Food Sci. Technol. Int. Tokyo, 3 (4), 329–335, 1997

Comparison of Essential Oil Components between Leaf and Peel in Citrus Hybrids ('Seto unshiu'×'Morita ponkan')

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Received January 29, 1997; Accepted June 23, 1997

The leaf oil components in hybrid seedlings of 'Seto unshiu' (*Citrus unshiu* MARC.) crossed with 'Morita ponkan' (*Citrus reticulata* BLANCO) as the pollen parent were examined. The leaf and peel oil components in the hybrid seedlings were also compared with those of their parents. Twenty eight of the 32 peaks found in the hydrocarbon fraction of the leaf oils were identified. The major compounds were γ-terpinene and β-caryophyllene in 'Seto unshiu' and sabinene and β-ocimene in 'Morita ponkan.' All 40 peaks found in the oxygenated compound fraction of the leaf oils were identified. The major compounds were linalool, (*Z*)-3-hexenal, and (*Z*)-2-hexenal. There was a correlation between the leaf oil and peel oil in the percentage of the oxygenated compound fraction in the hybrid seedlings. A similar correlation was found for the parents. When the hybrids were classified according to the major kinds of monoterpenes, such as sabinene and γ-terpinene, and thymol and α-sinensal, the same groupings were obtained with the leaf and peel oils. These results suggested that the flavor of the fruit can be predicted by analyzing the oil in the leaf, which can be sampled much earlier than the fruit.

Keywords: citrus hybrid seedling, leaf oil, Ponkan, Satsuma mandarin, essential oil component

Satsuma mandarin has a number of excellent characteristics related to productivity and quality, such as cold resistance, high productivity, and a skin that can be easily peeled. However, it has some drawbacks, such as a relatively weak aroma and the production of off-flavor when the juice is heated (Sawamura *et al.*, 1977, 1978). One way to solve these problems is to crossbreed satsuma mandarin with other citrus varieties to produce new citrus hybrids having both the desirable characteristics of satsuma mandarin and the good flavor of other citrus fruits. For effective crossbreeding, it is very important to clarify how the characteristic flavors of the parents are inherited by the hybrids. However, there have been only a few studies on the flavor of the citrus hybrids, mainly because the efficiency of crossbreeding is extremely low when polyembryonic varieties such as satsuma mandarin and sweet orange are used for seed parents.

However, using the current breeding method, it is necessary to wait for the first fruit of the hybrid seedling to determine its flavor. A more efficient method would be to predict the flavor of the fruit by analyzing the leaf oil at the early seedling stage, because the leaf of the hybrid can be sampled much earlier than the fruit. Many studies have been done on the citrus leaf oil composition for classification and identification of citrus species (Kesterson *et al.*, 1964; Pieringer *et al.*, 1964; Attaway *et al.*, 1966; Scora & Torrisi, 1966; Kamiyama & Amaha, 1972; Lund *et al.*, 1981), for identification of hybrids of polyembryonic citrus (Ikeda, 1976), and for utilization of leaf oil (Ekundayo *et al.*, 1991). These studies have shown that there is a correlation between leaf oil components and citrus species. However, the relationships between the peel and leaf oils in each of the individual hybrid seedlings have not been examined.

We (Sakamoto *et al.*, 1994) have previously examined the peel oil components in the hybrid seedlings of 'Seto unshiu' (*Citrus unshiu* MARC.) crossed with 'Morita ponkan' (*Citrus reticulata* BLANCO) and found that many of the hybrid seedlings had the characteristic flavors of the parents but some hybrid seedlings did not. The peel oils were composed of many compounds that were common to all hybrids and a few compounds that were specific for certain hybrids. In the present study, the leaf oil components in the hybrid seedlings of 'Seto unshiu' crossed with 'Morita ponkan' were examined as in the previous study, and the relationship between the peel oil and leaf oil components was examined to investigate which components of the peel oil can be predicted by the leaf oil.

