# The effects of rootstock on the flower components of Clementine Mandarin (*Citrus clementina*)

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## Abstract

Studies have shown the importance of oxygenated compounds in beverage and food products. Citrus rootstocks seem to have a profound influence on oxygenated compounds in plants. The goal of the present study was to investigate the effect of rootstocks on the oxygenated compounds in *Citrus clementina*. Flower oil components were extracted using an ultrasonic bath and eluted with n-pentane: diethyl ether (1:2). The oils were then analyzed using GC and GC-MS. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Forty-one, 41, and 39 flower components were identified in Sour orange, Swingle citromelo and Troyer citrange, respectively. These included aldehydes, alcohols, ketones, monoterpenes, and sesquiterpenes. The major flavor components identified included linalool and sabinene. Among the three rootstocks examined, Swingle citromelo showed the highest content of aldehydes. Since aldehyde content of citrus is one of the most important indicators of quality, it seems that Citrus rootstocks have a profound influence on this factor.

*Key words*: Citrus rootstock; flavor components; flower oil

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## Introduction

Mandarin is one of the most economically important crops in Iran. In the period 2011- 2012, the total mandarin production of Iran was estimated at around 825000 tonnes (FAO, 2012). *Citrus clementina* which is also called Yafa, is the most popular mandarin in the world. It is also one of the most important *Citrus* species cultivated in Iran and despite its importance, the flower components of *Citrus clementina* are relatively under-researched.

Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, and medicines. In addition, recent studies have identified antimicrobial properties for Citrus oil (Babazadeh, 2009).

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 (Verzera et al., 2003) and seasonal variation (Attaway et al., 1967) among other factors.

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Table 1
Common and botanical names for Citrus taxa used as scion and rootstock

| Common name | Botanical name | Parents | Category |
|---|---|---|---|
| Clementine(scion) | *C. clementina cv. Cadox* | Unknown | Mandarin |
| Sour orange (Rootstock) | *Citrus aurantium L.* | Mandarin ×Pomelo | Sour orange |
| Swingle citrumelo (Rootstock) | *Swingle citrumelo* | *C.paradisi var dancan × P.trifoliata (L.) Raf* | Poncirus hybrids |
| Troyer citrange (Rootstock) | *Troyer citrange* | *C.sinensis × P.trifoliata (L.) Raf* | Poncirus hybrids |

Aldehydes are important flavor compounds extensively used in food products (Hemming, 2011). The quality of honey can be calculated from the amount of oxygenated components present in it (Alissandraki et al., 2003). 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 established. Therefore, the presence of oxygenated compounds can encourage the agricultural yield (Kite et al., 1991).

In this paper, the flower compounds isolated from Clementine mandarin are compared with the aim of determining whether the quantity of oxygenated compounds is influenced by the rootstock.

## Material and Methods

### Clementine rootstocks

In 2007, rootstocks were planted at 4×4 m with three replications at an orchards in Ramsar (Latitude 36° 54′ N, longitude 50° 40′ E; Caspian Sea climate; average rainfall and temperature 970 mm and 16.25° C per year, respectively; soil was classified as loam-clay and 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 flower sample

Flowers were collected from many parts of the same trees in May 2015, early in the morning (6 to 8 am) and only during dry weather. The selection method of all samples was on a random basis.

### Flower extraction techniques

In order to obtain the volatile compounds from the flowers, 50 g of fresh flowers were placed in a 2000 ml spherical flask, along with 300 ml of n-pentane:diethyl ether (1:2). The flask was covered and then placed in an ultrasonic water bath for 20 min. Extraction experiments were performed with an ultrasound cleaning bath-Fisatom Scientific-FS14H (Frequency of 40 KHz, nominal power 90 W and 24 × 14 × 10 cm internal dimensions water bath). The temperature of the ultrasonic bath was held constant at 25° C. The extract was subsequently filtered through $MgSO_4$ monohydrate. The extract was finally concentrated under a gentle stream of nitrogen to 1 ml and placed in a vial. Vial was sealed and kept in the freezer at -4° C until the GC-MS analysis (Alissandraki et al., 2003).

### GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m $\times$0.25 mm i.d ; film thickness = 0.25 $\mu$m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60° C (3min) to 250 ° C (20 min) at a rate of 3° C/min. The injector and detector temperatures were 260° 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 based on 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.

