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# A Study on Peel Volatile Constituents and Juice Quality Parameters of Four Tangerine (Citrus reticulata) Cultivars from Ramsar, Iran

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# Abstract

The peel volatile constituents and juice quality parameters of four tangerine cultivars were investigated in this study. Peel flavor constituents were extracted by using cold-press and eluted by 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 and ash were determined in juice obtained from tangerine cultivars. Forty-six, Twenty- five, Forty and thirty-four peel constituents in Dancy, Cleopatra, Ponkan and Atabaki cultivars respectively including: aldehydes, alcohols, esters, monoterpenes, sesquiterpenes and other components were identified and quantified. The major flavor constituents were linalool, limonene,  $\gamma$ -terpinene, (E)- $\beta$ -ocimene,  $\beta$ -myrcene,  $\alpha$ -Pinene. Among the four cultivars examined, Dancy showed the highest content of aldehydes and Younesi showed the highest content of TSS. Since the aldehyde and TSS content of citrus peel are considered as two of the most important indicators of high quality, variety apparently has a profound influence on citrus quality.

Keywords: Flavor constituents, Peel oil, Cold-press, Juice quality, Tangerine cultivars.

# Introduction

The citrus is an economically important crop cultivated extensively in Iran. The total annual citrus production of Iran was about 87000 tonnes in 2010 [1]. Atabaki is a native variety of tangerine that grown in the Mazandaran province located in the north region of Iran. Younesi was produced from nucellar tissue of ponkan tangarine and it was cultured as a nucellar seedling by Ramsar research station in 1968 [2]. They are two of the most important tangerine cultivars used in Iran. Although

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they are as important cultivars, the flavor components of Atabaki and Younesi have not been investigated before.

Dancy tangerine was rediscovered by G. L. Dancy in Morocco and brought to Florida in 1867. It has been regarded as a Citrus fruit with potential commercial value because of its attractive and pleasant aroma [2]. 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 Essential oils of citrus are used residues. commercially for flavoring foods, beverages, perfumes, cosmetics, medicines, etc [3].

The insecticidal, antimicrobial, antioxidative and antitumor properties of Citrus peel oils have recently been reported [4]. Oxygenated compounds, mainly oxygenated terpenes, have been found to be responsible for the characteristic odor and flavor of Citrus fruits [4]. The quality of an essential oil may 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 [5, 6], cultivars or scions [7-9], seasonal variation [10], organ [11], method [12] and etc.

Branched aldehydes and alcohols are important

flavor compounds in many food products [3]. Various studies have shown that the tangerine-like smell is mainly based on carbonyl compounds, such as  $\alpha$ -sinensal, geranial, citronellal, decanal and perilaldehyde [13]. The quality of a honey may be calculated from the amount of oxygenated components present in the honey [14, 15] and various flowers may influence the quality of volatile flavor components present in the honey. It had been recognized previously that oxygenated compounds are important factor in deceiving and attracting the pollinators. These results may have consequences for yield in agricultural [16, 17].

Citrus juice is the most popular beverage in the world because of the fantastic flavor and abundant nutrition. The juice quality of citrus is an important economic factor in an industry that buys its fruit based on the juice sugar content and processes over 95% of its crop [18]. The greatest amounts of the high quality juices are consumed by the food and beverage industries. The quality of a juice may be calculated not only with the amount of oxygenated components present in the juice but also with concentration of compositions such as TSS, acids and vitamin C [5]. In citrus, fruit juice content, TSS and TA concentration are the main internal quality parameters used all over the world [19]. 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 [20]. The quantity 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 [20].

Various studies have shown that the scion or variety used may influence the quantity of chemical compositions (TSS, TA and vitamin C) present in the juice [21]. Compared with orange juice, very little research 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 paper, we compare the peel volatile compounds isolated from four different tangerines with the aim of determining whether the quantity of oxygenated compounds was influenced by the variety. Also the present study reports the effects of variety on the juice quality parameters with the aim of verifying if they were influenced by the variety.

