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Full Length Research Paper

A comparison of volatile components of flower, leaf and peel of *Citrus reticulata* Blanco (*Citrus nobilis* Lour var. deliciosa swingle)

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The volatile flavor components of flower, leaf and peel of *Citrus nobilis* Lour var. deliciosa swingle were investigated in this study. Flower, leaf and peel flavor components were extracted using water distillation method and then eluted using n-hexane solvent. Then they were all analyzed by Gas chromatography- flame ionization detector (GC-FID) and Gas chromatography–mass spectrometry (GC-MS). 39 flower components, 39 leaf components and 25 peel components including: aldehydes, alcohols, esters, ketons, monoterpenes and sesquiterpenes were identified and quantified. The major flavor components were linalool, limonene, sabinene, a-pinene, β-myrcene, terpinene-4-0l, (E)-β-ocimene and γ-terpinene. The flower oil showed the highest content of aldehydes and alcohols. Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, organ apparently has a profound influence on citrus oil quality.

Key words: Local tangerine, *Citrus reticulata* Blanco, tangerine flower oil, tangerine leaf oil, tangerine pee oil, flavor components, water-distillation.

INTRODUCTION

Citrus nobilis Lour var. deliciosa Swingle is a local tangerine and is widely planted on a large scale in Iran. It is probably a native of southern China, and is now found in all warm countries. Its fruit is characterized by a loose skin and pleasant flavor (Asgarpanah, 2002). In Citrus L. species essential oils occur in special oil glands in flowers, leaves, peel and juice. These valuable 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 used commercially for flavoring foods, beverages, perfumes, cosmetics, medicines, etc. (Salem, 2003). Up to now, numerous investigations have been performed aimed at identifying the aroma volatiles in the mandarin flower (Babazadeh Darjazi, 2011 a, b, c; Salem, 2003; Kharebava and Tsertsvadze, 1986; Yoshikawa et al., 1996), leaf (Babazadeh Darjazi, 2011 a, b; Salem, 2003; Lota et al., 2000, 2001; Ekundayo et al., 1990), peel (Babazadeh Darjazi, 2009; Babazadeh Darjazi et al., 2009; Lota et al., 2000, 2001) and juice (Babazadeh Darjazi, 2009; Babazadeh Darjazi et al., 2009; Yajima et al., 1979). The quality of an essential oil may be calculated from the quantity of oxygenated compounds present in the oil (Babazadeh Darjazi et al., 2009). Branched aldehydes and alcohols are important flavor compounds in many food products (Salem, 2003). Various studies have shown that the tangerine-like smell was also suggested to be mainly based on carbonyl compounds, such as α-sinensal, β-sinensal, geranial, citronellal and decanal (Babazadeh Darjazi, 2009; Salem, 2003; Asgarpanah, 2002). The quality of a honey may be calculated from the amount of oxygenated components present in the honey (Alissandrakis et al., 2003; Alistair et al., 1993) and various flowers may influence the quality of volatile flavor components present in the honey. It had been recognized previously that oxygenated compounds are important factor in deceiving and attracting the pollinators. These results may have consequences for yield in agricultural (Kite et al., 1991; Andrews et al., 2007). There have been very few studies on the essential oils of *C. nobilis* Lour var. deliciosa swingle, even though citrus oil compositions have been investigated in many areas

throughout the world. In this study, we compare the volatile compounds isolated from fresh flowers, leaves and peel of *C. nobilis* Lour var. deliciosa swingle with the aim of determining whether the quantity of oxygenated compounds was influenced by the organs.

