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Volatile Chemicals Identified in Extracts from Newly Hybrid Citrus, Dekopon (*Shiranuhi mandarin* Suppl. J.)

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Extracts from the peel and flesh of a citrus fruit, dekopon (*Shiranuhi mandarin* Suppl. J.), were obtained under reduced pressure followed by dichloromethane extraction. A total of 127 volatile chemicals were identified in the extracts using gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS). They included 11 monoterpenes, 32 monoterpenoids, 9 sesquiterpenes, 5 sesquiterpenoids, 20 aliphatic alcohols, 14 aliphatic esters, 15 aliphatic aldehydes and ketones, 7 aliphatic acids, and 10 miscellaneous compounds. The major volatile constituents of the extract from the peel were *d*-limonene (2380.33 mg/kg), myrcene (36.54 mg/kg), bisabolene (30.03 mg/kg), sabinene (21.12 mg/kg), *trans*-β-ocimene (16.96 mg/kg), valencene (12.84 mg/kg), decanal (8.14 mg/kg), β-phellandrene (4.53 mg/kg), citronellol (4.51 mg/kg), 4-terpineol (4.50 mg/kg), linalool (4.13 mg/kg), and citronellyl acetate (3.63 mg/kg). The major volatile constituents of the extract from the flesh were ethyl acetate (21.54 mg/kg), acetoin (7.23 mg/kg), 3-methylbutanol (2.79 mg/kg), *p*-mentha-*cis*-2,8-dien-1-ol (1.01 mg/kg), 3-methylbutanoic acid (0.95 mg/kg), isobutanol (0.59 mg/kg), *trans*-isopiperitenol (0.58 mg/kg), *p*-mentha-*trans*-2,8-dien-1-ol, and *trans*-carveol (0.44 mg/kg). Compositions of volatile chemicals in peel and flesh extract were considerably different: the peel extract was rich in terpenes, whereas the flesh extract was rich in aliphatic compounds.

KEYWORDS: Citrus flesh; citrus peel; volatile chemicals; gas chromatography; dekopon

INTRODUCTION

Essential oils of citrus peels have been used to create artificial flavors and fragrances for many years. There have been numerous studies on the constituents of volatile chemicals of citrus peel and flesh, such as orange (*1*, *2*), Mandarin orange (*3*), lemon and lime (*4*), and tangerine (*5*, *6*). Many new citrus species have been produced by hybridization, and their volatile constituents have also been reported. For example, 40 volatile compounds were reported in the leaf oil and peel oil of hybrid species of *Citrus unshiu* and *Citrus reticulata* (*7*). Volatile constituents of new citrus fruits prepared by hybridization have begun to receive much attention among flavor chemists today.

Dekopon (*Shiranuhi mandari*n Suppl. J.) is a hybrid of Kiyomi tangor (*Citrus unshiu* Marc. × *Citrus sinensis* Osb.) and ponkan (*Citrus reticutata* Bla.). It was developed by the Fruit and Vegetable Experimental Station of the Japanese Ministry of Forestry and Fishery in 1972. Kiyomi is a hybrid of Satsuma Mandarin (*C. unshiu* Marc.) and orange (*C. sinensis* Osb). Therefore, dekopon is a grandchild of the orange. A dekopon fruit is 7.5-8.8 cm and weighs 200-250 g. It is very rich in juice and has an orange-like aroma. It is now produced in the southern part of Japan and in Korea. The annual production of dekopon is now 14000 tons in Japan.

In the present study, volatile chemicals in extracts obtained from a dekopon fruit were identified by gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS).

MATERIALS AND METHODS

Materials. Dekopon fruits were bought from a local market. Authentic volatile compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI), Tokyo Kasei Organic Chemicals (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Fluka Chemical Co. (Ronkonkoma, NY) or obtained from Takata Koryo Co., Ltd. (Osaka, Japan) as a gift.

Isolation of Volatile Chemicals under Reduced Pressure. Peel (535 g) and flesh (868 g) of five dekopon fruits were homogenized separately in a blender with 1800 mL of distilled water and 200 mL of saturated sodium chloride solution. The homogenized sample was placed in a 3 L round-bottom flask. The solution was steam distilled at 32 °C under reduced pressure (32 mmHg). The distillate (900 mL) was extracted with 130 mL of dichloromethane using a liquid−liquid continuous extractor for 6 h. After the extract had been dried over anhydrous sodium sulfate, the solvent was removed by distillation with a Vigreux column. The distillation was stopped when the volume of extract was reduced to 1 mL, and then the solvent was further reduced under a purified nitrogen stream until the extract weight was 100 mg. The sample was stored at 5 °C until analysis.

