The effect of rootstocks on sugars, acids, carotenoids, chlorophylls, and ethylene of Satsuma mandarin (*Citrus unshiu*)

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Abstract

This study aimed to evaluate the effect of rootstocks on fruit sugars, organic acids, and carotenoids. The contents of sugars and organic acids in fruits were determined by HPLC. Total acidity (TA), total soluble solids (TSS), and pH value of the juice were also evaluated. Total carotenoids and chlorophylls contents were measured using a spectrophotometer. The content of ethylene in fruits was determined by gas chromatography. HPLC analysis of the juice allowed the detection of 3 sugars and 2 acids. Sucrose was the dominant sugar for all rootstocks. Total sugars ranged from 93.54 (mg/mL) (Flying dragon) to 111.54 (mg/mL) (*Swingle citrumelo*). Total acids changed from 9.50 (mg/mL) (Trifoliate orange) to 11.45(mg/mL) (Flying dragon). The ascorbic acid content varied from 0.20 (mg/mL) (Flying Dragon) to 0.32 (mg/mL) (Sour orange). The pH value ranged from 3.14 (Flying dragon) to 3.50 (Trifoliate orange), TSS content changed from 10.70 (%) (Flying dragon) to 11.10 (%) (*Swingle citrumelo*), TSS/TA varied from 11.75 (Flying dragon) to 13.45 (Trifoliate orange). The juice content ranged from 52.18 (%) (Flying Dragon) to 55.63 (%) (Sour orange). The amount of fruit production changed from 15 (Kg /tree) (Flying dragon) to 115 (Kg /tree) (*Swingle citrumelo*). Total carotenoids varied from 0.09 (*Swingle citrumelo*) to 0.15 (Sour orange and Flying dragon) (mg/gr DW). Among the four rootstocks evaluated, *Swingle citrumelo* demonstrated the maximum level of sugars and TSS. The study concluded that the rootstocks can affect sugars, acids, and, carotenoids contents of the fruits.

Keywords: carotenoids; citrus rootstocks; ethylene; organic acids; sugars

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Introduction

Satsuma mandarin (*Citrus unshiu*) is one of the most important mandarins that are widely cultivated in Iran. Although it is an important crop, little research has been done on individual sugars, acids, and carotenoids of Satsuma mandarin.

Fructose, glucose, and sucrose are three major sugars of citrus fruits. Sucrose is known as the dominant sugar in citrus fruits. Sugars usually display 80% of the total soluble solids of juice

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

Common name	Botanical name	Parents	Category
Satsuma mandarin (Scion)	*Citrus unshiu cv. Miyagawa*	Unknown	Mandarin
Sour orange (Rootstock)	*Citrus aurantium L.*	Mandarin ×Pomelo	Sour orange
Swingle citrumelo (Rootstock)	*Swingle citrumelo*	*C. paradisi cv. Duncan × P. trifoliata (L.) Raf*	Poncirus hybrids
Trifoliate orange (Rootstock)	*Poncirus trifoliata (L.) Raf*	Unknown	Poncirus
Flying dragon (Rootstock)	*Poncirus trifoliata (L.) Raf*	Unknown	Poncirus

(Varnamand Sutherland, 2012). Soluble solids are a mixture of organic acids and sugars that are used as an index of maturity and taste quality (Karadeniz, 2004). Ascorbic acid is an antioxidant and exhibits a key function in reduction of diseases. Carotenoids are known to reduce cancers, cataracts, and heart disease (Preedy et al., 2011). They are also widely used in the foodstuff, cosmetic, and medicine products as a natural coloring agent (Rostagno and Prado, 2013). In Citrus fruits, ethylene stimulates ripening by increasing the biosynthesis of carotenoids and chlorophylls destruction (Paliyath et al., 2009).

The ratio of sugars to acids affects the flavor of citrus fruit and has been considered as a quality indicator by both fresh consumption group and juice factories (Rees et al., 2012). Citrus juice is a fantastic resource of sugars and acids. The amount of citrus sugars is variable and depends on the rootstock (Barry et al., 2004), cultivar (Bermejo and Cano, 2012), etc. Several research studies have indicated that the rootstocks can influence the physicochemical traits of Satsuma mandarin (Cantuarias-Aviles et al., 2010).