MATERIALS AND METHODS

In 1989, *C. nobilis* Lour var. deliciosa swingle trees, grafted on Sour orange, were planted at 8×4m with three replication at Ramsar research station (Kotra) (Latitude 36°54' N, Longitude 50°40' E ; Caspian Sea climate, average rainfall 970 mm per year and average temperature 16.25°C; soil was classified as loam-clay, pH range 6.9 to 7). In the early week of June 2007, about 300 g of leaves and at least 200 g flower were collected from many parts of the same trees, located in Ramsar research station (Kotra), early in the morning (6 to 8 am) and only by dry weather. Also we collected at least 10 mature fruit from these trees in the last week of November 2007. In order to obtain the volatile compounds, about 200 g fresh mature peel, 300 g of fresh leaves and 200 g of fresh flower were subjected to hydro distillation for 3 h using a Clavenger-type apparatus. N-Hexane was used to isolate the oil layer from the aqueous phase. The hexane layer was dried over anhydrous sodium sulphate and stored at -4°C until used.

GC and GC-MS

An Agilent 6890N GC equipped with a DB-5 (30 m ×0.25 mm i.d; film thickness = 0.25 μm) fused silica capillary column (J and W Scientific) and a FID was used. The column temperature was programmed from 50°C (2 min) to 188°C (20 min) at a rate of 3°C/ min. The injector and detector temperatures were 220°C and helium was used as the carrier gas at a flow rate of 1.0 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 to C22) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. GC-MS 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 µl.

Identification of components

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

RESULTS

Flower components of the *C. nobilis* Lour var. deliciosa swingle

GC-MS analysis of the flavor compounds extracted from *C. nobilis* flower using water distillation allowed identification of 39 volatile components (Table 1):19 oxygenated terpenes (4 aldehydes, 9 alcohols, 5 ester and 1 ketone), and 20 non oxygenated terpenes (13 monoterpens and 7 sesqiterpens).

Leaf components of the *C. nobilis* Lour var. deliciosa swingle

GC-MS analysis of the flavor compounds extracted from *C. nobilis* leaf using water distillation allowed identification of 39 volatile components (Table 1 and Figure 1): 17 oxygenated terpenes (3 aldehydes, 14 alcohols), 22 non oxygenated terpenes (14 monoterpens, 8 sesqiterpens).

Peel components of the *C. nobilis* Lour var. deliciosa swingle

GC-MS analysis of the flavor compounds extracted from *C. nobilis* peel using water distillation allowed identification of 25 volatile components (Table 1): 9 oxygenated terpenes (3 aldehydes, 3 alcohols, 2 esters, 1 ketones), 16 non oxygenated terpenes (12 monoterpens, 4 sesqiterpens).

Aldehydes

Seven aldehyde components that were identified in this analysis were octanal, citronellal, decanal, neral, geranial, β-sinensal and α-sinensal (Table 2). In addition they were quantified (from 0.29 to 3.94%) that it was determined and reported as relative amount of those compounds in oil in this study. These findings were similar to the previous study undertaken by Salem (2003); Lota et al. (2001); Asgarpanah (2002). Tangerine oil is easily distinguished from other citrus oils by its content of various aliphatic aldehydes. Two main aliphatic aldehydes were β-sinensal and α-sinensal. In addition, tangerine oil also contained citronellal (Asgarpanah, 2002). β-sinensal has a woody aroma (Sawamura et al., 2004), and is considered as one of the major contributors to tangerine flavor (Asgarpanah, 2002). Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, organ apparently has a profound influence on *C. nobilis* oil quality. Among the three organs examined, flower showed the highest content of aldehydes (Table 2). Flower aldehyds were also compared to those of leaf and peel in this study. Neral, β-sinensal and α-sinensal were identified in flower and leaf oil, while they were not detected in peel oil. Compared with peel, the flower improved and increased aldehyde components about 13 times for *C. nobilis* Lour (Table 2).

Table 1. Chemical composition of essential oils of the flower, leaf and peel of *C. nobilis* Lour var. deliciosa Swingle.

