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# Comparison of peel volatile components of Page mandarin (*Citrus reticulata*) and its parents

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**Abstract:** The peel components and juice quality of Page mandarin and its parents were investigated in this study. Peel components were extracted using the cold-press and eluted using n-hexane. Then all analyzed using GC-FID and GC-MS. Total soluble solids, total acid, pH value, ascorbic acid as well as density were determined in juice obtained from Page mandarin and its parents. Forty-seven, Forty-six and twenty-seven peel components were identified in Page, Dancy and Celemantin cultivars respectively including: aldehydes, alcohols, esters, ketones, monoterpenes, sesquiterpenes and other components. The major components were limonene, (E)-β-ocimene, β-myrcene, sabinene**,** linalool and α-Pinene. Compared with its parents, Page mandarin showed the highest content of oxygenated compounds and TSS. Since the oxygenated compounds and TSS content of Citrus are considered as two of the most important indicators of high quality, Page mandarin and its parents apparently have a profound influence on these factors.

**Keywords:** Cold-press, Flavor components, Mandarin hybrids, Juice quality, Peel oil.

## Introduction

Citrus is one of the most economically important crops in Iran. In the period 2009- 2010, the total Citrus production of Iran was estimated at around 87000 tonnes [1]. Mandarin hybrids are so variable as the result of hybridization between many fine-quality mandarins and Citrus species. Many of these varieties or cultivars are now being used successfully for juice production and as fresh fruit. Page mandarin resulted from a cross between Minneola tangelo (Dancy tangerine× Duncan grapefruit) and Clementine mandarin. It is a hybrid citrus crop and has been regarded as a citrus fruit with potential commercial value because of its attractive and pleasant aroma [2]. It is one of the most important mandarin cultivars used in world. Although it is as important cultivar, the peel components of Page mandarin have been investigated very little previously.

Citrus oils occur naturally in special oil glands of 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 [3]. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc [4].

The quality of an oil or juice can be calculated from the quantity of oxygenated compounds present in them. The quantity of oxygenated compounds present in the oil, is variable and depends upon a number of factors including: polyploidy [5], hybridization [6-7], rootstock [8], scion *or* cultivar [9-10], climate [11] and etc.

In 1991, Moshonas et al [6] compared the peel and juice components of Amber sweet with those of its parent. This study showed similarity Amber sweet to the orange parent and its dissimilarity to other parent. In 2001, Shaw et al [7] compared the juice components of two new grapefruit hybrids with those of their

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parent. This study showed dissimilarity of hybrids to their parents. There are only a few references in literature dealing on the comparison of the volatile components of hybrids with those of their parents.

Citrus juice is the most popular beverage in the world because of the fantastic flavor and abundant nutrition. The quality of citrus juice is an important economic factor in an industry that buys its fruit based on the juice sugar content and processes over 95% [12]. The best juices are consumed by the food and beverage industries. The quality of citrus juice can be determined by the concentration of compositions such as TSS (total soluble solids), TA (total titratable acidity) and vitamin C [13]. Juice, TSS and TA content are the main internal parameters used to determine Citrus quality in the world [14]. 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 [15]. The amount of TSS present in the juice is variable and depends upon a number of factors including: hybridization [16], rootstock, variety, degree of maturity, seasonal effects, climate, nutrition, tree age and etc [15].

Several studies have shown that the Citrus hybrids, resulting from cross-breeding of two parents, may influence the quantity of chemical compositions (TSS, TA and vitamin C) present in the juice [16]. Very little research has been carried out on chemical compositions (TSS, TA and vitamin C) present in Citrus hybrids and their parents. Therefore, it is very important to be able to assess the differences between Citrus hybrids and their parents in terms of quantity of compositions (TSS, TA and vitamin C).

In this study, we compared the peel and juice composition isolated from Page mandarin with those of its parents.

## Results and discussion

*Flavor compounds of the Page mandarin peel:*

GC-MS analysis of the flavor compounds extracted from Page mandarin peel using cold-press led to the identification of 47 volatile components (Table **1**, Figure **1**) including 20 oxygenated terpenes [8 aldehydes , 10 alcohols , 1 ester, 1 ketone] and 27 non oxygenated terpenes [11 monoterpens, 16 sesqiterpens].

