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Original Article

The Effect of Rootstocks on the Peel Phenolic Compounds of Satsuma mandarin (*Citrus unshiu*)

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Abstract

The aim of this study was to determine total flavonoids and individually flavanone glycosides as well as antioxidant capacity in peel of Satsuma mandarin (Citrus unshiu) on different rootstocks. On other hand, the purpose of this study was to identify the rootstock that could produce the highest amount of flavonoids.Total flavonoids content was measured using aluminum chloride colorimetric method, whereas Folin-Ciocalteau colorimetric method was used to determine the total phenols content. The antioxidant activities were evaluated using reducing scavenging assays of DPPH radicals. HPLC-PDA detection was used for the analysis of individual flavanone glycosides (narirutin, naringin and hesperidin).The results showed that, the highest individually flavanone glycosides (27.73 mg/g DW), total flavonoids (10.74 mg/g DW), total phenol (3.91 mg/g DW) and DPPH scavenging activity (60.00 mg/g DW) were in the peel of Satsuma mandarin grafting on the flying dragon rootstock. According to results, the amount of phenolic acids (0.36 mg/g DW) of Satsuma mandarin grafted on trifoliate orange was higher than those of other rootstocks. Among the flavonone glycosides, hesperidin was determined in the highest concentration in all investigated peels. The results of correlation showed that there were a high positive correlation between the amount of total flavonoids and total phenols. Results showed that rootstock had an important role in increasing of concentration flavonoids, as well as antioxidant capacity. Finally based on the obtained results it can be concluded that although the concentration of flavonoid compounds is strongly related to the genotype of fruit, it seems that rootstocks affect the amount of flavonoids in the fruit.

Keywords: Citrus rootstocks, Phenolic compounds, Satsuma mandarin
Abbreviations: HPLC, High performance liquid chromatography; DPPH, 2, 2-diphenyl-1-picryhydrazyl radical.

Introduction

Mandarin is one of the most economically important crops in Iran. In the period 2011- 2012, the total mandarin production of Iran was estimated at around 825000 tones **[**1**]**. Satsuma mandarin (*Citrus unshiu*) is one of the most important mandarins are widely cultivated in Iran. Although it is an important crop, little research has been done on phenolic compounds of Satsuma mandarin.

Phenolic compounds have been classified into two major categories: phenolic acids and flavonoids. All flavonoids can be classified into flavanones, flavones, flavonols [2]. Flavanones are identified as the major flavonoid in citrus fruit and are the most abundant. Hesperetin and naringin are the most important flavanones in citrus fruit **[**3**]**.

Flavonoids play an important role in the prevention of cardiovascular diseases, cancers, and other degenerative diseases **[**4**]**. In addition, recent studies have identified antimicrobial and antifungal

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properties for flavonoids [5]. Flavonoids are important compounds extensively used in food and pharmaceutical industry [6].

Citrus peel is an excellent source of flavonoids. The quantity of flavonoids present in the Citrus fruit is variable and depends upon a number of factors, including: rootstock [7] cultivar [8], and etc.

Several studies have shown that the rootstocks used can influence the flavonoid compounds in Citrus [9, 10, 11, 12]. The aim of this research is to identify rootstock that can synthesize the maximum level of flavonoid compounds.

Material and Methods

Chemicals and Standards

Hesperidin, naringin, narirutin, diosmin, caffeic acid, p-coumaric acid, chlorogenic acid, gallic acid standards, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetonitrile, methanol and Folin–Ciocaltaeu's reagent were purchased from Sigma Chemical Co. (St. Louis, MO) . Rutin and Na2CO3 were purchased from Merck (Darmstadt, Germany).

Rootstocks

In 2001, rootstocks 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]. Sour orange, Swingle citrumelo, Trifoliate orange and Flying dragon were used as rootstocks in this experiment (Table 1).

Preparation of Peel 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 on the basis of completely randomized design.

Peel Extraction Technique

The peel was extracted according to the method of Chen *et al.* **[**13**]** with slight modifications. In order to obtain the phenolic compounds from the Peel, 0.2 g of dried peel (powder) were placed in a 200 ml spherical flask, along with 20 mL of methanol. The flask was covered and then placed in an ultrasonic water bath for 15 min. Extraction were performed with an ultrasound cleaning bath-Fisatom Scientific-FS14H (Frequency of 40 KHz, nominal power 90 W and 24×14×10 cm internal dimensions water bath). The temperature of the ultrasonic bath was held constant at 40°C. The extract was subsequently filtered through 0.45 mm filter paper. The concentration of the extract was finally reduced to 40 ml using methanol and placed in a vial. Vial sealed and was kept in the refrigerator at 4 °C until the HPLC analysis.