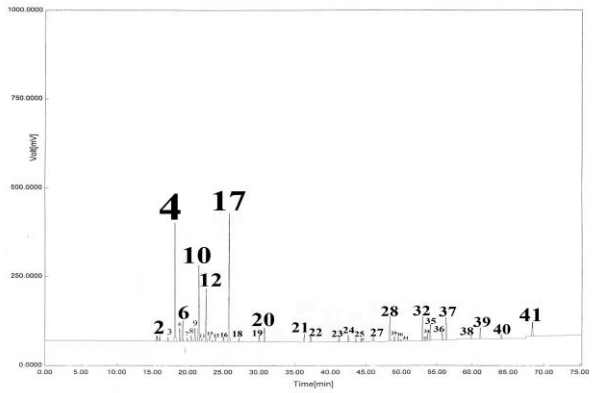


Fig. I. HRGC chromatogram of flower oil of Clementine mandarin on Sour orange

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 (Adams, 2001).

## Data analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 7 flower components. 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.

Table 2
Flower components of Clementine mandarin on three different rootstocks

| | Component | Sour orange | Swingle citrumelo | Troyer citrange | KI | | Component | Sour orange | Swingle citrumelo | Troyer citrange | KI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | α-thujene | * | * | * | 925 | 22 | β - elemene | * | * | * | 1343 |
| 2 | α-Pinene | * | * | * | 933 | 23 | Cis-jasmone | * | * | * | 1399 |
| 3 | Benzaldehyde | * | * | * | 954 | 24 | (Z)-β-caryophyllene | * | * | * | 1417 |
| 4 | Sabinene | * | * | * | 974 | 25 | (Z)-β-farnesene | * | * | * | 1451 |
| 5 | β-Pinene | * | * | * | 978 | 26 | α- humulene | * | * | * | 1461 |
| 6 | β-myrcene | * | * | * | 989 | 27 | E,E-α-farnesene | * | * | * | 1505 |
| 7 | α-phellandrene | * | * | * | 1003 | 28 | (E)-Nerolidol | * | * | * | 1562 |
| 8 | δ-3-carene | * | * | * | 1018 | 29 | Caryophyllene oxide | * | * | * | 1581 |
| 9 | p-cymene | * | * | * | 1024 | 30 | Hexadecane | * | * | * | 1593 |
| 10 | Limonene | * | * | * | 1030 | 31 | Tetradecanal | * | * | | 1610 |
| 11 | (Z)-β-ocimene | * | * | * | 1035 | 32 | 8-heptadecene | * | * | * | 1672 |
| 12 | (E)-β-ocimene | * | * | * | 1052 | 33 | Pentadecanal | * | * | | 1690 |
| 13 | γ- terpinene | * | * | * | 1057 | 34 | Heptadecane | * | * | * | 1692 |
| 14 | Octanol | * | * | * | 1065 | 35 | β -sinensal | * | * | * | 1699 |
| 15 | (E)-sabinene hydrate | * | * | * | 1068 | 36 | E,E-cis-farnesol | * | * | * | 1731 |
| 16 | α- terpinolene | * | * | * | 1088 | 37 | α-sinensal | * | * | * | 1755 |
| 17 | Linalool | * | * | * | 1100 | 38 | Caffeine | * | * | * | 1849 |
| 18 | Phenyl ethyl alcohol | * | * | * | 1110 | 39 | Nonadecane | * | * | * | 1892 |
| 19 | Terpinen-4-ol | * | * | * | 1179 | 40 | Eicosane | * | * | * | 1992 |
| 20 | α-terpineol | * | * | * | 1192 | 41 | Heneicosane | * | * | * | 2094 |
| 21 | Indol | * | * | * | 1296 | | | 41 | 41 | 39 | |

*There is in oil

Table 3
Statistical analysis of variation in flower components of Clementine mandarin on three different rootstocks