*Flavor compounds of the Dancy mandarin peel:*

GC-MS analysis of the flavor compounds extracted from Dancy tangerine peel using cold-press led to the identification of 46 volatile components (Table **1**, Figure **2**) including 23 oxygenated terpenes [13 aldehydes , 7 alcohols , 3 esters], 22 non oxygenated terpenes [11 monoterpens, 11 sesqiterpens] and 1 other compound.

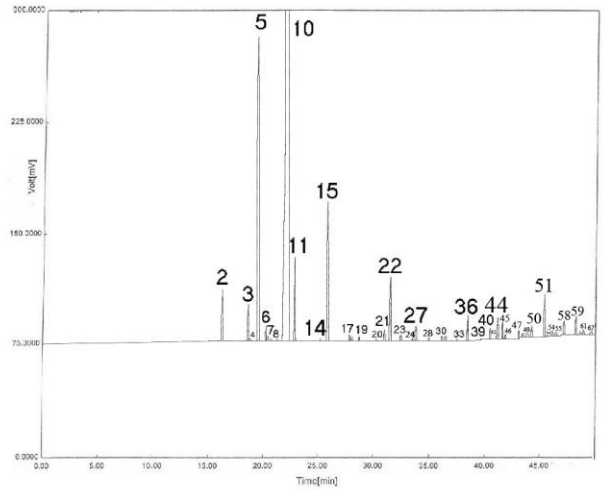


**Figure 1:** HRGC chromatograms of Page mandarin peel oil.

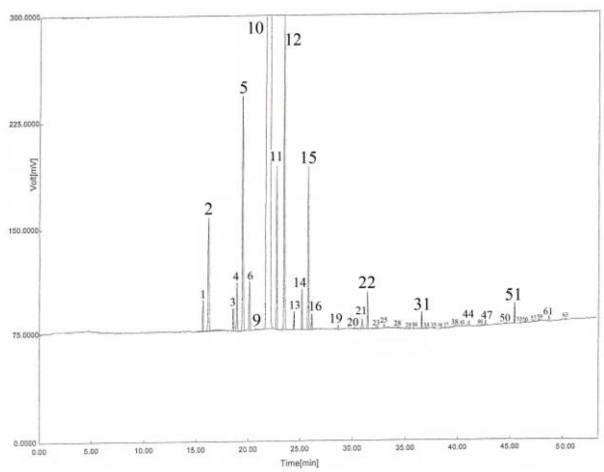


**Figure 2:** HRGC chromatograms of Dancy mandarin peel oil.

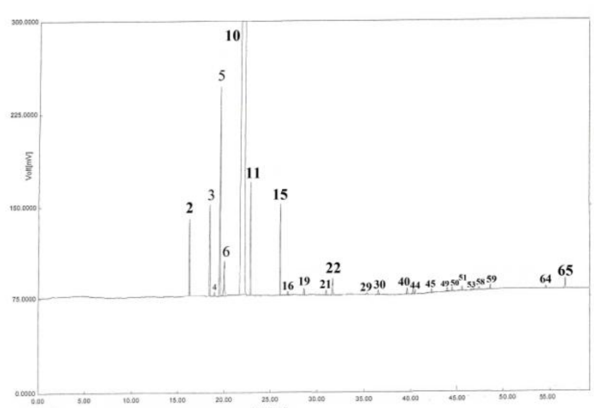

**Figure 3:** HRGC chromatograms of Celemantine mandarin peel oil.

*Flavor compounds of the Clementine mandarin peel:*

GC-MS analyze of the flavor compounds extracted from Clementine mandarin peel using cold-press led to the identification of 27 volatile components (Table **1**, Figure **3**) including 12 oxygenated terpenes [9 aldehydes , 3 alcohols] and15 non oxygenated terpenes [6 monoterpens, 9 sesqiterpens].

*Aldehydes:*

Fifteen aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, (E)-2-decenal, geranial, perillaldehyde, undecanal, (E)2,4-decadienal, (Z)-5-dodecanal, dodecanal, tetradecana, β-sinensal and $\alpha$-sinensal (Table **2**). In addition they were quantified from 0.77% to 0.84%. 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 mandarin flavor [17].

Among the three cultivars examined, Dancy showed the highest content of aldehydes. Page mandarin exhibited lower aldehydes content than the Dancy. Since the aldehydes content of Citrus oil is considered as one of the most important indicators of high quality, Page mandarin and its parents apparently have a profound influence on this factor.

Page aldehydes were also compared to those of its parents in this study. (Z)-5- dodecanal was identified in Page mandarin, while it was not detected in its parents. Compared with Clementine, the Page mandarin improved and increased aldehyde components about 1.05 times (Table **2**).