Instrumental Analyses of Components. All samples were analyzed using the Kovats GC retention index I (*8*) and GC-MS. The GC retention index and MS fragmentation pattern of each component were compared with those of the authentic compound for qualitative analysis.

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Table 1. Volatile Compounds Identified in Extracts from Peel and Flesh of Dekopon

compound	GC peak no.[a]	Kovats index	concn (mg/kg) peel	concn (mg/kg) flesh	compound	GC peak no.[a]	Kovats index	concn (mg/kg) peel	concn (mg/kg) flesh
				Monoterpenes					
α-pinene	9	1011	1.75	–[b]	γ-terpinene	29	1228	0.74	*c*
β-pinene	15	1093	1.03	–	*trans*-β-ocimene	31	1241	16.96	–
sabinene	17	1108	21.12	–	*p*-cymene	33	1255	0.13	–
myrcene	22	1150	36.54	–	terpinolene	34	1268	0.38	–
d-limonene	25	1181	2380.32	0.27					
β-phellandrene	27	1207	4.53	–	**total**			**2463.33**	**0.27**
cis-β-ocimene	28	1225	0.54	–					
				Monoterpenoids					
cis-limonene oxide	43	1430	0.17	–	citronellol	92	1759	4.51	0.02
trans-limonene oxide	45	1442	3.12	0.09	*p*-mentha-1(7),8-dien-2-yl acetate	93	1763	0.27	–
trans-sabinene hydrate	50	1459	–	*c*	L-perillaldehyde	94	1768	0.82	–
citronellal	51	1465	2.99	–	*p*-menthen-9-yl acetate	97	1808	0.21	–
linalool	59	1539	4.13	–	*trans*-carveol	98	1825	0.64	0.44
cis-sabinene hydrate	60	1540	0.53	0.02	geranylacetone	100	1840	–	0.06
trans-*p*-mentha-2-en-1-ol	62	1553	0.17	–	*p*-mentha-1,8-dien-9-yl acetate	101	1846	0.09	–
4-terpineol	66	1590	4.5	1.8	*cis*-carveol	102	1855	0.43	0.05
p-mentha-*cis*-2,8-dien-1-ol	69	1620	1.19	1.01	*p*-mentha-1(7),8-dien-2-ol	104	1877	–	0.02
citronellyl acetate	72	1649	3.63	*c*	*p*-menthen-9-ol	106	1924	–	0.03
p-mentha-*trans*-2,8-dien-1-ol	77	1664	0.80	0.58	5,6-epoxy-β-ionone	108	1967	–	0.01
trans-verbenol	79	1671	–	0.02	*p*-mentha-1,8-dien-9-ol	109	1979	–	0.01
p-mentha-1,8-dien-4-ol	80	1678	–	0.01	L-perillyl alcohol	111	1994	0.19	0.01
α-terpineol	81	1690	–	0.02	*p*-menth-8-ene-*cis*-1,2-diol	121	2250	–	0.03
neryl acetate	84	1706	0.95	–	*p*-menth-8-ene-*trans*-1,2-diol	122	2268	0.07	0.07
L-carvone	86	1721	1.44	0.09					
cis-isopiperitenol	88	1739	–	0.38	**total**			**43.17**	**3.55**
trans-isopiperitenol	90	1745	–	0.58					
				Sesquiterpenes					
δ-elemene	47	1452	0.54	–	α-humulene	74	1656	0.15	–
α-copaene	52	1472	0.47	–	valencene	82	1699	12.8	0.08
β-cubebene	57	1519	0.11	–	bisabolene	87	1736	30.03	–
β-elemene	64	1570	0.66	–	δ-cadinene	89	1739	0.87	–
β-caryophyllene	65	1575	0.23	–					
γ-elemene	70	1625	0.25	–	**total**			**45.57**	0.08
				Sesquiterpenoids					
caryophyllene oxide	107	1960	–	0.01	nootkatone	126	2504	1.86	0.14
caryophylla-4(12),8(13)-dien-5-one	113	2028	–	0.01					
trans-nerolidol	114	2029	0.52	–	**total**			**2.46**	**0.16**
elemol	116	2069	0.08	–					
				Aliphatic Alcohols					
ethanol	4	926	–	0.20	(*Z*)-3-hexenol	40	1376	0.55	0.16
2-butanol	10	1022	–	0.01	heptanol	46	1448	0.10	0.11
2-methyl-3-buten-2-ol	11	1036	–	0.09	2-ethylhexanol	53	1481	–	*c*
isobutanol	14	1087	–	0.59	octanol	61	1550	–	0.15
2-pentanol	19	1117	–	0.01	(*E*)-2-octenol	67	1603	–	0.01
butanol	21	1138	–	0.02	nonanol	73	1654	0.28	0.08
3-methylbutanol	26	1203	–	2.97	(*E*)-2-nonenol	83	1703	–	*c*
3-methyl-3-buten-1-ol	30	1240	–	0.01	decanol	91	1755	1.11	0.04
pentanol	32	1244	–	0.05	(*E*)-2-decenol	96	1808	–	0.05
(*Z*)-2-pentenol	37	1313	–	0.05					
hexanol	39	1347	–	0.14	**total**			**2.04**	**4.83**
				Aliphatic Esters					
methyl acetate	2	856	–	0.05	ethyl 3-hydroxy-3-methylbutanoate	42	1400	–	0.02
ethyl acetate	3	920	–	21.54	octyl acetate	49	1458	0.65	–
ethyl propanoate	5	949	–	0.05	ethyl 3-hydroxylbutanoate	55	1508	–	0.20
ethyl isobutanoate	6	957	–	0.01	ethyl 3-hydroxy-2-methylbutanoate	58	1537	–	0.01
propyl acetate	7	969	–	0.02	decyl acetate	78	1669	0.21	–
isobutyl acetate	8	1007	–	0.01	ethyl dodecanoate	99	1829	–	0.01
ethyl 2-methylbutanoate	12	1042	–	0.01					
ethyl crotonate	23	1151	–	0.04	**total**			**0.86**	**21.97**
				Aliphatic Aldehydes and Ketones					
acetone	1	845	–	0.11	nonanal	41	1380	0.16	–
hexanal	13	1070	–	0.02	(*E*,*Z*)-2,4-heptadienal	48	1457	–	0.01
(*Z*)-2-pentenal	16	1097	–	0.01	decanal	54	1485	8.14	–
(*E*)-2-pentenal	18	1117	–	0.01	(*E*)-2-decenal	71	1629	–	0.01
(*Z*)-3-hexenal	20	1130	0.29	0.08	(*E*,*E*)-2,4-decadienal	95	1795	0.17	–
2-heptanone	24	1169	–	0.02	4,5-epoxy-(*E*)-2-decenal	110	1985	–	0.01
acetoin	35	1277	–	7.23					
octanal	36	1277	0.11	–	**total**			**8.87**	**7.51**
6-methyl-5-hepten-2-one	38	1336	–	*c*					

