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Short Communication

The Effect of Sour Orange, Swingle Citrumelo and Troyer Citrange Rootstocks on the Peel Components of Kumquat (*Fortunella Margarita*)

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Abstract

Studies have shown that oxygenated compounds are important in beverage and food products. It seems that Citrus rootstocks have a profound influence on these factors. The goal of the present study is to investigate on rootstocks and these factors. Peel oil components were extracted using cold-press method and eluted using n-hexane. All compounds analyzed using GC and GC-MS. Data were analyzed using one-way analysis of variance and Duncan'smultiple range tests. Twenty-seven, Twenty-seven and Twenty-four peel components were identified in Sour orange, Swinglecitromelo and Troyer citrang respectively. They include aldehydes, alcohols, esters,monoterpenes and sesquiterpenes. The major flavor components identified included limonene, β-myrcene, α-pinene, linalool, (E)-β-ocimeneand γ-terpinene. Among the three rootstocks examined, Swinglecitromelo showed the highest content of aldehydes. Considering that aldehyde content of Kumquat is as one of indicators of high quality, it seems that Citrus rootstocks have a profound influence on this factor.

Keywords: Peel oil, Citrus rootstock, Flavor components

Introduction

Kumquats are believed to be native to China.Robert Fortunebrought them from China to Europe in1846 [1]. Today, there are a number of kumquat species that grow throughout the world, including*Fortunella japonica* (Marumi kumquat), *F. margarita* (Nagami kumquat), *F. crassifolia*(Meiwa kumquat), *F. hindsii*(Hong Kong Wild kumquat), and *F. polyandra*(Malayan kumquat) [2].

Fortunella margarita, that called Nagami kumquat or ovoid kumquat, is the most popular species in the world. It has an oval shape and a smooth, bright orange flavedo [2]. It is one of the most important *Fortunella*species cultivated in Iran. Although it is as important species, the peel components of *Fortunella margarita* have been investigated very little previously.

Kumquat essential oil plays an important role in the flavor and aroma of foods [3]. In addition, recent studies have identified antimicrobial propertiesfor kumquat oil.

The quality of an essential oil can be calculated from the quantity of aldehyde compounds present in the oil. The quantity of aldehyde compounds present in the oil, is variable and depends upon a number of factors including:rootstock [4] seasonal variation [5], and etc.

Aldehydes are important flavor compounds extensively used in food products [6]. The quality of a honey can be calculated from the amount of oxygenated components present in the honey [7]. In addition, type of flowers may influence the quality of volatile flavor components present in the honey. The effect of oxygenated compoundsin the attraction of the pollinators has been proven. Therefore, the presence of oxygenated compounds can encourage the agricultural yield [8].

In this paper, we compared the peel compounds isolated from Nagami kumquat with the aim of determining whether the quantity of oxygenated compounds influenced by the rootstock.

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Material and Methods

Kumquat Rootstocks

In 2010, rootstocks were planted at 2×2 m with three replication at orchards of the Ramsar[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, Swinglecitrumelo and Troyer citrange were used as rootstocks in this experiment (Table 1).

Preparation of Peel Sample

Fruits were collected from many parts of the same trees inDecember 2014, early in the morning (6 to 8 am) and only during dry weather. The selection method of all samples was on a random basis.

Cold-pressing Extraction Technique

About 150 g of *freshpeel* 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) [9].

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 *theirKovats*retention*indices* (RI), retention times (RT)and mass spectra with*those of reference compounds* [10].

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 6 peel component. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan'smultiple 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 Compounds of the Nagami Kumquat

GC-MS analysis of the flavor compounds extracted from Nagami Kumquat using cold-press allowed identification of 27 volatile components (Table 2, Fig. 1): 12 oxygenated terpenes[3 aldehydes, 7 alcohols,2 esters] and 15 non oxygenated terpenes [9 monoterpens, 6sesqiterpens].