*Alcohols:*

Eleven alcoholic components identified in this analysis were linalool, terpinene-4-ol, α-terpineol, β-citronellol, cis-carveol, thymol, perill alcohol, p-menth-1-en-9-ol, elemol, (E) nerolidol and germacrene D-4-ol (Table **2**).

The total amount of alcohols ranged from 0.63% to 1.35%. Linalool was identified as the major component in this study and was the most abundant. Linalool has been recognized as one of the most important components for mandarin flavor. Linalool has a flowery aroma [17] and its level is important to the characteristic favor of mandarin [4]. Among the three cultivars examined, Dancy showed the highest content of alcohols. Page exhibited lower alcohols contents than the Dancy.

**Table 1:** Peel volatile components of Page mandarin and its parents. (*There is in oil)

| | Component | Page | Dancy | Clementine | KI | | Component | Page | Dancy | Clementine | KI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | α- thujene | | * | | 928 | 34 | Undecanal | * | * | | 1307 |
| 2 | α - Pinene | * | * | * | 935 | 35 | (E)2,4-decadienal | | * | | 1322 |
| 3 | Sabinene | * | * | * | 975 | 36 | δ- elemene | * | * | | 1344 |
| 4 | β -pinene | * | * | * | 979 | 37 | Citronellyl acetate | | * | | 1349 |
| 5 | β -myrcene | * | * | * | 991 | 38 | Neryl acetate | | * | | 1356 |
| 6 | octanal | * | * | * | 1003 | 39 | Cis-carvyl acetate | * | | | 1365 |
| 7 | α-phellandrene | * | | | 1006 | 40 | α -copaene | * | * | * | 1373 |
| 8 | δ - 3 – carene | * | | | 1015 | 41 | Granyl acetate | | * | | 1382 |
| 9 | α -terpinene | | * | | 1017 | 42 | (Z)-5-dodecenal | * | | | 1392 |
| 10 | Limonene | * | * | * | 1036 | 43 | β -cubebene | | | * | 1396 |
| 11 | (E)- β - ocimene | * | * | * | 1049 | 44 | β -elemene | * | * | * | 1399 |
| 12 | γ - terpinene | | * | | 1061 | 45 | Dodecanal | * | * | * | 1409 |
| 13 | (E)sabinene hydrate | | * | | 1065 | 46 | (Z)- β -caryophyllene | * | * | | 1415 |
| 14 | α -terpinolene | * | * | | 1091 | 47 | γ - elemene | * | * | | 1440 |
| 15 | Linalool | * | * | * | 1100 | 48 | α -guaiene | * | | | 1447 |
| 16 | Nonanal | | * | * | 1109 | 49 | (Z)- β -farnesene | * | | * | 1453 |
| 17 | E-limonene | * | | | 1137 | 50 | α - humulene | * | * | * | 1464 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | oxide | | | | | | | | | | |
| 18 | Z-limonene oxide | * | | | 1141 | 51 | Germacrene D | * | * | * | 1493 |
| 19 | Citronellal | * | * | * | 1154 | 52 | Valencene | * | | | 1499 |
| 20 | Terpinene-4-ol | * | * | | 1182 | 53 | Bicyclogermacr ene | * | * | * | 1504 |
| 21 | α - terpineol | * | * | * | 1195 | 54 | α - muurolene | * | | | 1508 |
| 22 | Decanal | * | * | * | 1205 | 55 | (Z)-β-guaiene | * | | | 1511 |
| 23 | β -citronellol | * | * | | 1229 | 56 | E,E, α - farnesene | | * | * | 1514 |
| 24 | Cis-carveol | * | | | 1236 | 57 | γ - cadinene | * | | | 1522 |
| 25 | Thymol methyl ether | | * | | 1237 | 58 | δ-cadinene | * | * | * | 1532 |
| 26 | Neral | | * | | 1244 | 59 | Elemol | * | * | * | 1559 |
| 27 | Carvone | * | | | 1250 | 60 | (E) – nerolidol | * | | | 1567 |
| 28 | (E)-2-decenal | * | * | | 1263 | 61 | Germacrene B | * | * | | 1572 |
| 29 | Geranial | | * | * | 1275 | 62 | Germacrene D – 4 – ol | * | * | | 1585 |
| 30 | Perilla aldehyde | * | * | * | 1281 | 63 | Tetradecanal | | * | | 1612 |
| 31 | Thymol | | * | | 1291 | 64 | β - sinensal | | | * | 1704 |
| 32 | Perilla alcohol | * | | | 1296 | 65 | α -sinensal | | * | * | 1756 |
| 33 | P-menth-1-en-9-ol | * | | | 1301 | | | 47 | 46 | 27 | |

Page alcohols were also compared to those of its parents in this study. Perill alcohol, P-menth-1-en-9-ol and (E) nerolidol were identified in Page mandarin, while they were not detected in its parents. Compared with Clementine, the Page mandarin improved and increased alcohol components about 2.06 times (Table **2**).