Analysis of Phenolic Compounds by HPLC

HPLC analysis was performed with a Platin blue system (Knauer, Berlin, Germany) equipped with binary pump and a photodiode array (PDA) detector. The separation was carried out on a ODS-2 C-18 reversed phase column (250 mm × 4.6 mm, i.d.) 5 μm. 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 0.05% (v/v) aqueous phosphoric acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.6 mL/ min. The column was washed with 100% methanol and equilibrated to initial conditions for 15 min before each injection. UV–visible spectral measurements were made over the range of 210–400 nm. Chromatograms were recorded at 329 nm for caffeic acid, p-coumaric acid, chlorogenic acid. Chromatograms were also recorded at 283 nm for narirutin, naringin and hesperidin. Identification of phenolic acids and flavanone glycosides was based on retention times and UV–visible spectra of unknown peaks in comparison with standards. The concentration of the phenolic acids and flavanone glycosides was calculated from peak area according to calibration curves.

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 cv. flying dragon*	Unknown	Poncirus

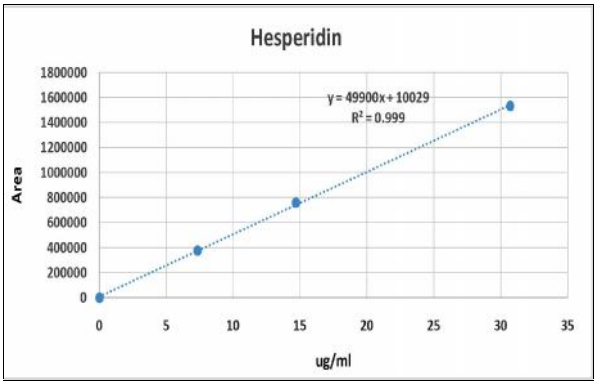

Fig. 1 The standard curve of hesperidin

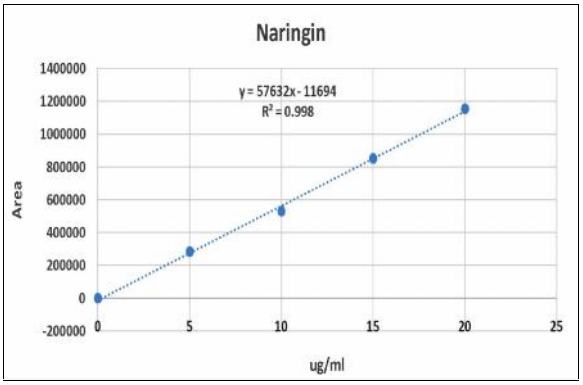

Fig. 2 The standard curve of naringin

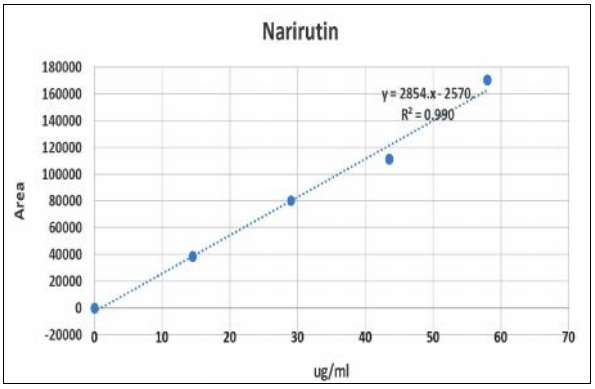

Fig. 3 The standard curve of narirutin

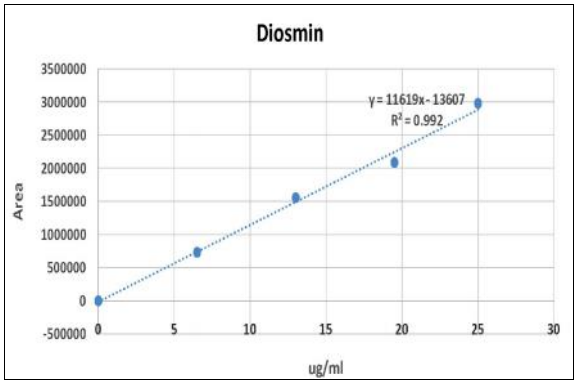

Fig. 4 The standard curve of diosmin

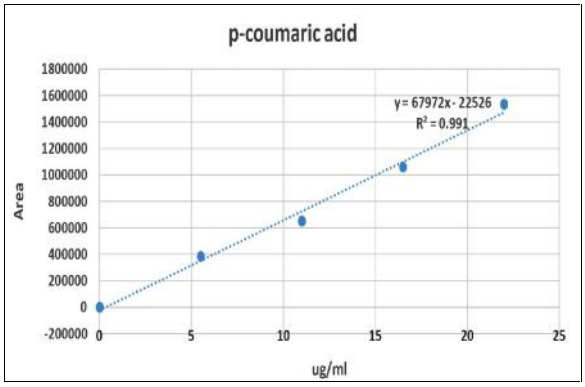

Fig. 5 The standard curve of p-coumaric acid

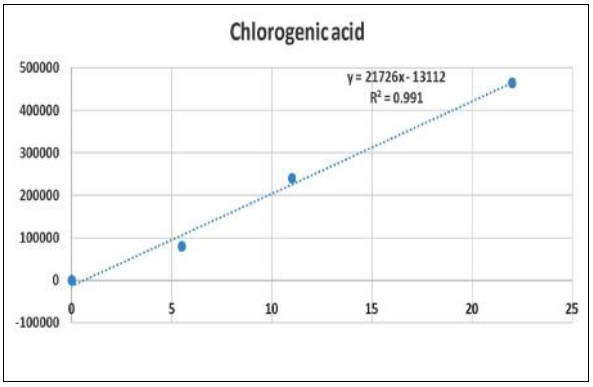

Fig. 6 The standard curve of chlorogenic acid

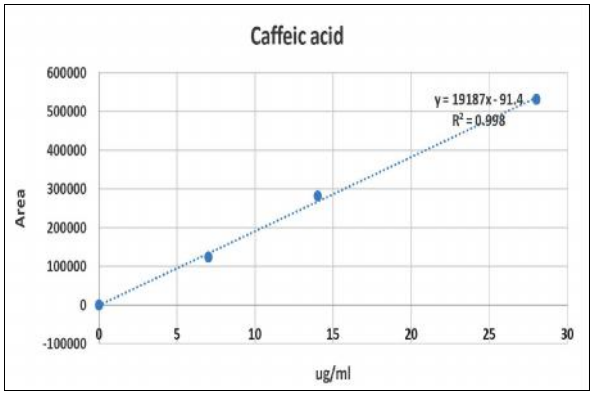

Fig. 7 The standard curve of caffeic acid

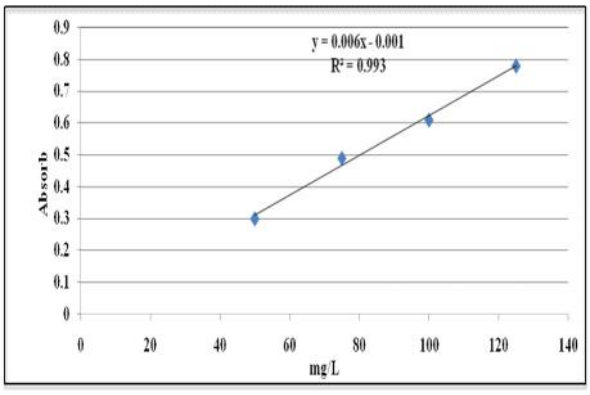

Fig. 8 The standard curve of rutin

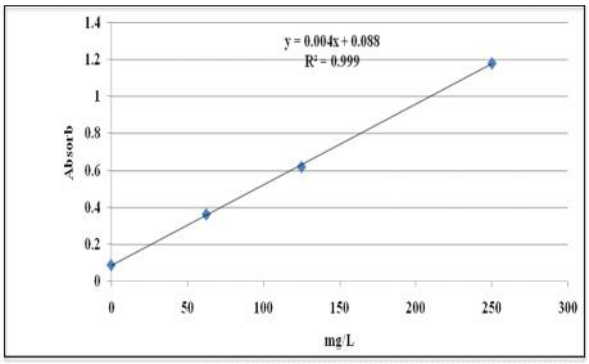