### Experimental

#### Tangerine scions

In 1989, tangerine scions that grafted on Sour orange rootstock, were planted at 8×4 m with three replication at Ramsar research Site [Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate, average rainfall 970 mm per year and average temperature16.25°C; soil was classified as loam-clay, pH range (6.9 to 7)]. Dancy, Cleopatra, Ponkan and Atabaki were used as cultivars in this experiment (Table 1).

| Common name                | botanical name  | Parents          | category    |
|----------------------------|---|------------------|-------------|
| Dancy(scion)               | Citrus reticulata cv. Dancy   | Unknown          | Tangerine   |
| Cleopatra (scion)          | <i>Citrus reticulata</i> (C.reshni<br>Hort.ex.Tan) <i>cv. Cleopatra</i> | Unknown          | Tangerine   |
| Younesi (scion)            | Citrus reticulata cv. Younesi   | Unknown          | Tangerine   |
| Atabaki(scion)             | Citrus reticulata cv. Atabaki   | Unknown          | Tangerine   |
| Sour orange<br>(Rootstock) | Citrus aurantium  | Mandarin ×Pomelo | Sour orange |

Table 1. Common and botanical names for citrus taxa used as scions and rootstock [2].

# 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 Site. 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 [22].

# 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 Site. 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. Each juice replicate was made with 10 tangerines. Three replicates were used for the quantitative analysis (n=3) [23].

### 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 [24].

# 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  $\mu$ 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 °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  $\mu$ L.

# Identification of Components

Components were identified by comparing their LRIs and matching their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search program (Chem. SW. Inc; NIST 98 version database) connected to a Varian Saturn 2000R MS. Identifications were also determined by comparing the retention time of each compound with that of known compounds [25, 26].

# Data analysis

SPSS 18 was used for analysis of the data

obtained from the experiments. Analysis of analyzed using analysis of variance (ANOVA)variations was based on the measurements of one way. Correlation between pairs of characters 11 peel component and 6 juice characteristics. and altitude was evaluated using Pearson's Variations among and within cultivars were 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. 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.

