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Original Article

The Effect of Rootstocks on Peel Components and Juice Quality of Clementine Mandarin (*Citrus clementina*)

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Abstract

Studies have shown that oxygenated compounds and TSS/TA are important for beverage and food industries. It seems that Citrus rootstocks have a profound influence on oxygenated compounds and TSS/TA. The aim of this research is to identify rootstock that can synthesize the maximum level of oxygenated compounds and TSS/TA. Peel oil was extracted using mechanical presses and eluted using n-hexane. Finally compounds were analyzed using GC-FID and GC-MS. Total soluble solids were determined using a refractometer. Twenty-seven, twenty-five and twenty-three compounds were identified in Sour orange, Swingle citromelo and Troyer citrang rootstocks, respectively. Limonene (92.87% to 93.16) and myrcene (1.57% to 1.79%) were the main compounds. Among the three rootstocks studied, Swingle citromelo demonstrated the maximum level of oxygenated compounds and TSS/TA. As a result of our research, we can express that the rootstocks can affect the amount of oxygenated compounds and TSS/TA.

Keywords: Citrus rootstocks, flavor components, juice quality, peel oil.

Abbreviations: TSS, Total soluble solids; TA, total acid; TSS/TA, Ratio total soluble solids to total acid; pH, potential of hydrogen; GC, Gas chromatograph; GC-MS, Gas chromatography- mass spectrometry; SPSS, Statistical package for social science; ANOVA, Analysis of variance; RI, Retention Index

Introduction

Citrus is one of the most economically important crops in Iran. In the period 2011- 2012, the total Citrus production of Iran was estimated at around 82500 tones [1]. Clementine (*Citrus clementina*) is one of the most important mandarins cultivated in Iran. It has been regarded as a mandarin fruit with potential commercial value because of its attractive and pleasant aroma [2]. Although it is as important mandarin, the flavor components of Clementine mandarin have been investigated very little previously.

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 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 [4,5], scions or varieties [6-8], seasonal variation [9], organ [10], method [11] and etc.

Aldehydes are important flavor compounds extensively used in food products [3]. Several studies have shown that the tangerine-like smell is mainly based on carbonyl compounds, such as α-sinensal, geranial, citronellal, decanal and peril

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aldehyde [12]. The quality of a honey can be calculated from the amount of oxygenated components present in the honey [13,14]. 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 [15,16].

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% [17]. 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 [18]. 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 [19]. 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 [19].

A number of studies have shown that the citrus rootstocks used may influence the quantity of chemical compositions *(*TSS, TA and vitamin C) present in the juice [20]. Compared with orange juice, very little research has been carried out on mandarin juice. Therefore, it is very important to be able to assess the differences between citrus rootstocks in terms of quantity of compositions (TSS, acids and vitamin C).

In this paper, we compared the peel compounds isolated from different rootstocks with the aim of determining whether the quantity of oxygenated compounds influenced by the rootstock. Also the present study reported the effects of rootstocks on the juice quality parameters with the aim of verifying if they were influenced by the rootstocks.

Material and Methods

Rootstocks

In 1989, citrus rootstocks 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]. Sour orange, Swingle citrumelo and Troyer citrange were used as rootstocks in this experiment (Table 1).

Preparation of Peel Sample

In the last week of January 2016, 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. Three replicates were carried out for the quantitative analysis (n=3) [4].

Preparation of Juice Sample

In the last week of January 2016, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. Juice was obtained by using the Indelicate Super Automatic, Type A2 104 extractor. After extraction, juice is 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. [4].

Table 1 Common and botanical names for citrus taxa used as rootstocks and scion [2].

Common name	botanical name	Parents	category
Clementine (scion)	*C. clementina cv. Cadox*	Unknown	Mandarin
Sour orange (Rootstock)	*C. aurantium(L.)*	Mandarin ×Pomelo	Sour orange
Swingle citrumelo (Rootstock)	*Swingle citrumelo*	Grapefruit × Poncirus	Poncirus hybrids
Troyer citrange (Rootstock)	*Troyer citrange*	Sweet orange × Poncirus	Poncirus hybrids

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 [21].

