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Original Article

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Comparison of Peel Components of Mandarin, Sour lemon and Sour orange (Citrus sp.)

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Abstract

The peel components of Mandarin, Sour lemon and Sour orange were analyzed. Peel flavor components were extracted using cold-press method and eluted using n-hexane. All compounds were analyzed using GC-FID and GC-MS. Twenty-seven, Twenty-four and Twenty-nine peel components were identified in Mandarin, Sour lemon and Sour orange respectively. They include aldehydes, alcohols, esters, monoterpenes and sesquiterpenes. The major flavor components identified included limonene, β-myrcene, linalool, (E)-β-ocimene, α-Pinene, β-Pinene and sabinene. Among the three species examined, Sour orange showed the highest aldehyde content. Considering that aldehyde content of Citrus oil is an indicator of high quality, it seems that Citrus species have a profound influence on oil quality.

Keywords: Flavor Components, Peel Oil, Cold Press, Citrus Species

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Introduction

The genus Citrus, belonging to the Rutaceae or Rue family, comprises about 140 genera and 1,300 species. Citrus sinensis (Orange), Citrus paradise (Grapefruit), Citrus limon (Lemon), Citrus reticulata (tangerine), Citrus grandis (shaddock), Citrus aurantium (Sour orange), Citrus medica (Citron), and Citrus aurantifolia (lime) are some important species of genus Citrus (Kamal et al., 2011).
Citrus is well known as one of the most economically important crops in world that is Cultivated in many countries with tropical or subtropical climate. By 2010, the total annual Citrus production in Iran has been 87000 tons (FAO, 2012). Sour lemon is a hybrid of the lime and citron that is extensively cultivated in Iran (Fotouhi and Fattahi, 2007). Clementine Mandarin is known as Citrus clementine hort (Verzera et al., 1998). It has been regarded as a Citrus fruit with commercial value because of its attractive and pleasant aroma.
In Citrus L. species essential oils occur in special oil glands in flowers, leaves, peel and juice. These essential oils are composed of many compounds including terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. They may also be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residues. Essential oils of Citrus are commercially used for flavoring foods, beverages, perfumes, cosmetics and medicines (Salem, 2003). In addition, recent studies have identified insecticidal, antimicrobial, antioxidative and antitumor properties for Citrus peel oils (Shahidi and Zhong, 2012). Although oxygenated compounds occurring in relatively small amounts, they have been found to be responsible for the specific odor and flavor of Citrus fruits. The quality of an essential oil can be calculated from the quantity of oxygenated compounds present in the oil. The quantity of oxygenated compounds in an oil is depends on a several factors including rootstock (Babazadeh et al., 2009; Bbazadeh, 2011a), Citrus species (Minh et al., 2002; Mondello et al., 2003; Gancel et al., 2005), seasonal variation (Babazadeh et al., 2011b), organ such as peel, juice, leaf, flower (Babazadeh, 2011c) and extraction method (Babazadeh, 2011d).
Branched aldehydes and alcohols are important flavor compounds, extensively used in food products (Babazadeh, 2009). Several studies have shown that the tangerine-like smell is mainly a result of the presence of carbonyl compounds such as octanal, citronellal, decanal, neral, geranial, dodecanal (Buettner et al., 2003). The quality of a honey can be calculated from the quantity of oxygenated components present in the honey (Alissandrakis et al., 2003; Alistair et al., 1993). In addition, type of flowers may influence the quality of volatile flavor components present in the honey. The effect of oxygenated compounds in attraction of the pollinators is well established. Therefore, presence of oxygenated compounds can encourage the agricultural yield (Kite et al., 1991; Andrews et al., 2007).
In this study, we compared the peel volatile components isolated from three Citrus species to determining whether the quantity of oxygenated compounds varies among different species.

Materials and methods

Citrus scions

In 1989, Citrus scions that was grafted on Sour orange rootstock, were planted at 8×4 m with three replication at Ramsar research station [Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate, average rainfall 970 mm per year and average temperature16.25°C; soil was classified as loam-clay, pH range (6.9 to 7)]. Clementine mandarin, Lisbon lemon and Sour orange were used as plant materials in this experiment (Table 1).