|  | Dancy |        | Cleopatra |        | Younesi |        | Atabaki |        |            |
|--|-------|--------|-----------|--------|---------|--------|---------|--------|------------|
| Compounds  | Mean  | St.err | Mean      | St.err | Mean    | St.err | Mean    | St.err | F<br>value |
| Oxygenated compounds   |       |        | •         |        | •       |        |         |        | •          |
| a) Aldehyds  |       |        |           |        |         |        |         |        |            |
| 1) Octanal   | 0.29  | 0.01   | 0.34      | 0.04   | 0.25    | 0.02   | 0.24    | 0.01   | F**        |
| 2) Nonanal   | 0.08  | 0      | 0.01      | 0.006  | 0.05    | 0.006  | 0.05    | 0.01   |            |
| 3) Citronellal   | 0.04  | 0      | 0         | 0      | 0.05    | 0.006  | 0.03    | 0.006  |            |
| 4) Decanal   | 0.2   | 0.006  | 0.18      | 0      | 0.2     | 0.01   | 0.11    | 0.01   | F**        |
| 5) Neral   | 0.01  | 0      | 0         | 0      | 0.04    | 0      | 0       | 0      |            |
| 6) (E)-2-decenal   | 0.01  | 0.006  | 0         | 0      | 0.007   | 0.003  | 0       | 0      |            |
| 7) Geranial  | 0.02  | 0.006  | 0.01      | 0      | 0.007   | 0.003  | 0.008   | 0.002  |            |
| 8) Perilla aldehyde  | 0.01  | 0.006  | 0.02      | 0.006  | 0.01    | 0      | 0.01    | 0.006  |            |
| 9) Undecanal   | 0.01  | 0.001  | 0         | 0      | 0.008   | 0.003  | 0       | 0      |            |
| 10) (E)2,4-decadienal  | 0.004 | 0.001  | 0         | 0      | 0.009   | 0.001  | 0       | 0      |            |
| 11) Dodecanal  | 0.01  | 0      | 0.03      | 0      | 0.02    | 0.01   | 0.01    | 0      |            |
| 12) Tetradecanal   | 0.02  | 0      | 0         | 0      | 0       | 0      | 0       | 0      |            |
| 13) β-sinensal   | 0     | 0      | 0         | 0      | 0.009   | 0.001  | 0.009   | 0.002  |            |
| 14) α-sinensal   | 0.14  | 0.02   | 0         | 0      | 0.01    | 0.01   | 0.02    | 0.01   |            |
| total  | 0.84  | 0.05   | 0.59      | 0.05   | 0.67    | 0.07   | 0.48    | 0.05   |            |
| b) Alcohols  |       |        |           |        |         |        |         |        |            |
| 1) linalool  | 1.130 | 0.100  | 0.770     | 0.006  | 0.860   | 0.090  | 0.560   | 0.060  | F**        |
| 2) Terpinen-4-ol   | 0.009 | 0.001  | 0         | 0      | 0.010   | 0.000  | 0.010   | 0.000  |            |
| 3) α-terpineol   | 0.080 | 0.000  | 0.080     | 0.000  | 0.070   | 0.006  | 0.070   | 0.000  |            |
| 4) β-citronellol   | 0.030 | 0.000  | 0         | 0      | 0.010   | 0.006  | 0.007   | 0.003  |            |
| 5) Nerol   | 0     | 0      | 0.007     | 0.003  | 0       | 0      | 0       | 0      |            |
| 6) Thymol  | 0.090 | 0.000  | 0         | 0      | 0       | 0      | 0.020   | 0.000  |            |
| 7) Elemol  | 0.010 | 0.006  | 0         | 0      | 0       | 0      | 0       | 0      |            |
| 8) Germacrene D-4-ol   | 0.003 | 0.001  | 0         | 0      | 0       | 0      | 0       | 0      |            |
| total  | 1.35  | 0.10   | 0.85      | 0.01   | 0.95    | 0.10   | 0.66    | 0.06   |            |
| c) Esters  | 0.007 | 0.001  | 0         | 0      | 0.007   | 0.002  | 0       | 0      |            |
| 1) Citronellyl acetate   | 0.00/ | 0.001  | 0         | 0      | 0.007   | 0.003  | 0       | 0      |            |
| 2) Neryl acetate   | 0.01  | 0      | 0.01      | 0      | 0.009   | 0.002  | 0       | 0      |            |
| 3) Granyl acetate  | 0.009 | 0 001  | 0         | 0      | 0.007   | 0.003  | 0       | 0      |            |
| total<br>Monotormonog  | 0.02  | 0.001  | 0.01      | 0      | 0.02    | 0.008  | 0       | 0      |            |
| 1) g thuisno   | 0.16  | 0.006  | 0         | 0      | 0.26    | 0.006  | 0.16    | 0.006  |            |
| 1) a-titujene  | 0.10  | 0.000  | 0.59      | 0.03   | 0.20    | 0.000  | 0.10    | 0.000  | E**        |
| 2) Q-philene<br>3) Sabinana  | 0.00  | 0.00   | 1.01      | 0.03   | 0.41    | 0.07   | 0.9     | 0.03   | Г<br>Г**   |
| A) B pipopo  | 0.12  | 0.02   | 0.04      | 0.08   | 0.41    | 0.03   | 0.39    | 0.03   | Г<br>Г**   |
| 5) B-myrcone   | 1.68  | 0.01   | 1.76      | 0.01   | 1.82    | 0.04   | 1.67    | 0.05   | I<br>NS    |
| $6) a_{terninene}$   | 0.02  | 0.00   | 0         | 0.11   | 0.03    | 0.01   | 0.06    | 0.03   | 115        |
| The second secon | 0.02  | 0.01   | 0         | 0      | 0.05    | 0.01   | 0.00    | 0.02   | E**        |
| /) Limonene  | 87.07 | 0.7    | 92.01     | 0.55   | 84.24   | 2.19   | 87.22   | 1.06   | F**        |
| 8) (E)-β-ocimene   | 1.13  | 0.42   | 1.33      | 0.55   | 1.14    | 0.31   | 1.03    | 0.25   | NS         |
| 9) γ-terpinene   | 4.68  | 0.51   | 0.18      | 0.03   | 7.43    | 1.02   | 5.29    | 0.01   | F**        |
| 10) (E)-sabinene hydrate   | 0.09  | 0.01   | 0.05      | 0.01   | 0.11    | 0.02   | 0.09    | 0.02   |            |

