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Research Paper

Peel Oil Components of Clementine Mandarin on Different Rootstocks

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Abstract
The aim of this research is to identify rootstock that can synthesize the maximum level of oxygenated compounds. Peel oil was extracted using mechanical presses and eluted using n-hexane. Finally compounds were analyzed using GC-FID and GC-MS. Twenty-four, Twenty-seven and twenty-six compounds were identified in Trifoliate orange, Orlando tangelo and Murcott rootstocks, respectively Limonene (% 92.55 to 93.09) and myrcene (%1.59 to %1.70) were the main compounds. Among the three rootstocks studied, Orlando tangelo demonstrated the maximum level of oxygenated compounds. As a result of our research, we can express that the rootstocks can affect the amount of oxygenated compounds.

Keywords: Citrus rootstocks, flavor components, peel oil.

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1. Introduction

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. 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 [1]. 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 [2], scions or varieties [3] and etc.
Aldehydes are important flavor compounds extensively used in food products. Several studies have shown that the tangerine-like smell is mainly based on carbonyl compounds, such as α-sinensal, geranial, citronellal, decanal and peril aldehyde [4]. The quality of a honey can be calculated from the amount of oxygenated components present in the honey [5]. 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 [6].
In this paper, we compared the peel compounds isolated from different rootstocks with the aim of

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determining whether the quantity of oxygenated compounds influenced by the rootstock.

2. MATERIALS AND METHODS

2-1. 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]. Trifoliate orange, Orlando tangelo and Murcott were used as rootstocks in this experiment (Table 1)

Table 1: Common and botanical names for citrus taxa used as rootstocks and scion.

Common name	botanical name	Parents	category
Clementine (scion)	*C. clementina cv. Cadox*	Unknown	Mandarin
Trifoliate orange (Rootstock)	*Poncirus trifoliata*	Unknown	Poncirus
Orlando tangelo (Rootstock)	*Citrus sp. cv. Orlando*	*Citrus reticulata cv. Dancy × Citrus paradisi cv. Duncan*	Tangelo
Murcott (Rootstock)	*Citrus sp. cv. Murcot*	*C.reticulata× C.sinensis*	Tangor

2-2. 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) [2].

2-3. 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 L.

2-4. Identification of components

Components were identified by comparison of their Kovats retention indices (KI), retention times (RT) and mass spectra with those of reference compounds [7].

2-5. Data analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurement of 9 peel component. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.01$.

3. RESULTS

3-1. Peel flavor compounds of the Clementine mandarin on Trifoliate orange rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Trifoliate orange rootstock allowed identification of 24 volatile components (Table 2, fig 1): 10 oxygenated terpenes [7 aldehydes , 3 alcohols], 14 non oxygenated terpenes [6 monoterpens, 8 sesqiterpens].

3-2. Peel flavor compounds of the Clementine mandarin on Orlando tangelo rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Orlando tangelo rootstock allowed identification of 27 volatile components (Table 2, fig 1): 12 oxygenated terpenes [9 aldehydes , 3 alcohols], 15 non oxygenated terpenes [6 monoterpens, 9 sesqiterpens].

3-3. Peel flavor compounds of the Clementine mandarin on Murcott rootstock

GC-MS analysis of the flavor compounds extracted from Clementine mandarin peel on Murcott rootstock allowed identification of 26 volatile components (Table 2, fig 1): 12 oxygenated terpenes [9 aldehydes , 3 alcohols], 14 non oxygenated terpenes [6 monoterpens, 8 sesqiterpens].

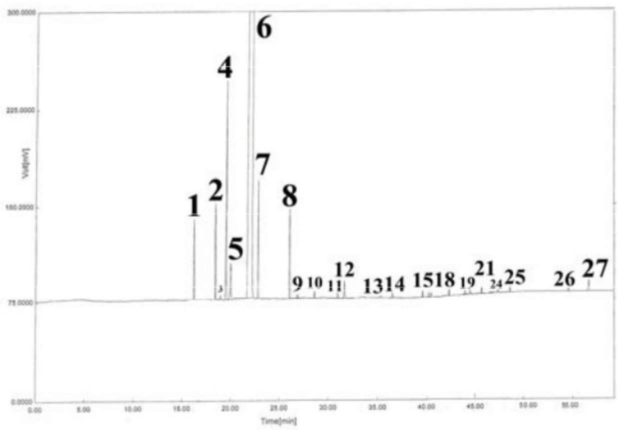

Fig 1: HRGC chromatogram of Clementine mandarin peel oil

3-4. 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 0.51 % to 0.78 %. 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 [4]. For all the dominant aldehydes, the differences among rootstocks were found significant on the 1% level. Among the three rootstocks examined, Orlando tangelo 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.

Orlando tangelo aldehydes were also compared to those of Murcott and Trifoliate orange in this study. n- Nonanal and perillaldehyde were identified in Orlando tangelo and Murcott, while they were not detected in Trifoliate orange. Compared with Trifoliate orange, the Orlando tangelo improved and increased aldehyde components about 1.52 times (Table 2).