Table 1 Common and botanical names for Fortunella or Citrustaxa used as scion and rootstock

Common name	Botanical name	Parents	Category
Nagami Kumquat (scion)	*Fortunella margarita*	Unknown	Kumquat
Sour orange (Rootstock)	*Citrus aurantium L.*	Mandarin ×Pomelo	Sour orange
Swinglecitrumelo (Rootstock)	*Swinglecitrumelo*	*C.paradisivardancan × P.trifoliata (L.) Raf*	Poncirus hybrids
Troyer citrange (Rootstock)	*Troyer citrange*	*C.paradisivardancan × P.trifoliata (L.) Raf*	Poncirus hybrids

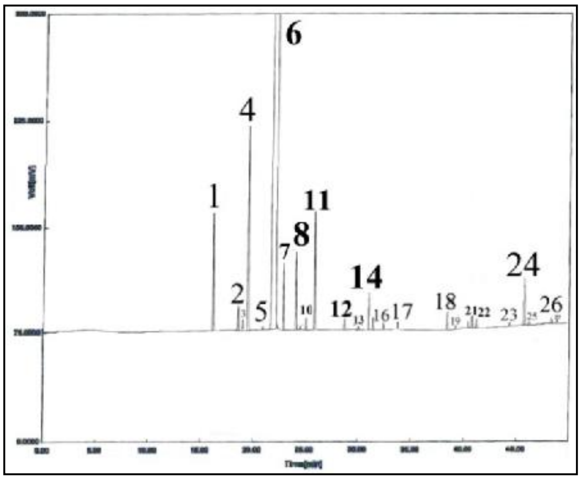

Fig. 1 HRGC chromatogram of peel oil of Nagami Kumquat

Aldehydes

Threealdehyde components that identified in this analysis were n-decanal, citronellal, and neral (Table 3). In addition they were quantified from 0.07% to 0.23%. The concentration of n-decanal was higher in our samples. Among three rootstocks examined, Swinglecitrumelo showed the highest content of aldehydes. Since the aldehyde content of Kumquat oil is considered as one of the most important indicators of high quality, rootstock apparently has a profound influence on this factor (Table 3).

Alcohols

Sevenalcoholic components identified in this analysis were n-octanol, linalool, terpinen-4-o1, -αterpineol, geraniol, elemol and (E)-nerolidol(Table 3). The total amount of alcohols ranged from 0.65% to 1.19%.Linalool was identified as the major component in this study and was the most abundant. Linalool has a fruity-like aroma [9] and its level is important to the*characteristic favor*of Kumquat. Among three rootstocks examined, Swinglecitrumelo showed the highest content of alcohols (Table 3).

Esters

Two ester components identified in this analysis were neryl acetate andgeranyl acetate. The total amount of esters ranged from 0.08% to 0.14%. Among three rootstocks examined, Swinglecitrumelo and Sour orange showed the highest content of esters (Table 3).

Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 90.14 % to 93.79 %. Limonene was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Troyer citrangeshowed the highest content of monoterpenes (Table 3).

Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.62% to 0.77 %. Germacren D was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Swinglecitrumelo showed the highest content of sesquiterpenes (Table 3).

Results of Statistical Analyses

Differences were considered to be significant at $P<$ 0.01. These differences on the 1% level occurred in linalool,α-pinene,β-myrcene, limonene,ocimene and γ-terpinene (Table 3).

Results of Correlation

Simple intercorrellations between 6 components are presented in a correlation matrix (Table 4). The highest positive values or r (correlation coefficient) were observed betweenocimene and α-pinene. γ-terpinenealso showed a high positive correlation with β-myrcene . α-pinene showed a high negative correlation with linalool (Table 4)

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.

Discussion

Our observation that rootstocks had an effect on some of the components of Kumquat oil was in accordance with previous findings [4]. The compositions of the peel oils obtained by Cold-press from three rootstocks of Kumquatwere 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 [11]. 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 and irrigation affects the content of oil present in Citrus [12].