*Esters:*

Four ester components identified in this analysis were citronellyl acetate, neryl acetate, cis-carvyl acetate and granyl acetate. The total amount of esters ranged from 0.00% to 0.02%. Among the three cultivars examined, Dancy showed the highest content of esters (Table **2**).

*Ketones:*

One ketone component identified in this analysis was carvone. The total amount of ketones ranged from 0.00% to 0.11%. Compared with its parents, Page mandarin showed the highest content of ketones (Table **2**).

*Monoterpene hydrocarbons:*

The total amount of monoterpene hydrocarbons ranged from 95.01 % to 97.91%. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak Citrus-like aroma [17] and is considered as one of the major contributors to mandarin flavor. Among the three cultivars examined, Clementine showed the highest content of monoterpenes (Table **2**).

*Sesquiterpene hydrocarbons:*

The total amount of sesquiterpene hydrocarbons ranged from 0.15 % to 1.36%. Germacrene D was identified as the major component in this study and was the most abundant. Compared with its parents, Page mandarin showed the highest content of sesquiterpenes (Table **2**).

**Table 2:** Statistical analysis of variation in peel flavor Components of Page mandarin and its parents (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.

| Compounds | Page | | Parents | | | | F value |
|---|---|---|---|---|---|---|---|
| | | | Dancy | | Clementine | | |
| | Mean | St.err | Mean | St.err | Mean | St.err | |
| Oxygenated compounds | | | | | | | |
| a) Aldehyds | | | | | | | |
| 1) Octanal | 0.09 | 0.005 | 0.29 | 0.01 | 0.27 | 0.02 | F** |
| 2) Nonanal | | | 0.08 | 0 | 0.01 | 0.006 | |

