Physicochemical characteristics in the fruits of Page mandarin (*Citrus reticulata*) on different rootstocks

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Abstract

The aim of this study was to determine physicochemical characteristics in the fruit of Page mandarin (*Citrus reticulata*) on different rootstocks. The study was conducted at Ramsar Research Institute in a completely randomized design with 4 treatments and 3 replications during 2015. The content of individual sugars in fruits was determined by High performance liquid chromatography (HPLC). Total acidity (TA) and pH value of the juice was also evaluated. In addition, total carotenoids and chlorophylls content was measured using a spectrophotometer. Crude fat was extracted using a Soxhlet`s apparatus. Crude protein was measured by Kjeldahl's method. Total dry matter was determined by dehumidifying the fruits in an oven at 80 ℃. Results showed that the highest levels of total sugars (107.35 mg/ mL), pH (3.50), and carotenoid (0.14 mg/ g) were in fruits of Page mandarin (*Citrus reticulata*) grafting on the Troyer citrange. According to the findings, the amount of total acid (0.73%) of Page mandarin grafted on sour orange was higher than those of other rootstocks.

Keywords: chemical traits, physical traits, page mandarin, rootstocks, sugars

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Introduction

Page mandarin (*Citrus reticulata*) is a hybrid resulting from a cross between Clementine and Minneola tangelo that was made in 1942 in USA and was released in 1963 (Fotouhi and Fattahi, 2007). It is one of the most important mandarins cultivated in the world. Despite its importance, little research has been done on individual sugars of Page mandarin (White, 1990).

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Sugars and organic acids are important components of citrus fruit, and 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 (Rees et al, 2012).

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 (Varnam and Sutherland, 2012). Bermejo and Cano (2012) stated that the ratios of fructose, glucose, and sucrose in citrus juice (except in citrons, limes, and lemons) were generally about 1:1:2 and sucrose was the predominant sugar. They reported that mandarins and hybrid groups had the highest amounts of sucrose (52.88 to 64.88 to g /L). Generally, sugar levels in mandarins range from 1-3% fructose, 1-2% glucose, and 2-6% sucrose (Lado et al, 2015). White (1990) reported the ratios of fructose: glucose: sucrose as about 2.64:2.50:5.80% W/V for Page mandarin.

An important compound in citrus fruits, carotenoids are known to reduce cancers, cataracts, and heart disease (Preedy et al, 2011). These compounds are also widely used in the foodstuff, cosmetic, and medicine products as natural coloring agent (Rostagno and Prado, 2013).

The amount of citrus sugars is variable and is dependent on the rootstock (Babazadeh-Darjazi et al., 2019), cultivar (Bermejo and Cano, 2012), etc. A number of studies have indicated that the rootstocks can influence the physicochemical traits of Page mandarin (Hayat Bakhsh et al., 2004; Babazadeh-Darjazi et al., 2009).

Legua et al. (2014) showed that rootstocks can influence sucrose, glucose, fructose, citric acid, and ascorbic acid contents of Clemenules mandarin. They found that the juice of Clemenules mandarin grafted on Cleopatra mandarin had a much higher sucrose and fructose contents than the others rootstocks. On the other hand, they reported the highest levels of citric acid and ascorbic acid in Clemenules mandarin grafted on Volkameriana. 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 was with trees grafted on *C. macrophylla* and Cleopatra mandarin. Legua et al. (2013) found that rootstocks can affect total sugars and TA contents of Lane late navel orange. They observed that the highest content of total sugars was in fruits from trees on F&A 418 rootstock. Emmanouilidou and Kyriacou (2017) reported that rootstocks can affect total sugar, glucose, and fructose contents of Lane Late and Delta orange cultivars. They mentioned that total sugar contents in Lane Late orange grafted on Cleopatra mandarin was higher than that in other trees. Asim et al. (2015) also showed that rootstocks can influence crud fat, crude protein, and crude fiber of Kinnow mandarin. They found that the highest percentages of crud fat, crude protein, and crude fiber were in the peel of Kinnow mandarin grafted on rough lemon.

The aim of this study was to determine physicochemical characteristics of Page mandarin fruit (*Citrus reticulata*) on different rootstocks.

Material and Methods

Chemicals and standards

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

Rootstocks

In 1989, rootstocks were planted at 8×4 m with three replications at Ramsar Research Station (Latitude 36° 54′ N, longitude 50° 40′ E, Caspian Sea climate with an average rainfall and temperature of 970 mm and 16.25 ℃ per year, respectively, and the soil classified as loam-clay with pH range 6.9-7). Sour orange, Swingle

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

Common name	Botanical name	Parents	Category
Page mandarin (Scion)	*Citrus reticulate cv Page*	Minneola tangelo ×clementine	Mandarin hybrid
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
Troyer citrange (Rootstock)	*Troyer citrange*	*C.sinensis × P.trifoliata (L.) Raf*	Poncirus hybrids

citrumelo, Trifoliate orange, 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 early in the morning (6 to 8 am), in January 2015 and only during dry weather. The selection method was on the basis of completely randomized design with 4 treatments and 3 replications. The juice was extracted using a juicer before they were centrifuged at 15,000 rpm for 20 min at 4 ℃. (Legua et al., 2014).