Table2 Peel components of Nagami Kumquat on three different rootstocks

	Component	Sour orange	Swinglecit rumelo	Troyer citrange	KI		Component	Sour orange	Swinglecit rumelo	Troyer citrange	KI
1	α - Pinene	*	*	*	935	15	n- Decanal	*	*	*	1202
2	Sabinene	*	*	*	974	16	Neral	*	*		1242
3	β - Pinene	*	*	*	980	17	Geraniol	*	*		1258
4	β -myrcene	*	*	*	992	18	δ -elemene	*	*	*	1344
5	α -phellandrene	*	*	*	1006	19	Neryl acetate	*	*	*	1364
6	Limonene	*	*	*	1032	20	α - copaene	*	*	*	1380
7	(E)-β-ocimene	*	*	*	1052	21	Geranyl acetate	*	*	*	1384
8	γ- terpinene	*	*	*	1062	22	β - elemene	*	*	*	1396
9	*n*- Octanol	*	*	*	1070	23	α -humulene	*	*	*	1458
10	α- terpinolene	*	*	*	1090	24	Germacren D	*	*	*	1491
11	Linalool	*	*	*	1102	25	Bicyclogermacren	*	*	*	1501
12	Citronellal	*	*		1155	26	Elemol	*	*	*	1556
13	Terpinene-4-ol	*	*	*	1183	27	(E)-nerolidol	*	*	*	1567
14	α-terpineol	*	*	*	1195			27	27	24	

*There is in oil

Table 3 Statistical analysis of variation in peel flavor components of Nagami Kumquat on three different rootstocks

Compounds	Sour orange		Swinglecitrumelo		Troyer citrange		
	Mean	St.err	Mean	St.err	Mean	St.err	F value
a) Aldehyds	-	-	-	-	-	-	-
1) *n*- Decanal	0.1	0.01	0.12	0.01	0.07	0.01	
2) Citronellal	0.06	0.006	0.07	0.01	-	-	-
3) Neral	0.04	0.006	0.04	0.006	-	-	-
total	0.2	0.02	0.23	0.02	0.07	0.01	-
b) Alcohols	-	-	-	-	-	-	-
1) *n*- Octanol	0.03	0.006	0.03	0.006	0.01	0.00	-
2) Linalool	0.6	0.10	0.70	0.06	0.40	0.04	F**
3) Terpinen-4-ol	0.04	0.006	0.04	0.00	0.03	0.006	-
4) α-terpineol	0.23	0.02	0.29	0.02	0.16	0.01	-
5) Geraniol	0.05	0.006	0.07	0.01	-	-	-
6) Elemol	0.04	0.01	0.04	0.006	0.04	0.006	-
7) (E)-nerolidol	0.03	0.006	0.02	0.006	0.01	0.00	-
total	1.02	0.15	1.19	0.10	0.65	0.06	-
d) Esteres	-	-	-	-	-	-	-
1) Neryl acetate	0.05	0.006	0.06	0.01	0.03	0.006	-
2) Granyl acetate	0.09	0.01	0.08	0.01	0.05	0.006	-
total	0.14	0.01	0.14	0.02	0.08	0.01	-
Monoterpenes							
1) α-pinene	0.65	0.05	0.62	0.04	0.77	0.07	F**
2) Sabinene	0.17	0.02	0.16	0.02	0.15	0.01	-
3) β- pinene	0.11	0.01	0.11	0.01	0.16	0.02	-
4) β-myrcene	1.46	0.14	1.38	0.11	1.48	0.12	F**
5) α- phellandrene	0.02	0.006	0.02	0.00	0.02	0.006	-
6) Limonene	90.03	0.46	87.06	0.37	90.19	0.52	F**
7) (E)-β-ocimene	0.35	0.05	0.34	0.05	0.45	0.06	F**
8) γ-terpinene	0.45	0.03	0.37	0.04	0.49	0.07	F**
9) α-terpinolene	0.1	0.01	0.08	0.01	0.09	0.01	-
total	93.34	0.77	90.14	0.65	93.79	0.88	-
Sesquiterpenes	-	-	-	-	-	-	-
1) δ -elemene	0.16	0.020	0.22	0.030	0.22	0.030	-
2) α - copaene	0.05	0.01	0.05	0.01	0.02	0.00	-
3) β - elemene	0.07	0.01	0.06	0.006	0.04	0.006	-
4) α- humulene	0.03	0.006	0.04	0.006	0.06	0.01	-
5) Germacren D	0.26	0.02	0.34	0.03	0.30	0.03	-
6) Bicyclogermacren	0.05	0.006	0.06	0.01	0.05	0.006	-
Total	0.62	0.07	0.77	0.09	0.69	0.08	-
Total oxygenated compounds	1.36 -	0.19 -	1.56 -	0.15 -	0.8 -	0.08 -	-