Mashayekhi et al. (2013) showed that rootstocks can influence total sugar, glucose, and sucrose contents in fruits of Parson Brown and Mars Oranges. They found that the highest total sugar was with trees of Parson Brown and Mars Oranges grafted on Rough lemon rootstock. Navarro et al. (2010) showed that rootstocks can influence total sugar, glucose, and sucrose content of Clemenules mandarin. They reported that the content of total sugar, glucose, and sucrose of Clemenules mandarin grafted on 'Carrizo' citrange rootstocks was higher than that grafted on 'Cleopatra' mandarin rootstocks. Legua et al. (2014) showed that rootstocks can influence sucrose, glucose, fructose, citric acid, and ascorbic acid content of 'Clemenules' mandarin. They found that juice of 'Clemenules' mandarin grafted on Cleopatra mandarin had a much higher content of sucrose and fructose than the other rootstocks. However, the highest citric and ascorbic acid contents were found in 'Clemenules' mandarin grafted on Volkameriana rootstocks. Legua et al. (2011) showed that rootstocks can influence sucrose, glucose, fructose, citric acid, and ascorbic acid contents of 'Lane Late' navel orange. They found that the highest total sugar in trees grafted on *C. macrophylla* and Cleopatra. Castle (1995) reported that rootstocks can influence TSS: TA (total soluble solids to total acids ratio) contents of fruits of sweet orange. He reported that fruits of sweet orange on trifoliate orange had higher TSS: TA and lower acidity. Gonzatto et al. (2011) showed that rootstocks can influence TA content of 'Oneco' mandarin. They found that the highest percentage of TA was in fruits from trees on Flying Dragon rootstock. Cantuarias-Aviles et al. (2010) compared Satsuma mandarin fruits grafted on 12 rootstocks and found that 'Flying Dragon' induced higher TA.

Rootstocks have also been found to affect the pigments in citrus fruit peels. Valencia and Joppa (Jaffa) oranges grown on trifoliate and sweet orange rootstocks had a significantly higher concentration of total carotenoids in the juice than on rough lemon rootstock (Ladaniya, 2008). The aim of this research was to identify the rootstock that can synthesize the maximum level of sugars and carotenoids.