| Compounds | Page | | Parents | | | | F value |
|---|---|---|---|---|---|---|---|
| | | | Dancy | | Clementine | | |
| | Mean | St.err | Mean | St.err | Mean | St.err | |
| 3) Citronellal | 0.03 | 0.005 | 0.04 | 0 | 0.07 | 0.006 | |
| 4) Decanal | 0.50 | 0.01 | 0.2 | 0.006 | 0.19 | 0.02 | F** |
| 5) Neral | | | 0.01 | 0 | | | |
| 6) (E)-2-decenal | 0.009 | 0.001 | 0.01 | 0.006 | | | |
| 7) Geranial | | | 0.02 | 0.006 | 0.01 | 0 | |
| 8) Perilla aldehyde | 0.04 | 0.005 | 0.01 | 0.006 | 0.01 | 0 | |
| 9) Undecanal | 0.03 | 0.01 | 0.01 | 0.001 | | | |
| 10) (E)2,4-decadienal | | | 0.004 | 0.001 | | | |
| 11) (Z)-5- dodecanal | 0.01 | 0.01 | | | | | |
| 12) Dodecanal | 0.11 | 0.01 | 0.01 | 0 | 0.03 | 0.006 | |
| 13) Tetradecanal | | | 0.02 | 0 | | | |
| 14) β-sinensal | | | | | 0.01 | 0.006 | |
| 15) α-sinensal | | | 0.14 | 0.02 | 0.17 | 0.01 | |
| total | 0.81 | 0.05 | 0.84 | 0.05 | 0.77 | 0.07 | |
| b) Alcohols | | | | | | | |
| 1) linalool | 0.92 | 0.02 | 1.130 | 0.100 | 0.570 | 0.020 | F** |
| 2) Terpinen-4-ol | 0.01 | 0.00 | 0.009 | 0.001 | | | |
| 3) α-terpineol | 0.09 | 0.01 | 0.080 | 0.000 | 0.030 | 0.000 | |
| 4) β-citronellol | 0.05 | 0.01 | 0.030 | 0.000 | | | |
| 5)Cis-carveol | 0.009 | 0.0005 | | | | | |
| 6) Thymol | | | 0.090 | 0.000 | | | |
| 7)Perill alcohol | 0.04 | 0.01 | | | | | |
| 8)P-menth-1-en-9-ol | 0.009 | 0.001 | | | | | |
| 9) Elemol | 0.14 | 0.04 | 0.010 | 0.006 | 0.030 | 0.010 | |
| 10) (E)nerolidol | 0.02 | 0.01 | | | | | |
| 11) Germacrene D-4-ol | 0.02 | 0.01 | 0.003 | 0.001 | | | |
| total | 1.30 | 0.11 | 1.35 | 0.10 | 0.63 | 0.03 | |
| c) Esters | | | | | | | |
| 1) Citronellyl acetate | | | 0.007 | 0.001 | | | |
| 2) Neryl acetate | | | 0.01 | 0 | | | |
| 3)Cis-carvyl acetate | 0.007 | 0.0005 | | | | | |
| 4) Granyl acetate | | | 0.009 | 0 | | | |
| total | 0.007 | 0.0005 | 0.02 | 0.001 | | | |
| d) Ketones | | | | | | | |
| 1) Carvone | 0.11 | 0.002 | | | | | |
| Monoterpenes | | | | | | | |
| 1) α-thujene | | | 0.16 | 0.006 | | | |
| 2) α-pinene | 0.34 | 0.01 | 0.88 | 0.06 | 0.51 | 0.01 | F** |
| 3) Sabinene | 0.21 | 0.005 | 0.12 | 0.02 | 0.6 | 0.09 | F** |
| 4) β- pinene | 0.01 | 0.005 | 0.33 | 0.01 | 0.03 | 0 | |
| 5) β-myrcene | 2.02 | 0.005 | 1.68 | 0.08 | 1.72 | 0.07 | F** |
| 6) α-phellandrene | 0.03 | 0 | | | | | |
| 7) δ-3-carene | 0.01 | 0 | | | | | |
| 8) α-terpinene | | | 0.02 | 0.01 | | | |
| 9) Limonene | 91.84 | 0.24 | 87.07 | 0.7 | 94.19 | 0.71 | F** |
| 10) (E)-β-ocimene | 0.50 | 0.02 | 1.13 | 0.42 | 0.86 | 0.35 | NS |
| 11) γ-terpinene | | | 4.68 | 0.51 | | | |
| 12) (E)-sabinene hydrate | | | 0.09 | 0.01 | | | |
| 13) α-terpinolene | 0.01 | 0 | 0.23 | 0.05 | | | |
| 14)Cis- limonene oxide | 0.02 | 0.005 | | | | | |
| 15) Trans-limonene oxide | 0.02 | 0.005 | | | | | |
| total | 95.01 | 0.29 | 96.39 | 1.87 | 97.91 | 1.23 | |
| Sesquiterpenes | | | | | | | |
| 1) δ-elemene | 0.20 | 0.02 | 0.01 | 0 | | | |
| 2) α-copaene | 0.07 | 0.005 | 0.008 | 0.002 | 0.04 | 0 | |
| 3) β-cubebene | | | | | 0.02 | 0.006 | |
| 4) β-elemene | 0.19 | 0.02 | 0.05 | 0.006 | 0.01 | 0.01 | |
| 5) (Z)-β-caryophyllene | 0.02 | 0.005 | 0.01 | 0 | | | |

| Compounds | Page | | Parents | | | | F value |
|---|---|---|---|---|---|---|---|
| | | | Dancy | | Clementine | | |
| | Mean | St.err | Mean | St.err | Mean | St.err | |
| 6) γ-elemene | 0.05 | 0.005 | 0.05 | 0.01 | | | |
| 7) α-guaiene | 0.05 | 0.01 | | | | | |
| 8) α-humulene | 0.09 | 0.005 | | | 0.01 | 0 | |
| 9) (Z)-β-farnesene | 0.07 | 0.01 | 0.01 | 0 | 0.01 | 0.006 | |
| 10) Germacrene D | 0.35 | 0.02 | 0.1 | 0.006 | 0.02 | 0 | F** |
| 11) Valencene | 0.004 | 0.002 | | | | | |
| 12) Bicyclogermacrene | 0.008 | 0.002 | 0.02 | 0 | 0.01 | 0 | |
| 13) α-muurolene | 0.04 | 0 | | | | | |
| 14) (Z)-β-guaiene | 0.05 | 0.005 | | | | | |
| 15) E,E-α-farnesene | | | 0.01 | 0 | 0.01 | 0 | |
| 16) γ-cadinene | 0.01 | 0 | | | | | |
| 17) δ-cadinene | 0.11 | 0.005 | 0.02 | 0.01 | 0.02 | 0.006 | |
| 18) Germacrene B | 0.05 | 0.01 | 0.05 | 0.006 | | | |
| total | 1.36 | 0.12 | 0.33 | 0.04 | 0.15 | 0.02 | |
| Other compounds | | | | | | | |
| 1)Thymol methyl ether | | | 0.04 | 0.006 | | | |
| Total oxygenated compounds | 2.22 | 0.16 | 2.21 | 0.15 | 1.4 | 0.1 | |
| Total | 98.59 | 0.57 | 98.97 | 2.06 | 99.46 | 1.35 | |