Juice analyses technique

The total titratable acidity was determined by titration with sodium hydroxide (0.1 N) and displayed as percentage of citric acid. The pH value was determined using a digital pH meter (Jenway, Model: 3510). Sugars 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. The separation was carried out on a Shodex Asahipak NH2P-50 4E column (250 × 4.6 mm). Column temperature was maintained at 25 ℃, 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 the retention times of unknown peaks in comparison with standards. The concentration of sugars was calculated from peak area according to the calibration curves. Standard solutions of sugars (fructose, glucose, and sucrose) were prepared by dissolving the required amount of each standard in deionized water. Calibration was performed by injecting three times of the standards at four different concentrations (Figs. I-III).

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

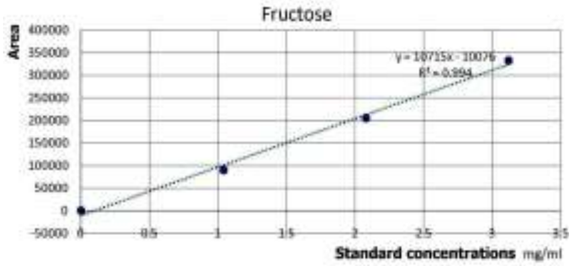

Fig I. The standard curve of fructose

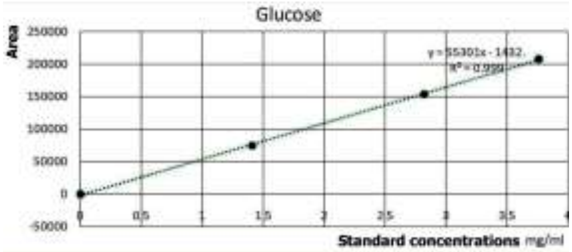

Fig II. The standard curve of glucose

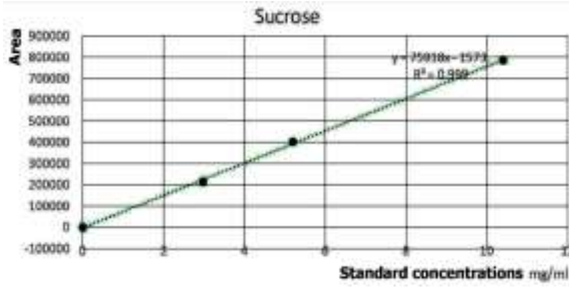

Fig III. The standard curve of sucrose

Determination of total carotenoid and chlorophylls

The method applied in this study was explained by Van-Wyka et al. (2009). Peels were freeze-dried at -56 ℃ for 4 days to lose all their moisture and then were powdered by a mill. Samples were frozen at -80 ℃ until analyzed. All extractions were carried out under low light conditions to reduce photo destruction.

Briefly, 0.2 g freeze-dried sample was mixed with 10 mL 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 ℃. Then, the samples were passed through an ashless filter paper. The filtrates were placed in a spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan) and the 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 chlorophyll or carotenoid per g dry weight (mg g^{-1} dry weight).

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

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

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

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

where C_a, C_b, C_{a+b}, and C_{x+c} represent chlorophyll *a* chlorophyll *b*, total chlorophyll, and total carotenoids, respectively.

Crude fat extraction technique

Crude fat was extracted using Soxhlet's apparatus. The method applied in this study, was explained by Fanali et al. (2013). Three grams of dried sample were placed in a thimble. The thimble was placed in an extraction chamber, which was suspended above a flask containing the solvent (petroleum ether) and below a condenser. The flask was heated and the solvent was evaporated and moved up into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the sample. As the solvent passed through the sample, it extracted the fats and carried them into the flask. The solvent was evaporated and the weight of remaining fat was measured (W_{fat}). The weight of the initial sample was also measured (W_{sample}). Then, the percentage of crude fat was calculated as below:

$$\%\text{Crude fat} = (W_{fat}/W_{sample}) \times 100.$$

Crude protein extraction technique

Crude protein was measured by Kjeldahl's method in 3 steps, namely digestion, distillation, and titration. Two grams of dried sample were digested by sulfuric acid and a catalyst, e.g. selenium or copper sulphate (to speed up the reaction). The solution in the digestion flask was then distilled by addition of sodium hydroxide, which converted the ammonium sulfate into ammonia gas. Liberated ammonia moved out of the digestion flask into the receiving flask which contained boric acid. It was condensed and collected in boric acid solution as a receiver and produced ammonium borate. The ammonium borate was titrated with 0.1 N HCL until a purple color change by using methyl red as an indicator. Crude protein was calculated by multiplying nitrogen percentage by a factor 6.25 as:

$$N = (V \times N \times 0.014 \times 100) / w$$

$$\text{Crude protein\%} = N\% \times 6.25$$

where V is the volume of acid used for sample titration, (0.1N), N denotes normality of acid, and W shows sample weight in grams.