S/no	Component	Flower	Leaf	Peel	KI	S/no	Component	Flower	Leaf	Peel	KI
1	α- thujene	*	*	*	925	30	Tymol		*		1270
2	α - Pinene	*	*	*	933	31	Geranial	*			1275
3	Sabinene	*	*	*	974	32	Terpinyl acetate	*			1313
4	β - Pinene	*	*	*	977	33	δ -elemen		*		1342
5	β -myrcene	*	*	*	989	34	Citronellyl acetate	*			1353
6	Octanal			*	1003	35	Neryl acetate	*		*	1356
7	α - phellandrene	*	*	*	1003	36	Geranyl acetate	*		*	1381
8	α - terpinene		*	*	1017	37	β - elemene	*	*	*	1389
9	P-cymene		*		1023	38	(Z)- β -caryophyllene	*	*		1428
10	Limonene	*	*	*	1029	39	Geranyl acetone	*			1434
11	(Z)- β - ocimene	*	*		1035	40	γ-elemen		*		1437
12	(E)- β - ocimene	*	*	*	1046	41	α -guaiene	*			1450
13	γ- terpinene	*	*	*	1062	42	Allo aromadendrene	*			1456
14	(E)-sabinene hydrate	*	*		1068	43	(Z)- β - farnesene		*		1457
15	(Z)- linalool oxide	*			1072	44	Germacrene D	*	*	*	1490
16	α -terpinolene	*	*	*	1088	45	E,E, α - farnesene	*	*	*	1505
17	Linalool	*	*	*	1100	46	δ -cadinene		*	*	1522
18	Cis-p-menth-2-en-1-ol	*	*		1114	47	Elemol		*		1557
19	Trans-p-Menth-2-en-1-ol	*	*		1127	48	(E) – nerolidol	*	*		1562
20	(Z)-limonene oxid			*	1141	49	Spathulenol	*	*		1588
21	(E)-β -terpineol		*		1144	50	Caryophyllene oxide	*			1596
22	Citronellal			*	1154	51	Globulol	*			1605
23	Terpinen-4-ol	*	*	*	1185	52	α -muurolol		*		1650
24	α -terpineol	*	*	*	1194	53	α -cadinol		*		1665
25	Decanal			*	1205	54	β -sinensal	*	*		1700
26	Thymol methyl ether		*		1235	55	E,E-cis-farnesol	*			1725
27	Neral	*	*		1242	56	α-sinensal	*	*		1728
28	Carvone			*	1250	57	Phytol		*		1949
29	Linalyl acetate	*			1253						

*There is in oil.

Alcohols

Sixteen alcohol components identified in this study were linalool, cis-p-menth -2-en-1-ol, trans-p-menth -2-en-1-ol, (E)- β -terpineol, terpinene-4-ol, α -terpineol, thymol methyl ether, tymol, elemol, (E) nerolidol, spathulenol, α -muurolol, α -cadinol, phytol, globulol and E,E-cis-farnesol (Table 2).

The total amount of alcohols ranged from (0.70 to 39.11%) that it was determined and reported as relative amount of those compounds in *C. nobilis* oil. Linalool was the major component in this study and it was the most abundant. Linalool, the most significant alcohol compound of tangerine oil, is recognized as being very important to good tangerine flavor (Babazadeh Darjazi, 2009; Salem, 2003). Linalool has a flowery (rose-like) aroma (Sawamura et al., 2004) and its level is important to flavor character in tangerine flower, leaf and peel (Salem, 2003). Among the three organs examined, flower showed the highest content of alcohols (Table 2). Flower alcohols were also compared to those of leaf and peel in this study. Cis-p-menth -2-en-1-ol, trans-p-menth -2-en-1-ol, (E)-nerolidol and spathulenol were identified in flower and leaf oil, while they were not detected in peel oil. Compared with peel, the flower improved and increased alcohol components about 56 times for *C. nobilis* Lour (Table 2).

Esters

Five ester components identified in the analysis were linalyl acetate, terpinyl acetate, citronellyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged (from 0.00 to 0.13%) in oil and linalyl acetate was the most abundant. Among the three organs examined, flower showed the highest content of esters in oil (Table 2).