Table 1. (Continued)

compound	GC peak no.[a]	Kovats index	concn (mg/kg) peel	concn (mg/kg) flesh	compound	GC peak no.[a]	Kovats index	concn (mg/kg) peel	concn (mg/kg) flesh
				Aliphatic Acids					
acetic acid	44	1437	–	0.06	nonanoic acid	119	2155	–	0.05
isobutanoic acid	63	1554	–	0.11	dodecanoic acid	125	2470	–	0.02
3-methylbutanoic acid	75	1658	–	0.95	tetradecanoic acid	127	2682	–	0.02
2-methylbutanoic acid	76	1664	–	0.04					
octanoic acid	115	2047	–	0.05	**total**			**0**	**1.25**
				Miscellaneous Compounds					
2-(methylthio)ethanol	56	1516	–	0.03	γ-decalactone	118	2132	–	0.01
γ-butyrolactone	68	1610	–	0.11	docosane	120	2200	–	0.03
3-(methylthio)propanol	85	1708	–	0.02	tricosane	123	2300	–	0.05
benzyl alcohol	103	1864	–	0.11	1,3-acetylbenzene	124	2333	–	0.01
phenylethyl alcohol	105	1899	–	0.07					
γ-nonalactone	112	2015	–	0.02	**total**			**0**	**0.37**
heneicosane	117	2100	–	0.02					

[a] Refer to **Figures 1** and **2**. [b] –, not detected. [c] Less than 0.01 mg/kg.

