Journal of Medicinal Plants and By-products (2019) 2: 105-114

Original Article

Physicochemical Characteristics of Kumquat (*Fortunella margarita*) on Citrus Rootstocks

Behzad Babazadeh-Darjazi[1*] and Kamkar Jaimand[2]

[1]*Department of Horticulture, Roudehen Branch, Islamic Azad University, Roudehen, Iran*
[2]*Phytochemistry Group, Department of Medicinal Plants & By-products, Research Institute of Forest and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran*

Article History: Received: 02 July 2018 /Accepted in revised form: 10 August 2018

Abstract

The aim of this study was to determine organic acids and individual sugars as well as carotenoids in fruit of Kumquat [*Fortunella margarita* (Lour.) Swingle] on different rootstocks. On other hand, the purpose of this study was to identify the rootstock that could produce the highest amount of sugars. The content of individual sugars and organic acids in fruits were determined by HPLC, whereas spectrophotometer was used to determine the total carotenoids and chlorophylls. Total acidity (TA), total soluble solids (TSS) and pH value of juice was also evaluated. The content of ethylene in fruits was determined by Gas chromatograph. The results showed that, the highest of total sugars (51.63 mg/mL), pH (2.43), TSS (11.06%) and TSS/TA (3.71) were in fruit of Kumquat (Fortunella Margarita) grafting on the Troyer citrange rootstock. According to results, the amount of citric acid (45.07 mg/mL), ascorbic acid (0.41 mg/mL), juice (26.78 (%) and carotenoids (0.10 mg/gr DW) of Kumquat grafted on Sour orange was higher than those of other rootstocks. Among the sugars, Sucrose was determined in the highest concentration in all investigated fruits. The results of correlation showed that there were a high positive correlation between the amount of sucrose and glucose. Results showed that Troyer citrange rootstock had an important role in increasing of sugars, pH, TSS, as well as TSS/TA. Finally based on the obtained results it can be concluded that although the concentration of sugars and organic acids is strongly related to the genotype of fruit, it seems that rootstocks affect the amount of sugars and organic acids.

Keywords: Chemical traits, Kumquat, Physical traits, Rootstocks
Abbreviations: HPLC, High performance liquid chromatography

Introduction

Kumquats are believed to be native to China. Robert Fortune brought them from China to Europe in1846 [1]. Today, there are a number of kumquat species that have grown throughout the world, including *Fortunella japonica* (Thunb.) Swingle (Marumi kumquat), *Fortunella margarita* (Lour.) Swingle (Nagami kumquat), *F. crassifolia* (Meiwa kumquat), *F. hindsii* (Hong Kong Wild kumquat), and *F. polyandra* (Malayan kumquat) [2].
Fortunella margarita, that called Nagami kumquat or ovoid kumquat, is the most popular species in the world. It has an oval shape and a smooth, bright orange flavedo [2]. It is one of the most important *Fortunella* species cultivated in Iran. Although it is as important species, no studies have been done on individual sugars, acids and carotenoids of *Fortunella margarita*.
Fructose, glucose and sucrose are three major sugars of citrus fruits. Sucrose is known as the dominant sugar in citrus fruit and is plentiful. Sugars usually display 80% of the total soluble solids of juice [3]. Soluble solids are mixture of organic acids and sugars that applied as an index of maturity and taste quality [4]. Ascorbic acid is an antioxidant and exhibits a key function in the reduction of diseases. Carotenoids are

*Corresponding author: Department of Horticulture, Roudehen Branch, Islamic Azad University, Roudehen, Iran
Email Address: babazadeh@riau.ac.ir

also known to reduce cancers, cataracts, and heart disease [5]. Carotenoids are also widely used in the foodstuff, cosmetic and medicine products as natural coloring agent [6]. In Citrus fruits, ethylene will be able to stimulate ripening by increasing the biosynthesis of carotenoids and chlorophylls destruction [7].

Ratio of sugars to acids affects the flavor of citrus fruit and has been considered as quality indicator by both fresh consumption group and juice factories [8]. Kumquat juice is a fantastic resource of sugars and acids. The amount of kumquat acid is changeable and is dependent on the rootstock [9], varieties [10] and etc. A number of researches have indicated that the varieties can influence the physicochemical traits of kumquat fruit [11].

Mashayekhi *et al.* [12] showed that rootstocks can influence total sugar, glucose and sucrose content 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.* [13] showed that rootstocks can influence on total sugar, glucose and sucrose content of Clemenules mandarin. They mentioned that content of total sugar, glucose and sucrose of Clemenules mandarin grafted on 'Carrizo' citrange was higher than those on 'Cleopatra' mandarin rootstock. Legua *et al* [14] showed that rootstocks can influence on 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 others rootstocks. However, the highest of citric acid and ascorbic acid were found in 'Clemenules' mandarin grafted on Volkameriana rootstock. Yesiloglu *et al.* [15] reported that rootstocks can influence on TSS, TA content of fruits of Henderson grapefruit. They mentioned that TSS content of Henderson grapefruit on Troyer citrange was higher than those on other rootstocks. Yildiz *et al.* [16] showed that rootstocks can influence on TA content of 'Rio Red' grapefruit. They found that the highest percentage of total acids (TA) was in fruits from trees on Sour orange rootstock.

