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Investigation on Peel Components and Juice Quality of Two Local Cultivars of Tangerine (Citrus reticulata) from Iran

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ABSTRACT: The peel components and juice quality of two local cultivars of tangerine were investigated in this study. Peel components were extracted using cold-press and eluted using n-hexane. Then all analyzed by GC-FID and GC-MS. Total soluble solids, total acids, pH value, ascorbic acid as well as density were determined in juice obtained from tangerine cultivars. Thirty and forty-one peel components were identified in Moallem-kooh and adib cultivars respectively including: aldehydes, alcohols, esters, monoterpenes and sesquiterpenes. The major flavour components were limonene, linalool γ-terpinene, (E)-β-ocimene, β-myrcene, α-pinene and sabinene. Between two cultivars examined, Adib showed the highest content of aldehydes and TSS. Since the aldehyde and TSS content of citrus are considered as two of the most important indicators of high quality, cultivar apparently has a profound influence on these factors.
Key Words: Cold-press, Flavor Components, Juice Quality, Peel Oil, Tangerine Cultivars.

INTRODUCTION

Citrus is one of the most economically important crops in Iran. In the period 2009- 2010, the total Citrus production of Iran was estimated at around 87000 tons [1]. Moallem-kooh and Adib are local cultivars of tangerine that cultivated in the Mazandaran province located in the north region of Iran [2]. They are two of the most important tangerine cultivars used in the north region of Iran. Although they are as important cultivars, the peel components of Moallem-kooh and adib have not been investigated before.

Citrus oils occur naturally in special oil glands of 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. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc. [3].

The quality of an essential oil can be calculated from the quantity of oxygenated compounds present in the oil. The quantity of oxygenated compounds present in the oil is variable and depends upon a number of factors including: rootstock [4], cultivars or scions [5, 6], seasonal variation [7], organ [8], extraction method [9] and etc.

Branched aldehydes and alcohols are important flavor compounds extensively used in food products [3]. Several studies have shown that the tangerine-like smell is mainly a result of the presence of carbonyl compounds, such as -sinensal, geranial, citronellal, decanal and peril-aldehyde [10]. The quality of a honey can be calculated from the quantity of oxygenated components present in the honey [11, 12]. In addition, type of flowers may influence the quality of volatile flavor components present in the honey. The effect of oxygenated compounds in the attraction of the pollinators has been proven. Therefore, the presence of oxygenated compounds can encourage the agricultural yield [13, 14].

Citrus juice is the most popular beverage in the world because of the fantastic flavor and abundant nutrition. The quality of citrus juice is an important economic factor in an industry that buys its fruit based on the sugar content and processes over 95% [15]. The best juices are consumed by the food and beverage industries. The quality of citrus juice may be determined not only by the amount of oxygenated components present in the juice but also by the concentration of compositions such as TSS, acids and vitamin C [4]. Juice, TSS and TA content are the main internal parameters used to determine Citrus quality in the world [16]. TSS content also forms the basis of payment for fruit by some juice processors in a number of countries, especially where the trade in juice is based on frozen concentrate [17]. The amount of TSS present in the juice is variable and depends upon a number of factors including: rootstock, scion or variety, degree of maturity, seasonal effects, climate, nutrition, tree age and etc. [17].

Several studies have shown that the cultivars used as scion may influence the quantity of chemical compositions (TSS, TA and vitamin C) present in the juice [18]. Compared with orange juice, very little research

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has been carried out on tangerine juice. Therefore, it is very important to be able to assess the differences between tangerine cultivars in terms of quantity of compositions (TSS, acids and vitamin C).

In this study, we compare the peel components isolated from two tangerine with the aim of determining whether the quantity of oxygenated compounds influenced by the cultivars. Also the present study reports the effects of cultivars on the juice quality parameters.

MATERIALS AND METHODS

Tangerine scions

In 1989, tangerine scions that 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 and temperature were 970 mm and 16.25°C per year, respectively; soil was classified as loam-clay, pH ranged from 6.9 to 7]. Moallem-kooh and Adib were used as scions in this experiment (Table 1).