| 11) α-terpinolene          | 0.23  | 0.05  | 0     | 0     | 0.39  | 0.11  | 0.26  | 0.05  |     |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| 12) Trans-limonene oxide   | 0     | 0     | 0     | 0     | 0.005 | 0     | 0     | 0     |     |
| total                      | 96.39 | 1.87  | 97.87 | 1.37  | 97.62 | 3.9   | 97.46 | 1.61  |     |
| Sesquiterpenes             |       |       |       |       |       |       |       |       |     |
| 1) δ-elemene               | 0.01  | 0     | 0.15  | 0     | 0.03  | 0.006 | 0.04  | 0.006 |     |
| 2) α-copaene               | 0.008 | 0.002 | 0     | 0     | 0     | 0     | 0     | 0     |     |
| 3) β-elemene               | 0.05  | 0.006 | 0.05  | 0.006 | 0.01  | 0.006 | 0.01  | 0.006 |     |
| 4) (Z)-β-caryophyllene     | 0.01  | 0     | 0     | 0     | 0     | 0     | 0     | 0     |     |
| 5) γ-elemene               | 0.05  | 0.01  | 0.06  | 0.01  | 0.01  | 0     | 0.01  | 0.006 |     |
| 6) α-humulene              | 0     | 0     | 0     | 0     | 0.01  | 0.006 | 0.01  | 0     |     |
| 7) (Z)-β-farnesene         | 0.01  | 0     | 0.01  | 0.006 | 0     | 0     | 0     | 0     |     |
| 8) Germacrene D            | 0.1   | 0.006 | 0.11  | 0.006 | 0.03  | 0     | 0.04  | 0     | F** |
| 9) Bicyclogermacrene       | 0.02  | 0     | 0     | 0     | 0     | 0     | 0     | 0     |     |
| 10) E,E-α-farnesene        | 0.01  | 0     | 0     | 0     | 0.006 | 0     | 0.007 | 0.002 |     |
| 11) δ-cadinene             | 0.02  | 0.01  | 0.01  | 0     | 0.009 | 0.001 | 0.01  | 0     |     |
| 12) Germacrene B           | 0.05  | 0.006 | 0.05  | 0.006 | 0.01  | 0     | 0.01  | 0     |     |
| total                      | 0.33  | 0.04  | 0.44  | 0.03  | 0.11  | 0.01  | 0.13  | 0.02  |     |
| Other compounds            |       |       |       |       |       |       |       |       |     |
| 1)Thymol methyl ether      | 0.04  | 0.006 | 0     | 0     | 0     | 0     | 0.03  | 0.006 |     |
| Total oxygenated compounds | 2.21  | 0.15  | 1.45  | 0.06  | 1.64  | 0.17  | 1.14  | 0.11  |     |
| Total                      | 98.97 | 2.06  | 99.76 | 1.46  | 99.37 | 4.08  | 98.76 | 1.74  |     |

**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. 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.

| Cultivars | TSS (%) | Total Acids<br>(%) | TSS /TA<br>rate | Ascorbic acid<br>(%) | РН   | Juice (%)   | Total dry<br>matter (%) | Ash (%) |
|-----------|---------|--------------------|-----------------|----------------------|------|-------------|-------------------------|---------|
| Dancy     |         |                    |                 |                      |      |             |                         |         |
| (scion)   | 9       | 0.87               | 10.34           | 43.82                | 3.42 | 71.61       | 14.79                   | 1       |
| Cleopatra |         |                    |                 |                      |      |             |                         |         |
| (scion)   | 7.6     | 2.81               | 2.70            | 27.81                | 2.93 | 64          | 17.56                   | 3       |
| Younesi   |         |                    |                 |                      |      |             |                         |         |
| (scion)   | 10.4    | 0.71               | 14.64           | 29.04                | 3.53 | 71.18       | 14.80                   | 3       |
| Atabaki   |         |                    |                 |                      |      |             |                         |         |
| (scion)   | 8       | 1.11               | 7.20            | 36.61                | 3.31 | 55.55       | 13.21                   | 3       |
|           | F**     | <b>F</b> **        | F**             | F**                  | F**  | <b>F</b> ** |                         |         |

# **Results and discussion**

*Flavor compounds of the 'Dancy' tangerine peel* GC-MS analyze of the flavor compounds extracted from 'Dancy' tangerine peel by using cold-press allowed identification of 46 volatile components (Table 4, Figure 1): 23 oxygenated terpenes [13 aldehydes, 7 alcohols, 3 esters], 22 non oxygenated terpenes [11 monoterpens, 11 sesqiterpens] and 1 other compound. *Flavor compounds of the 'Cleopatra' tangerine peel* 

GC-MS analyze of the flavor compounds extracted from 'Cleopatra' tangerine peel by using cold-press allowed identification of 25 volatile components (Table 4) : 10 oxygenated terpenes [6 aldehydes , 3 alcohols, 1 esters], 15 non oxygenated terpenes [8 monoterpens, 7 sesqiterpens].