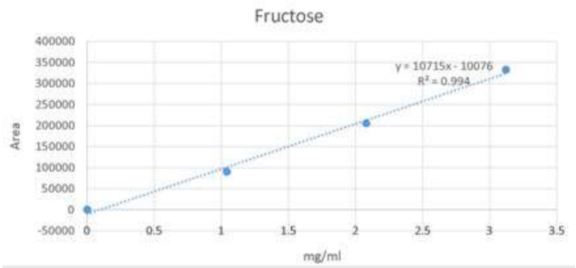

Fig. I. The standard curve of fructose

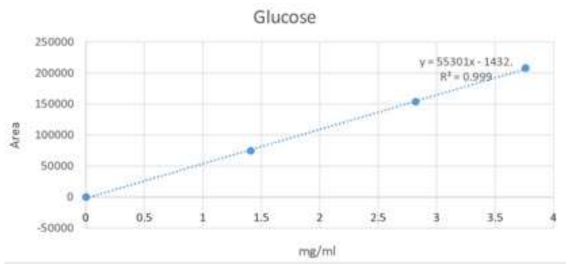

Fig. II. The standard curve of glucose

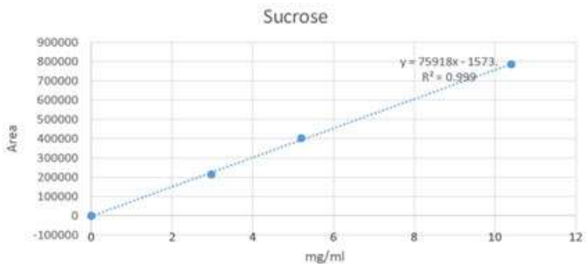

Fig. III. The standard curve of sucrose

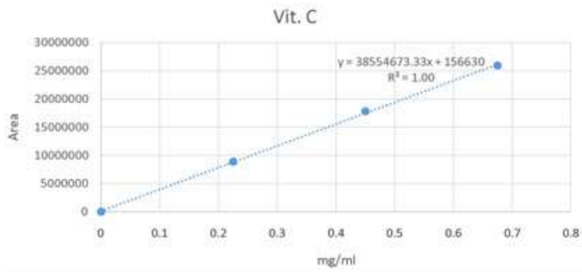

Fig. IV. The standard curve of ascorbic acid

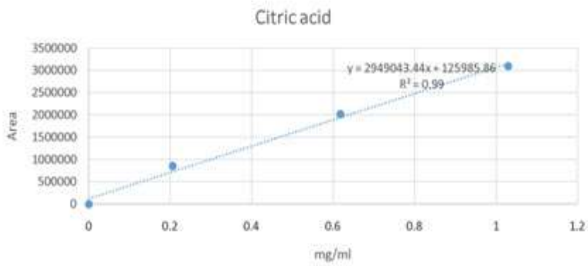

Fig. V. The standard curve of citric acid

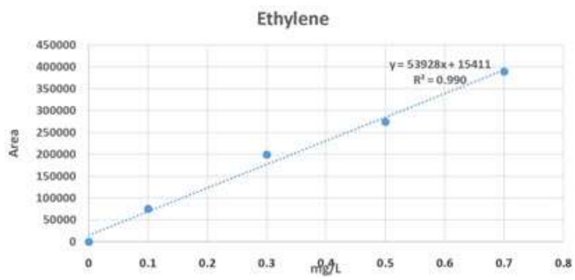

Fig. VI. The standard curve of ethylene

Material and Methods

Chemicals and standards

Standards of fructose, glucose, sucrose, ascorbic acid, citric acid, ethylene, acetonitrile, butylated hydroxyl toluene (BHT) and diethyl dithiocarbamate (DDC) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium hydroxide and phosphoric acid were purchased from Merck (Darmstadt, Germany).

Rootstocks

Rootstocks were planted at an 8 × 4 m plot with three replications at Ramsar research station [Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate in 2001. The average rainfall and temperature were 970 mm and 16.25˚ C per year respectively and the soil was classified as loam-clay with pH ranging from 6.9 to 7. Sour orange, Swingle citrumelo, Trifoliate orange, and Flying dragon were used as rootstocks in this experiment (Table 1).

Preparation of juice sample

Fruits were collected from different parts of the same trees in January 2016, early in the morning (6 to 8 am) and only during dry weather. The selection method was based on a completely randomized design. Fruits juice was extracted using a juicer. Then, Juices were centrifuged at 15,000 rpm for 20 min at 4 ˚C. Three replicates were done for this research (n = 3) (Legua et al., 2014).

Juice analyses technique

The total titratable acid was determined by titration with sodium hydroxide (0.1 N) and displayed as % citric acid. Total soluble solids were measured using a refractometer (Kruss, Germany). The pH value was determined using a digital pH meter (Jenway, Model: 3510). Sugars, citric acid, and ascorbic acid were measured by HPLC (Legua et al., 2014).

Analysis of sugars by HPLC

HPLC analysis was performed with a Platin blue system (Knauer, Berlin, Germany) equipped with a binary pump and a Refractive Index (RI) detector. Separation was carried out on a Shodex Asahi pak NH2P-50 4E column (250 × 4.6 mm). The column temperature was maintained at 25˚ C and the injection volume for all samples was 10 μL. Elution was performed isocratically with the mobile phase consisting of 75% (v/v) acetonitrile (eluent A) and 25% (v/v) water (eluent B) at a flow rate of 1 mL/ min. Identification of sugars was based on retention times of unknown peaks in comparison with the standards. The concentration of the sugars was calculated from peak area according to calibration curves. Standard solutions of sugars (fructose, glucose, and sucrose) and organic acids (ascorbic and citric acids) were prepared by dissolving the required amount of each standard in deionized water. Calibration was performed by injecting the standard three times at four different concentrations. Standard solutions of fructose at concentrations of 0, 1.04, 2.08, and 3.12 mg/mL were used to obtain a standard curve. Standard solutions of glucose at concentrations of 0, 1.41, 2.82, and 3.76 mg/mL, were used to obtain a standard curve. Standard solutions of sucrose at concentrations of 0, 2.97, 5.20, and 10.40 mg/mL were used to obtain a standard curve. Standard solutions of ascorbic acid at concentrations of 0, 0.22, 0.45, and 0.67 mg/mL were used to obtain a standard curve. Standard solutions of citric acid at concentrations of 0, 0.20, 0.61, and 1.03 mg/mL were used to obtain a standard curve (Figs. I-V). Sugars concentration was estimated from the calibration curve and the result was expressed as milligrams of compound per milliliter (mg/ mL).