Table 1: Common and botanical names for Citrus taxa used as scions and rootstock (Fotouhi and Fattahi, 2007).

Common name	botanical name	Parents	category
Clementine(scion)	Citrus clementina	Unknown	Mandarin
Lisbon(scion)	Citrus limon (L.) Burm.	Citrus aurantifolia L. × Citrus medica L.	Sour lemon
Sour orange (scion)	Citrus aurantium	Citrus reticulata × Citrus grandis	Sour orange
Sour orange (Rootstock)	Citrus aurantium	Citrus reticulata × Citrus grandis	Sour orange

Preparation of peel sample

In the last week of January 2012, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. About 150 g of fresh peel was cold-pressed and then the oil was separated from the crude extract by centrifugation (at 4000 rpm for 15 min at 4 °C). The supernatant was dehydrated with anhydrous sodium sulfate at 5 °C for 24h and then filtered. The oil was stored at -25 °C until analyzed.

GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m 0.25 mm i.d; film thickness = 0.25 m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60 °C (3min) to 250 °C (20 min) at a rate of 3 °C/min. The injector and detector temperatures were 260 °C and helium was used as the carrier gas at a flow rate of 1.00 mL/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C22) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. Gas chromatography-mass spectrometry was used to identify the volatile components. The analysis was carried out with a Varian Saturn 2000R. 3800 GC linked with a Varian Saturn 2000R MS.

The oven condition, injector and detector temperatures, and column (DB-5) were the same as those given above for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s. Injection volume was 1 mL.

Identification of components

Components were identified by comparing their LRIs and also comparing their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search program (Chem. SW. Inc; NIST 98 version database) connected to a Varian Saturn 2000R MS. Identification of compounds were also determined by comparing the retention time of each compound with that of known compounds (Adams, 2001; McLafferty and Stauffer, 1991).

Data analysis

Variation of target nine peel compounds among Citrus species was analyzed using inferential statistics. ANOVA was used for comparing amount of compounds among and within Citrus species. Correlation between pairs of components was evaluated using Pearson's correlation coefficient (Table 3 and 4). All statistical analyses were carried out using SPSS Version 18 Software.

Results

Flavor compounds of the Clementine mandarin peel

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel by using cold-press led to identification of 27 volatile components (Table 2, Figure 1) including 12 oxygenated terpenes [9 aldehydes, 3 alcohols] and 15 non oxygenated terpenes [6 monoterpens, 9 sesqiterpens].

Table 2: Peel volatile components of Citrus species

	Component	Man-darin	Sour lemon	Sour orange	KI
1	α - Pinene	*	*	*	935
2	Camphene			*	951
3	Sabinene	*		*	975
4	β -pinene	*	*	*	979
5	β -myrcene	*		*	991
6	octanal	*		*	1003
7	α -terpinene		*		1014
8	Limonene	*	*	*	1036
9	(E)- β - ocimene	*		*	1049
10	γ - terpinene		*		1061
11	α -terpinolene		*		1091
12	Linalool	*	*	*	1100
13	Nonanal	*		*	1109
14	Camphor		*		1150
15	Citronellal	*	*		1154
16	α - terpineol	*	*	*	1195
17	Decanal	*	*	*	1205
18	Nerol		*		1228
19	Neral		*	*	1244
20	Linalyl acetate			*	1255
21	(E)-2-decenal		*	*	1263
22	Geranial	*		*	1275
23	Perilla aldehyde	*			1282
24	Undecanal			*	1307
25	Nonanyl acetate			*	1312
26	Terpinyl acetate			*	1345
27	Citronellyl acetate		*		1349
28	Neryl acetate		*	*	1356
29	α -copaene	*			1385
30	Granyl acetate		*	*	1389
31	β -cubebene	*			1396
32	β -elemene	*			1399
33	Dodecanal	*			1409
34	(Z)- β -caryophyllene		*	*	1415
35	α -bergamotene		*		1419
36	Linalyl butanat			*	1427
37	(Z)- β - farnesene	*	*	*	1453
38	α - humulene	*			1466
39	(E)-2-dodecenal			*	1477
40	Germacrene D	*		*	1493
41	Bicyclogermacrene	*		*	1499
42	β -bisabolene		*		1506
43	E,E, α - farnesene	*			1514
44	(Z)- α -bisabolene		*		1516
45	δ-cadinene	*			1532
46	Elemol	*			1559
47	(E)-nerolidol			*	1567
48	Germacrene D-4-ol		*		1576
49	Tetradecanal			*	1613
50	α -bisabolol		*		1686
51	β - sinensal	*			1700
52	α –sinensal	*			1757
		27	24	29	