Materials and Methods

Plant materials Six hybrids (A–F) were obtained from crossing 'Seto unshiu' (*Citrus unshiu* MARC.) with 'Morita ponkan' (*Citrus reticulata* BLANCO) as the pollen parent. The seedlings were grafted onto Trifoliate orange

(*Poncitrus trifoliata* RAF.) in September 1985 in the experimental field of the Fruit Tree Research Institute, Hiroshima Prefectural Agricultural Research Center. The leaves of each hybrid and their parents were collected in December 1991.

Preparation of leaf oil from citrus leaves The leaves (10 g) were mixed with liquid nitrogen and homogenized with an electric blender. The finely divided leaves (5 g) and 500 ml of deionized water were placed in a 1000-ml round-bottom flask. The leaf oils were separated from the leaves with 80 ml of diethyl ether by simultaneous distillation-extraction at 70°C under reduced pressure (approximately 70 mmHg) for 1 h, using a modified Likens-Nickerson apparatus. The total amount of extract was dried for 18 h over anhydrous sodium sulfate and concentrated to about 50 μl under a purified nitrogen stream. The concentrates were kept in the dark at −80°C until gas chromatograph-mass spectrometer (GC-MS) analysis. Constituents were separated for calculation of peak area percent and identification by injecting 1 μl concentrate of leaf oil into a capillary GC-MS.

Capillary GC-MS A Varian 3400 gas chromatograph interfaced to a Finnigan MAT Model 800 ion trap detector was used for calculation of peak area percent and mass spectral identification of the GC components. Peak area percent was calculated using a GC-MS total ion chromatogram. The scanning rate was 1 scan/s. Leaf oil components were identified by comparison of their Kovats gas chromatographic retention indices and mass spectra with those of authentic compounds or with those in the *Wiley/NBS Registry of Mass Spectral Data*. Separation was achieved on a 60 m×0.25 mm i.d. fused silica capillary column, coated with cross-linked polyethylene glycol 20 M, film thickness 0.25 μm (DB-WAX, J&W Scientific, Folsom, CA). The column temperature was maintained at 40°C and then programmed to increase 3°C/min up to 230°C (30-min hold). The injector temperature was 230°C. The column flow rate of helium, the carrier gas, was 30 cm/s. An injector splitter was used at a split ratio of 30 : 1.

Results and Discussion

Characteristics of the hydrocarbon compounds in leaf oil Table 1 shows the essential oil compositions of the hydrocarbon fractions of the leaf oils from 'Seto unshiu,' 'Morita ponkan,' and six hybrids therefrom, along with their Kovats indices obtained with a DB-WAX column. Of the 32 peaks detected, 28 were identified or inferred. All 19 of the hydrocarbons that had been identified in peel oil (Sakamoto *et al.*, 1994) were detected in the leaf oil. The numbers of components detected were 25 in 'Seto unshiu,' 29 in 'Morita ponkan,' and 21 to 25 in the hybrids. No components were detected in the hybrids that were not found in the parents.

Table 1. Percentages of hydrocarbons in leaf oil from 'Seto unshiu,' 'Morita ponkan' and their hybrid seedlings.