# *Flavor compounds of the 'Younesi' tangerine peel*

GC-MS analyze of the flavor compounds extracted from 'Younesi' tangerine peel by using cold-press allowed identification of 40 volatile components (Table 4): 20 oxygenated terpenes [13 aldehydes, 4 alcohols, 3 esters], 20 non oxygenated terpenes [12 monoterpens, 8 sesqiterpens].

# *Flavor compounds of the 'Atabaki' tangerine peel*

GC-MS analyze of the flavor compounds extracted from 'Atabaki' tangerine peel by using cold-press allowed identification of 34 volatile components (Table 4): 14 oxygenated terpenes [9 aldehydes, 5 alcohols], 19 non oxygenated terpenes [11 monoterpens, 8 sesqiterpens] and 1 other compound.



Figure 1. HRGC chromatograms of 'Dancy' tangerine peel oil.

|    | Component               | Dancy | Cleopatra | Younesi | Atabaki | KI   | Component |                             | Dancy                | Cleopatra | Younesi | Atabaki | KI   |
|----|-------------------------|-------|-----------|---------|---------|------|-----------|-----------------------------|----------------------|-----------|---------|---------|------|
| 1  | α- thujene              | *     |           | *       | *       | 928  | 28        | Thymol                      | *                    |           |         | *       | 1291 |
| 2  | α - Pinene              | *     | *         | *       | *       | 935  | 29        | Undecanal                   | *                    |           | *       |         | 1307 |
| 3  | Sabinene                | *     | *         | *       | *       | 975  | 30        | (E)2,4-decadienal           | *                    |           | *       |         | 1322 |
| 4  | β -pinene               | *     | *         | *       | *       | 979  | 31        | δ- elemene                  | *                    | *         | *       | *       | 1344 |
| 5  | β -myrcene              | *     | *         | *       | *       | 991  | 32        | Citronellyl acetate         | *                    |           | *       |         | 1349 |
| 6  | octanal                 | *     | *         | *       | *       | 1003 | 33        | Neryl acetate               | *                    | *         | *       |         | 1356 |
| 7  | $\alpha$ -phellandrene  |       |           |         |         | 1006 | 34        | α -copaene                  | *                    |           |         |         | 1385 |
| 8  | $\alpha$ -terpinene     | *     |           | *       | *       | 1021 | 35        | Granyl acetate              | *                    |           | *       |         | 1389 |
| 9  | Limonene                | *     | *         | *       | *       | 1036 | 36        | β -cubebene                 |                      |           |         |         | 1396 |
| 10 | (E)- $\beta$ - ocimene  | *     | *         | *       | *       | 1049 | 37        | β -elemene                  | *                    | *         | *       | *       | 1399 |
| 11 | γ - terpinene           | *     | *         | *       | *       | 1061 | 38        | Dodecanal                   | *                    | *         | *       | *       | 1409 |
| 12 | (E)sabinene<br>hydrate  | *     | *         | *       | *       | 1070 | 39        | (Z)- $\beta$ -caryophyllene | - β -caryophyllene * |           |         |         | 1415 |
| 13 | $\alpha$ -terpinolene   | *     |           | *       | *       | 1091 | 40        | γ - elemene                 | *                    | *         | *       | *       | 1440 |
| 14 | Linalool                | *     | *         | *       | *       | 1100 | 41        | (Z)- β - farnesene          |                      |           | *       | *       | 1453 |
| 15 | Nonanal                 | *     | *         | *       | *       | 1109 | 42        | $\alpha$ - humulene         | *                    | *         |         |         | 1466 |
| 16 | Trans-limonene<br>oxide |       |           | *       |         | 1141 | 43        | Germacrene D                | *                    | *         | *       | *       | 1493 |
| 17 | Citronellal             | *     |           | *       | *       | 1154 | 44        | Valencene                   |                      |           |         |         | 1499 |
| 18 | Terpinene-4-ol          | *     |           | *       | *       | 1182 | 45        | Bicyclogermacrene           | *                    |           |         |         | 1504 |
| 19 | $\alpha$ - terpineol    | *     | *         | *       | *       | 1195 | 46        | E,E, $\alpha$ - farnesene   | *                    |           | *       | *       | 1514 |
| 20 | Decanal                 | *     | *         | *       | *       | 1205 | 47        | δ-cadinene                  | *                    | *         | *       | *       | 1532 |
| 21 | $\beta$ -citronellol    | *     |           | *       | *       | 1229 | 48        | Elemol                      | *                    |           |         |         | 1559 |
| 22 | Nerol                   |       | *         |         |         | 1231 | 49        | Germacrene B                | *                    | *         | *       | *       | 1572 |
| 23 | Thymol methyl ether     | *     |           |         | *       | 1236 | 50        | Germacrene D-4-ol           | *                    |           |         |         | 1588 |
| 24 | Neral                   | *     |           | *       |         | 1244 | 51        | Tetradecanal                | *                    |           |         |         | 1612 |
| 25 | (E)-2-decenal           | *     |           | *       |         | 1263 | 52        | β - sinensal                |                      |           | *       | *       | 1704 |
| 26 | Geranial                | *     | *         | *       | *       | 1275 | 53        | α -sinensal                 | *                    |           | *       | *       | 1756 |
| 27 | Perilla aldehyde        | *     | *         | *       | *       | 1282 |           |                             | 46                   | 25        | 40      | 34      |      |