| | Sour orange | | Swingle citrumelo | | Troyer citrange | | |
|---|---|---|---|---|---|---|---|
| Compounds | Mean | St.err | Mean | St.err | Mean | St.err | F value |
| a) Aldehyds | | | | | | | |
| 1) Benzaldehyde | 0.20 | 0.02 | 0.30 | 0.03 | 0.20 | 0.02 | |
| 2) Tetradecanal | 0.05 | 0.006 | 0.06 | 0.01 | | | |
| 3) Pentadecanal | 0.03 | 0.006 | 0.03 | 0.006 | | | |
| 4) β -sinensal | 1.97 | 0.12 | 2.24 | 0.09 | 1.41 | 0.10 | F** |
| 5) α -sinensal | 2.45 | 0.11 | 2.63 | 0.10 | 2.13 | 0.13 | F** |
| total | 4.70 | 0.26 | 5.26 | 0.23 | 3.74 | 0.25 | |
| b) Alcohols | | | | | | | |
| 1) Octanol | 0.10 | 0.006 | 0.10 | 0.01 | 0.10 | 0.01 | |
| 2) Linalool | 20.91 | 0.27 | 26.00 | 0.20 | 15.72 | 0.18 | F** |
| 3) Phenyl ethyl alcohol | 0.24 | 0.03 | 0.19 | 0.01 | 0.19 | 0.01 | |
| 4) Terpinen-4-ol | 0.55 | 0.05 | 0.48 | 0.04 | 0.34 | 0.04 | |
| 5) α-terpineol | 1.32 | 0.10 | 1.67 | 0.05 | 1.08 | 0.08 | |
| 6) Indol | 0.90 | 0.05 | 0.80 | 0.05 | 0.80 | 0.05 | |
| 7) (E)-Nerolidol | 2.78 | 0.10 | 3.11 | 0.11 | 2.48 | 0.09 | F** |
| 8) E,E-cis-farnesol | 0.91 | 0.05 | 0.82 | 0.04 | 1.04 | 0.05 | |
| total | 27.71 | 0.65 | 33.17 | 0.52 | 21.75 | 0.52 | |
| d) Ketones | | | | | | | |
| 1) Cis-jasmone | 0.27 | 0.02 | 0.23 | 0.02 | 0.17 | 0.02 | |
| Monoterpenes | | | | | | | |
| 1) α-thujene | 0.07 | 0.006 | 0.07 | 0.01 | 0.08 | 0.01 | |
| 2) α-pinene | 0.92 | 0.05 | 0.81 | 0.05 | 1.03 | 0.08 | |
| 3) Sabinene | 21.52 | 0.20 | 19.23 | 0.23 | 24.84 | 0.20 | F** |
| 4) β-Pinene | 1.47 | 0.09 | 1.54 | 0.10 | 1.87 | 0.09 | |
| 5) β-myrcene | 2.34 | 0.10 | 2.19 | 0.11 | 2.48 | 0.11 | |
| 6) α-phellandrene | 0.14 | 0.02 | 0.15 | 0.01 | 0.15 | 0.01 | |
| 7) δ-3-carene | 0.75 | 0.05 | 0.78 | 0.04 | 0.88 | 0.07 | |
| 8) p-cymene | 1.45 | 0.05 | 1.57 | 0.07 | 1.66 | 0.11 | |
| 9) Limonene | 9.85 | 0.10 | 9.91 | 0.10 | 11.00 | 0.11 | F** |
| 10) (Z)-β-ocimene | 0.18 | 0.02 | 0.18 | 0.01 | 0.20 | 0.02 | |
| 11) (E)-β-ocimene | 6.31 | 0.10 | 7.04 | 0.09 | 8.96 | 0.10 | F** |
| 12) γ-terpinene | 0.70 | 0.05 | 0.60 | 0.06 | 0.60 | 0.05 | |
| 13) (E)-sabinene hydrate | 0.41 | 0.02 | 0.58 | 0.04 | 0.54 | 0.04 | |
| 14) α-terpinolene | 0.37 | 0.02 | 0.31 | 0.02 | 0.36 | 0.04 | |
| total | 46.48 | 0.88 | 44.96 | 0.96 | 54.65 | 1.06 | |
| Sesquiterpenes | | | | | | | |
| 1) β-elemene | 0.52 | 0.02 | 0.55 | 0.05 | 0.41 | 0.03 | |
| 2) (Z)-β-caryophyllene | 0.99 | 0.05 | 1.15 | 0.08 | 1.64 | 0.08 | |
| 3) (Z)-β-farnesene | 0.37 | 0.03 | 0.96 | 0.04 | 0.91 | 0.07 | |
| 4) α-humulene | 0.07 | 0.01 | 0.06 | 0.01 | 0.06 | 0.01 | |
| 5) E,E-α-farnesene | 0.26 | 0.02 | 0.27 | 0.02 | 0.24 | 0.02 | |
| 6) Caryophyllene oxide | 0.38 | 0.04 | 0.41 | 0.03 | 0.35 | 0.05 | |
| total | 2.59 | 0.17 | 3.40 | 0.24 | 3.61 | 0.26 | |
| 1) Hexadecane | 0.21 | 0.02 | 0.18 | 0.01 | 0.26 | 0.02 | |
| 2) 8-heptadecene | 2.79 | 0.09 | 2.92 | 0.10 | 3.22 | 0.13 | |
| 3) Heptadecane | 0.98 | 0.08 | 0.85 | 0.05 | 1.13 | 0.08 | |
| 4) Caffeine | 0.11 | 0.01 | 0.22 | 0.02 | 0.13 | 0.01 | |
| 5) Nonadecane | 1.26 | 0.05 | 0.97 | 0.07 | 1.36 | 0.08 | |
| 6) Eicosane | 0.48 | 0.04 | 0.44 | 0.02 | 0.33 | 0.02 | |
| 7) Heneicosane | 1.43 | 0.07 | 1.20 | 0.04 | 1.62 | 0.08 | |
| total | 7.26 | 0.38 | 6.78 | 0.31 | 8.05 | 0.43 | |
| Total oxygenated compounds | 32.68 | 0.94 | 38.66 | 0.77 | 25.66 | 0.79 | |
| Total | 89.01 | 2.38 | 93.80 | 2.28 | 91.97 | 2.54 | |