Compounds	Seto unshiu	Morita ponkan	Hybrid seedlings						Kovats index
			A	B	C	D	E	F	
α-Pinene	1.84	2.03	1.52	2.91	2.60	3.18	1.35	2.94	1025
α-Thujene	0.87	0.42	0.38	1.58	1.57	1.96	0.34	1.65	1029
β-Pinene	5.52	3.21	2.67	4.03	4.00	4.74	2.44	4.01	1114
Sabinene	1.11	37.3	30.2	0.56	0.56	0.67	34.6	0.58	1125
Myrcene	0.72	4.33	3.52	1.74	1.22	1.44	3.52	1.25	1168
α-Phellandrene	—	0.04	—	—	—	0.07	0.08	0.07	1170
α-Terpinene	0.34	0.24	0.31	0.65	0.58	0.48	0.37	0.58	1182
d-Limonene	4.34	4.20	3.82	5.92	7.64	6.33	4.82	6.79	1205
β-Phellandrene	—	0.69	0.67	—	—	—	0.55	—	1211
cis-β-Ocimene	0.09	0.78	0.37	0.19	0.37	0.31	0.63	0.24	1240
γ-Terpinene	22.1	2.49	3.86	34.4	26.8	28.4	3.11	28.6	1255
trans-β-Ocimene	3.88	9.71	5.46	3.86	6.79	6.76	9.72	4.57	1256
p-Cymene	13.4	1.34	1.44	18.2	17.7	22.3	1.41	16.2	1277
Terpinolene	1.34	0.39	0.49	2.45	2.39	1.98	0.48	2.31	1288
δ-Elemene	—	0.84	—	—	0.82	0.13	—	—	1478
α-Copaene	0.35	—	0.20	—	—	0.13	—	—	1500
β-Cubebene	0.35	—	—	—	—	—	—	—	1548
trans-α-Bergamotene	—	0.18	—	—	—	—	—	—	1566
Sesquiterpene M.W. 204	0.42	—	0.38	0.30	0.15	0.23	—	0.24	1586
β-Elemene	9.41	4.70	7.31	6.51	3.55	5.77	0.14	4.97	1599
β-Caryophyllene	16.2	5.01	13.6	7.10	7.98	8.01	8.73	8.47	1610
Sesquiterpene M.W. 204	—	0.12	—	—	0.13	—	—	—	1645
β-Farnesene	—	5.55	—	—	—	—	—	—	1670
α-Caryophyllene	5.42	0.82	3.84	3.04	2.07	2.95	0.73	2.63	1685
Sesquiterpene M.W. 204	0.11	0.07	—	—	—	—	—	0.79	1694
Germacrene D	0.74	1.78	0.31	—	2.14	0.12	0.19	0.28	1724
Valencene	0.05	0.22	0.36	—	0.19	—	0.37	—	1732
β-Bisabolene	2.97	8.27	13.1	2.58	6.82	1.34	15.1	8.35	1748
α-Farnesene	6.90	1.43	4.78	3.33	0.76	2.06	10.1	3.77	1752
δ-Cadinene (T)	0.84	0.59	1.17	0.21	0.41	0.31	0.94	0.50	1769
Sesquiterpene M.W. 204	0.64	0.57	0.19	0.38	0.11	0.33	0.25	0.19	1777
γ-Elemene	—	2.66	—	0.06	2.68	—	—	—	1849

(T): Tentative compound.

—: Not detected.

d-Limonene, which was the major component of peel oil, constituted only 7.6% of the oils in leaf oil. 'Seto unshiu' was rich in *γ*-terpinene, *p*-cymene, and *β*-caryophyllene, followed by *β*-elemene and *β*-pinene in that order. 'Morita ponkan' was rich in sabinene and *β*-ocimene, followed by *β*-bisabolene and *β*-farnesene in that order, showing a considerably different hydrocarbon composition from that of 'Seto unshiu.' Most notably, *γ*-terpinene and *p*-cymene in 'Seto unshiu' and sabinene in 'Morita ponkan' accounted for over 10% of the total hydrocarbon constituents, and they are expected to provide important clues in assessing the resemblance between the hybrids and their parents. Kamiyama and Amaha (1972) also showed high contents of *γ*-terpinene and *p*-cymene in the leaf oil of *Citrus unshiu* and a high content of sabinene in that of *Citrus reticulata* (Ponkan).

Characteristics of the oxygenated compounds in leaf oil Table 2 shows the essential oil compositions of the oxygenated compound fractions of the leaf oils from 'Seto unshiu,' 'Morita ponkan,' and six hybrids therefrom. Forty oxygenated compounds were identified or inferred. Of the 81 compounds identified or inferred in the peel oil (Sakamoto *et al.*, 1994), 29 were also found in leaf oil. While there were more peaks of hydrocarbons detected in the leaf oil than in the peel oil, the number of peaks of oxygenated compounds was larger in the peel oil than in the leaf oil. Components that constituted more than 10% of the total oxygenated compounds in the leaf oil in both 'Seto unshiu' and 'Morita ponkan' were linalool, (*Z*)-3-hexenal, and (*E*)-2-hexenal,

Table 2. Percentages of oxygenated-compounds in leaf oil from 'Seto unshiu,' 'Morita ponkan' and their hybrid seedlings.