*Juice quality parameters:*

Juice quality parameters are given in Table **3**. The content of total acid ranged from 0.44% (Clementine) to 0.87% (Dancy), and Brix (total soluble solids) ranged from 9 % (Dancy) to 10.9% (Page). TSS/TA rate ranged from 10.34 (Dancy) to 22.27 (Clemantine). Ascorbic acid ranged from 43.82 % (Dancy) to 60.37% (Page). The pH value ranged from 3.42 (Dancy) to 4.02 (Clemantine). The juice yield ranged from 38 % (Page) to 71.61% (Dancy). Ash ranged from 1 % (Dancy) to 3 % (Clemantine). Total dry matter ranged from 14.79% (Dancy) to 16.01% (Clemantine).

Among the three cultivars examined, Clemantine showed the highest content of TSS /TA and pH. Page mandarin exhibited a lower TSS /TA and pH content than the Clemantine (Table **3**).

**Table 3:** Statistical analysis of variation in juice quality parameters of Page mandarin and its parents. 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 (%) | TSS /TA rate | Ascorbic acid (%) | PH | Juice (%) | Total dry matter (%) | Ash (%) |
|---|---|---|---|---|---|---|---|---|
| Page (scion) | 10.9 | 0.74 | 14.82 | 60.37 | 3.91 | 38 | 15.1 | 2 |
| Dancy (scion) | 9 | 0.87 | 10.34 | 43.82 | 3.42 | 71.61 | 14.79 | 1 |
| Clementine(scion) | 9.8 | 0.44 | 22.27 | 53.33 | 4.02 | 70.33 | 16.01 | 3 |
| | F** | F** | F** | F** | F** | F** | | |

*Results of statistical analyses:*

Differences were considered to be significant at $P <$ 0.01. These differences on the 1% level occurred in octanal, decanal, linalool, α-pinen, sabinene, β-myrcen, limonene, germacrene D, TSS, TA, TSS /TA, ascorbic acid, pH and juice**.** **The** non affected oil component was (E)-β-ocimene (Tables **2** and **3**).

*Results of correlation:*

Simple intercorrellations between 9 peel components are presented in a correlation matrix (Table **4**). The highest positive values or r (correlation coefficient) were observed between germacrene D and decanal (96%); β-myrcen and decanal (94%). The highest significant negative correlations were observed between decanal and octanal (97%); sabinene and linalool (93%); limonene and linalool (93%) (Table **4**).

Also simple intercorrellations between 6 juice characteristics are presented in a correlation matrix (Table **5**). The highest positive values or r (correlation coefficient) were observed between Ascorbic acid and TSS (98%); pH and TSS /TA (85%). The highest significant negative correlations were observed between TSS /TA and TA (98%); Juice and TSS (91%) (Table **5**).

Our observation that citrus hybrids and their parents have different effects on some of the components is in accordance with previous findings [6-7].

The compositions of the peel oils obtained by cold pressing from Page mandarin were very similar to its parents. However, the relative concentration of compounds was different according to the type of parent.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies [9].