Table 1. Common and botanical names for citrus taxa used as scions and rootstock

Common name	botanical name	Parents	category
Moallem-kooh (scion)	*Citrus reticulata cv. Moallem-kooh*	*Unknown*	Tangerine
Adib (scion)	*Citrus reticulata cv. Adib*	*Unknown*	Tangerine
Sour orange (Rootstock)	*Citrus aurantium*	Mandarin × Pomelo	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.

Preparation of juice 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. Juice was obtained using the Indelicate Super Automatic, Type A2 104 extractor. After extraction, juice was screened to remove peel, membrane, pulp and seed pieces according to the standard operating procedure. Three replicates were carried out for the quantitative analysis (n=3). Ten fruits were used for each replicate.

Chemical methods

The total titratable acidity was assessed by titration with sodium hydroxide (0.1 N) and expressed as % citric acid. Total soluble solids, expressed as Brix, were determined using a Carl Zeiss, Jena (Germany) refractometer. The pH value was measured using a digital pH meter (WTW Inolab pH-L1, Germany). Ascorbic acid was determined by titration with Potassium iodide. The density of the juice was measured using a pycnometer and ash was determined by igniting a weighed sample in a muffle furnace at 550 c to a constant weight [19].

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 to C (3min) to 250 o C (20 min) at a rate of 3 o C/ min. The injector and detector temperatures were 260 o 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 μL.

Identification of Components

Components were identified by comparison of their Kovats retention indices (RI), retention times (RT) and mass spectra with those of reference compounds [20, 21].

Data analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 11 peel components and 6 juice characteristics. Variations between cultivars were analyzed using one-way analysis of variance (ANOVA). The correlation between pairs of characters was evaluated using Pearson's correlation coefficient (Table 2 and 3).

Table 2. Statistical analysis of variation in peel flavor Components of tangerine cultivars (see Materials and methods). Mean is average composition in % over the different cultivars used with three replicates.

Compounds	Moallem-kooh		Adib		
	Mean	St.err	Mean	St.err	F value
Oxygenated compounds					
a) Aldehyds					
1) Octanal	0.03	0.01	0.59	0.12	F**
2) Citronellal	0	0	0.02	0	
3) Decanal	0.06	0.006	0.19	0.02	F**
4) Neral	0	0	0.1	0.01	
5) (E)-2-decenal	0	0	0.009	0.002	
6) Geranial	0	0	0.14	0.01	
7) Perilla aldehyde	0	0	0.006	0.002	
8) Dodecanal	0.01	0	0.02	0.006	
9) β-sinensal	0.02	0	0.02	0.006	
10) α-sinensal	0.02	0.01	0.16	0.01	
Total	0.14	0.02	1.25	0.18	
b) Alcohols					
1) Linalool	0.05	0.01	2.96	0.27	F**
2) α-terpineol	0.01	0.006	0.05	0.006	
3) Cis -carveol	0	0	0.01	0	
4) Nerol	0	0	0.02	0.01	
5) Elemol	0.09	0.01	0.05	0.006	
6) (E)-nerolidol	0	0	0.01	0	
7) Germacrene D-4-ol	0	0	0.005	0.001	
Total	0.15	0.02	3.10	0.29	
c) Esters					
1) Neryl acetate	0.07	0.01	0.03	0.006	
2) Granyl acetate	0.05	0.01	0	0	
Total	0.12	0.02	0.03	0.006	
Monoterpenes					
1) α-thujene	0.02	0.006	0.01	0	
2) α-pinene	0.49	0.006	0.49	0.07	NS
3) Sabinene	0.16	0.04	1.55	0.06	F**
4) β- pinene	0.1	0.01	0.03	0.01	F**
5) β-myrcene	1.52	0.12	1.54	0.21	NS
6) Limonene	94.46	1.12	88.4	0.27	F**
7) (E)-β-ocimene	1.32	0.21	1.36	0.18	NS
8) γ-terpinene	0.78	0.11	0.35	0.15	F**
9) (E)-sabinene hydrate	0	0	0.13	0.01	
10) α-terpinolene	0.04	0.006	0.02	0.006	
total	98.89	1.628	93.88	0.96	
Sesquiterpenes					
1) δ-elemene	0.09	0.006	0.008	0.003	
2) α-copaene	0.07	0.006	0.05	0.006	
3) β -cubebene	0.08	0.02	0.05	0.006	
4) β-elemene	0.02	0.006	0	0	
5) (Z)-β-caryophyllene	0.03	0	0.02	0.006	
6) (Z)-β-farnesene	0.03	0.01	0.03	0	
7) α-humulene	0	0	0.01	0	
8) Germacrene D	0.13	0.02	0.03	0.006	F**
9) Valencene	0	0	0.02	0	
10) Bicyclogermacrene	0.01	0.01	0.02	0.01	
11) (Z)-β- guaiene	0	0	0.01	0	
12) E,E-α-farnesene	0.01	0	0.01	0.006	
13) δ - amorphene	0	0	0.007	0.001	
14) δ-cadinene	0.03	0.006	0.02	0.006	
15) Germacrene B	0.01	0.006	0	0	
Total	0.51	0.09	0.28	0.05	
Total oxygenated compounds	0.41	0.06	4.38	0.47	
Total	99.81	1.79	98.55	1.50	