Compounds	Seto unshiu	Morita ponkan	Hybrid seedlings						Kovats index
			A	B	C	D	E	F	
Alcohols	44.0	75.9	85.9	68.1	69.1	87.5	86.4	81.4	
Ethanol	1.15	0.71	0.99	0.97	0.92	0.75	0.85	0.36	933
1-Penten-3-ol	1.55	0.59	0.31	1.05	0.66	0.24	0.54	0.58	1159
(*Z*)-2-Penten-1-ol	1.25	0.55	0.33	0.47	0.69	0.24	0.30	0.53	1321
1-Hexanol	0.39	—	0.07	0.10	0.08	0.06	0.12	0.09	1355
(*E*)-3-Hexenol	—	—	—	—	—	—	—	0.12	1361
(*Z*)-3-Hexenol	2.05	0.28	0.60	0.58	0.60	0.53	0.54	0.57	1387
trans-Sabinene hydrate	0.40	3.45	2.77	0.40	0.23	0.42	3.41	0.28	1469
Linalool	34.7	67.2	74.6	62.2	64.6	82.3	73.0	76.6	1550
cis-Sabinene hydrate	0.29	0.60	0.56	0.20	0.11	0.35	0.91	0.28	1554
1-Octanol	0.13	—	0.09	0.07	—	0.12	0.11	0.08	1560
α-Terpineol	1.23	1.15	2.39	1.17	0.78	1.60	2.26	1.18	1702
Citronellol	—	0.12	—	—	—	—	—	—	1768
Nerol	—	—	0.12	0.08	—	0.14	0.23	0.09	1802
Myrtenol	—	0.15	0.05	—	—	—	—	—	1807
Nerolidol	—	0.36	0.21	0.31	0.16	0.31	0.37	0.27	2040
β-Elemol (T)	0.61	0.16	0.11	0.15	0.22	0.18	—	0.10	2086
Thymol	0.28	0.56	2.74	0.34	—	0.30	3.74	0.22	2185
Isothymol	—	—	—	0.05	—	—	—	—	2215
Aldehydes	55.0	23.5	13.6	31.2	30.3	12.1	13.1	18.3	
Hexanal	3.08	1.16	0.76	1.94	2.09	0.44	0.87	1.43	1081
(*E*)-2-Pentenal	0.23	—	0.04	0.06	0.06	—	0.04	0.05	1129
(*E*)-3-Hexenal	2.42	0.53	0.49	0.86	0.90	0.38	0.22	0.53	1135
(*Z*)-3-Hexenal	12.9	7.23	4.87	9.99	9.36	2.31	3.37	4.13	1140
(*E*)-2-Hexenal	35.7	6.75	6.81	11.1	13.9	4.75	8.04	9.13	1218
Octanal	—	0.23	0.33	0.19	0.29	0.55	0.22	0.21	1290
Nonanal	—	0.09	—	—	—	0.21	—	0.21	1395
(*E, E*)-2-Hexadienal	0.42	0.09	0.07	0.24	0.21	0.11	0.07	0.18	1403
Citronellal	—	0.20	—	—	—	0.06	—	—	1482
Decanal	0.22	0.23	0.14	0.36	0.50	0.54	0.22	0.21	1501
Neral	—	—	—	0.32	0.24	0.82	—	—	1685
Dodecanal	—	0.05	—	0.24	0.13	0.10	—	0.10	1712
β-Sinensal	—	5.03	0.05	0.04	0.16	—	0.03	0.04	2236
α-Sinensal	—	1.94	—	5.85	2.41	1.79	—	2.09	2340
Esters	0.32	0.29	0.35	0.35	0.36	0.15	0.36	0.13	
Ethyl formate	0.11	0.07	0.06	0.07	0.08	0.03	0.04	0.03	821
Ethyl acetate	0.20	0.21	0.22	0.28	0.28	0.12	0.12	0.10	886
Neryl acetate	—	—	0.07	—	—	—	0.20	—	1728
Ketones	0.25	0.11	0.07	0.16	0.13	0.06	0.04	0.07	
2-Butanone	—	—	—	0.05	—	0.02	—	—	902
2-Pentanone	—	—	—	—	—	—	0.03	—	975
1-Penten-3-one	0.25	0.11	0.07	0.11	0.13	0.04	0.01	0.07	1019
Others	0.42	0.18	0.09	0.25	0.22	0.18	0.12	0.15	
cis-linalool oxide	—	0.10	0.09	0.06	—	—	0.12	0.04	1447
Caryophyllene oxide	0.42	0.08	—	0.18	0.22	0.18	—	0.10	2003

(T): Tentative compound.