*** There is in oil.**

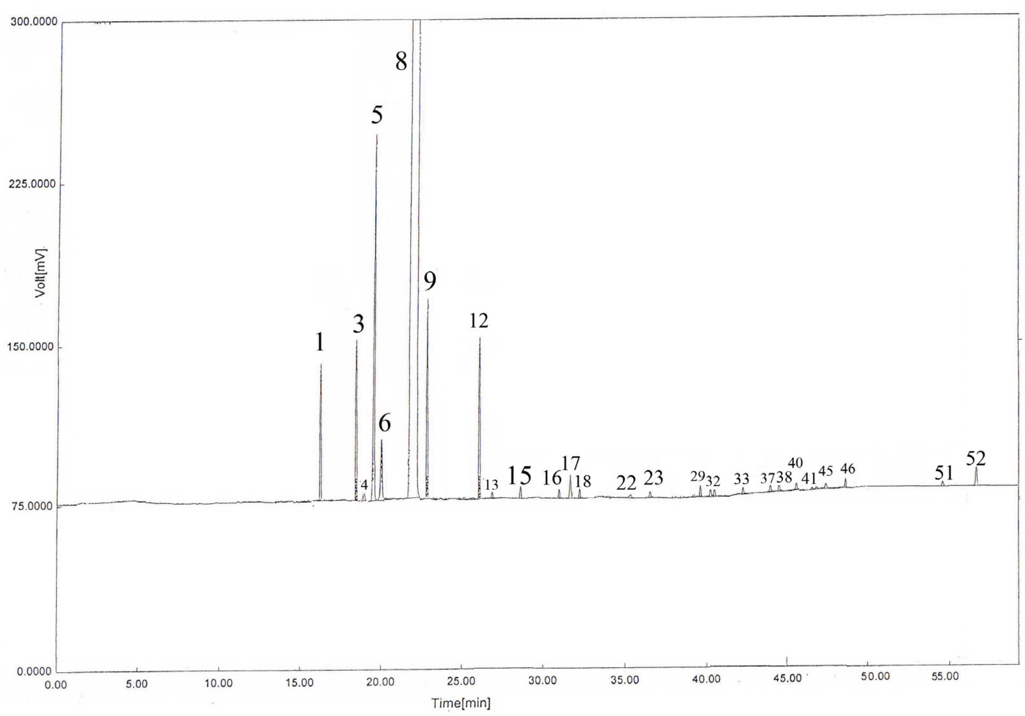

Figure 1: HRGC chromatogram of 'Celemantine' mandarin peel oil.

Flavor compounds of the Lisbon lemon peel

GC-MS analysis of the flavor compounds extracted from Lisbon lemon peel by using cold-press led to identification of 24 volatile components (Table 2) including 12 oxygenated terpenes [4 aldehydes, 5 alcohols, 3 esters] and 12 non oxygenated terpenes [7 monoterpens, 5 sesqiterpens].

Flavor compounds of the Sour orange peel

GC-MS analysis of the flavor compounds extracted from Sour orange peel by using cold-press led to identification of 29 volatile components (Table 2) including 18 oxygenated terpenes [9 aldehydes, 3 alcohols, 6 esters] and 11 non oxygenated terpenes [7 monoterpens, 4 sesqiterpens].