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.

Table 3. Statistical analysis of variation in juice quality parameters of tangerine cultivars. Mean is average parameter in % over the different cultivars used with three replicates.

Cultivars	TSS (%)	Total Acids (%)	TSS /TA rate	Ascorbic acid (%)	PH	Juice (%)	Total dry matter (%)	Ash (%)
Moallem-kooh (scion)	8.2	2.76	2.97	41.54	2.84	55.48	16.07	4
Adib (scion)	9.4	1.97	4.77	49.28	2.98	69.71	17.80	3
	F**	**F****	**F****	**F****	**F***	**F****		

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

RESULTS

Flavor compounds of the Moallem-kooh tangerine peel

GC-MS analysis of the flavor compounds extracted from Moallem-kooh tangerine peel using cold-press allowed identification of 30 volatile components (Table 4, Figure1) : 10 oxygenated terpenes [5 aldehydes , 3 alcohols, 2 esters] and 20 non oxygenated terpenes [9 monoterpens, 11 sesqiterpens].

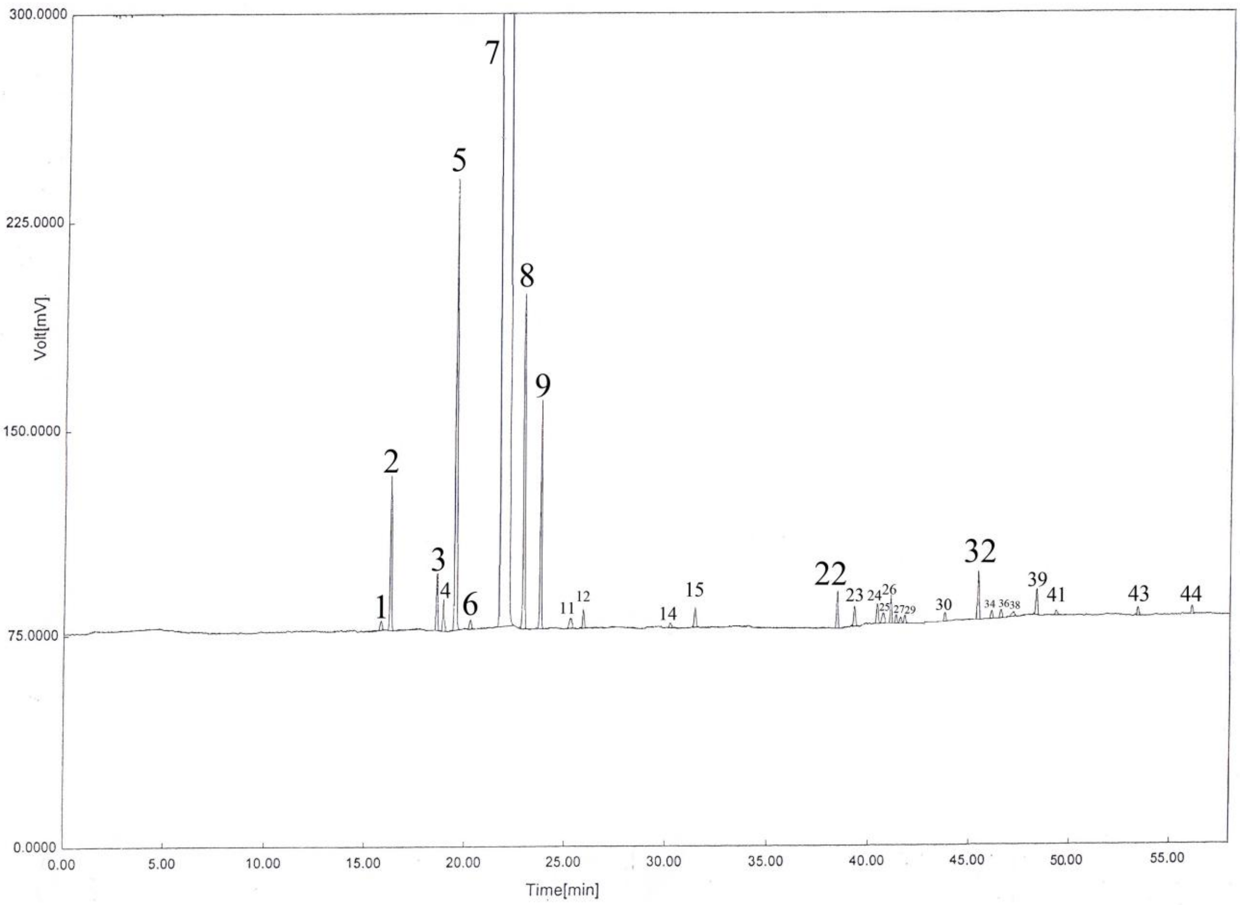

Figure 1. HRGC chromatograms of Moallem-kooh tangerine peel oil

Flavor compounds of the Adib tangerine peel

GC-MS analysis of the flavor compounds extracted from Adib tangerine peel using cold-press allowed identification of 41 volatile components (Table 4): 18 oxygenated terpenes [10 aldehydes , 7 alcohols , 1 esters] and 23 non oxygenated terpenes [10 monoterpens, 13 sesqiterpens].

Aldehydes

Ten aldehyde components that identified in this analysis were octanal, citronellal, decanal, neral, (E)-2-decenal, geranial, perillaldehyde, dodecanal, -sinensal and -sinensal (Table 3). In addition they were quantified from 0.14% to 1.25%. 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 tangerine flavor [10]. Between two cultivars examined, Adib showed the highest content of aldehydes (Table 3). Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, cultivar apparently has a profound influence on this factor.