—: Not detected.

which together amounted to over 80% of the total oxygenated compounds of both parents. The percentages of linalool and (E)-2-hexenal were almost the same (35.7% and 34.7%, respectively) in 'Seto unshiu' while they differed considerably (67.2% and 6.75%, respectively) in 'Morita ponkan.' In the hybrid seedlings, the percentage of linalool was over 60% in all the hybrids, while that of (E)-2-hexenal was low in all the hybrids, the highest being 13.9% in the seedlings of hybrid C, which had compositions closer to that of 'Morita ponkan.' Other than these three components, *trans*-sabinene hydrate, thymol, β-sinensal, and α-sinensal constituted over 3% of the total oxygenated compounds in the hybrid seedlings. In 'Morita ponkan' and hybrids A and E in which the percentage of sabinene in the hydrocarbon fraction was relatively high, the percentage of *trans*-sabinene hydrate was also high. The same tendency was noted in *cis*-sabinene hydrate. Thymol was not detected in hybrid C and was lower than 1% in 'Seto unshiu,' 'Morita ponkan,' and hybrids B, D, and F, whereas it was 4.8-fold and 13.3-fold higher than that of the parents in hybrids A (2.74%) and E (3.74%), respectively. While β-sinensal and α-sinensal were not detected in 'Seto unshiu,' they constituted 5.03% and 1.94%, respectively, in 'Morita ponkan.' In the hybrid seedlings, β-sinensal was not detected in hybrid D and it was lower than 0.16% in the other hybrids, while α-sinensal was not detected in hybrids A and E but was high in hybrids B, C, D, and F. The percentage of α-sinensal in hybrid B was 3-fold higher (5.85%) than that in 'Morita ponkan.'

Classification patterns of the hybrids based on the hydrocarbon components of the leaf and peel oils We examined the percentages of the hydrocarbon components in the leaf oils and found that the hybrids could be classified into two groups, one resembling 'Morita ponkan' in which sabinene constituted over 30% of the total hydrocarbon (hybrids A and E) and the other resembling 'Seto unshiu' in which sabinene and γ-terpinene constituted over 20% and 10%, respectively, of the total hydrocarbon (hybrids B, C, D, and F). Clear differences, though not as large as in γ-terpinene and p-cymene, were noted between these two groups in the percentages of a number of monoterpenes including α-pinene, β-pinene, myrcene, β-phellandrene, and terpinolene. However, there were no significant differences between the groups in most sesquiterpenes such as α-copaene, β-caryophyllene, β-elemene, α-caryophyllene, and β-bisabolene.

We then examined whether this classification according to the leaf oil could be applied to the peel oil. The hybrid seedlings were classified into two groups, hybrids A and E, which will be referred to as group 1, and hybrids B, C, D and F, which will be referred to as group 2, according to the principal component in the leaf oil. Of the 13 hydrocarbon components showing 3% or higher percentages in the leaf oil of the hybrid seedlings, 6 components which are also contained in the peel oil (α-pinene, β-pinene, sabinene, myrcene, d-limonene and γ-terpinene) were chosen. The results are summarized in Table 3.

Of the 6 hydrocarbon compounds, all except myrcene exhibited the same classification pattern in the leaf and peel oils of the hybrid seedlings. Group 2 which showed higher percentages of α-pinene, β-pinene and γ-terpinene in the leaf oil than group 1, also had higher percentages of these compounds in the peel oil. In contrast, group 1 had higher percentages of sabinene and d-limonene than group 2 in both the leaf and peel oils. Sabinene showed the largest difference in percentage between group 1 and group 2, and the difference was more than 54-fold in the leaf oil and more than 3.4-fold in the peel oil. In d-limonene which showed the smallest difference between the two groups, there was a clear difference in the leaf oil, but little difference in the peel oil. On the other hand, the percentage of myrcene in the leaf oil showed a clear difference between the two groups, while the percentage of myrcene in the peel oil did not. In summary, for 5 of the 6 major hydrocarbon components (α-pinene, β-pinene, sabinene, d-limonene and γ-terpinene), there were distinct relationships between the leaf and peel oils.