Investigations have shown that fruits grown on Rough lemon rootstock have lower acidity than those grown on Poncirus trifoliata. Generally, Percent of juice, acidity and TSS is also influenced by rootstock [17]. Studies have shown (in Australia) that trees on trifoliate orange, tangelo and Cleopatra mandarin rootstocks produced fruits with high in acidity and soluble solids but low in ascorbic acid, while trees on Rough lemon and lime rootstocks produced fruits with low in acidity and soluble solids but high in ascorbic acid. Higher TSS, acidity and TSS to Acid ratio are recorded in fruits of Blood Red orange on trifoliate orange, sweet and sour oranges. Fruits of Kinnow mandarin on Seville kimb rootstock contained the highest TSS, acidity, vitamin 'C', reducing sugars, sucrose and total sugars [18]. Some researchers have stated that rootstock is the most important factor affecting the composition of citrus fruits. Shamouti oranges on sour orange rootstocks contained 10% more sugars and 18% more citric acid than fruit grown on sweet lime rootstocks. In South Africa also, Valencia oranges and Marsh grapefruit on rough lemon rootstocks had lower soluble solids contents than fruit on the other rootstocks, but were different in acid. The citric acid yields from lemons on 8 different rootstocks were reported by some researchers [19]. The Rough lemon produced the lowest citric acid content and the trifoliate showed a marked tendency to produce the highest acid content. Similar data apparently taken from studies of fruits from the same trees were reported later by other researchers, who also included data on the influence of the same rootstocks on fruits of the Satsuma and Dancy mandarins and the Rough lemon. Some researchers found that the Pineapple orange in Florida, produced higher concentrations of citric acid, total solids, and total sugars when grown on sour orange rootstocks than when grown on Rough lemon rootstocks. Other researchers, also working in Florida, have reported similar results in investigations made with Parson Brown and Valencia oranges on various rootstocks. They found that rootstocks can influence on degree of acidity, amount of sugar, proportion of acidity to sugars and aroma of orange juice. Higher quality resulted when the fruit was grown on sour orange rootstocks than when it was grown on Rough lemon. Probably the most extensive and reliable data showing the influence of rootstocks on the sugar and acid content of citrus fruits are the analyses reported by some researchers [20]. 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 significantly higher concentration of total carotenoids in the juice than on rough lemon rootstock [21].

The aim of this research is to identify rootstock that can synthesize the maximum level of sugars and carotenoids.

Material and Methods

Chemicals and Standards

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

Rootstocks

In 2010, rootstocks were planted at 2×2 m with three replication at orchards of the Ramsar [Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate, average rainfall and temperature were 970 mm and 16.25 °C per year, respectively; soil was classified as loam-clay, pH ranged from 6.9 to 7]. Sour orange, Swingle citrumelo and Troyer citrange 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 experimental design was completely randomized design with three replicates. Then, Juices were centrifuged at 15,000 rpm for 20 min at 4 ˚C [14].

Juice Analyses Technique

The total titrable 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 [14].

Analysis of Sugars by HPLC

HPLC analysis was performed with a Platin blue system (Knauer, Berlin, Germany) equipped with binary pump and a Refractive Index (RI) detector. The separation was carried out on a Shodex Asahipak NH2P-50 4E column (250×4.6 mm). 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 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 acid and citric acid) 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 solution of fructose at concentrations of 0, 1.04, 2.08 and 3.12 mg/mL, used to obtain a standard curve. Standard solutions of glucose at concentrations of 0, 1.41, 2.82 and 3.76 mg/mL, used to obtain a standard curve. Standard solutions of sucrose at concentrations of 0, 2.97, 5.20 and 10.40 mg/mL, used to obtain a standard curve. Standard solutions of ascorbic acid at concentrations of 0, 0.22, 0.45 and 0.67 mg/mL, used to obtain a standard curve. Standard solutions of citric acid at concentrations of 0, 0.20, 0.61 and 1.03 mg/mL, used to obtain a standard curve (Fig. 1 to 5).

Sugars concentration was estimated from calibration curve and the result was expressed as milligrams of compound per milliliter (mg/ mL).

Table 1 Common and botanical names for Fortunella Swingle or Citrus L. taxa used as scion and rootstock.

Common name	Botanical name	Parents	Category
Nagami Kumquat (Scion)	*Fortunella margarita* (Lour.) Swingle	Unknown	Kumquat
Sour orange (Rootstock)	*Citrus aurantium* L.	Mandarin × Pomelo	Sour orange
Swingle citrumelo (Rootstock)	*X Citroncirus* spp.	*Citrus paradisi* Macfad × *Poncirus trifoliata (*L.) Raf.	Poncirus hybrids
Troyer citrange (Rootstock)	*X Citroncirus* sp.	*Citrus Sinensis* (L.) Osbeck. × *Poncirus trifoliata* (L.) Raf.	Poncirus hybrids