Aldehydes

Fourteen aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, (E)-2-decenal, geranial, perillaldehyde, undecanal, dodecanal, (E)-2-dodecenal, tetradecanal, α-sinensal and β-sinensal (Table 3). In addition they were quantified from 0.66% to 0.85%. The concentrations of octanal and decanal were higher in our samples. Octanal has a Citrus-like aroma, and is considered as one of the major contributors to mandarin flavor (Buettner et al., 2003). Among the three species examined, Sour orange showed the highest content of aldehydes (Table 3). Since the aldehyde content of Citrus oil is considered as an important indicator of high quality, Citrus species apparently has a profound influence on oil quality.

Sour orange aldehydes were also compared to those of mandarin and Sour lemon in this study. undecanal, (E)-2-dodecenal and tetradecanal were identified in Sour orange, while they were not detected in mandarin and Sour lemon. Amount of aldehydes in Sour orange was 1 times higher than Sour lemon (Table 3).

Table 3: Statistical analysis of variation in peel flavor components of Citrus species

Compounds	Mandarin		Sour lemon		Sour orange		F value
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	
Oxygenated compounds							
a) Aldehyds							
1) Octanal	0.27	0.02	0	0	0.44	0.03	F**
2) Nonanal	0.01	0.006	0	0	0.08	0.01	
3) Citronellal	0.07	0.006	0.05	0.01	0	0	
4) Decanal	0.19	0.02	0.01	0	0.2	0.03	F**
5) Neral	0	0	0.37	0.02	0.03	0.006	
6) (E)-2-decenal	0	0	0.23	0.05	0.005	0.001	
7) Geranial	0.01	0	0	0	0.06	0.01	
8) Perilla aldehyde	0.01	0	0	0	0	0	
9) Undecanal	0	0	0	0	0.01	0	
10) Dodecanal	0.03	0.006	0	0	0	0	
11) (E)-2-dodecenal	0	0	0	0	0.02	0	
12) Tetradecanal	0	0	0	0	0.01	0	
13) β-sinensal	0.01	0.006	0	0	0	0	
14) α-sinensal	0.17	0.01	0	0	0	0	
Total	0.77	0.07	0.66	0.08	0.85	0.08	
b) Alcohols							
1) Linalool	0.57	0.02	0.27	0.04	0.5	0.09	F**
2) α-terpineol	0.03	0	0.01	0	0.09	0.02	
3) Nerol	0	0	0.26	0.02	0	0	
4) Elemol	0.03	0.01	0	0	0	0	
5) (E) - nerolidol	0	0	0	0	0.07	0.01	
6) Germacrene D - 4 - ol	0	0	0.01	0	0	0	
7) α- bisabolol	0	0	0.02	0	0	0	
Total	0.63	0.03	0.57	0.06	0.66	0.12	
c) Esters							
1) Linalyl acetate	0	0	0	0	0.23	0.05	
2) Nonanyl acetate	0	0	0	0	0.007	0.001	
3)Terpinyl acetate	0	0	0	0	0.007	0.002	
4) Citronellyl acetate	0	0	0.02	0	0	0	
5) Neryl acetate	0	0	0.25	0.03	0.03	0	
6) Granyl acetate	0	0	0.11	0.02	0.11	0.02	
7) Linalyl butanat	0	0	0	0	0.007	0.001	
Total	0	0	0.38	0.05	0.39	0.07	