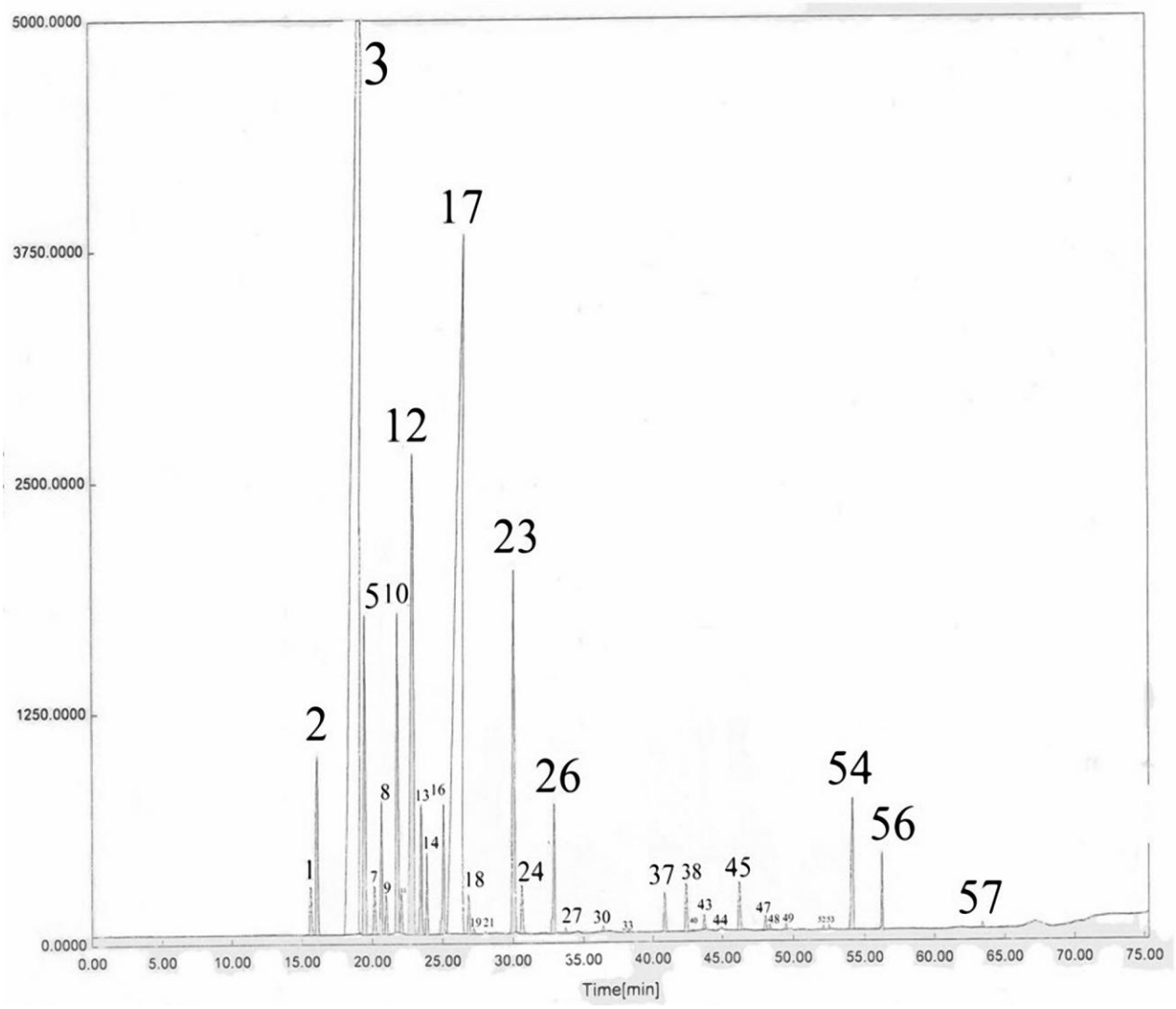

Figure 1. HRGC chromatogram of *C. nobilis* Lour var. deliciosa Swingle leaf oil.

ketones

Two ketone compounds identified in the analysis were carvone and geranyl acetone. Among the three organs examined flower showed the highest content of ketones (Table 2). Flower ketones were also compared to those of leaf and peel in this study. Geranyl acetone was identified in flower oil, while it was not detected in leaf and peel (Table 2).

Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged (from 51.87 to 95.62%). Sabinene was the major component among the monoterpene hydrocarbons of *C. nobilis* flower and leaf oil while limonene was the major component among the monoterpene hydrocarbons of *C. nobilis* peel oil. Limonene has a weak citrus-like aroma (Sawamura et al., 2004) and is considered as one of the major contributors to tangerine flavor (Salem, 2003). Among the three organs examined, peel had the highest monoterpenes hydrocarbons in oil (Table 2).

Sesquiterpene hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged (from 0.31 to 2.01%). β-elemene, (Z)-β-caryophyllene and E, E-α- farnesene were the major components among the sesquiterpene hydrocarbons of *C.nobilis* flower and leaf oil. Among the three organs examined,

Table 2. Statistical analysis of variation in volatile components of flower, leaf and peel of Citrus nobilis Lour var. deliciosa Swingle. Mean is average composition (%) in the different organs used with three replicates. St. err. = standard error. F value is accompanied by its significance, indicated by: NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

S/No	Compounds	Flower		Leaf		Peel		
		Mean	St.err	Mean	St.err	Mean	St.err	
	Oxygenated compounds							
a	Aldehyds							
1	Octanal	0.00	0.00	0.00	0.00	0.14	0.006	
2	Citronellal	0.00	0.00	0.00	0.00	0.03	0.005	
3	Decanal	0.00	0.00	0.00	0.00	0.12	0.005	
4	Neral	0.07	0.00	0.07	0.001	0.00	0.00	
5	Geranial	0.009	0.001	0.00	0.00	0.00	0.00	
6	β-sinensal	2.08	0.16	1.78	0.08	0.00	0.00	
7	α-sinensal	1.79	0.19	0.85	0.04	0.00	0.00	
	Total	3.94	0.35	2.70	0.12	0.29	0.01	
b	b) Alcohols							
1	linalool	24.91	1.35	23.47	0.10	0.58	0.01	F**
2	Cis-p-menth-2-en-1-ol	0.26	0.11	0.31	0.01	0.00	0.00	
3	Trans-p-menth-2-en-1-ol	0.29	0.03	0.10	0.01	0.00	0.00	
4	(E)- β-terpineol	0.00	0.00	0.01	0.00	0.00	0.00	
5	Terpinene-4-ol	4.00	0.20	4.28	0.02	0.02	0.005	F**
6	α-terpineol	0.80	0.10	0.50	0.05	0.10	0.006	
7	Thymol methyl ether	0.00	0.00	1.10	0.01	0.00	0.00	
8	Tymol	0.00	0.00	0.14	0.01	0.00	0.00	
9	Elemol	0.00	0.00	0.20	0.001	0.00	0.00	
10	(E)-nerolidol	7.51	0.29	0.10	0.01	0.00	0.00	
11	Spathulenol	0.46	0.06	0.14	0.01	0.00	0.00	
12	α-muurolol	0.00	0.00	0.04	0.005	0.00	0.00	
13	α-cadinol	0.00	0.00	0.05	0.005	0.00	0.00	
14	Phytol	0.00	0.00	0.12	0.005	0.00	0.00	
15	Globulol	0.09	0.001	0.00	0.00	0.00	0.00	
16	E,E-cis-farnesol	0.79	0.03	0.00	0.00	0.00	0.00	
	Total	39.11	2.17	30.56	0.24	0.70	0.02	
C	Esters							
1	Linalyl acetate	0.09	0.01	0.00	0.00	0.00	0.00	
2	Terpinyl acetate	0.01	0.00	0.00	0.00	0.00	0.00	
3	Citronellyl acetate	0.01	0.005	0.00	0.00	0.00	0.00	
4	Neryl acetate	0.009	0.00	0.00	0.00	0.03	0.005	
5	Granyl acetate	0.02	0.005	0.00	0.00	0.04	0.005	
	Total	0.13	0.02	0.00	0.00	0.07	0.01	
d	Ketones							
1	Carvone	0.00	0.00	0.00	0.00	0.01	0.00	
2	Geranyl acetone	0.04	0.01	0.00	0.00	0.00	0.00	
	Total	0.04	0.01	0.00	0.00	0.01	0.00	
	Monoterpenes							
1	α -thujene	0.007	0.00	0.54	0.004	0.008	0.00	
2	α -pinene	0.93	0.16	2.04	0.03	0.46	0.01	F**
3	sabinene	36.33	1.02	38.91	0.23	0.19	0.006	F**
4	β- pinene	1.42	0.45	1.75	0.06	0. 30	0.02	