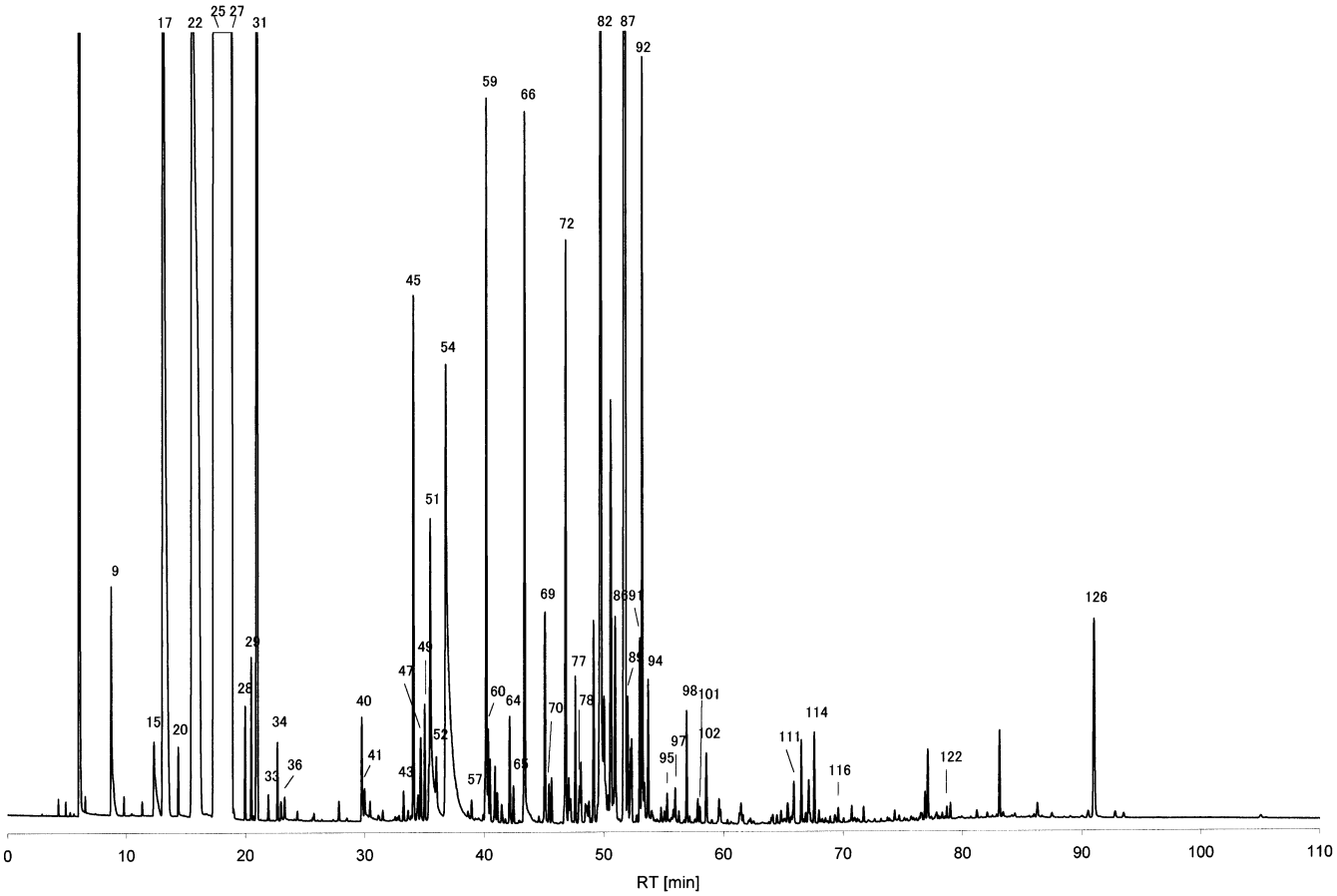

Figure 1. Typical gas chromatogram of an extract from peels of dekopon.

A Hewlett-Packard (HP) 6890 GC equipped with a 60 m × 0.25 mm (d_f = 0.25 μm) DB-Wax bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used for routine quantitative analysis. The oven temperature was held at 40 °C for 2 min and then programmed to 200 °C at 2 °C/min. Detector and injector temperatures were 250 °C. The linear velocity of the helium carrier gas flow rate was 30 cm/s at a split ratio of 30/1.