Physical traits of fruit

Fifty fruits were randomly sampled and evaluated for each tree. Total dry matter was determined by dehumidifying the fruits in an oven at 80 ℃. Ash was measured by placing the weighed fruits in a furnace at 560 ℃. The weight of fresh fruit was determined using a scale.

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 12 traits. The data were statistically analyzed and the means were compared by Duncan's Multiple Rang Test (DMRT). Significance of the differences between means was considered at $p<0.05$. The correlation between pairs of characters was evaluated using Pearson's Correlation Coefficient.

Results

Determination of sugars

HPLC analyses of juice allowed to identify 3 sugars, namely fructose, glucose, and sucrose (Fig. IV and Table 2). Moreover, the amount of total sugars ranged between 88.56 and 107.35 mg/mL. Sucrose was the dominant sugar in this study. For all the sugars, the differences among rootstocks were found significant at 1% probability level. Fruits on Troyer citrange showed significant increase in fructose, glucose, and sucrose. Among

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

	Sour orange		Swingle citrumelo		Trifoliate orange		Troyer citrange		
Compounds	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value
Sugars									
1) Fructose (mg/mL)	21.65bc	0.61	23.43ab	0.69	20.27c	0.66	24.14a	0.73	**
2) Glucose (mg/mL)	19.46b	0.71	21.41a	0.64	17.66c	0.55	22.86a	0.67	**
3) Sucrose (mg/mL)	54.19b	1.04	59.19a	1.18	50.63c	1.11	60.35a	1.21	**
Total	95.30	2.36	104.03	2.51	88.56	2.32	107.35	2.61	
Total titratable acid (%)	0.73a	0.09	0.72a	0.08	0.70 a	0.06	0.68 a	0.09	ns
pH	3.20c	0.08	3.25bc	0.06	3.40ab	0.07	3.50a	0.08	**
Total dry matter (%)	12.95ab	0.67	13.80a	0.55	12.26b	0.33	12.99ab	0.35	*
Ash (%)	3.33a	0.58	4.00a	0.00	2.67a	0.57	3.33a	0.57	ns
Fresh fruit weight (g)	119bc	5.00	131a	6.00	123bc	3.90	118bc	4.10	*
Dry fruit weight [z] (g)	7.79ab	0.30	8.27a	0.43	7.20b	0.28	7.75ab	0.45	*
Crude fat (%)	2.00b	0.10	2.12ab	0.20	2.18ab	0.20	2.05ab	0.10	*
Crude protein (%)	6.06 a	0.57	5.95 a	0.81	5.78 a	0.78	5.9 a	0.62	ns
Carotenoids (mg/gr DW)	0.11bc	0.01	0.10c	0.00	0.13ab	0.01	0.14a	0.01	**
Chlorophyll A (mg/gr DW)	0.01b	0.00	0.02a	0.001	0.002c	0.00	0.004c	0.00	**
Chlorophyll B (mg/gr DW)	0.005b	0.00	0.01a	0.00	0.003c	0.00	0.005b	0.00	**
Total chlorophyll (mg/gr DW)	0.01	0.00	0. 03	0.001	0.005	0.00	0.009	0.00	

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≤0.05, ** significant difference at P≤0.01. Any two means within a row not followed by the same letter are significantly different at P≤0.01 or P≤ 0.05. Z = Dry fruit weight for 7.00 g fruit.

four rootstocks evaluated, Troyer citrange indicated the maximum level of sugars (Table 2).

Total titratable acid (TA), pH, crude fat, and crude protein

The amount of TA, pH, crude fat, and crude protein are given in Table 2. There was significant difference (p≤0.01) in the pH of Page mandarin on different rootstocks. The highest pH was recorded for Troyer citrange which was significant over those from Swingle citrumelo and Sour orange.

Despite the little differences recorded for crude fat on different rootstocks, fruits from Trifoliate orange contained the highest crude fat (2.18 %) while those from Sour orange contained the least crude fat (2.00 %).

Total acid (TA) and crude protein were not significantly affected by the rootstocks. The amount of total acid ranged between 0.68 and 0.73%. The highest percentage of total acid (TA) was recorded in the fruits from trees on Sour orange whereas the lowest TA was detected in fruits from trees on Troyer citrange. Although no significant differences for crude protein were observed among the four rootstocks, fruits from trees on Sour orange had the highest crude protein content.