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 ^{o} 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 [22,23].

Data Analysis

Statistical package for social science 18 (SPSS 18) was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 9 peel component and 6 juice characteristics. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.01$. The correlation between pairs of characters was evaluated using Pearson's correlation coefficient.

Results

Peel Flavor Compounds of the Clementine Mandarin on Sour Orange Rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Sour orange rootstock allowed identification of 27 volatile components (Table 2): 12 oxygenated terpenes [9 aldehydes , 3 alcohols], 15 non oxygenated terpenes [6 monoterpens, 9 sesqiterpens].

Peel Flavor Compounds of the Clementine Mandarin Peel on Swingle Citrumelo Rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Swingle citrumelo rootstock allowed identification of 25 volatile components (Table 2): 12 oxygenated terpenes [9 aldehydes , 3 alcohols], 13 non oxygenated terpenes [6 monoterpens, 7 sesqiterpens].

Peel Flavor Compounds of the Clementine Mandarin Peel on Troyer Citrange Rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Troyer citrange rootstock allowed identification of 23 volatile components (Table 2): 9 oxygenated terpenes [6 aldehydes , 3 alcohols], 14 non oxygenated terpenes [6 monoterpens, 8 sesqiterpens].

Aldehydes

Nine aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, geranial, perillaldehyde, dodecanal, β-sinensal and α-sinensal. In addition they were quantified from 0.58% to 0.81%. The concentrations of n-octanal and n-decanal were higher in our samples. n-Octanal has a citrus-like aroma, and is considered as one of the major contributors to mandarin flavor [12]. Among the three rootstocks examined, Swingle citrumelo showed the highest content of aldehydes. Since the aldehyde content of Citrus oil is considered as one of the most important indicators of high quality, rootstock apparently has a profound influence on this factor.
Swingle citrumelo aldehydes were also compared to those of Sour orange and Troyer citrange in this study. n-Nonanal, geranial and perillaldehyde were

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identified in Swingle citrumelo and sour orange, while they were not detected in Troyer citrange. Compared with Troyer citrange, the Swingle citrumelo improved and increased aldehyde components about 1.39 times (Table 2).

Alcohols

Three alcoholic components identified in this analysis were linalool, α-terpineol and elemol. The total amount of alcohols ranged from 0.51% to 0.69%. Linalool was identified as the major component in this study and was the most abundant. Linalool has a flowery aroma [12] and its level is important to the characteristic favor of mandarin [3]. Among three rootstocks examined, Swingle citrumelo showed the highest content of alcohols. Compared with Troyer citrange, the Swingle citrumelo improved and increased alcohol components about 1.35 times. (Table 2)

Monoterpenes Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 96.86 % to 97.13%. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak citrus-like aroma [12] and is considered as one of the major contributors to mandarin flavor [3]. Among three rootstocks examined, Troyer citrange showed the highest content of monoterpenes (Table 2).

Table 2 Statistical analysis of variation in peel flavor components of clementine mandarin on three different rootstocks