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.

## Results

### Flower components of the Clementine mandarin

GC-MS analysis of the flavor components extracted from Clementine mandarin using the ultrasonic bath allowed identification of 41 volatile components (Table 2, Fig. I) including 14 oxygenated terpenes (5 aldehydes, 8 alcohols, 1 ketone), 20 non-oxygenated terpenes (14 monoterpenes, 6 sesqiterpenes), and 7 other components.

## Aldehydes

Five aldehyde components identified in this analysis were benzaldehyde, tetradecanal, pentadecanal, β-sinensal, and α-sinensal (Table 3). In addition, they were quantified from 3.74% to 5.26%. The concentration of α-sinensal was higher in the study samples. Among 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 quality, rootstock apparently has a profound influence on this factor (Table 3).

## Alcohols

Eight alcoholic components identified in this analysis were octanol, linalool, Phenyl ethyl alcohol, terpinen-4-o1, α-terpineol, indol, (E)-nerolidol and E,E-cis-farnesol (Table 3). The total concentration of alcohols ranged from 21.75% to 33.17%. Linalool was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Swingle citrumelo showed the highest alcohol content (Table 3).

## Ketones

One component identified in this analysis was cis-jasmone. The total amount of ketones ranged from 0.17% to 0.27%. Among three rootstocks examined, Sour orange showed the highest ketone content (Table 3).

## Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 44.96 % to 54.65 %. Limonene was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Troyer citrange showed the highest content of monoterpenes (Table 3).

## Sesquiterpene hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 2.59% to 3.61 %. (Z)-β-caryophyllene was identified as the major component in this study and was the most abundant. Among three rootstocks examined, Troyer citrange 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 β-sinensal, α-sinensa, linalool, (E)-nerolidol, sabinene, limonene and (E)-β-ocimene (Table 3).

## Results of correlation

Simple correlations between 6 components are presented in a correlation matrix (Table 4). The highest positive values of correlation coefficient (r) were observed between α-sinensal and β-sinensal. The highest significant negative correlations were observed between sabinene and linalool (Table 4).

## Discussion

Our observation that rootstocks had an effect on some of the components of Clementine oil was in accordance with previous findings

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

| | β -sinensal | α -sinensal | Linalool | (E)-Nerolidol | Sabinene | Limonene |
|---|---|---|---|---|---|---|
| α -sinensal | 0.98** | | | | | |
| Linalool | 0.97** | 0.92** | | | | |
| (E)-Nerolidol | 0.97** | 0.97** | 0.91** | | | |
| Sabinene | 0.94** | -0.94** | -0.97** | -0.91** | | |
| Limonene | -0.85** | -0.75* | -0.94** | -0.27* | 0.89** | |
| (E)-β-ocimene | -0.78* | -0.70* | -0.87** | -0.26* | 0.77* | 0.97** |

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

(Verzera et al., 2003). Compositions of the flower oils obtained by ultrasonic bath 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 literature revealed some inconsistencies with previous studies (Miguel. et al., 2008). This could be attributed 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. Fertilizers and irrigation affect the content of oil present in Citrus (Kesterson et al., 1974). As fertilization, irrigation, and other operations were controlled in this study, this variability cannot be due to 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 is catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively (Hay and Waterman, 1995). The pronounced enhancement in the amount of oxygenated compounds, when Swingle citrumelo was used as the rootstock, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

Cytokinins can influence the essential oil components (Stoeva and Iliev, 1997). It is generally accepted that Cytokinins in higher plants are synthesized mainly in the root system and transported via the transpiration stream in xylem to the shoot. In addition, cytokinin level in the xylem sap also can vary by rootstock and exhibit a source-sink relationship by making strong sink tissues for mineral elements and other metabolites including amino acids (Gordon et al., 1984).

High positive correlations between pairs of terpenes suggest a genetic control (Scora et al., 1976) 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 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.

## Conclusion

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

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