Compounds	Mandarin		Sour lemon		Sour orange		F value
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	
Monoterpenes							
1) α-pinene	0.51	0.01	2.57	0.07	1.2	0.04	F**
2) Camphene	0	0	0	0	0.01	0	
3) Sabinene	0.6	0.09	0	0	0.7	0.1	F**
4) β- pinene	0.03	0	9.58	0.09	1.74	0.21	F**
5) β-myrcene	1.72	0.07	0	0	3.21	0.27	F**
6) α-terpinene	0	0	0.1	0.01	0	0	
7) Limonene	94.19	0.71	69.02	2.01	81.37	1.62	F**
8) (E)-β-ocimene	0.86	0.35	0	0	5.86	0.2	F**
9) γ-terpinene	0	0	9.76	0.15	0	0	
10) α-terpinolene	0	0	0.68	0.03	0	0	
11) Camphor	0	0	0.01	0	0	0	
Total	97.91	1.23	91.72	2.36	94.09	2.44	
Sesquiterpenes							
1) α-copaene	0.04	0	0	0	0	0	
2) β-cubebene	0.02	0.006	0	0	0	0	
3) β-elemene	0.01	0.01	0	0	0	0	
4) (Z)-β-caryophyllene	0	0	0.36	0.02	0.07	0.006	
5) α -bergamotene	0	0	0.65	0.04	0	0	
6) (Z)-β-farnesene	0.01	0	0.06	0.01	0.01	0	
7) α - humulene	0.01	0.006	0	0	0	0	
8) Germacrene D	0.02	0	0	0	0.29	0.04	
9) Bicyclogermacrene	0.01	0	0	0	0.01	0	
10) β -bisabolene	0	0	0.9	0.08	0	0	
11) E,E-α-farnesene	0.01	0	0	0	0	0	
12) (Z)-α -bisabolene	0	0	0.01	0	0	0	
13) δ-cadinene	0.02	0.006	0	0	0	0	
Total	0.15	0.02	1.98	0.15	0.38	0.04	
Total oxygenated compounds	1.4	0.1	1.61	0.19	1.9	0.27	
Total	99.46	1.35		2.7	96.37	2.75	

Alcohols

Seven alcohol components identified in this analysis were linalool, -terpineol, nerol, elemol, (E)-nerolidol, germacrene D-4-ol, -bisabolol (Table 3). The total amount of alcohols ranged from 0.57% to 0.66%. Linalool was identified as the major alcohol component in this study and it was the most abundant. Linalool has been recognized as one of the most important components for Citrus peel oil flavor. Linalool has a flowery aroma (Buettner et al., 2003) and its level is important to flavor characteristics of Citrus peel oil. Among the three species examined, Sour orange showed the highest content of alcohols (Table 3).

Sour orange alcohols were also compared to those of mandarin and Sour lemon in this study. (E)-nerolidol was identified in Sour orange, while it was not detected in mandarin and Sour lemon.

Amount of alcohols in Sour orange was 1 time higher than Sour lemon (Table 3).

Esters

Seven ester components identified in the analysis were linalyl acetate, nonanyl acetate, terpinyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, linalyl butanat. The total amount of esters ranged from 0.00% to 0.39%. Among the three species examined, Sour orange showed the highest content of esters in oil (Table 3).

Monoterpenes hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 91.72% to 97.91%. Limonene was the major component among the monoterpene hydrocarbons of Citrus peel oil. Limonene has a weak Citrus-like aroma (Buettner et al., 2003) and is considered as one of the major contributors to citrus flavor. Among the three species examined, mandarin had the highest monoterpenes hydrocarbons in oil (Table 3).

Sesquiterpenes hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.15 % to 1.98 %. (Z)-β-caryophyllene, α-bergamotene, and β-bisabolene were the major components among the sesquiterpen hydrocarbons of Sour lemon peel oil. Among the three species examined, Sour lemon showed the highest sesquiterpenes content in oil (Table 3)

Results of statistical analyses

Statistical analysis was performed on the peel oil data using SPSS 18. Comparisons were made using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered to be significant at $P < 0.01$. All nine individual oil components analyzed showed statistically significant differences (Table 3).

Results of correlation

Intercorrellations between nine components are presented in Table 4. The highest positive correlations were between β-myrcene and octanal (98%), β- pinene and α-pinene (98%), sabinene and decanal (97%). The highest significant negative correlations were between limonene and α-pinen (97%), β- pinene and decanal (96%), β- pinene and sabinene (94%) (Table 4).