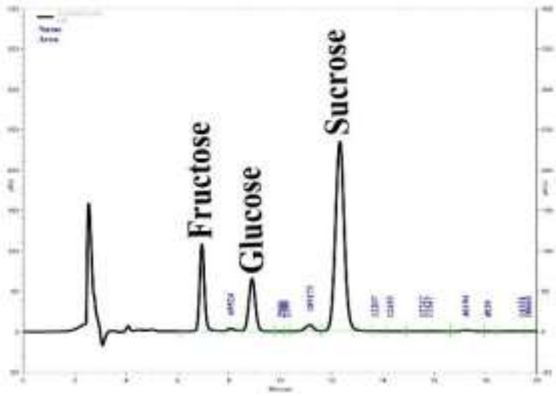

Fig. VII. HPLC chromatogram of sugars of Satsuma mandarin

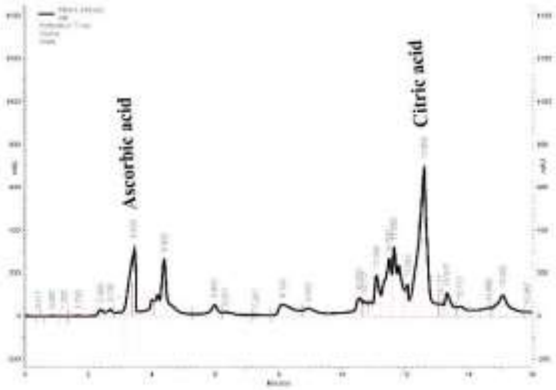

Fig. VIII. HPLC chromatogram of acids of Satsuma mandarin.

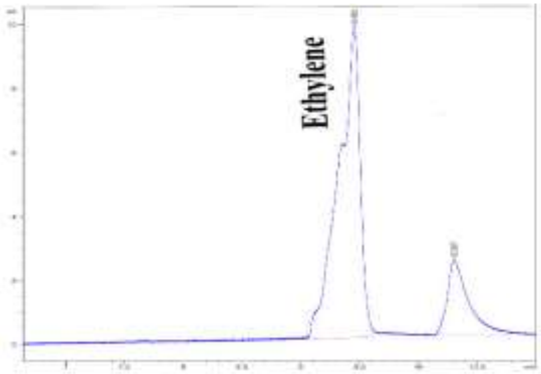

Fig. IX. GC ethylene chromatogram of Satsuma mandarin

Analysis of acids by HPLC

The same HPLC was applied for this study. It was fitted with an ODS-2 C-18 reversed phase column (250 mm × 4 mm) and a photodiode array (PDA) detector. The column temperature was set on 25˚ C. Elution was performed isocratically with the mobile phase consisting of 0.05% (v/v) aqueous phosphoric acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.6 mL/

min. Chromatograms were recorded at 254 nm for citric acid and ascorbic acid. Acid concentrations were estimated from the calibration curve and the result was displayed as milligrams of compound per milliliter (mg/ mL).

Identification of sugars and acids components

Identification of sugars and organic acids was based on retention times of unknown peaks in comparison with standards.