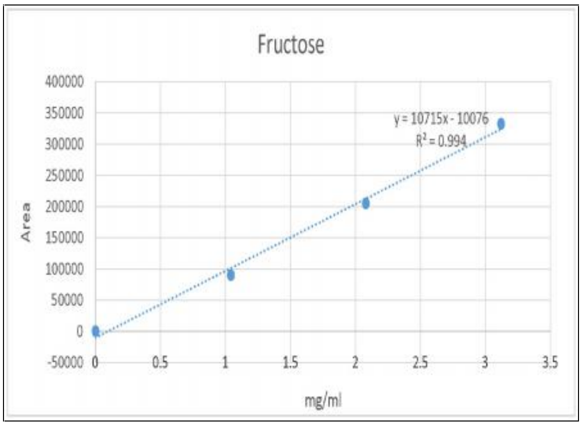

Fig. 1 The standard curve of fructose

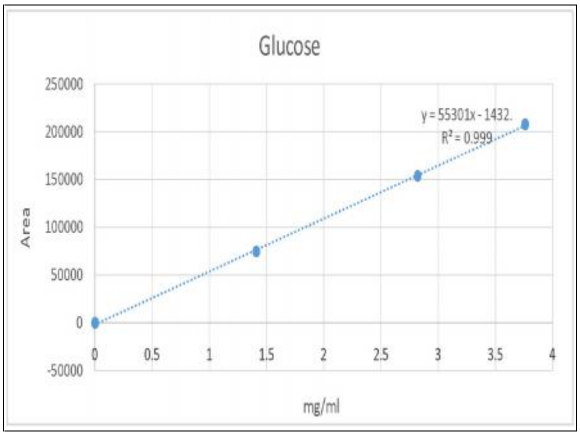

Fig. 2 The standard curve of glucose

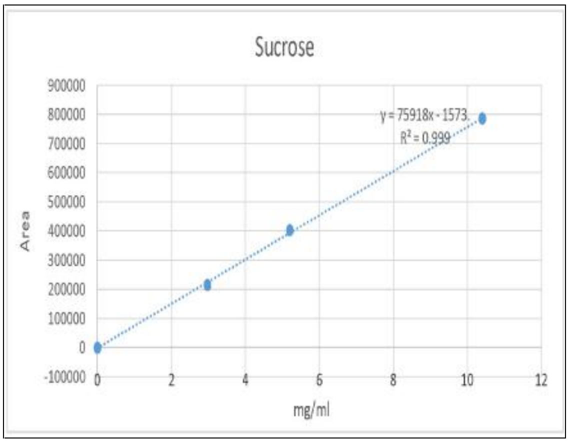

Fig. 3 The standard curve of sucrose

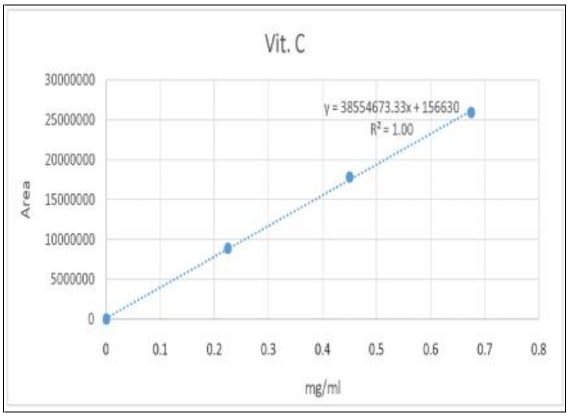

Fig. 4 The standard curve of ascorbic acid

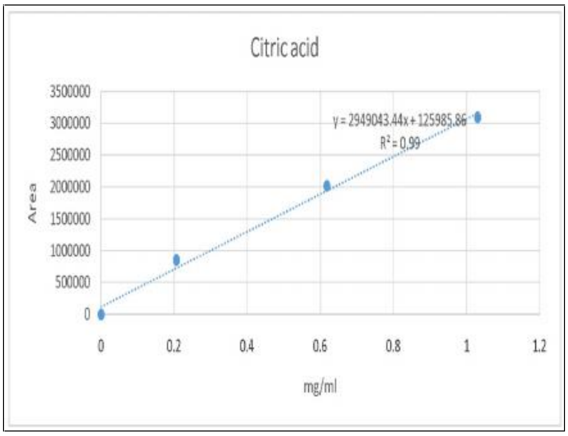

Fig. 5 The standard curve of citric acid

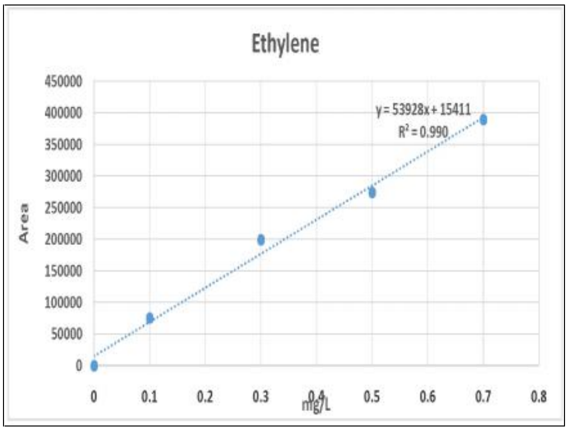

Fig. 6 The standard curve of ethylene

Analysis of Organic Acids by HPLC

A same HPLC was applied for this study. It 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. Acids concentration was estimated from calibration curve and the result was displayed as milligrams of compound per milliliter (mg/ mL).