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

| | Octanal | Decanal | Linalool | α-pinene | Sabinene | β -myrcene | Limonene | (E)-β-ocimene |
|---|---|---|---|---|---|---|---|---|
| Decanal | -0.97** | | | | | | | |
| Linalool | -0.01 | 0.16 | | | | | | |
| α-pinene | 0.80** | -0.71* | 0.57 | | | | | |
| Sabinene | 0.20 | -0.35 | -0.93** | -0.37 | | | | |
| β -myrcene | -0.90** | 0.94** | 0.10 | -0.70* | -0.22 | | | |
| Limonene | -0.32 | 0.16 | -0.93** | -0.80** | 0.83** | 0.21 | | |
| (E)-β-ocimene | 0.73* | -0.60 | 0.34 | 0.75* | -0.10 | -0.39 | -0.52 | |
| Germacrene D | -0.92** | 0.96** | 0.37 | -0.53 | -0.55 | 0.88** | -0.06 | -0.54 |

*=significant at 0.05
**=significant at 0.01

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

| | TSS (%) | TA (%) | TSS /TA | Ascorbic acid(%) | pH |
|---|---|---|---|---|---|
| TA (%) | -0.19 | | | | |
| TSS /TA | 0.28 | -0.98** | | | |
| Ascorbic acid(%) | 0.98** | -0.37 | 0.45 | | |
| pH | 0.69* | -0.78* | 0.85** | 0.78* | |
| Juice (%) | -0.91** | -0.18 | 0.10 | -0.83** | -0.34 |

*=significant at 0.05
**=significant at 0.01

**Table 6**: Common and botanical names for Citrus taxa used as scions and rootstock [2].

| Common name | botanical name | Parents | category |
|---|---|---|---|
| Page (scion) | *Citrus reticulata cv Page* | *(Citrus reticulata cv. Dancy × Citrus paradisi cv. Duncan) × Citrus clementina cv. Cadox* | Mandarin hybrid |
| Dancy(scion) | *Citrus reticulata cv. Dancy* | *Unknown* | Tangerine |
| Clementine(scion) | *Citrus clementina cv. Cadox* | *Unknown* | Mandarin |
| Sour orange (Rootstock) | *Citrus aurantium* | *Mandarin ×Pomelo* | Sour orange |

It may be related to rootstock and environmental factors that can influence the compositions. However, it should be noticed that the extraction methods also may influence the results. Fertilizer [18] and irrigation [19] 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 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 [20]. The pronounced enhancement in the amount of oxygenated compounds, when Page mandarin used as the scion, Indicate that either the synthesis of geranyl pyrophosphate is enhanced or activities of both enzymes increased.

High positive correlations between pairs of terpenes such as germacrene D and decanal (96%); β-myrcen and decanal (94%) suggest a genetic control [21] and such dependence between pairs of terpenes is due to their derivation of one from another that is not known. Similarly, high negative correlations observed between decanal and octanal (97%); sabinene and linalool (93%); limonene and linalool (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 an extended insight into the biosynthetic pathway of each terpenoid compound, the true significance of these observed correlations is not clear. The highest positive value (correlation) was observed between germacrene D and decanal (96%). This result indicates that these compounds should be under the control of a single dominant gene [21] (Table **3**).

Considering that acetate is necessary for the synthesis of terpenes, it can be assumed that that there is a specialized function for this interesting molecule and it may be better served by Page mandarin. Our results showed that there was a positive correlation between pH and TSS/TA (sugars). This finding was similar to previous studies [22].

## Conclusion

Present study showed that there was a great variation in most of the measured characters between Page mandarin and its parents. Studies like this is very important to determine the amount of chemical compositions existing in the new hybrids that we want to use, before their fruits can be used in food industries, aromatherapy, pharmacy, cosmetics, hygienic products and other areas. Further research on the relationship between mandarin hybrids and their parents' compositions is necessary.

## Experimental

*Mandarin scions:*

In 1989, mandarin scions that grafted on sour orange rootstock, 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]. Page, Dancy and Clementine mandarin were used as plant materials in this experiment (Table **6**).

*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 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.

*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 station. Juice was obtained using the Indelicate Super Automatic, Type A2 104 extractor. After extraction, juice was screened to remove peel, membrane, pulp and seed pieces according to the standard operating procedure. Three replicates were carried out for the quantitative analysis (n=3). Ten fruits were used for each replicate.

*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). 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 [23]. Ascorbic acid was determined by titration with Potassium iodide [23-24]

*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 $^o$ C and helium was used as the carrier gas at a flow rate of 1.00 ml/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C22) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. Gas chromatography-mass spectrometry was used to identify the volatile components. The analysis was carried out with a Varian Saturn 2000R. 3800 GC linked with a Varian Saturn 2000R MS.

The oven condition, injector and detector temperatures, and column (DB-5) were the same as those given above for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s. Injection volume was 1 μL.