Next, we examined the degree of similarity in the percentages of these 5 components between the hybrid seedlings and their parents. There were marked differences between the parents in the percentages of sabinene and γ-terpinene in the leaf oil. Group 1 hybrids were similar to 'Morita ponkan' in the percentages of these compounds, and group 2 hybrids were similar to 'Seto unshiu.' α-Pinene and β-pinene showed

Table 3. Comparison in major hydrocarbons between leaf and peel oils.

Compounds	Seto unshiu	Morita ponkan	Hybrid seedlings					
			A	B	C	D	E	F
Leaf oil								
α-Pinene	1.84	2.03	1.52	2.91	2.6	3.18	1.35	2.94
β-Pinene	5.52	3.21	2.67	4.03	4.00	4.74	2.44	4.01
Sabinene	1.11	37.3	30.2	0.56	0.56	0.67	34.6	0.58
Myrcene	0.72	4.33	3.52	1.74	1.22	1.44	3.52	1.25
d-Limonene	4.34	4.20	3.82	5.92	7.64	6.33	4.82	6.79
γ-Terpinene	22.1	2.49	3.86	34.4	26.8	28.4	3.11	28.6
Peel oil								
α-Pinene	1.03	1.21	1.24	1.66	1.60	1.77	1.06	1.69
β-Pinene	0.61	0.91	0.78	1.24	1.30	1.55	0.70	1.41
Sabinene	0.26	1.27	2.29	0.28	0.29	0.34	1.17	0.31
Myrcene	3.36	3.52	3.14	3.10	2.80	2.84	2.50	2.79
d-Limonene	89.2	86.8	84.2	82.1	80.6	78.7	87.9	80.1
γ-Terpinene	3.54	4.58	5.28	9.52	10.0	11.3	4.73	10.6

a similar tendency, although to a lesser degree. On the other hand, the hydrocarbon composition was similar between the peel oils of the parents, making it impossible to clearly distinguish which of the seed or pollen parents the hybrid seedlings resemble.

These results showed that the hybrids could be classified into the 'Seto unshiu' type and into 'Morita ponkan' type from the leaf components. Although the percentages of the hydrocarbons in the leaf oils were different from those of the peel oil, the same tendency in the classification patterns of the hybrids was seen between the leaf and peel oils.

Classification pattern of the hybrids based on the oxygenated components of the leaf and peel oils Of the 6 components showing 3% or higher percentages in the leaf oil of the hybrid seedlings, 5 components which are also contained in the peel oil (*trans*-sabinene hydrate, linalool, (*E*)-2-hexenal, thymol and α-sinensal) were chosen. The results are summarized in Table 4.

We found that the classification pattern of the hybrids using the oxygenated components was the same as that obtained using the hydrocarbon components. That is, the hybrids could be classified according to the 3 components, *trans*-sabinene hydrate, thymol and α-sinensal, into the two groups, one consisting of hybrids A and E and the other consisting of hybrids B, C, D, and F. As in the case of the hydrocarbons, hybrids A and E resembled 'Morita ponkan' while hybrids B, C, D, and F resembled 'Seto unshiu' when the percentages of *trans*-sabinene hydrate and thymol were compared, whereas the opposite tendency was noted when α-sinensal was compared.