Determination of total carotenoids and chlorophylls

The method applied in this study was explained by Van-Wyka et al. (2009). Peels were freeze-dried at -56˚ C for 4 days to lose all their moisture and then powered by a mill. Samples were frozen at -80˚ C until analyzed. All extractions were carried out under low light conditions to decrease photo-destruction. Briefly, 0.2 g freeze-dried sample was mixed with 10 mL ethanol solvent (95% v/v), butylatedhydroxytoluene (BHT) (100 mg L^{-1}) and diethyldithiocarbamate (DDC) (200 mg L^{-1}). The samples were inverted for two mins. and kept at 4˚ C before they were passed through an ashless filter paper. The filtrates were put in a spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan) and absorbance was determined at 470 nm, 649 nm, and 664 nm. The concentration of chlorophylls and total carotenoids were calculated by the following formula. Results were displayed as mg of chlorophyll or carotenoid

$$C_a = \frac{(13.36 A_{664} - 5.19 A_{649}) * 8.1}{DW} \quad [mg\,g^{-1} dw]$$

$$C_b = \frac{(27.43 A_{649} - 8.12 A_{664}) * 8.1}{DW} \quad [mg\,g^{-1} dw]$$

$$C_{a+b} = \frac{(5.24 A_{664} + 22.24 A_{649}) * 8.1}{DW} \quad [mg\,g^{-1} dw]$$

$$C_{x+c} = \frac{(4.785 A_{470} + 3.657 A_{664} - 12.76 A_{649}) * 8.1}{DW} \quad [mg\,g^{-1} dw]$$

Chlorophyll *a* (C_a), chlorophyll *b* (C_b), total chlorophylls (C_{a+b}) and total carotenoids (C_{x+c}):

per gram dry weight (mg g^{-1} dry weight).

Ethylene extraction technique

To obtain the ethylene, fruits were weighed and placed in a jar. The jar was covered and placed at room temperature for 1 h. The temperature of the room was kept constant at 25˚ C. The volume of headspace around the fruits was measured. Ethylene was extracted with a 50 mL plastic syringe through the septum of the jar. One (1) mL of the extracted gas was injected into a gas chromatograph. Standard solutions of ethylene at concentrations of 0, 0.1, 0.3, 0.5, and 0. 7 mg/L were used to obtain a standard curve (Fig.VI). Results were displayed as nano-liter per kilogram fresh weight of fruit per hour (NL kg^{-1} h^{-1}).

Analysis of ethylene by GC

An Agilent 7890A gas chromatograph (USA) was employed for this study. It was fitted with a CP-Sil 88 column (100 m × 250 µm × 0.2 µm). The column temperature was set on 70˚ C. The injector temperature was set on 160˚ C. The detector temperature was set on 135˚ C. Nitrogen was applied as the carrier gas at a flow rate of 27 mL/min. Ethylene concentration was estimated from the calibration curve and the result was displayed as nano-liter per kilogram fresh weight of fruit per hour (NL kg^{-1} h^{-1}).

Physical traits of fruit and fruit production (yield)

Fifty fruits were randomly sampled and evaluated for each tree. Fruit physical traits are presented in Table 2. Total dry matter was determined by dehumidifying of the fruits in an oven at 80˚ C. Ash was measured by placing the weighed fruits in a furnace at 560˚ C. The weight of fresh fruit was determined using a scale. The weight of dried fruit was evaluated after drying with the oven. Fruit length, fruit diameter, and rind thickness were determined using a caliper. Fruit shape index was explained as the ratio of fruit diameter to length. The fruit yield was measured separately for each tree. Fruits for each tree were measured using a digital scale.

Table 2
Statistical analysis of variation in juice compositions and fruit physical traits of Satsuma mandarin on four different rootstocks