Adib aldehydes were also compared to those of Moallem-kooh in this study. Citronellal, neral, (E)-2-decenal, geranial and perilla aldehyde were identified in Adib while they were not detected in the Moallem-kooh. Compared with Moallem-kooh, the Adib improved and increased aldehyde components about 8.92 times. (Table 3)

Table 4. Peel volatile components of tangerine cultivars (*There is in oil)

	Component	Moallem-kooh	Adib	KI		Component	Moallem-kooh	Adib	KI
1	α- thujene	*	*	928	24	α -copaene	*	*	1385
2	α - Pinene	*	*	935	25	Granyl acetate	*		1389
3	Sabinene	*	*	975	26	β -cubebene	*	*	1396
4	β -pinene	*	*	979	27	β -elemene	*		1399
5	β -myrcene	*	*	991	28	Dodecanal	*	*	1409
6	octanal	*	*	1003	29	(Z)- β -caryophyllene	*	*	1415
7	Limonene	*	*	1036	30	(Z)- β - farnesene	*	*	1453
8	(E)- β - ocimene	*	*	1049	31	α - humulene		*	1466
9	γ - terpinene	*	*	1061	32	Germacrene D	*	*	1493
10	(E)sabinene hydrate		*	1070	33	Valencene		*	1499
11	α -terpinolene	*	*	1091	34	Bicyclogermacrene	*	*	1504
12	Linalool	*	*	1100	35	(Z)- β-guaiene		*	1509
13	Citronellal		*	1154	36	E,E, α - farnesene	*	*	1512
14	α - terpineol	*	*	1195	37	δ - amorphene		*	1514
15	Decanal	*	*	1205	38	δ-cadinene	*	*	1532
16	Cis -carveol		*	1231	39	Elemol	*	*	1559
17	Nerol		*	1236	40	(E)-nerolidol		*	1567
18	Neral		*	1244	41	Germacrene B	*		1572
19	(E)-2-decenal		*	1263	42	Germacrene D-4-ol		*	1586
20	Geranial		*	1275	43	β - sinensal	*	*	1704
21	Perilla aldehyde		*	1282	44	α -sinensal	*	*	1756
22	δ- elemene	*	*	1344			30	41	
23	Neryl acetate	*	*	1356					

Alcohols

Seven alcoholic components identified in this analysis were linalool, -terpineol, cis-carveol, nerol, elemol, (E)-nerolidol and germacrene D-4-ol (Table 3).