3-5. Alcohols

Three alcoholic components identified in this analysis were linalool, α- terpineol and elemol. The total amount of alcohols ranged from 0.50% to 0.81%. Linalool was identified as the major component in this study and was the most abundant. Linalool has a flowery aroma [4] and its level is important to the characteristic favor of mandarin. There was statistically significant difference on the 1% level in linalool. Among three rootstocks examined, Orlando tangelo showed the highest content of alcohols. Compared with Trifoliate orange, the Orlando tangelo improved and increased alcohol components about 1.62 times. (Table 2).

3-6. Monoterpenes hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 96.48 % to 97.45 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak citrus-like aroma [4] and is considered as one of the major contributors to mandarin flavor. There was statistically significant difference on the 5% level in α-pinen and sabinene . The non affected oil components were β -myrcene and limonen. Among three rootstocks examined, Trifoliate orange showed the highest content of monoterpenes (Table 2).

3-7. Sesquiterpenes hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.13 % to 0.25 %. Germacren D was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Orlando tangelo showed the highest content of sesquiterpenes (Table 2).

Table 2: Statistical analysis of variation in peel flavor components of Clementine mandarin on different rootstocks

		Trifoliate orange			Orlando tangelo		Murcott		
									F
1	α - Pinene	935	0.42	0.04	0.51	0.04	0.40	0.03	F*
2	Sabinene	975	0.85	0.09	0.67	0.08	0.90	0.09	F*
3	β -pinene	979	0.13	0.01	0.07	0.01	0.09	0.01	
4	β -myrcene	991	1.70	0.10	1.59	0.10	1.66	0.11	NS
5	n-octanal	1003	0.19	0.02	0.32	0.03	0.25	0.02	F**
6	Limonene	1036	93.09	0.40	92.55	0.31	93.02	0.37	NS
7	(E)- β - ocimene	1049	1.26	0.10	1.09	0.12	1.10	0.09	NS
8	Linalool	1100	0.40	0.03	0.71	0.05	0.55	0.04	F**
9	n-Nonanal	1109			0.01	0	0.03	0	
10	Citronellal	1154	0.08	0.01	0.07	0	0.04	0	
11	α - terpineol	1195	0.08	0.01	0.09	0.01	0.04	0.006	
12	n-Decanal	1205	0.06	0.01	0.19	0.01	0.11	0.01	F**
13	Geranial	1275	0.01	0	0.02	0	0.02	0	
14	Perilla aldehyde	1282			0.01	0	0.02	0	
15	α -copaene	1373	0.02	0	0.03	0	0.01	0	
16	β -cubebene	1388			0.01	0			
17	β -elemene	1399	0.02	0	0.05	0.01	0.01	0	
18	n-Dodecanal	1409	0.01	0	0.03	0	0.01	0	
19	(Z)- β - farnesene	1458	0.01	0	0.02	0	0.01	0	
20	α - humulene	1466	0.01	0	0.01	0	0.02	0	
21	Germacrene D	1493	0.04	0.006	0.09	0.01	0.04	0	
22	Bicyclogermacrene	1504	0.02	0	0.01	0	0.01	0	
23	E,E, α - farnesene	1523	0.01	0	0.02	0	0.01	0	
24	δ-cadinene	1532	0.01	0	0.01	0	0.02	0	
25	Elemol	1559	0.02	0	0.01	0	0.03	0.006	
26	β - sinensal	1704	0.01	0	0.02	0	0.01	0	
27	α -sinensal	1756	0.15	0.01	0.11	0.01	0.08	0.01	F**
	Aldehyds		0.51	0.05	0.78	0.05	0.57	0.04	
	Alcohols		0.50	0.04	0.81	0.06	0.62	0.05	
	Monoterpenes		97.45	0.74	96.48	0.66	97.17	0.70	
	Sesquiterpenes		0.14	0.006	0.25	0.02	0.13	0	
	oxygenated compounds		1.01	0.09	1.59	0.11	1.19	0.09	
	Total		98.60	0.83	98.32	0.79	98.49	0.79	

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.

4. DISCUSSION

Data obtained from this experiment revealed that oxygenated compounds in peel significantly impressed by rootstocks that it was in accordance with previous studies [2]. However, it should be kept in mind that the environmental factors and extraction methods also may influence the results. Fertilizer and irrigation affects the content of compositions present in citrus. 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 [8]. The pronounced enhancement in the amount of oxygenated compounds, when Orlando tangelo used as the rootstock, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

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 Orlando tangelo.

5. CONCLUSIONS

In the present study we found that the amount of peel compositions was 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 can vary when different rootstocks are utilized. Among the three rootstocks examined, Orlando tangelo showed the highest content of oxygenated compounds. The lowest of oxygenated compounds were produced by Trifoliate orange. Further research on the relationship between rootstocks and oxygenated compounds is necessary.

6. ACKNOWLEDGMENT

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