Compounds	Sour orange		Swingle citrumelo		Trifoliate orange		Flying Dragon		F value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Sugars									
1) Fructose (mg/mL)	13.38b	0.30	16.10a	0.38	12.02c	0.27	9.01d	0.25	**
2) Glucose (mg/mL)	21.88b	0.41	24.34a	0.39	19.96c	0.33	17.94d	0.27	**
3) Sucrose (mg/mL)	75.81a	0.60	71.10c	0.68	74.02b	0.64	66.59d	0.58	**
Total	111.07	1.31	111.54	1.45	106.00	1.24	93.54	1.10	
Organic acids									
1) Citric acid (mg/mL)	10.11ab	0.68	10.09ab	0.54	9.27b	0.72	11.25a	0.60	*
2)Ascorbic acid (mg/mL)	0.32a	0.03	0.26ab	0.02	0.23b	0.02	0.20b	0.02	**
Total	10.43	0.71	10.35	0.56	9.50	0.74	11.45	0.62	
Total titratable acid (%)	0.85ab	0.16	0.84ab	0.12	0.81b	0.10	0.91a	0.14	NS
pH	3.30ab	0.09	3.35ab	0.11	3.50a	0.07	3.14b	0.10	**
TSS (%)	11.00a	0.10	11.10a	0.10	10.90ab	0.08	10.70b	0.12	**
TSS/TA	12.94a	0.24	13.21a	0.30	13,45a	0.27	11.75b	0.22	**
Juice (%)	55.63a	0.78	55.60a	0.75	53.51ab	0.91	52.18b	0.81	**
Total dry matter (%)	12.62ab	0.24	12.88a	0.22	12.55ab	0.26	12.00b	0.20	**
Ash (%)	3.33a	0.58	3.00a	0.00	2.00b	0.00	2.00b	0.00	**
Fresh fruit weight (g)	89.00ab	6.00	96.98a	5.20	78.66b	5.27	74.24b	4.20	**
Dry fruit weight ^{z} (g)	7.64a	0.10	7.72a	0.10	7.56a	0.10	7.25b	0.10	**
Fruit diameter (mm)	57.70ab	2.80	61.40a	2.18	54.30ab	2.10	50.70b	2.80	**
Fruit length (mm)	46.16b	2.23	52.93a	2.23	45.25b	2.70	40.84b	2.64	**
Fruit shape index (Fd/Fl)	1.25a	0.08	1.16a	0.07	1.20a	0.09	1.23a	0.08	NS
Rind fruit weight ^{z} (g)	14.62b	1.00	14.68b	1.05	16.58ab	1.20	18.63a	1.28	**
Rind thickness (mm)	2.00c	0. 1	2.10c	0. 1	2.50b	0.1	3.00a	0.1	**
Fruit production (Kg/tree)	109a	6	115a	5	90b	5	15c	2	**
Carotenoids (mg/g DW)	0.15a	0.01	0.09c	0.006	0.11bc	0.01	0.15a	0.01	**
Chlorophyll A (mg/g DW)	0.00	0.00	0.001a	0.00	0.00	0.00	0.00	0.00	**
Chlorophyll B (mg/g DW)	0.00	0.00	0.01a	0.00	0.01a	0.00	0.00	0.00	**
Total chlorophyll (mg/g DW)	0.13	0.01	0.10	0.006	0.12	0.01	0.15	0.01	
Ethylene (NL kg^{-1} h^{-1})	4.24ab	0.48	0.71c	0.10	3.81b	0.74	5.70a	0.93	**

The mean is the average of traits applied with three replicates. SD = standard deviation. Results of analysis of variance: NS: not significant, *: significant difference at $P \leq 0.05$, **: significant difference at $P \leq 0.01$; any two means within a row not followed by the same letter are significantly different at $P \leq 0.01$ or $P \leq 0.05$.
^{z} For 7.00g fruit

Data Analysis

SPSS 18 was used for the analysis of the data obtained from the experiments. Analysis of variance was based on the measurements of 24 traits. Comparisons were made using a 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.

Results

HPLC analyses

HPLC analyses of juice allowed the identification of 3 sugars (fructose, glucose, and sucrose) and 2 acids (citric acid and ascorbic acid) (Figs. VII-VIII, Table 2).

Determination of sugars

Fructose, glucose, and sucrose were three sugars recognized in this study. Moreover, the amount of total sugars ranged from 93.54 to 111.54 mg/mL. Sucrose was the dominant sugar in this study. For all sugars, the differences among rootstocks were found significant on the 1% level. Fruits on Swingle citrumelo showed a significant increase in fructose and glucose but showed decreased sucrose. Among the four rootstocks evaluated, Swingle citrumelo indicated the maximum level of sugars (Table 2).