We then examined whether the classification according to the leaf oil could be applied to the peel oil. There were close relationships in the percentages of thymol and α-sinensal between the leaf and peel oils. Group 1, which showed a high percentage of thymol in the leaf oil, also showed a high percentage of thymol in the peel oil. On the other hand, group 2, which showed a high percentage of α-sinensal in the leaf oil, also had a high percentage of α-sinensal in the peel oil. The percentage of thymol in group 1 and the percentage of α-sinensal in group 2 were markedly higher than those in the parents. Interestingly, such markedly higher percentages were observed in both leaf and peel oils. Based on the percentage of thymol, group 1 hybrids were similar to 'Morita ponkan,' and group 2 hybrids were similar to 'Seto unshiu.' Based on the percentage of α-sinensal, however, group 1 hybrids were similar to 'Seto unshiu,' and group 2 hybrids were similar to 'Morita ponkan.' Thus, the pattern of similarity in α-sinensal between the hybrid seedlings and their parents was the opposite of that in the other components, but the similarity between the hybrid seedlings and their parents showed the same pattern in the leaf and peel oils.

On the other hand, because there was only a small difference in the percentage of *trans*-sabinene hydrate in the peel oil between the hybrid groups, they could not be classified by the percentage in the peel oil. Linalool, the most abundant oxygenated compound, showed high percentages in both the leaf and peel oils of all of the 6 hybrid seedlings as did 'Morita ponkan.' Linalool was not used in the classification of the hybrids. However, in 'Morita ponkan' and the hybrids in which the percentages of linalool were high in the peel oils, those in the leaf oils were also high. Attaway *et al.* (1967) also reported that linalool was the major constituent of most leaf oils and was a precursor in the biogenesis of many of the citrus flavor constituents. (*E*)-2-hexenal, which showed a relatively high percentage in the leaf oil, was scarce in the peel oil (0.1–0.33%), making the comparison of this component between the leaf and peel oils meaningless.

These results showed that although the similarity between each hybrid group and the parents was different depending on the components, the hybrids could be classified into the 'Seto unshiu' type and into 'Morita ponkan' type from the leaf oil components. The same tendency in the classification patterns of the hybrids was also seen between the leaf and peel oils. *trans*-Sabinene hydrate showed no relationship between the leaf and peel oils, but the classification pattern of the hybrids by their percentages in the leaf oil was the same as that by the other components.

Comparison of percentages of the oxygenated compound fraction between the leaf and peel oils The percentages of the oxygenated compound fraction in the leaf and peel oils were determined. The percentages of the oxygenated compound fraction in the leaf oil of the 'Seto unshiu,' 'Morita ponkan' parents and 6 kinds of their hybrid seedlings were more than 40%, while that of the leaf oil was

Table 4. Comparison in major oxygenated compounds between leaf and peel oils.

Compounds	Seto unshiu	Morita ponkan	Hybrid seedlings					
			A	B	C	D	E	F
Leaf oil								
trans-Sabinene hydrate	0.40	3.45	2.77	0.40	0.23	0.42	3.41	0.28
Linalool	34.7	67.2	74.6	62.2	64.6	82.3	73.0	76.6
(*E*)-2-Hexenal	35.7	6.75	6.81	11.1	13.9	4.75	8.04	9.13
Thymol	0.28	0.56	2.74	0.34	—	0.30	3.74	0.22
α-Sinensal	—	1.94	—	5.85	2.41	1.79	—	2.09
Peel oil								
trans-Sabinene hydrate	—	0.25	2.71	0.84	1.05	0.98	1.47	1.38
Linalool	23.6	32.0	32.8	26.3	34.8	31.7	35.1	37.3
(*E*)-2-Hexenal	0.19	0.18	0.17	0.33	0.17	0.10	0.21	0.10
Thymol	—	2.58	11.0	0.16	0.06	0.13	2.36	0.39
α-Sinensal	—	0.93	0.02	3.15	3.38	4.45	—	6.20

—: Not detected.

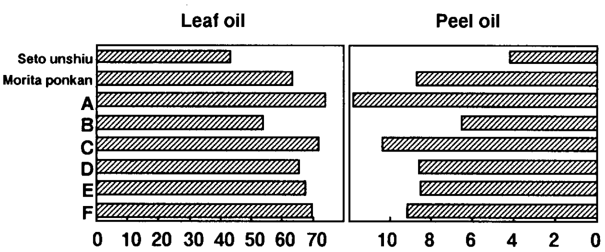

Fig. 1. Percentage of oxygenated compounds in leaf and peel oils.

lower than 12% (Fig. 1). Thus, the percentage of the oxygenated compound fraction in the leaf oil was different from that in the peel oil. However, a correlation between the leaf and peel oils can be seen from a graphical illustration in Fig. 1.