Table 2 cont.

5	β-myrcene	2.65	0.53	3.84	0.05	2.06	0.02	F**
6	α -phellandrene	0.24	0.01	0.43	0.05	0.04	0.006	
7	α-terpinene	0.00	0.00	1.04	0.04	0.007	0.00	
8	P-cymene	0.00	0.00	0. 30	0.02	0.00	0.00	
9	limonene	7.46	0.35	3.20	0.03	87.45	0.49	F**
10	(Z)- β -ocimene	0.23	0.01	0.29	0.01	0.00	0.00	
11	(E)- β -ocimene	1.34	0.24	7.44	0.02	0.30	0.02	F**
12	γ - terpinene	0.55	0.14	1.29	0.007	5.10	0.20	F**
13	(E) Sabinene hydrate	0.41	0.03	0.60	0.006	0.00	0.00	
14	(Z)-linalool oxide	0.02	0.006	0.000	0.00	0.00	0.00	
15	α-terpinolene	0.29	0.03	1.10	0.01	0. 20	0.00	
16	Trans-limonene oxide	0.00	0.00	0.00	0.00	0.01	0.006	
	Total	51.87	2.97	62.48	0.56	95.62	0.77	
	Sesquiterpenes							
1	δ-elemene	0.00	0.00	0.01	0.00	0.00	0.00	
2	β-elemene	0.53	0.06	0.33	0.02	0.04	0.005	
3	(Z)- β-caryophyllene	0.64	0.03	0.51	0.03	0.00	0.00	
4	γ -elemene	0.00	0.00	0.01	0.00	0.00	0.00	
5	(Z)- β-farnesene	0.00	0.00	0.23	0.05	0.00	0.00	
6	δ -guaiene	0.07	0.009	0.00	0.00	0.00	0.00	
7	allo aromadendrene	0.03	0.006	0.00	0.00	0.00	0.00	
8	Germacrene D	0.09	0.001	0.01	0.00	0.17	0.01	
9	E,E- α-farnesene	0.36	0.06	0.50	0.05	0.10	0.005	
10	δ -cadinene	0.00	0.00	0.007	0.00	0.007	0.00	
11	Caryophyllene oxide	0.29	0.10	0.00	0.00	0.00	0.00	
	Total	2.01	0.26	1.60	0.15	0.31	0.02	
	Total oxygenated compounds	43.22	2.55	33.26	0.36	1.07	0.04	
	Total	97.10	5.78	97.34	1.07	97	0.83	

flower had the highest sesquiterpenes content (Table 2).

Result of correlation

Correlations of the main components were evaluated with Pearson correlation analysis. Simple intercorrellations between 8 components are presented in a correlation matrix (Table 3). The highest positive values or r (correlation coefficient) were between [sabinene and terpinene-4-ol (1.00%)]; [sabinene and linalool (99%)]; [terpinene-4-ol and linalool (98%)].

The highest significant negative correlations were between [limonene and linalool (99%)]; [limonene and terpinene-4-ol (99%)]; [limonene and sabinene (99%)]; [γ-terpinene and linalool (99%)]. When 8 components were cluster analyzed, there was clustering of only 3 components into 2 two-compound factors above the 98% level of function. These 2 factors resulted from the clustering of highly positively interrelated compounds such as [sabinene and terpinene-4-ol (1.00%)]; [sabinene and linalool (99%)] (Table 3).