Identification of Sugars and Organic Acids

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* [22]. Peels were freeze-dried at –56 ˚C for 4 days to lose all their moisture and then powdered by a mill. Samples were frozen at –80 ˚C until analyzed. All extractions were carried out under low light conditions to decrease of photo destruction. Briefly, 0.2 g freeze-dried sample was mixed with 10mL of ethanol solvent (95% v/v), butylated hydroxytoluene (BHT) (100 mg L^{-1}) and diethyldithiocarbamate(DDC) (200 mg L^{-1}). The samples were inverted for two min and kept at 4 ˚C. The samples were passed through an ashless filter paper. The filtrates were putted in a spectrophotometer (UV 1600 PC,Shimadzu, Tokyo, Japan) and absorbances were determined at 470 nm, 649 nm and 664 nm. The concentration of chlorophylls and total carotenoid were calculated by the following formula. Results were displayed as mg of chlorophyll or carotenoid per g dry weight (mg g^{-1} dry weight).

$$C_a = \frac{(13.36A_{664.2} - 5.19A_{648.6}) * 8.1}{DW} \quad [mg\,g^{-1}dw]$$

$$C_b = \frac{(27.43A_{648.6} - 8.12A_{664.2}) * 8.1}{DW} \quad [mg\,g^{-1}dw]$$

$$C_{a+b} = \frac{(5.24A_{664.2} + 22.24A_{648.6}) * 8.1}{DW} \quad [mg\,g^{-1}dw]$$

$$C_{x+c} = \frac{(4.785A_{470} + 3.657A_{664.2} - 12.76A_{648.6}) * 8.1}{DW} \quad [mg\,g^{-1}dw]$$

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

Ethylene Extraction Technique

In order to obtain the ethylene, fruits were weighed and were placed in a jar. The jar was covered and placed at room temperature for 1h. The temperature of room was holding constant at 25 ˚C. The volume of headspace around the fruits was measured. Ethylene was extracted with a 50mL plastic syringe through the septum of jar. 1 mL of extracted gas was injected to Gas Chromatograph. Standard solutions of ethylen at concentrations of 0, 0.1, 0.3, 0.5 and 0. 7 mg/L, used to obtain a standard curve (Fig. 6). Results were displayed as nanoliter 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 applied for this study. It fitted with a CP-Sil 88 column (100m×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 **109** calibration curve and the result was displayed as nanoliter per kilogram fresh weight of fruit per hour (nL kg^{-1} h^{-1}).

Physical Traits of Fruit and Fruit Production (yield)

Twenty fruits were randomly sampled and evaluated for each tree. Fruit physical traits were presented in Table 2. Total dry matter was determined by dehumidify of 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 evaluated with 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.

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. One-way analysis of variance was done for measured traits. Comparisons were made using 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

Result of the HPLC Analyses

HPLC analyses of juice allowed to quantification of 3 sugars (fructose, glucose and sucrose) and 2 acids (citric acid and ascorbic acid) (Fig. 7 to 8, Table 2).

Determination of Sugars

Fructose, glucose and sucrose were three sugars that recognized in this study. Moreover, the amount of total sugars ranged from 46.22 to 51.63 mg/ mL. Sucrose was the dominant sugar in this study. For all the sugars, the differences among rootstocks were found significant on the 1% level. Fruits on Troyer citrange showed significantly increase of sucrose and glucose but decreased fructose. Among three rootstocks evaluated, Troyer citrange indicated the maximum level of sugars (Table 2).

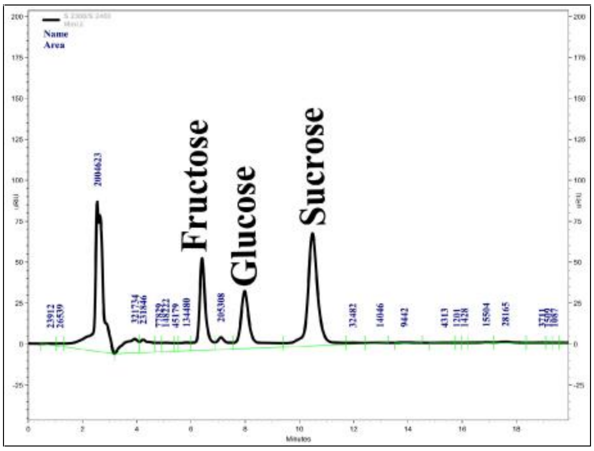

Fig. 7 HPLC chromatogram of sugars of Nagami Kumquat on Troyer citrange

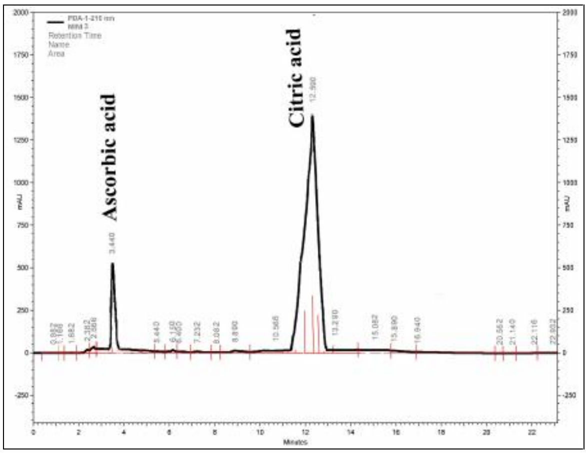

Fig. 8 HPLC chromatogram of organic acids of Nagami Kumquat on Troyer citrange

Determination of Organic Acids

Citric acid and ascorbic acid were two acids that recognized in this study. Moreover, the amount of total acids ranged from 44.06 to 45.48 mg/ mL. There was statistically significant difference on the 5% level in citric acid and ascorbic acid.