The total amount of alcohols ranged from 0.15% to 3.10%. Linalool was identified as the major component in this study and was the most abundant. Linalool has been recognized as one of the most important components for mandarin flavor [10]. Linalool has a flowery aroma [10] and its level is important to the characteristic favor of tangerine [3]. Between two cultivars examined, Adib showed the highest content of alcohols (Table 3). Adib alcohols were also compared to those of Moallem-kooh in this study. Cis-carveol, nerol, (E)-nerolidol and germacrene D-4-ol were identified in Adib, while they were not detected in Moallem-kooh. Compared with Moallem-kooh, the Adib improved and increased alcohol components about 20.66 times. (Table 3)

Esters

Two ester components identified in this analysis were neryl acetate and geranyl acetate. The total amount of esters ranged from 0.03% to 0.12%. Between two cultivars examined, Moallem-kooh showed the highest content of esters (Table 3).

Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 93.88 % to 98.89%. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak Citrus-like aroma [10] and is considered as one of the major contributors to tangerine flavor. Between two cultivars examined, Moallem-kooh showed the highest content of monoterpenes (Table 3).

Sesquiterpene hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.28 % to 0.51 %. Germacrene D was identified as the major component in this study and was the most abundant. Between two cultivars examined, Moallem-kooh showed the highest content of sesquiterpenes (Table, 3).

Juice quality parameters

Juice quality parameters are given in table 4. The content of total acids ranged from 1.97 % (Adib) to 2.76 % (Moallem-kooh) and Brix (total soluble solids) ranged from 8.2 % (Moallem-kooh) to 9.4% (Adib). TSS/TA rate ranged from 2.97 (Moallem-kooh) to 4.77 (Adib). Ascorbic acid ranged from 41.54 % (Moallem-kooh) to 49.28% (Adib). The pH value ranged from 2.84 (Moallem-kooh) to 2.98 (Adib). The juice yield ranged from 55.48 % (Moallem-kooh) to 69.71% (Adib). Ash ranged from 3 % (Adib) to 4 % (Moallem-kooh). Total dry matter ranged from 16.07% (Moallem-kooh) to 17.80 % (Adib).

Between two cultivars examined, Adib showed the highest content of TSS, TSS /TA and pH. (Table 4).

Results of statistical analyses

Statistical analysis was performed on the peel and juice data using SPSS 18. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in octanal, decanal, linalool, β-pinene, sabinene, limonene, γ-terpinene, germacrene D, TSS, TA, TSS /TA, ascorbic acid and juice. This difference on the 5% level occurred in pH. The non-affected oil components were α-pinen, -myrcen and (E)- - ocimene (Table 3 and 4).

Results of correlation

Simple intercorrellations between 11 peel components are presented in a correlation matrix (Table 5).

Table 5. 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	(E)-β-ocimene	γ-terpinene
decanal	0.99**									
linalool	0.99**	0.99**								
α-pinene	0.20	0.18	0.06							
sabinene	0.98**	0.98**	0.99**	0.01						
β- pinene	-0.98**	-0.98**	-0.98**	-0.13	-0.97**					
B-myrcene	0.27	0.26	0.15	0.89*	0.09	-0.28				
limonene	-0.96**	-0.96**	-0.97**	0.004	-0.98**	0.92**	-0.007			
(E)-β-ocimene	0.28	0.29	0.18	0.65	0.16	-0.19	0.47	-0.19		
γ-terpinene	-0.77	-0.79	-0.84*	0.37	-0.86*	0.85*	0.10	0.79	0.17	
germacrene D	-0.94**	-0.94**	-0.95**	0.004	-0.96**	0.89*	0.02	0.99**	-0.18	**0.75**