			Sour orange		Swingle citrumelo		Troyer citrange		
	Compounds	RI	Mean	St.err	Mean	St.err	Mean	St.err	F value
1	α - Pinene	935	0.54	0.04	0.48	0.04	0.62	0.05	F*
2	Sabinene	975	0.70	0.04	0.74	0.04	0.64	0.05	NS
3	β -pinene	979	0.04	0.006	0.04	0.00	0.04	0.006	-
4	β -myrcene	991	1.66	0.04	1.57	0.04	1.79	0.06	F**
5	n-octanal	1003	0.24	0.01	0.27	0.01	0.23	0.01	F**
6	Limonene	1036	93.04	0.32	93.16	0.22	92.87	0.18	NS
7	(E)-β-ocimene	1049	0.92	0.10	0.87	0.06	1.17	0.07	F**
8	Linalool	1100	0.54	0.04	0.63	0.05	0.43	0.03	F**
9	n-Nonanal	1109	0.01	0.006	0.01	0.00	-	-	-
10	Citronellal	1154	0.06	0.01	0.05	0.006	0.06	0.01	-
11	α-terpineol	1195	0.04	0.01	0.02	0.006	0.04	0.006	-
12	n-Decanal	1205	0.17	0.01	0.21	0.01	0.14	0.01	F**
13	Geranial	1275	0.01	0.00	0.01	0.006	-	-	-
14	Perilla aldehyde	1282	0.01	0.006	0.01	0.00	-	-	-
15	α -copaene	1373	0.03	0.006	0.03	0.00	0.01	0.00	-
16	β-cubebene	1388	0.01	0.00	-	-	-	-	-
17	β-elemene	1399	0.02	0.006	0.02	0.006	0.01	0.00	-
18	n-Dodecanal	1409	0.04	0.006	0.04	0.006	0.05	0.006	-
19	(Z)- β - farnesene	1458	0.02	0.00	0.03	0.006	0.03	0.006	-
20	α - humulene	1466	0.01	0.00	0.02	0.006	0.02	0.00	-
21	Germacrene D	1493	0.04	0.006	0.05	0.01	0.04	0.006	-
22	Bicyclogermacrene	1504	0.02	0.006	0.03	0.00	0.02	0.006	-
23	E,E, α - farnesene	1523	0.02	0.00	-	-	0.03	0.006	-
24	δ-cadinene	1532	0.03	0.006	0.03	0.006	0.02	0.006	-
25	Elemol	1559	0.04	0.006	0.04	0.01	0.04	0.00	-
26	β-sinensal	1704	0.02	0.006	0.03	0.006	0.02	0.00	-
27	α-sinensal	1756	0.15	0.01	0.18	0.01	0.13	0.01	F**
28	Aldehyds	-	0.64	0.02	0.81	0.05	0.58	0.04	-
29	Alcohols	-	0.62	0.05	0.69	0.06	0.51	0.03	-
30	Monoterpenes	-	96.90	0.54	96.86	0.40	97.13	0.41	-
31	Sesquiterpenes	-	0.18	0.03	0.21	0.03	0.13	0.01	-
32	Oxygenated compounds	-	1.26	0.07	1.50	0.11	1.09	0.07	-
33	Total	-	98.34	0.64	98.57	0.54	98.35	0.49	-

Mean is average composition (%) in three different rootstocks 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.

Sesquiterpenes Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.13% to 0.21%. Germacren D was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Swingle citrumelo showed the highest content of sesquiterpenes (Table 2).

Juice Quality Parameters

Juice quality parameters are given in Table 3. Brix (total soluble solids) was from 11.90% (Troyer citrange) to 12.20% (Swingle citrumelo) and the content of total acidity was from 0.66% (Swingle citrumelo) to 0.71% (Sour orange). TSS/TA rate was from 16.90% (Sour orange) to 18.48% (Swingle citrumelo). Ascorbic acid was from 58.72% (Troyer citrange) to 73.04% (Sour orange). The pH value was from 3.70% (Sour orange) to 3.90% (Swingle citrumelo). The juice yield was from 54.20% (Troyer citrange) to 54.76% (Sour orange). Total dry matter was from 13.51% (Troyer citrange) to 14.82% (Swingle citrumelo). Ash was from 3% (Troyer citrange) to 4% (Swingle citrumelo and sour orange).

Among the three rootstocks examined, Swingle citrumelo showed the highest content of TSS, TSS /TA and pH. The lowest of TSS /TA and pH content were produced by Sour orange. (Table 3).

Results of Statistical Analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in octanal, decanal, α-sinensal, linalool, β-myrcen, (E)-β-cimene, TSS, TA, TSS /TA, ascorbic acid, pH and juice yield. This difference on the 5% level occurred in α-pinen. The non affected oil components were sabinene and limonen (Table 2 and 3).

Results of Correlation

Simple intercorrellations between 9 components are presented in a correlation matrix (Table 4). Not only α-sinensal demonstrated a significant positive correlation with n-octanal but also it demonstrated a significant positive correlation with decanal. Linalool also demonstrated a significant positive correlation with n-decanal.