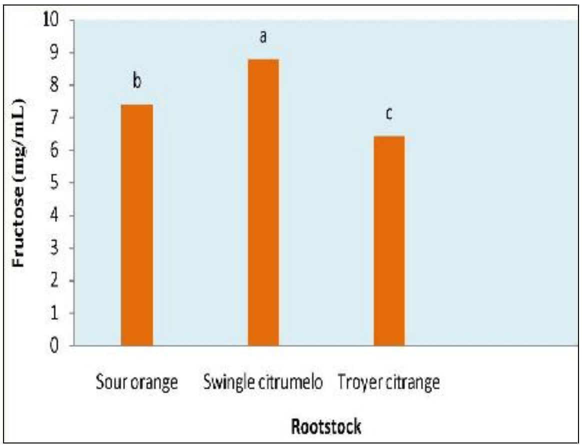

Fig. 9 The effect of rootstocks on fructose

The highest citric acid content was found in fruits from trees on sour orange (45.07 mg/mL) and Swingle citrumelo (44.84 mg/mL) while the lowest was found on fruits of Troyer citrange (42.53 mg/mL).

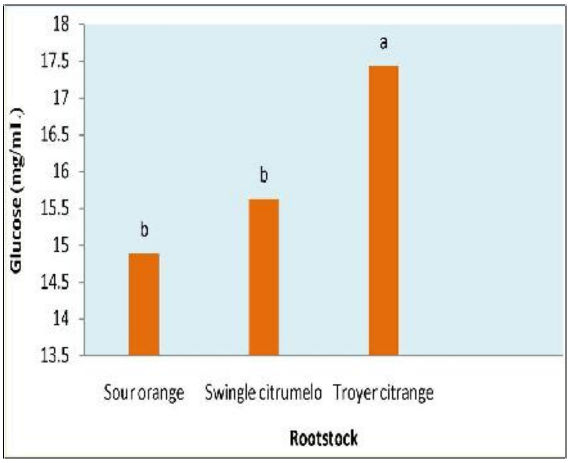

Fig. 10 The effect of rootstocks on glucose

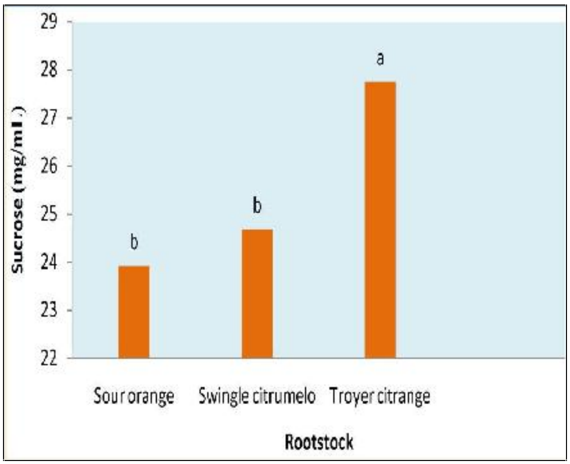

Fig. 11 The effect of rootstocks on sucrose

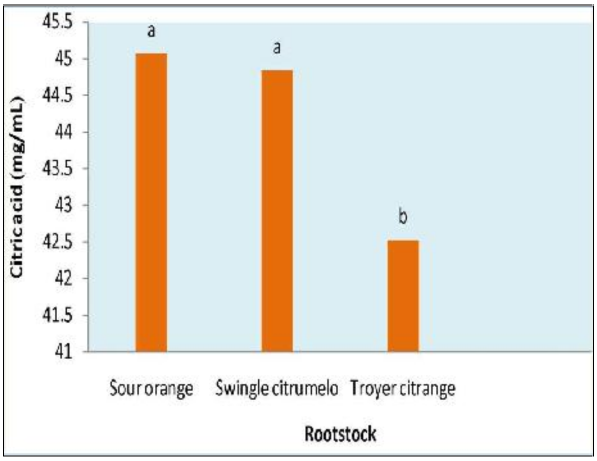

Fig. 12 The effect of rootstocks on citric acid

The fruits from trees on Troyer citrange showed ascorbic acid content significantly lower than those on Sour orange and Swingle citrumelo (Table 2).

Results of Total Titratable Acid (TA)

The amount of total titratable acid ranged from 2.98 to 3.43%. Total acid content (TA) was significantly affected by the rootstocks. The highest percentage of total acids (TA) was in fruits from trees on Sour orange, followed by Swingle citrumelo, whereas the lowest TA was detected in fruits from trees on Troyer citrange (Table 2).

