1457	*trans*-Limonene oxide	-	t	0.24	0.23	t	RI, MS	12.71
18	1476	Citronellal	-	0.90	-	-	-	RI, MS	12.96
19	1493	Copaene	-	-	0.33	t	-	RI, MS	13.19
20	1496	Decanal	-	0.46	-	-	0.62	RI, MS	13.24
21	1546	Linalool	21.94	3.34	7.27	0.31	1.66	RI, MS, CO	13.88
22	1555	Linalyl acetate	4.05	-	-	-	-	RI, MS, CO	14.03
23	1587	β-Elemene	-	0.92	-	t	-	RI, MS	14.48
24	1587	Thymyl methyl ether	-	-	0.47	t	-	RI, MS	14.48
25	1597	β-Caryophyllene	-	0.51	-	-	t	RI, MS	14.61

26	1601	4-Terpineol	0.64	t	0.60	0.21	0.70	RI, MS	14.66
27	1658	Citronellyl acetate	-	0.39	-	-	-	RI, MS	15.31
28	1661	(*E*)-β-Farnesene	-	-	-	0.32	1.75	RI, MS	15.34
29	1675	α-Humulene	t	0.20	t	-	t	RI, MS	15.51
30	1682	Neral	t	0.73	t	-	-	RI, MS	15.60
31	1700	α-Terpinol	11.62	0.56	0.93	0.30	0.87	RI, MS	15.80
32	1715	Germacrene D	t	t	0.22	-	0.16	RI, MS	15.97
33	1724	Neryl acetate	4.12	2.18	t	1.40	-	RI, MS	16.08
34	1733	Geranial	0.22	1.03	t	-	-	RI, MS	16.19
35	1741	Carvone	t	t	t	-	-	RI, MS	16.28
36	1744	α-Farnesene	-	-	-	0.43	-	RI, MS	16.31
37	1755	Geranyl acetate	5.92	6.12	0.64	0.64	-	RI, MS	16.43
38	1761	δ-Cadinene	t	-	0.84	0.27	0.43	RI, MS	16.50
39	1764	β-Citronellol	-	0.89	-	-	-	RI, MS	16.54
40	1771	β-Sesquiphellanderene	-	-	-	-	t	RI, MS	16.62
41	1791	Perillaldehyde	-	-	-	t	-	RI, MS	16.85
42	1799	Nerol	2.37	1.46	0.30	0.37	-	RI, MS	16.95
43	1838	Carveol	t	t	0.17	t	-	RI, MS	17.35
44	1847	Geraniol	5.35	2.18	1.24	t	-	RI, MS	17.45
45	1868	*trans*-Carveol	-	-	t	t	-	RI, MS	17.68
46	2065	Octanoic acid	-	t	-	-	t	RI, MS	19.63
47	2143	Spathulenol	-	-	1.95	t	0.37	RI, MS	20.35
48	2192	Thymol	-	-	1.76	t	1.36	RI, MS	20.80
49	2215	Not identified	-	2.30	-	-	-		21.02
50	2223	Carvacrol	-	t	0.23	t	t	RI, MS	21.09
51	2252	α-Cadinol	-	-	0.43	-	-	RI, MS	21.36
52	2263	Not identified	-	0.99	-	1.00	-		21.46
53	2281	*n*-Decanoic acid	-	t	t	t	-	RI, MS	21.63
		Total Identified (%)	99.8	96.2	99.5	96.4	99.6		
		Compounds Identified	28	40	37	35	30		

a. SD (±) were insignificant and excluded from the Table to avoid congestion.
b. Identification by Kovats index (RI = retention index).
c. Identification by GC-MS library search.
d. Standard compound co-injected.
e. Detected < 0.1 %.
f. Not detected.

4. Conclusions

The volatile components of the essential oils of five major citrus cultivars in Kikai-jima were investigated. The oil extracted from 'Shiiku' contained high levels of linalool and other specific constituents, showing a composition similar to that of bergamot. GC-MS analysis of the 'Shiiku' oil is reported for the first time. The compositional patterns of the essential oils of the other four citrus peels of Kikai-jima Island were very similar to those grown in Okinawa Island.

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