*=significant at 0.05
**=significant at 0.01

The highest positive values or r (correlation coefficient) were observed between [decanal and octanal (99%)]; [linalool and octanal (99%)]; [linalool and decanal (99%)]; [sabinene and linalool (99%)]; [germacrene D and limonene (99%)]. The highest significant negative correlations were observed between [β-pinene and octanal (98%)]; [β-pinene and decanal (98%)]; [β-pinene and linalool (98%)]; [limonene and sabinene (98%)] (Table 5). Also simple intercorrellations between 6 juice characteristics are presented in a correlation matrix (Table 6). The highest positive values or r (correlation coefficient) were observed between [Juice and Ascorbic acid (100%)]; [TSS /TA and TSS (0.99%)]; [Ascorbic acid and TSS (0.99%)]; [Ascorbic acid and TSS /TA (0.99%)] ; [Juice and TSS (0.99%)]; [Juice and TSS /TA (0.99%)].The highest significant negative correlations were observed between [Ascorbic acid and TA (99%)]; [Juice and TA (99%)] (Table 6).

Table 6. Correlation matrix (numbers in this table correspond with juice quality parameters mentioned .

	TSS (%)	TA (%)	TSS /TA	Ascorbic acid (%)	pH
TA (%)	-0.98**				
TSS /TA	0.99**	-0.98**			
Ascorbic acid(%)	0.99**	-0.99**	0.99**		
pH	0.95**	-0.87*	0.94**	0.91*	
Juice (%)	0.99**	-0.99**	0.99**	1.00**	**0.91***

*=significant at 0.05
**=significant at 0.01

DISCUSSION

Our observation that different cultivars have an effect on some of the components of tangerine oil is in accordance with previous findings [5, 6]. The compositions of the peel oils obtained by cold pressing from different cultivars of tangerine were very similar. However, the relative concentration of compounds was different according to the type of cultivar.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies [6]. It may be related to cultivar, rootstock and environmental factors that can influence the compositions. However, it should be noticed that the extraction methods also may influence the results. Fertilizer [22] and irrigation [23] affects the content of compositions present in citrus juice. 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 biosynthetic pathway of oxygenated compounds. The biosynthetic pathway of oxygenated compounds in higher plants is as below:

Mevalonic acid → Isopentenyl Pyrophosphate → 3.3-dimethylallylpyrophosphate→ geranyl pyrophosphate→ Alcohols and Aldehyds

This reaction pathway catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively [24]. The pronounced enhancement in the amount of oxygenated compounds, when Adib used as the scion, Indicate that either the synthesis of geranyl pyrophosphate is enhanced or activities of both enzymes increased.

High positive correlations between pairs of terpenes such as [decanal and octanal (99%)]; [linalool and octanal (99%)]; [linalool and decanal (99%)]; [sabinene and linalool (99%)]; [germacrene D and limonene (99%)] suggest the presence of a genetic control [25] and such dependence between pairs of terpenes is due to derivation of one from another that is not known. Similarly, high negative correlations observed between [β-pinene and octanal (98%)]; [β-pinene and decanal (98%)]; [β-pinene and linalool (98%)]; [limonene and sabinene (98%)] 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 an extended insight into the biosynthetic pathway of each terpenoid compound, the true significance of these observed correlations is not clear. The highest positive value (correlation) was observed between [decanal and octanal (99%)]; [linalool and octanal (99%)]; [linalool and decanal (99%)]; [sabinene and linalool (99%)]; [germacrene D and limonene (99%)]. This result indicates that these compounds should be under the control of a single dominant gene [25] (Table 5).

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 by Adib. Our results showed that there was a positive correlation between pH and TSS /TA (sugars). This finding was similar to previous studies [26 .[

CONCLUSION

In the present study we found that the amount of peel and juice compositions were significantly affected by cultivars and there was a great variation in most of the measured characters between two cultivars. The present study demonstrated that volatile compounds in peel and quality parameters in juice can vary when different cultivars are utilized. Between two cultivars examined, Adib showed the highest content of TSS /TA and pH. The lowest of TSS /TA and pH were produced by Moallem-kooh. These results show that there is a positive correlation between pH and TSS /TA (sugars). Studies like this is very important to determine the amount of chemical compositions existing in the cultivars that we want to use, before their fruits can be used in food industries, aromatherapy, pharmacy, cosmetics, hygienic products and other areas. Further research on the relationship between cultivars and quality parameters is necessary.

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