Results of pH, TSS, TSS/TA and Juice Content

The amount of pH, TSS, TSS/TA and juice content were given in Table 2. Among three rootstocks evaluated, Troyer citrange indicated the maximum level of pH, TSS and TSS/TA. The highest levels of juice found in Sour orange. There was significant difference on the 1% level in the content of pH, TSS, TSS/TA of Nagami Kumquat juices on different rootstocks. Despite the little differences recorded for juice percentage, Kumquat fruits from Sour orange gave the highest juice percentage (26.78%) while those from Troyer citrange gave the least juice percentage (24.92%). The highest percentage of TSS was for fruits from Troyer citrange (11.06%), while the least was for those from both Swingle citrumelo and Sour orange (10.90 and 10.80%, respectively). The highest pH was recorded for Troyer citrange which was significant over those from Swingle citrumelo and Sour orange. TSS: TA ratio was lowest for sour orange.

Results of Fruit Physical Traits and Fruit Production (yield)

The amount of fruit physical traits and fruit production were given in Table 2. For more the physical traits, the differences among rootstocks were found significant. The results indicated that trees grafted on Swingle citrumelo significantly gave the heaviest fruit (14.00 g), while those grafted on Troyer citrange gave the lightest fruit (9.20 g). With respect to fruit length and diameter, fruits from the trees on Swingle citrumelo significantly gave the longest fruit (38.80 mm) and diameter (27.32 mm), while the least values were recorded for those grafted on Sour orange (35.00, 25.30 mm) and Troyer citrange (31.27, 22.35mm). Although no significant differences for fruit shape index (Fd/Fl) were observed among the three rootstocks, fruits from trees on Sour orange and Troyer citrange gave the highest content. In addition, fruits from the trees on Sour orange significantly gave the thickest rind (2.20 mm) followed by those from Swingle citrumelo (2.00 mm) and Troyer citrange (1.70mm). Yield of trees grafted on Swingle citrumelo was significantly higher than those of trees grafted on the other rootstocks. Trees grafted on sour orange gave intermediate yield, whereas trees on Troyer citrange rootstock gave the lowest yield.

Determinations of Total Carotenoids and Chlorophylls Content

The amount of total carotenoids and chlorophylls were given in Table 2. Fruits on Sour orange had significantly more carotenoids and no chlorophylls than fruits on others rootstocks.

Result of the Ethylene Analysis

GC analyses of fruits allowed identification of ethylene in retention time of 9.30 minutes (Fig. 17). Among three rootstocks evaluated, Sour orange indicated the maximum level of ethylene (Table 2).

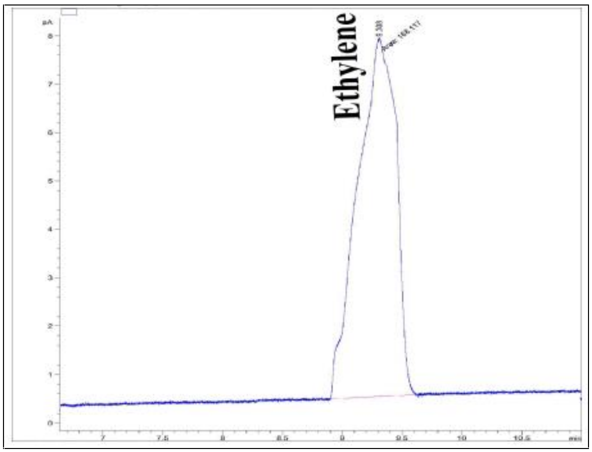

Fig. 13 GC ethylene chromatogram of Nagami Kumquat on Troyer citrange

Results of Correlation

Not only sucrose and glucose showed a high positive correlation with each other but also they showed a high positive correlation with pH and TSS. TSS also showed a high positive correlation with pH.

Table 2 Statistical analysis of variation in juice compositions and fruit physical traits of Nagami kumquat on three different rootstocks.

Compounds	Sour orange Mean	SD	Swingle citrumelo Mean	SD	Troyer citrange Mean	SD	F value
Sugars							
1) Fructose (mg/mL)	7.41b	0.32	8.80a	0.25	6.44c	0.20	**
2) Glucose (mg/mL)	14.89b	0.34	15.63b	0.39	17.44a	0.32	**
3) Sucrose (mg/mL)	23.92b	0.43	24.68b	0.44	27.75a	0.51	**
Total	46.22	0.64	49.11	0.64	51.63	0.61	
Organic acids							
1) Citric acid (mg/mL)	45.07a	1.01	44.84a	1.15	42.53b	0.97	*
2) Ascorbic acid (mg/mL)	0.41a	0.04	0.35ab	0.03	0.30b	0.03	*
Total	45.48	0.73	45.19	0.86	44.06	0.65	
Total titratable acid (%)	3.43a	0.14	3.38b	0.16	2.98b	0.22	*
pH	2.20b	0.06	2.25b	0.07	2.43a	0.03	**
TSS (%)	10.80b	0.06	10.90ab	0.05	11.06a	0.07	**
TSS/TA	3.14c	0.03	3.22b	0.03	3.71a	0.01	**
Juice (%)	26.78a	0.51	26.40ab	0.51	24.92b	0.56	*
Total dry matter (%)	12.99ab	0.26	13.28a	0.28	12.23b	0.30	**
Ash (%)	3.67a	0.58	4.00a	0.00	2.67b	0.58	*
Fresh fruit weight (g)	12.00ab	1.30	14.00a	1.30	9.20b	1.00	**
Dry fruit weight ^{z} (g)	0.90b	0.06	1.10a	0.08	0.79b	0.10	**
Fruit diameter (mm)	25.30ab	1.40	27.32a	1.47	22.35b	1.06	**
Fruit length (mm)	35.00ab	1.73	38.80a	1.93	31.27b	1.78	**
Fruit shape index (Fd/Fl)	0.72a	0.01	0.70a	0.01	0.71a	0.01	ns
Rind fruit weight ^{z} (g)	2.63a	0.10	2.67a	0.10	2.29b	0.12	**
Rind thickness (mm)	2.20a	0. 10	2.00ab	0. 10	1.70b	0.10	**
Fruit production (g /tree)	300ab	5	312a	5	291b	4	**
Carotenoids (mg/gr DW)	0.10a	0.01	0.06b	0.006	0.08ab	0.006	**
Chlorophyll A (mg/gr DW)	0.00	0.00	0.001a	0.00	0.001a	0.001	**
Chlorophyll B (mg/gr DW)	0.00	0.00	0.002a	0.001	0.00	0.00	**
Total chrophyll (mg/gr DW)	0.00	0.00	0.003	0.00	0.001	0.00	
Ethylene (nL kg−1 h−1)	1.36a	0.27	0.29b	0.06	0.90a	0.16	**