*Identification of components:*

Components were identified by comparison of their Kovats retention indices (RI), retention times (RT) and mass spectra with those of reference compounds [25-26]

*Data analysis:*

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 9 peel components and 6 juice characteristics. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.01$. The correlation between pairs of characters was evaluated using Pearson's correlation coefficient (Tables 2 and **3**).

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**References**

[1] FAO. Available from <http://www.fao.org> **2012**.

[2] Fotouhi- Ghazvini, R.; Fattahi- moghadam, J. *Citrus growing in Iran; Guilan University*: Rasht, Iran, **2007**.

[3] Nagy, S.; Shaw, P.; Veldhuis, M. K. *Citrus Science and Technology*; the AVI Publishing Company: Westport, USA, **1977**.

[4] Babazadeh- Darjazi, B.; Rustaiyan, A.; Talaei, A.; Khalighi, A.; Larijani, K.; Golein, B.; Taghizad, R. *J. Chem. Chem. Eng*. **2009**, *28*, 99.

[5] Gancel, A. L.; Ollitrault, P.; Froelicher, Y.; Tomi, F.; Jacquemond, C.; Luro, F.; Brillouet, J. M. *J. Agric. Food. Chem*. **2003**, *51*, 6006.

[6] Moshonas, M.G.; Shaw, P.E.; Carter, R.D. *J. Agric. Food. Chem*. **1991**, *39*, 1416.

[7] Shaw, P. E.; Goodner, K. L.; Moshonas, M. G.; Hearn , C. J. *Scientia. Hort*. **2001**, *91*, 71.

[8] Verzera, A.; Trozzi, A.; Gazea, F.; Cicciarello, G.; Cotroneo, A. *J. Agric. Food. Chem*. **2003**, *51*, 206.

[9] Lota, M.L.; Serra, D.; Tomi, F.; Casanova, J. *Biochem. Syst. Ecol*. **2001**, *29*, 77.

[10] Babazadeh-Darjazi, B.; Golein, B. *J. Med. Plant. Products.* **2012**, *2*, 157.

[11] Scora, R. W.; Torrisi, S. *Proc. Am. Soc. Hort. Sci*. **1966**, *88*, 262.

[12] Rouse, R. E. *Proc. Fla. State. Hort. Soc*. **2000**, *113*, 112.

[13] Babazadeh Darjazi, B. Ph.D thesis, Islamic Azad University. Science and Research Branch, **2009**.

[14] Antonucci, F.; Pallottino, F.; Paglia, G.; Palma, A.; Aquino, S.D.; Menesatti, P. *Food. Bioprocess. Technol*. **2011**, *4*, 809.

[15] Hardy, S.; Sanderson, G. *Primefact.* **2010**, *980*, 1.

[16] Rapisarda, P.; Fabroni, S.; Peterek, S.; Russo, G.; Mock, H.P. *Food. Chem*. **2009**, *117*, 212.

[17] Buettner, A.; Mestres, M.; Fischer, A.; Guasch, J.; Schieberie, P. *Eur. Food. Res. Technol*. **2003**, *216*, 11.

[18] Rui, W.; Xue-gen, S.; You-zhang1, W.; Xiao-e1, Y. *J. Zhejiang. Univ. Science B*. **2006**, *7*, 696.

[19] Al-Rousan, W. M. M.; Ajo, R.Y.; Angor, M.M.; Osaili, T.; Bani-Hani, N. M. *J. Food. Agric. Environ*. **2012**, *10*,115.

[20] Hay, R. K. M.; Waterman, P. *Volatile oil crops; their biology, biochemistry and production*; Wiley-Blackwell: New York, USA, **1995**.

[21] Scora, R.W.; Esen, A.; Kumamoto, J. *Euphytica*.**1976**, *25*, 201.

[22] Knee, M. *Fruit quality and its biological basis*; CRC Press LLC Publication: Florida, USA, **2002**.

[23] Majedi, M. *Food chemical analysis methods*;University Jahad: Tehran, Iran, **1994**.

[24] Mazaheri-Tehrani, M.; Salari, A.; Heidari, A. *Iran. Food. Sci. Technol. Res J*. **2006**, 2, 53.

[25] Adams, R.P. *Identification of essential oil components by gas chromatography / mass spectrometry; Allured Publishing Corporatio*n, Carol Stream: Illinois, USA, **2001**.

[26] McLafferty, F.W.; Stauffer, D. B. *The important peak index of the registry of mass spectral data*; Wiley: New York, USA, **1991**.