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

# Aldehydes

Fourteen aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, (E)-2-decenal, geranial, perillaldehyde, undecanal, (E)-2,4decadienal, dodecanal, tetradecanal,  $\beta$ -sinensal and  $\alpha$ -sinensal (Table 2). In addition they were quantified [from 0.48% to 0.84%] that it was determined and reported as relative amount of those compounds in oil. 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 [13]. Among the four cultivars examined, Dancy showed the highest content of aldehydes (Table 2). Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, cultivars apparently has a profound influence on tangerine oil quality. Dancy aldehydes were also compared to those of Cleopatra, Younesi and Atabaki in this study. Tetradecanal was identified in Dancy, while it was not detected in the Cleopatra, Younesi and Atabaki. Compared with Atabaki, the Dancy improved and increased aldehyde components about1.75 times (Table 2).

### Alcohols

Eight alcohol components identified in this analysis were linalool, terpinene-4-ol,  $\alpha$ -terpineol,  $\beta$ -citronellol, Nerol, thymol, elemol, Germacrene D-4-ol (Table 2). The total amount of alcohols ranged [from 0.66% to 1.35%] that it was determined and reported as relative amount of those compounds in oil. Linalool was the major component in this study and it was the most abundant. Linalool has been recognized as one of the most important components for tangerine peel oil flavor. Linalool has a flowery aroma [13] and its level is important to flavor character in tangerine peel oil [3]. Among the four cultivars examined, Dancy showed the highest content of alcohols (Table 2). Dancy alcohols were also compared to those of Cleopatra, Younesi and Atabaki in this study. Elemol and germacrene D-4-ol were identified in Dancy, while they were not detected in Cleopatra, Younesi and Atabaki. Compared with Atabaki, the Dancy improved and increased alcohol components about 2 times (Table 2).

### Esters

Three ester components identified in the analysis were citronellyl acetate, neryl acetate, geranyl acetate. The total amount of esters ranged [from 0.00% to 0.02%]. Among the four cultivars examined, Dancy showed the highest content of esters in oil (Table 2).

#### Monoterpenes hydrocarbons

The total amount of monoterpene hydrocarbons ranged [from 96.39 % to 97.87%]. Limonene was the major component among the monoterpene hydrocarbons of tangerine peel oil. Limonene has a weak citrus-like aroma [13] and is considered as one of the major contributors to tangerine flavor [3]. Among the four cultivars examined, Cleopatra had the highest monoterpenes hydrocarbons in oil (Table 2)

# Sesquiterpenes hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged [from 0.11 % to 0.44 %]. Germacrene D was the major component among the sesquiterpen hydrocarbons of tangerine peel oil. Among the four cultivars examined, Cleopatra had the highest sesquiterpenes content in oil (Table 2).