Mean is 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.

Table 3 Intercorrellations between 5 compositions in a correlation matrix

	Fructose	Glucose	Sucrose	Citric acid	Ascorbic acid	pH
Glucose	-0.52	-	-	-	-	-
Sucrose	-0.60	0.99**	-	-	-	-
Citric acid	0.72*	-0.60	-0.64	-	-	-
Ascorbic acid	0.37	-0.62	-0.62	0.89**	-	-
pH	-0.50	0.98**	0.97**	-0.46	-0.46	-
TSS	-0.40	0.98**	0.97**	-0.49	-0.56	0.98**

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

Discussion

The results indicated that the sugars and organic acids were affected by rootstocks. These findings were consistent with the results of other researchers [14]. The composition of sugars obtained from three rootstocks of Nagami kumquat was very similar. However, the relative concentration of sugars was different according to the type of rootstock.

Based on the results, the highest glucose, sucrose and total sugar were detected in the fruits from the trees on Troyer citrange rootstock, followed by Swingle citrumelo. These findings are in contrast with those of Mashayekhi *et al*. [12] who found that the highest total sugar was with trees of Parson Brown and Mars Oranges grafted on Rough lemon rootstock. Yesiloglu *et al.* [15] reported that TSS content of Henderson grapefruit on Troyer citrange was higher than those on other rootstocks. These findings are in agreement with the results of this study. Our results showed that the highest percentage of total acids (TA) was detected in the fruits from the trees on Sour orange rootstock. These results are in agreement with work of Yildiz *et al.* [16] where the trees on sour orange have the highest TA.

Differences among rootstocks could be attributed to the differential ability of the rootstocks to absorb water and nutrients and related to the physical differences among the root systems [23] and inability to produce, conduct or utilize some endogenous growth promoters such as auxins and giberlins [24].

The amount of physicochemical traits in kumquat fruits obtained in presented investigation was not in accordance with previously published data [9]. It might be related to rootstock and environmental factors that could influence the traits.

It was observed that the application of fertilizer and irrigation affected the content of sugars present in crops [25]. 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 → Fructos- 6-phosphate→ Glucose- 6- phosphate→ Glucose- 1-phosphate→UDP- Glucose→ Sucrose -6- phosphate →Sucrose → Glucose and Fructose [26].

Reaction pathway catalyzed by sucrose-6-phosphate synthase and sucrose-6-phosphate phosphatase respectively [27]. An increase in the amount of sugars, when Troyer citrange, used as the rootstock, showed that either the synthesis of Triose-P was enhanced or activities of both enzymes increased.

Studies have shown that plant hormones affect sugars of fruit [28]. On the other hand, the level of plant hormones can also be changed by rootstocks [29].

Research has shown that ethylene can stimulate the biosynthesis of carotenoids and can reduce chlorophylls of citrus peel [30]. On the other hand, the level of ethylene can also be changed by rootstocks [29].

It is commonly accepted that carotenoids in higher plants are originated from acetyl-CoA via the mevalonic acid pathway [Acetyl-CoA →Mevalonic acid→ Geranylgeranylpyrophosphate → Phytoene→Lycopene→ α-caroten or β-caroten] [31].

Considering that 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 Troyer citrange.

Conclusion

In the present study we found that the amount of sugars and acids were significantly impressed by rootstocks and there was a great variation in most of the measured characters among three rootstocks. The present study demonstrated that the relative concentration of sugars and acids was different according to the type of rootstock. Among three rootstocks examined, Troyer citrange showed the highest content of sugars, pH, TSS and TSS/TA. The highest content of citric acid, ascorbic acid, juice and carotenoids were produced by Sour orange. Further research on the relationship between rootstocks and sugars is necessary.

Acknowledgements

The author thanks Roudehen Branch, Islamic Azad University for the financial support of the present research.

References

1. Spiegel-Roy P, Goldschmidt EE. The Biology of Citrus. Cambridge University Press. 1996.
2. Dugo G, Mondello L. Citrus Oils: Composition, Advanced Analytical Techniques, Contaminants and Biological Activity. CRC Press, Taylor and Francis group, Boca Raton. 2010.
3. Varnam A, Sutherland JM. Beverages: Technology, Chemistry and Microbiology, Springer Science & Business Media, New York. 2012.
4. Karadeniz F. Main organic acid distribution of authentic citrus juices in Turkey. Turk J Agric Fore. 2004;28:267-271.