Table 4: Correlation matrix (numbers in this table correspond with main components mentioned in Table 3).

	octanal	decanal	linalool	α-pinene	sabinene	β- pinene	B-myrcene	limonene
decanal	0.90**							
linalool	0.73*	0.94**						
α-pinene	-0.74*	-0.91**	-0.91**					
sabinene	0.92**	0.97**	0.89**	-0.87**				
β- pinene	-0.84**	-0.96**	-0.93**	0.98**	-0.94**			
B-myrcene	0.98**	0.88**	0.73*	-0.67*	0.92**	-0.79*		
limonene	0.59	0.81**	0.85**	-0.97**	0.76*	-0.92**	0.51	
(E)-β-ocimene	0.86**	0.62	0.40	-0.31	0.68*	-0.47	0.90**	0.12

**significant at 0.05 level   **significant at 0.01 level*

Discussion

Our observation that Citrus species have an effect on some of the components of oil is in accord with other the finding in previous studies (Minh et al., 2002; Mondello et al., 2003; Gancel et al., 2005). The compositions of the peel oils obtained by cold pressing from different species of Citrus were quite similar. However, relative concentration of compounds was different according to type of species.
Comparison of our data with those in the literatures revealed some inconsistencies with previous studies (Lota et al., 2001). It may be related to rootstock and environmental factors that can influence the compositions. However, it should be noticed that the extraction methods also may influence the results. Fertilizer (Kesterson et al., 1974) and irrigation (Kesterson et al., 1974) affect the content of peel oil present in Citrus. Fertilization, irrigation, and other operations were carried out uniform in this study so we do not believe that this variability is a result of these factors.
The discovery of geranyl pyrophosphate (GPP) as an intermediate between mevalonic acid and oxygenated compounds (Alcohols and aldehyds) led to a rapid description of the oxygenated compounds biosynthetic pathway. The major pathway of oxygenated compounds biosynthesis in higher plants is as below:
Mevalonic acid → Isopentenyl Pyrophosphate → 3.3-dimethylallylpyrophosphate → geranyl pyrophosphate → Alcohols and Aldehyds
The reactive steps in the pathway are catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively (Baghalian and Naghdabadi, 2000). The pronounced enhancement in the amount of oxygenated compounds, when Sour orange was used as the plant material, indicate that either the synthesis of geranyl pyrophosphate is enhanced or activities of both enzymes increased.
High positive correlations between pairs of terpenes suggest presence of a genetic control (Scora et al., 1976) whether such dependence between pairs of terpenes is due to their derivation of one from another is not known. Similarly, high negative correlations observed between limonene and α-pinen; β-pinene and decanal; β- pinene and sabinene suggest that one of the two compounds is being synthesized at the expense of the other or of its precursor. The observed insignificant correlations imply genetic and/or biosynthetic independence. However, without an extended insight into the biosynthetic pathway of each terpenoid compound, the true significance of these correlations is not clear. The highest positive correlation was between β-myrcene and octanal. This result indicates that these compounds should be under the control of a single dominant gene (Scora et al., 1976).
Considering that acetate is necessary for the synthesis of terpenes, it can be assumed that there is a specialized function for this molecule and it may be better served and utilized in Sour orange. Results showed that the some of the species analyzed, can be distinguished by particular characteristics that is available in their essential oils. For example, tangerine oil can be easily distinguished from other Citrus oils by its various aliphatic aldehydes (β-sinensal and α-sinensal). Among the three species examined, Lemon showed a high percentage in neral, β-pinene, β-bisobolene, trans-α-bergamotene and trans-β-farnesene. Therefore, it is easily recognizable from the other species with this pattern of concentrations. These findings were similar to previous studies (Shaw, 1979; Mondello et al., 1995).

Conclusion

In the present study we found that the amounts of peel compositions were significantly affected by Citrus species and there is a great variation in most measured characteristics among different species. The present study demonstrated that volatile compounds in peel can vary when different species is utilized. Among the three species examined, Sour orange showed the highest content of aldehydes, alcohols and esters. Studies like this is very important to determine the amount of chemical compositions existing in the Citrus species before their fruits can be used in food industries, aromatherapy, pharmacy, cosmetics, hygienic products and other areas. Further research on the relationship between Citrus species and essential oil (oxygenated terpenes) is necessary.

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