An HP 6890 GC interfaced to an HP 5973 mass spectrometer was used for MS identification of the GC components. The column and oven conditions for GC-MS analysis were identical to those used for the GC analysis. MS was operated at a scan range of *m*/*z* 35−350, an ionization voltage of 70 eV, and an ion source temperature of 230 °C.

RESULTS AND DISCUSSION

The total yields of volatile chemicals from peel and flesh were 2555.26 mg/kg (0.26%, w/w) and 40.24 mg/kg (0.0042%, w/w), respectively. **Table 1** shows the compounds identified in extracts obtained from the peel and flesh of dekopon with their calculated concentrations and Kovats indices on a DB-Wax column. The concentration of each chemical was calculated using a method previously reported (*9*).

Typical gas chromatograms of extracts from peel and flesh are shown in **Figures 1** and **2**, respectively. A total of 127 components were identified in the present study. The composi-

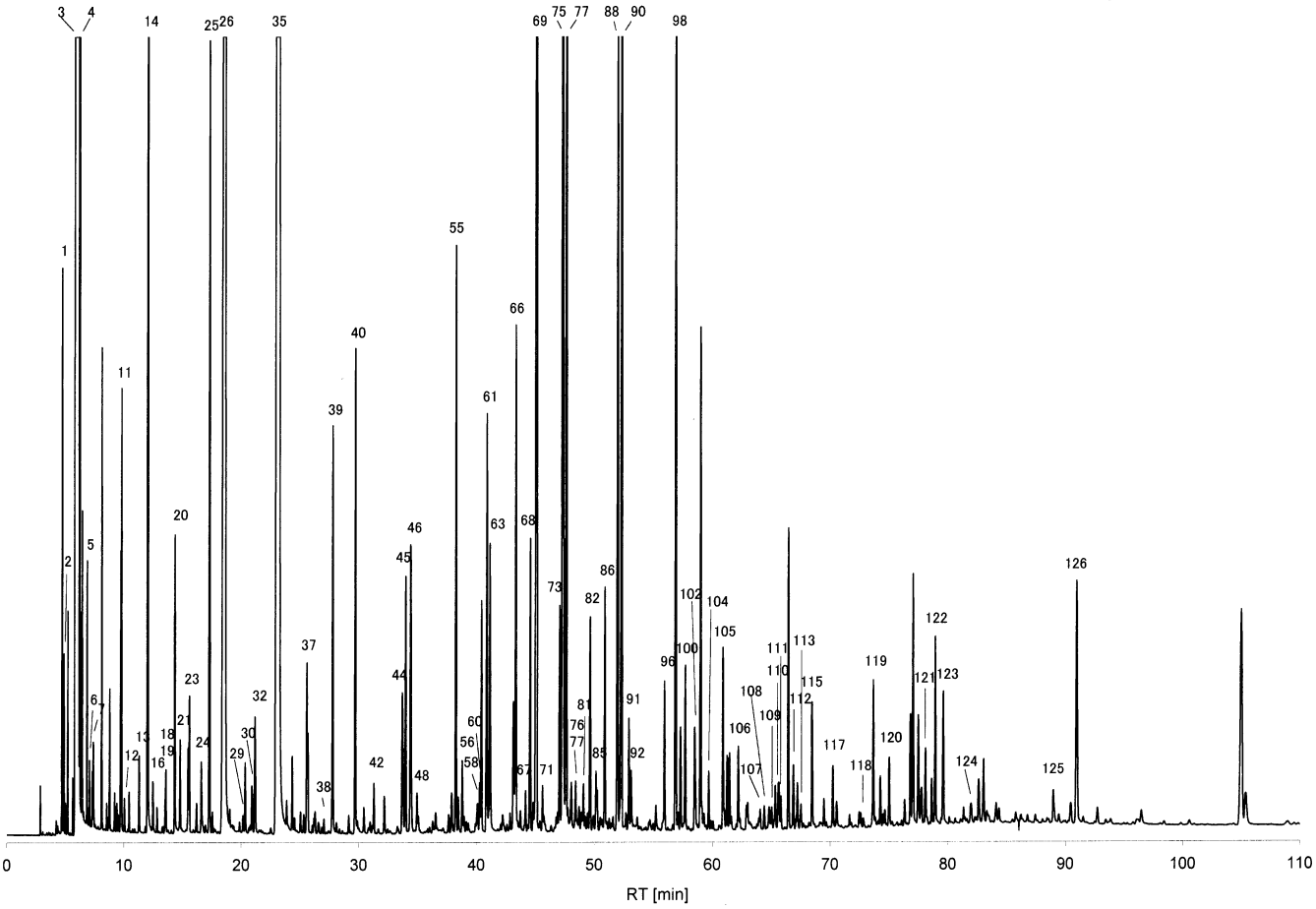

Figure 2. Typical gas chromatogram of an extract from the flesh of dekopon.