5. Preedy VR, Watson RR, Pate VB. Nuts and Seeds in Health and Disease Prevention, Academic Press, London. 2011.
6. Rostagno MA, Prado JM. Natural Product Extraction: Principles and Applications, Royal Society of Chemistry, London. 2013.
7. Barry GH, Castle WS, Davies FS. Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. J Am Soc Hortic Sci. 2004;129:881-889.
8. Rees D, Farrell G, Orchard J. Crop Post-harvest: Science and Technology, Perishables, Vol 3, John Wiley & Sons, Uk. 2012.
9. Zhi-long XU, Shi-ying SU, Hua-lin YI. Effects of different rootstocks on tree growth and fruit quality of kumquat . J Huazhong Agric Univ. 2014;33:32-35.
10. Koh JS, Kim CS, Ko MS, Yang YT. Manufacture of processed foods and its quality characteristics from kumquats, a Citrus variety produced in Cheju. Korean J Food Sci Technol. 1993;25:33-38.
11. Tang CH, Lu MH, Tsai WH. A new variety of Kumquat *(*Fortunella crassifolia swingle) tainung No.1 Citrin . J Taiwan Agric Rec. 2013;62:83-91.
12. Mashayekhi K, Sadeghi H, Akbarpour V, Atashi S, Mousavizadeh SJ, Abshaei M, Nazari Z. Effect of some citrus rootstocks on the amount of biochemical composition of Parson Brown and Mars Oranges in Jiroft. J Hortic Sci. 2013;27:9-17.
13. Navarro JM, Perez-Perez JG, Romero P, Botla P. Analysis of the changes in quality in mandarin fruit, produced by deficit irrigation treatments. Food Chem. 2010;119:1591–1596.
14. Legua P, Fornerb JB, Hernandeza FCA, Forner-Giner MA. Total phenolics, organic acids, sugars and antioxidant activity of mandarin (*citrus clementina Hort. ex Tan*.) variation from rootstock. Sci Hort. 2014;174:60-64.
15. Yesiloglu T, Yılmaz B, Cimen B, Incesu M. Influences of rootstocks on fruit quality of ‘Henderson’ grapefruit. Turk Agric Nat Sci. 2014;1:1322-1325.
16. Yildiz E, Kaplankiran M, Demirkeser TH, Toplu C, Uysal-Kamiloglu M. Performance of ‟Rio Red’’ grapefruit on seven rootstocks in the eastern mediterranean region of Turkey. J Agr Sci Tech. 2014;16:897-908.
17. Siddiq M. Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging. John Wiley & Sons. 2012.
18. NPCS Board of Consultants & Engineers. Handbook on Citrus Fruits Cultivation and Oil Extraction. Asia Pacific Business Press Inc. 2009.
19. Chichester CO, Mrak EM, Stewart GF. Advances in Food Research, Volume 9. Academic Press. 1960.
20. Webber HJ, Batchelor LD. The Citrus Industry. University of California Press. 1965.
21. Ladaniya M. Citrus Fruit: Biology, Technology and Evaluation. Academic Press. 2008.
22. Van-Wyka AA, Huysamera M, Barry GH. Extended low-temperature shipping adversely affects rind colour of ‘Palmer Navel’ sweet orange [*Citrus sinensis* (L.) Osb.] due to carotenoid degradation but can partially be mitigated by optimising post-shipping holding temperature. Postharvest Biol Technol. 2009;53:109–116.
23. Al-Jaleel A, Zekri M, Hammam Y. Yield, fruit quality, and tree health of ‘Allen Eureka’ lemon on seven rootstocks in Saudi Arabia. Sci Hort. 2005;105:457–465.
24. Muhtaseb J, Ghnaim H. Effect of four rootstocks on fruit quality of sweet orange c.v. 'Shamouti' under Jordan valley conditions. Emir J Agric Sci. 2006;18:33-39.
25. Kumar D, Singh BP, Kumar P. An overview of the factors affecting sugar content of potatoes. Ann Appl Biol. 2004;145:247-256.
26. Salter A, Wiseman H, Tucker G. Phytonutrients, John Wiley & Sons. New York. 2012.
27. Maloney VJ, Park JiY, Unda F, Mansfield SD. Sucrose phosphate synthase and sucrose phosphate phosphatase interact in planta and promote plant growth and biomass accumulation. J Exp Bot. 2015;1-12.
28. Roa AR, Garcia-Luis A, Barcena JLG, Huguet CM. Effect of 2,4-D on fruit sugar accumulation and invertase activity in sweet orange cv. Salustiana. Aust J Crop Sci. 2015;9:105-111.
29. Tomala K, Andziak J, Jeziorek K, Dziuban R . Influence of rootstock on the quality of 'Jonagold' apples at harvest and after storage . J Fruit Ornam Plant Res. 2008;16:31-38.
30. Hutchings JB. Food Colour and Appearance, Springer Science & Business Media, New York. 2011.
31. Gross J. Pigments in Vegetables: Chlorophylls and Carotenoids. Springer Science & Business Media, New York. 2012.