### Juice quality parameters

Juice quality parameters are given in Table 3. The content of total acids was from 0.71 % (Younesi) to 2.81 % (Cleopatra), and Brix (total soluble solids) was from 7.6 % (Cleopatra) to 10.4% (Younesi). TSS/TA rate was from 2.70 % (Cleopatra) to 14.64% (Younesi). Ascorbic acid was from 27.81 % (Cleopatra) to 43.82% (Dancy). The pH value was from 2.93 % (Cleopatra) to 3.53% (Younesi). The juice yield was from 55.55 % (Atabaki) to 71.61% (Dancy). Ash was from 1 % (Dancy) to 3 % (Cleopatra, Younesi and Atabaki). Total dry matter was from 13.21% (Atabaki) to 17.56 % (Cleopatra). Among the four cultivars examined, Younesi showed the highest content of TSS, TSS /TA and pH. The lowest of TSS, TSS /TA and pH were produced by Cleopatra. Among cultivars, Dancy had the highest juice content and Ascorbic acid. (Table 4).

# Statistical analyses

Statistical analysis was performed on the peel and juice data using SPSS 18. The Duncan's multiple range tests was used to separate the significant cultivars. Among all analyzed, 15 showed statistically significant differences due to the influence of different cultivars. These differences on the 1% level occurred in Octanal, decanal, linalool,  $\alpha$ -pinen,  $\beta$ - pinene, sabinene, limonene,  $\gamma$ -terpinene, Germacrene D, TSS, TA, TSS /TA, Ascorbic acid, pH, Juice. The non affected oil components were  $\beta$ -myrcen and (E)- $\beta$ -ocimene that they are provided only for convenience of the reader (Table 3 and 5).

# Results of correlation

Simple intercorrellations between 11 peel components are presented in a correlation matrix (Table 5). The highest positive values or r (correlation coefficient) were between [ $\gamma$ -terpinene and  $\beta$ -pinene (98%)]; [ $\beta$ -pinene and  $\alpha$ -pinene (97%)]; [ $\gamma$ -terpinene and  $\alpha$ -pinene (97%)]. The highest significant negative correlations were between [limonene and  $\alpha$ -pinene (96%)]; [ $\gamma$ -terpinene and  $\beta$ -pinene (93%)] (Table 3).

|               | Octanal | decanal | linalool | α-pinene | sabinene | β- pinene | B-myrcene | limonene | (E)-β-<br>ocimene | γ-terpinene |
|---------------|---------|---------|----------|----------|----------|-----------|-----------|----------|-------------------|-------------|
| decanal       | 0.39    |         |          |          |          |           |           |          |                   |             |
| linalool      | 0.33    | 0.83**  |          |          |          |           |           |          |                   |             |
| α-pinene      | -0.65*  | 0.15    | 0.17     |          |          |           |           |          |                   |             |
| sabinene      | 0.68*   | 0.02    | -0.26    | -0.74**  |          |           |           |          |                   |             |
| β- pinene     | -0.75** | 0.07    | 0.07     | 0.97**   | -0.76**  |           |           |          |                   |             |
| B-myrcene     | 0.38    | 0.39    | 0.22     | 0.33     | 0.21     | 0.20      |           |          |                   |             |
| limonene      | 0.64*   | -0.15   | -0.25    | -0.96**  | 0.78**   | -0.93**   | -0.26     |          |                   |             |
| (E)-β-ocimene | 0.08    | 0.13    | 0.18     | -0.15    | 0.30     | -0.13     | 0.05      | 0.04     |                   |             |
| γ-terpinene   | -0.75** | 0.02    | 0.12     | 0.97**   | -0.84**  | 0.98**    | 0.16      | -0.96**  | -0.14             |             |
| Germacrene D  | 0.78**  | 0.38    | 0.44     | -0.80**  | 0.51     | -0.83**   | -0.18     | 0.71**   | 0.22              | -0.80**     |

Table 5. Correlation matrix (numbers in this table correspond with main components mentioned in Table 2.