Determination of organic acids

Citric acid and ascorbic acid were two acids determined in this study. The amount of total acids ranged from 9.50 to 11.45 mg/ mL. There was a statistically significant difference between the 5% level in citric acid. The highest citric acid content was found in fruits from trees on Flying Dragon (11.25 mg/mL) and sour orange (10.11 mg/mL) while the lowest content of this acid was found on fruits of Trifoliate orange (9.27). The fruits from trees on Flying Dragon showed ascorbic acid content significantly lower than those on Sour orange and Swingle citrumelo (Table 2).

Results of total titratable acid (TA)

Total titratable acid ranged from 0.81 to 0.91%. Total acid content (TA) was not significantly affected by the rootstocks. The highest percentage of total acids (TA) was in fruits from trees on Flying Dragon, followed by Sour orange whereas the lowest TA was detected in fruits from trees on Trifoliate orange (Table 2).

Results of pH, TSS, TSS/TA, and juice content

The values of pH, TSS, TSS/TA, and juice content are given in Table 2. Among the four rootstocks evaluated, Trifoliate orange indicated the maximum level of pH and TSS/TA. The highest levels of juice were found in Sour orange. There was a significant difference between the 1% level in the content of pH, TSS, and TSS/TA of Satsuma mandarin juice on different rootstocks. Despite the little differences recorded for juice percentage on some rootstocks, Satsuma mandarin fruits from Sour orange yielded the highest juice percentage (55.63 %) while those from Flying Dragon had the least juice percentage (52.18 %). The highest percentage of TSS was for the fruits from Swingle citrumelo (11.10%) while the least was for those from both Trifoliate orange and Flying Dragon (10.90 and 10.70%, respectively). The highest pH was recorded for Trifoliate orange which was significantly more than those from Sour orange and Flying Dragon. TSS: TA ratio was lowest for Flying Dragon.

Results of fruit physical traits and fruit production (yield)

The amount of fruit physical traits and fruit production are given in Table 2. For more physical traits, the differences among rootstocks were found significant. Results indicated that trees grafted on Swingle citrumelo significantly yielded the heaviest fruit (96.98 g) while those grafted on Flying dragon had the lightest fruit (74.24 g). Concerning fruit length and diameter, fruits from the trees on Swingle citrumelo had significantly the longest fruit (52.93mm) and diameter (61.40 mm) while the least values were recorded for those grafted on Trifoliate orange (45.25, 54.30 mm) and Flying dragon (40.84, 50.70 mm). Although no significant differences for fruit shape index (Fd/Fl) were observed among the four rootstocks, fruits from trees on Sour orange and the Flying dragon gave the highest content. Also, fruits from the trees on Flying dragon significantly had the thickest rind (3.00 mm) followed by those from Trifoliate orange (2.50 mm) and Sour orange (2.10 mm). The yields of trees grafted on Swingle citrumelo and sour orange were significantly higher than those of trees grafted on the other rootstocks. Trees grafted on Trifoliate orange had medium yield whereas trees on Flying dragon rootstock had the lowest yield.

Determination of total carotenoids and chlorophylls content

Total carotenoids and chlorophylls are given in Table 2. Fruits on sour orange and the Flying dragon had significantly more carotenoids and no chlorophylls than fruits on other rootstocks.

Result of the ethylene analysis

GC analyses of fruits allowed the identification of ethylene in the retention time of 9.45 minutes (Fig. IX). Among the four rootstocks evaluated, the Flying dragon indicated the maximum level of ethylene (Table 2).

Table 3
Correlations between 8 traits in a correlation matrix

	Fructose	Glucose	Sucrose	Citric acid	Ascorbic acid	pH	TSS
Glucose	0.99**						
Sucrose	0.51	0.45					
Citric acid	-0.35	-0.25	-0.54				
Ascorbic acid	0.61*	0.64*	0.78**	-0.02			
pH	0.46	0.37	0.65*	-0.38	0.34		
TSS	0.90**	0.90**	0.61*	-0.12	0.76**	0.64*	
TSS/TA	0.72**	0.65*	0.78**	-0.55	0.52	0.92**	0.79**

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

Results of correlation

Not only glucose and fructose showed a high positive correlation with each other but also they showed a high positive correlation with TSS. TSS/TA also showed a high positive correlation with pH. (Table 3).