Statistical analysis

Differences for main components among organs were analyzed by performing separate one-way analysis of variance (ANOVA). The Duncan's Multiple Range test was used to separate the significant organs. Of the 8 individual oil components analyzed, all showed statistically significant differences due to the influence of individual organs. These differences on the 1% level occurred in linalool, terpinene-4-ol, α -pinene, sabinene, β -myrcene, limonene, (E)-β- ocimene and γ- terpinene (Table 2).

DISCUSSION

Observations were made that changing organ has an effect on some of the components of tangerine oil accord with other observations (Babazadeh Darjazi, 2011 b;

Table 3. Correlation matrix (numbers in this table correspond with main components mentioned in Table 2).

	Linalool	Terpinene-4-ol	α-pinene	Sabinene	β-myrcene	Limonene	(E)- β -ocimene	γ - terpinene
Terpinene-4-ol	0.98**							
α-pinene	0.68*	0.76*						
Sabinene	0.99**	1.00**	0.76*					
β-myrcene	0.69*	0.73*	0.90**	0.74*				
Limonene	-0.99**	-0.99**	-0.75*	-0.99**	-0.74*			
(E)- β -ocimene	0.57	0.65	0.97**	0.65	0.93**	-0.64		
γ – terpinene	-0.99**	-0.97**	-0.60	-0.97**	-0.62	0.97**	-0.48	

*=significant at 0.05. **=significant at 0.01.

Salem, 2003; Asgarpanah, 2002; Lota et al., 2000). The compositions of *C. nobilis* Lour obtained from flower, leaf and peel were very similar. However, relative concentration of compounds differed according to type of materials. Compared with peel, the flower improved and increased alcohol components about 56 times for *C. nobilis* Lour. The amount of alcohol components obtained from peel were low probably because of decrease in endogenous enzymes activity [isopentenyl pyrophosphate isomerase (IPI) and geranylpyrophosphate synthase (GPS) (Hay and Waterman, 1995) resulting in decreased of labile compounds. Also the lower proportion of the detected alcohol components in peel was probably due to seasonal temperature (Sekiya et al., 1984) that it is the most important environmental factor in the control of endogenous enzymes.

According to our results, it appears that the relative percentages of the identified compounds depend on the plant part studied. However, it should be kept in mind that the isolation method has an effect on some of the components of oil (Babazadeh Darjazi, 2011c). The pronounced enhancement in the amount of oxygenated compounds, when flower was used as the organ, showed that either the synthesis of geranyl pyrophosphate (GPP) is enhanced or activities of both enzymes (IPI and GPS) increased (Hay and Waterman, 1995). High positive correlations between two terpens such as (sabinene and terpinene-4-ol (1.00%)); (sabinene and linalool (99%)); [terpinene-4-ol and linalool (98%)) suggest a genetic control (Scora et al., 1976). Whether such dependence between two terpenes is due to their derivation of one from another is not known. Similarly, high negative correlations observed between (limonene and linalool (99%)); (limonene and terpinene-4-ol (99%)); (limonene and sabinene (99%)); (γ- terpinene and linalool (99%)) suggest that one of the two compounds is being synthesized at the expense of the other or of its precursor. Non-significant negative and positive correlations can imply genetic and /or biosynthetic independence. However, without a thorough knowledge of the biosynthetic pathway leading to each terpenoid compound, the true significance of these observed correlations is not clear.

Conclusion

In the present study we found that the percent of flavor compounds was significantly affected by organ. The essential oil obtained from flower contained more oxygenated compounds and fewer monoterpene components than those isolated from leaf and peel. It is easy to observe the significant variations among flower and other organs, mainly in terms of the quantities of oxygenated compounds. The essential oils and their aroma compounds are very important and widely used in hygienic products, aromatherapy, pharmacy, food industries, cosmetics and other areas. Therefore, many studies, such as this study is very crucial in order to identify what type of chemical constituents existing in the materials that we want to use, before the essential oil can be utilized in those industries. Further research on the relationship between organ and essential oil (oxygenated terpenes) is necessary.

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