Physical traits of the fruits

The values of fruit physical traits are given in Table 2. For most of the physical traits, the differences among rootstocks were significant. Results indicated that trees grafted on Swingle citrumelo significantly had the heaviest fruits (131 g) while those grafted on Troyer citrange had the lightest fruits (118 g).

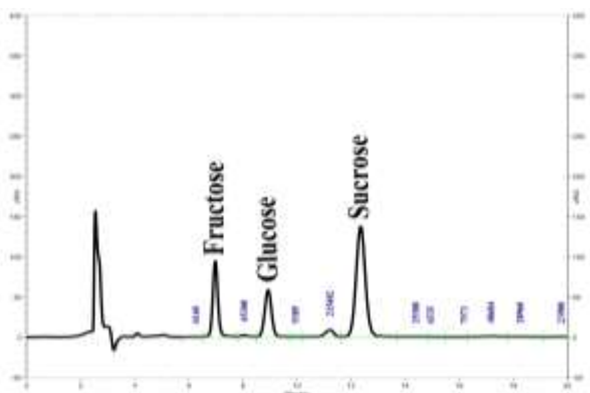

Fig. IV. HPLC chromatogram of sugars of Page mandarin

Table 3
Correlations between four traits in a correlation matrix

Variables	Fructose	Glucose	Sucrose	TA	pH	Total dry matter	Fresh fruit weight
Glucose	0.99**						
Sucrose	0.99**	0.98**					
TA	0.24	0.14	0.14				
pH	0.34	0.36	0.26	0.17			
Total dry matter	0.74**	0.66*	0.73**	0.63*	-0.05		
Fresh fruit weight	0.71**	0.63*	0.69*	0.61*	-0.03	0.91**	
Dry fruit weight	0.26	0.14	0.24	0.65*	-0.01	0.73**	0.64*

* and ** show significant differences at p≤0.05 and p≤0.01, respectively.

With regard to total dry matter and dry fruit weight, the highest values (13.80% and 8.27 g, respectively) were obtained from the trees on Swingle citrumelo showing significant differences from the other fruits while the least values were recorded for those grafted on Trifoliate orange (12.26% and 7.20 g, respectively). Although no significant differences for ash were observed among the four rootstocks, ash percent in fruits from trees grafted on Swingle citrumelo was more than those grafted on the other rootstocks. Trees grafted on Sour orange and Troyer citrange resulted in an intermediate ash percent whereas trees on Trifoliate orange had the lowest ash percent (Table 2).

Total carotenoids and chlorophylls contents

The amount of total carotenoids and chlorophylls are given in Table 2. Fruits on Troyer citrange had significantly more carotenoid and less chlorophyll than fruits on others rootstocks. The highest chlorophyll a content was recorded in Swingle citrumelo which was significantly different from that in Trifoliate orange and Troyer citrange (Table 2).

Results of correlation

Glucose showed a high positive correlation with fructose at about 0.99. Moreover, sucrose also showed a high positive correlation with fructose and glucose about 0.99 and 0.98, respectively (Table 3).

Discussion

The data obtained from this experiment revealed that the amount of sugars was significantly affected by rootstocks, which is in accordance with the previous studies (Babazadeh-Darjazi et al., 2019). However, it should be kept in mind that the environmental factors and extraction methods also may influence the results. Fertilizer and irrigation affects the content and compositions in citrus. Fertilization, irrigation, and other operations were controlled in this study so we did not believe that this variability was a result of these factors.

The mean concentrations of fructose, glucose, and sucrose ranged as 20.27-24.14, 17.66-22.86, and 50.63-60.35 mg/g, respectively. Bermejo and Cano (2012) stated that the ratios of fructose: glucose: sucrose in citrus juice (except in citrons, limes, and lemons) were generally about 1:1:2 and sucrose was the predominant sugar. They reported that mandarins and hybrids groups had the highest amounts of sucrose (52.88-64.88 g/L). These ratios were similar for the cultivar under study, and sucrose content was the highest.

Based on our results, fruits of trees grafted on different rootstocks had TA value ranging between 0.68% and 0.73%. These values are lower than those reported by Rafat et al. (2009) found TA value of 0.93% for Page mandarin. Different results may be related to rootstock, harvesting time, alternate bearing, etc.

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 (Salter et al., 2012).

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

Studies have shown that plant hormones affect sugar contents of the fruits (Roa et al., 2015). On the other hand, the level of plant hormones can also be changed by rootstocks (Tomala et al., 2008). 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 fruit sugar contents were significantly influenced by rootstocks and there was a great variation in most of the measured traits among the four rootstocks. Among the four rootstocks examined, Troyer citrange showed the highest content of sugars, pH, and carotenoids. The lowest sugar contents were recorded in Trifoliate orange. Further research on the relationship between rootstocks and fruit sugar contents is recommended.

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