Total	95.32	1.04	92.47	0.89	95.29	1.05	-

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

	Linalool	α-pinene	β-myrcene	Limonene	Ocimene
α-pinene	-0.97**	-	-	-	-
β-myrcene	-0.83**	0.78*	-	-	-
Limonene	-0.78*	0.68*	0.97**	-	-
Ocimene	-0.94**	0.99**	0.72*	0.59	-
γ-terpinene	-0.90**	0.86**	0.99**	0.94**	0.81**

*=significant at 0.05, **=significant at 0.01

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 byisopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively [13]. The pronounced enhancement in the amount of oxygenated compounds, when Swinglecitrumelo 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 terpenessuggest a genetic control [14] 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.

Considering that acetate is necessary for thesynthesis ofterpenes, it can be assumed that there is a specialized function for this molecule and it may be better served in Swinglecitrumelo.

Conclusion

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 three rootstocks examined, Swinglecitrumelo showed the highest content of oxygenated compounds. The lowest of oxygenated compounds content were produced by Troyer citrange. Further research on the relationship between rootstocks and oxygenated compounds is necessary.

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References

1. Spiegel-Roy P, Goldschmidt EE.The biology of Citrus. Cambridge University Press, 1996.
2. Dugo G, Mondello L.Citrus oils: composition, advanced analytical techniques, contaminants and biological activity. CRC Press, Taylor and Francis group, Boca Raton, 2010.
3. Maarse H. Volatile compounds in foods and beverages. CRC Press, Boca Raton, 1991.
4. Verzera A, Trozzi A, Gazea F, Cicciarello G, Cotroneo A. Effect of rootstock on the composition of bergamot(*Citrus bergamiaRisso et Poiteau*) essential oil. J Agric Food. Chem. 2003;51:206-210.
5. Attaway JA, Pieringer AP, Barabas LJ. The origin of citrus flavor components. III. A study of the percentage variation in peel and leaf oil terpenes during one season. Photochemistry. 1967;6:25-32.

6. Hemming D. Plant sciences review. CAB Reviews, CABI UK. 2011.
7. Alissandrakis E, Daferera D, Tarantilis PA, Polissiou M, Harizanis PC. Ultrasound assisted extraction of volatile compounds from *Citrus* flowers and *Citrus* honey. Food. Chem. 2003; 82: 575-582.
8. Kite G, Reynolds T, Prance T. Potential pollinator–attracting chemicals from Victoria (Nymphaeaceae). Biochem. Syst. Ecol. 1991;19:535-539.
9. Choi HS. Characteristic odor components of kumquat (*Fortunella japonica* Swingle) peel oil. J Agric Food Chem. 2005; 53:1642-1647.
10. Adams RP. Identification of essential oil components by gas chromatography / mass spectrometry.Allured Publishing Corporation, Carol Stream,2001.
11. Koyasako A, Bernhard A. Volatile constituents of the essential oil of kumquat. J Food Sci. 1983;48:1807-1812.
12. Kesterson JW, Braddock RJ, Koo RCJ. The effect of bud wood, rootstock, irrigation and fertilization on the yield of Florida lemon oil. Proc Fla State Hort Soc. 1974; 87: 6-9.
13. Hay RKM, Waterman P. Volatile oils crops; their biology, biochemistry and production. Wiley. 1995.
14. Scora RW, Esen A, Kumamoto J. Distribution of essential oils in leaf tissue of an F2 population of *Citrus*. Euphytica. 1976;25:201-209.