Also simple intercorrellations between 6 juice characteristics are presented in a correlation matrix (Table 5). PH demonstrated a significant positive correlation with TSS /TA.

Discussion

Our observation that rootstocks had an effect on some of the components of Clementine mandarin oil was in accordance with previous findings [4, 5]. The compositions of the peel oils obtained by Cold-press from three rootstocks were very similar. However, the relative concentration of compounds was different according to the type of rootstock.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies [20]. It may be related to rootstock and environmental factors that can influence the compositions. However, it should be kept in mind that the extraction methods also may influence the results. Fertilizer [24] and irrigation [25] affects the content of compositions present in citrus juice. Fertilization, irrigation and other operations were carried out uniform in this study so we did not believe that this variability was 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 [26]. The pronounced enhancement in the amount of oxygenated compounds, when Swingle citrumelo used as the rootstock, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

High positive correlations between pairs of terpenes suggest a genetic control [27] and such dependence between pairs of terpenes was due to derivation of one from another that was not known. Similarly, high negative correlations between pairs of terpenes indicated that one of the two compounds had been 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 [27].

Table 3 Statistical analysis of variation in juice quality parameters of clementine mandarin on three different rootstocks

Rootstocks	TSS (%)	Total Acids (%)	TSS /TA rate	Ascorbic acid (%)	PH	Juice (%)	Total dry matter (%)	Ash (%)
Sour orange	12	0.71	16.90	73.04	3.7	54.76	13.56	4
Swingle citrumelo	12.2	0.66	18.48	64.24	3.9	54.30	14.82	4
Troyer citrange	11.9	0.68	17.50	58.72	3.8	54.20	13.51	3
	F**	F**	F**	F**	F**	F**		

Mean is average composition (%) in three different rootstocks 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.

Table 4 Correlation matrix (numbers in this table correspond with main components mentioned in Table 2).

	n-octanal	n-decanal	α-sinensal	linalool	α-pinene	sabinene	β-myrcene	limonene
n-decanal	0.96**	-	-	-	-	-	-	-
α-sinensal	0.98**	0.99**	-	-	-	-	-	-
linalool	0.94**	0.98**	0.98**	-	-	-	-	-
α-pinene	-0.48	-0.66	-0.58	-0.59	-	-	-	-
sabinene	0.89**	0.88**	0.91**	0.93**	-0.33	-	-	-
β-myrcene	-0.59	-0.76*	-0.69*	-0.71*	0.98**	-0.46	-	-
limonene	0.78*	0.70*	0.76*	0.77*	.007	0.88**	-0.14	-
β-ocimene	-0.46	-0.66*	-0.58	-0.63	0.96**	-0.43	0.96**	-0.05

*=significant at 0.05 **=significant at 0.01

Table 5 Correlation matrix (numbers in this table correspond with juice quality parameters mentioned in Table 3).

	TSS (%)	TA (%)	TSS /TA	Ascorbic acid(%)	pH
TA (%)	-0.49	-	-	-	-
TSS /TA	0.75*	-0.87**	-	-	-
Ascorbic acid (%)	0.20	0.64	-0.49	-	-
pH	0.65	-0.88**	0.98**	-0.60	-
Juice (%)	-0.02	0.79*	-0.67*	0.97**	-0.76*

*=significant at 0.05 **=significant at 0.01

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 Swingle citrumelo. Our results showed that there was a positive correlation between TSS/TA and pH. It was consistent with previous published [28].

Conclusion

In the present study we found that the amount of peel and juice compositions were significantly affected by rootstocks and there was a great variation in most of the measured characters among three rootstocks. The present study demonstrated that volatile compounds in peel and quality parameters in juice can vary when different rootstocks are utilized. Among the three rootstocks examined, Swingle citrumelo showed the highest content of oxygenated compounds and TSS /TA. The lowest of oxygenated compounds were produced by Troyer citrange. Further research on the relationship between rootstocks and oxygenated compounds is necessary.

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