\*=significant at 0.05

\*\*=significant at 0.01

Also simple intercorrellations between 6 juice characteristics are presented in a correlation matrix (Table 6). The highest positive values or r (correlation coefficient) were between [TSS /TA and TSS (96%)]; [pH and TSS /TA (94%)]; [pH and TSS (0.84%)]. The highest significant negative correlations were between [pH and TA (97%)]; [TSS /TA and TA (88%)] (Table 6).

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

|                  |         | 5.      |         |                     |      |
|------------------|---------|---------|---------|---------------------|------|
|                  | TSS (%) | TA (%)  | TSS /TA | Ascorbic<br>acid(%) | рН   |
| TA (%)           | -0.73** |         |         |                     |      |
| TSS /TA          | 0.96**  | -0.88** |         |                     |      |
| Ascorbic acid(%) | -0.02   | -0.51   | 0.17    |                     |      |
| pH               | 0.84**  | -0.97** | 0.94**  | 0.40                |      |
| Juice (%)        | 0.71**  | -0.28   | 0.59*   | 0.09                | 0.42 |
|                  |         |         |         |                     |      |

\*=significant at 0.05

\*\*=significant at 0.01

Our observations that different scions or cultivars have an effect on some of the components of tangerine oil are accord with other observations [7-9]. The compositions of the peel oils obtained by cold pressing from different cultivars of tangerine were very similar. However, relative concentration of compounds differed according to type of variety. A comparison of our data with those in the literatures revealed that some of the peel components identified in our study are not compatible with the published one for Dancy and Cleopatra [7]. Also comparisons of our data with those in the literatures revealed that content of the juice compositions in our study are not agree with previously published for Younesi [21]. It may be related to rootstock and environmental factors that can influence compositions. However, it should be kept in mind that the chemical methods also have an effect on content of the juice and peel

compositions[23]. Fertilizer [27] and irrigation [28] 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 results from 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 oxygenated compounds biosynthetic pathway. The major pathway of oxygenated compounds biosynthesis in higher plants is as below:

Mevalonic acid  $\rightarrow$ Isopentenyl Pyrophosphate  $\rightarrow$ 3.3-dimethylallylpyrophosphate  $\rightarrow$  geranyl pyrophosphate  $\rightarrow$  Alcohols and Aldehyds

The steps in the pathway are catalyzed by isopentenyl pyrophosphate isomerase and

geranyl pyrophosphate synthase, respectively [29]. The pronounced enhancement in the amount of oxygenated compounds, when Dancy was used as the variety, showed that either the synthesis of oxygenated compounds is enhanced or activities of both enzymes increased.

High positive correlations between two terpens such as [ $\gamma$ -terpinene and  $\beta$ -pinene (98%)]; [ $\beta$ -pinene and  $\alpha$ -pinene (97%)]; [ $\gamma$ -terpinene and  $\alpha$ -pinene (97%)] suggest a genetic control [30].Whether such dependence between two terpenes is due to their derivation of one from another is not known. Similarly, high negative correlations observed between [limonene and  $\alpha$ -pinene (96%)]; [ $\gamma$ -terpinene and limonene (96%)]; [limonene and  $\beta$ -pinene (93%)] 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 a thorough knowledge of the biosynthetic pathway, leading to each terpenoid compound, the true significance of these observed correlations is not clear. The highest positive value (correlation) was between [ $\gamma$ -terpinene and  $\beta$ -pinene (98%)]. This result indicated which these compounds were under the control of a single dominant gene [30] (Table 3).

Due to the fact that acetate is necessary for the synthesis of terpenes, leads us to believe that there is a specialized function for this interesting molecule and that this molecule may be better served and utilized when Dancy is used as the variety. Our results show that there is a positive correlation between TSS (sugars) and pH. These doses agree with previously published [31].

# Conclusion

In the present study we found that there is a great variation in most of the measured characters among Dancy and other cultivars. Dancy was distinguished from other cultivars by its higher oxygenated compounds. These volatile differences among different cultivars provide fundamental information for improved genetic understanding and future improvement in tangerine aroma and flavor. Differentiation of different cultivars based on their aroma profiles may lead to better understanding of genetic control of aroma production.

Many studies, such as this study is very crucial in order to determine the amount of chemical compositions existing in the cultivars that we want to use, before the compositions can be utilized in industries. Further research on the relationship between cultivars and quality parameters is necessary.

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