tions of extracts recovered from flesh and peel were considerably different. Totals of 54 and 89 chemicals were identified in the extracts from the peel and the flesh, respectively.

The major group of chemicals found in the peel extract was 11 monoterpenes (hydrocarbon), which composed 96.43% of the total GC peak area; a large amount (2380.32 mg/kg) of *d*-limonene composed 93.15% of the total GC peak area. Myrcene (36.54 mg/kg) and *trans*-β-ocimene (16.96 mg/kg), respectively, made up the second and third greatest amounts among the monoterpenes found in peel extract. Twenty monoterpenoids (oxygenated monoterpenes), which composed 1.03% of the total GC peak area, were identified in the peel extract. The major volatile constituents of monoterpenoids in this extract were citronellol (4.51 mg/kg)—which is the greatest amount among the monoterpenoids found—linalool 4.13 mg/kg), citronellyl acetate (3.63 mg/kg), *trans*-limonene oxide (3.12 mg/kg), and citronellal (2.99 mg/kg). Among nine sesquiterpenes found in the peel extract, bisabolene (30.03 mg/kg) made up the greatest amount, followed by valencene (12.8 mg/kg).

The major group of chemicals identified in the flesh extract were aliphatic compounds, including 20 alcohols (total = 4.83 mg/kg), 12 esters (total = 21.97 mg/kg), 11 aldehydes and ketones (total = 7.51 mg/kg), and 7 acids (total = 1.25 mg/kg). The major volatile constituents in this extract were ethyl acetate (21.54 mg/kg)—which is the greatest amount—acetoin (7.23 mg/kg), 3-methylbutanol (2.97 mg/kg), *p*-mentha-*cis*-2,8-dien-1-ol (1.01 mg/kg), and 3-methylbutanoic acid (0.95 mg/kg).

The peel extract contained a total 43 terpene compounds that composed 99.97% of the total GC peak area, whereas the flesh extract contained a total 29 terpene compounds, which composed only 9.59% of the total GC peak area. The number of aliphatic compounds found in the peel extract was 11, which composed only 0.46% of the total GC peak area. On the other hand, the flesh extract contained 40 aliphatic compounds, which composed 88.37% of the total GC peak area. Seven aliphatic acids (total = 1.25 mg/kg) were found in the flesh extract, but none were found in the peel extract. The flesh extract contained 10 miscellaneous compounds, including 3 γ-lactones and 2 sulfur-containing compounds, but none of them were detected in the peel extract.

The level of terpenes in the extract from flesh was rather low. However, this may be due to the low content of *d*-limonene compared to the extract from peel.

Myrcene was second greatest amount after *d*-limonene, which is consistent with Uruguayan Satsuma Mandarin (grandparent of dekopon) oil. On the other hand, *trans*-β-ocimene, which is in third greatest amount in the peel oil of dekopon, was found only in trace amount in Uruguayan Satsuma Mandarin oil. γ-Terpenene, which was in second greatest amount in Uruguayan Satsuma Mandarin oil, was not a major constituent of dekopon (*10*). Mandarin hybrid juices also contained a high level of γ-terpenene (*11*).

These 14 aliphatic alcohols and 7 esters were also found in headspace samples of orange (grandparent of dekopon) (*12*). However, a comparison of the composition of volatiles between the samples in the present study and in headspace samples is not relevant because of different sampling methods used.

It is well-known that *d*-limonene is the major constituent of citrus peel oils. Generally, *d*-limonene composes >90% of citrus

peel oils. The content of *d*-limonene in the dekopon peel seems to be considerably higher than it is in other citrus peels, suggesting that dekopon is a good source of *d*-limonene. The composition and quantity of flavor chemicals, such as aldehydes, ketones, and esters, in the flesh extract seem to be well balanced, which may be why dekopon fruit has gained popularity.

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