The percentage of the oxygenated compound fraction in the leaf oil was more than 70% in hybrids A and C, between 60 and 70% in 'Morita ponkan' and hybrids D, E and F, between 50 and 60% in hybrid B, and about 40% (the lowest) in 'Seto unshiu.' On the other hand, the percentage of the oxygenated compound fraction in the peel oil was more than 10% in hybrids A and C, between 8 and 9% in 'Morita ponkan' and hybrids D, E and F, between 6 and 7% in hybrid B, and about 4% (the lowest) in 'Seto unshiu.' Thus, the hybrid seedlings showing high percentages of the oxygenated compound fraction in the leaf oil also had high percentages in the peel oil, and the hybrid seedlings showing low percentages in the leaf oil also had low percentages in the peel oil.

Possibility of predicting the peel oil components in citrus hybrids based on the leaf oil components As described above, of the 11 major components, all but 4 (myrcene, *trans*-sabinene hydrate, linalool and (*E*)-2-hexenal) resulted in the same classification of the 6 hybrid seedlings into the two groups consisting of hybrids A, E and hybrid B, C, D, and F, and the classification pattern of the hybrids was the same using the peel oils as it was with the leaf oils. Although the similarity between each hybrid group and the parents was different depending on the components, their patterns were similar regardless of whether the composition of the leaf oil or peel oil was used. Myrcene and *trans*-sabinene hydrate showed no relationship between the leaf and peel oils, but the classification pattern of the hybrids by their percentages in the leaf oil was the same as that by the other components.

The percentage of oxygenated compound fraction showed a similar tendency in the leaf and peel oils. Therefore, the percentages of the oxygenated compound fraction in the peel oil can be predicted from the percentages in the leaf oil.

Our findings demonstrated a correlation between the leaf oil and peel oil in the percentage of the oxygenated compound fraction and correlations between the major components such as sabinene, γ-terpinene, thymol and α-sinensal. Because the percentage of the oxygenated compound fraction and the major components in the leaf oil reflected those in the peel oil, it may be possible to predict the percentages of those in the peel oil based on the leaf oil. Although there were similarities in the compositions of the leaf oil between the hybrid seedlings and their parents, the nature of the correlation differed depending on the individual components, and the patterns of correlation could also be predicted by the components of the leaf oil. In particular, it is very important that the percentages of thymol and α-sinensal in the leaf were related to those in the peel oil. Thymol (Wilson & Shaw, 1981) and α-sinensal (Moshonas & Lund, 1969) are characteristic flavor compounds of mandarin and orange, respectively. Our previous study showed that the percentages of thymol and α-sinensal in the peel oil of the hybrid seedlings were considerably higher than those of the parents. As shown in Table 3, the percentages of thymol and α-sinensal in the leaf oil of the hybrid seedlings were also much higher than those of the parents. Therefore, the hybrids which had extraordinarily high percentages of thymol and α-sinensal in peel oils could be predicted from the leaf oils.

These results suggested that the characteristic of the flavor of fruit can be predicted by analyzing the major compounds (α-pinene, β-pinene, sabinene, *d*-limonene, γ-terpinene, thymol and α-sinensal) in the oil of the leaf, which can be sampled much earlier than the fruit. The peel oils are composed of many compounds that are common to all hybrids and a few compounds that are specific for certain hybrids. Some hybrids have the characteristic flavors of the parents. If seedlings of the desired characteristics can be selected in the seedling stage, the efficiency of crossbreeding will become markedly higher. However, the above results were obtained from the first hybrid generation of 'Seto unshiu' crossed with 'Morita ponkan,' and hybrid seedlings of other combinations and the second generation of hybrids crossed with Tangor have not been examined. Furthermore, while the characteristic pattern in the composition of the leaf oil remains almost unchanged throughout the year, the composition of the peel oil changes as the fruit matures (Attaway *et al.*, 1967; Kamiyama, 1968). The number of hybrid seedlings used in this study (6) was not large, therefore, it is necessary to confirm these results using many different kinds of hybrid seedlings.

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