Discussion

Results indicated that sugars and organic acids were affected by rootstocks. These findings were consistent with the results of other researchers (Legua et al., 2014). The composition of sugars obtained from four rootstocks of Satsuma mandarin was very similar. However, the relative concentration of sugars was different according to the type of rootstock.

Based on the findings, the highest fructose, glucose, and total sugar were detected in the fruits from the trees on Swingle citrumelo rootstock, followed by Sour orange. These findings are in contrast with those of Mashayekhi et al. (2013) who found the highest total sugar was in trees of Parson Brown and Mars Oranges grafted on Rough lemon rootstock. Castle et al. (1995) reported that fruits of sweet orange on trifoliate orange had higher TSS/TA ratio and lower acidity, which is in agreement with the results of this study. The highest percentage of total acids (TA) was recorded in the fruits from the trees on Flying Dragon rootstock. These results are in agreement with the work of Gonzatto et al. (2011) where the trees on Flying Dragon had the highest TA.

Differences among rootstocks could be attributed to the differential ability of the rootstocks to absorb water and nutrients and to the physical differences among the root systems (Al-Jaleel et al., 2005) and also inability to produce, conduct, or utilize some endogenous growth promoters such as auxins and gibberellins (Muhtaseb and Ghnaim, 2006).

The number of physicochemical traits in Satsuma mandarin fruits obtained in the present study was not consistent with the previously published data (Cantuarias-Aviles et al., 2010). This might be related to rootstock and environmental factors that could influence the traits.

The application of fertilizer and irrigation affects the content of sugars present in crops (Kumar et al., 2004). Fertilization, irrigation, and other operations were carried out uniform in this study so we did not believe that these variations might be due to the variation in environmental conditions.

The discovery of sucrose -6- phosphate, as an intermediate between UDP- glucose and sucrose, led to a rapid description of the biosynthetic pathway of sugar compounds. The biosynthetic pathway of sugar compounds in higher plants is as follows: Photosynthesis → Triose-P → Fructose- 6- phosphate → Glucose- 6- phosphate → Glucose- 1- phosphate → UDP-Glucose → Sucrose -6- phosphate → Sucrose → Glucose and Fructose (Salter et al., 2012).

Reaction pathway catalyzes bysucrose-6-phosphate synthase and sucrose-6-phosphate phosphatase respectively (Maloney et al., 2015). An increase in the amount of sugars, when Swingle citrumelo is used as the rootstock, shows that either the synthesis of Triose-P was enhanced or the activities of both enzymes were increased.

Studies have shown that plant hormones affect sugars content of the fruit (Roa et al., 2015). On the other hand, the level of plant hormones can also be changed by rootstocks (Tomala et al., 2008).

Research has shown that ethylene can stimulate the biosynthesis of carotenoids and can reduce the chlorophylls of citrus peel (Hutchings, 2011). On the other hand, the level of ethylene can also be changed by rootstocks (Tomala et al., 2008).

It is commonly accepted that carotenoids in higher plants are originated from acetyl-CoA via the mevalonic acid pathway [Acetyl-CoA → Mevalonic acid → Geranylgeranyl pyrophosphate → Phytoene → Lycopene → α-carotene or β-caroten](Gross, 2012).

Sinve Triose-P is necessary for the synthesis of sugars, 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 sugars and acids were significantly affected by rootstocks and there was a great variation in most of the measured characteristics among four rootstocks. The present study demonstrated that the relative concentrations of sugars and acids were different according to the type of rootstock. Among the four rootstocks examined, Swingle citrumelo showed the highest content of sugars, TSS, total dry matter, fresh fruit weight, dry fruit weight, fruit diameter, fruit length, and fruit production. The lowest sugars, ascorbic acid, pH, TSS, TSS/TA, and juice content, as well as the lowest total dry matter, fresh fruit weight, dry fruit weight, fruit diameter, fruit length, and fruit production were produced by Flying dragon. Further research on the relationship between rootstocks and sugars is necessary.

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