Fig. 9 The standard curve of gallic acid

Standard solutions of phenolic compounds were prepared by dissolving hesperidin, narirutin, naringin, diosmin, caffeic acid, p-coumaric acid, chlorogenic acid in HPLC grade methanol and stored at -20 °C between analyses. Calibration was performed by injecting the standard three times at five different concentrations. Standard solution of hesperidin that diluted in methanol at concentrations of 0, 7.33, 14.67, 22 and 30.69 ug/mL, used to obtain a standard curve.

Standard solutions of naringin at concentrations of 0, 5, 10, 15 and 20 ug/mL, used to obtain a standard curve. Standard solutions of narirutin at concentrations of 0, 14.5, 29, 43.5 and 58 ug/mL, used to obtain a standard curve. Standard solutions of diosmin at concentrations of 0, 6.5, 13, 19.5 and 25 ug/mL, used to obtain a standard curve. Standard solutions of *p*-coumaric acid at concentrations of 0, 5.5, 11, 16.5 and 22 ug/mL, used to obtain a standard curve. Standard solutions of caffeic acid at concentrations of 0, 7, 14 and 28 ug/mL, used to obtain a standard curve. Standard solutions of chlorogenic acid at concentrations of 0, 5.5, 11 and 22 ug/mL, used to obtain a standard curve. (Fig 1 to 7)

The amount of each phenolic acid and flavanone glycosides was expressed as milligrams of compound per gram of dry weight (mg/g DW).

Identification of Flavonoid Components

Phenolic acids and flavonoids were identified by comparing the retention times, absorption spectra (210–400 nm) and mass spectra of unknown peaks with those of reference compounds.

Determination of Total Flavonoid Content

The basic principle of colorimetric method is aluminium chloride that forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, respectively. In addition, aluminum chloride also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids resulting in pink colour formation [14].

The total flavonoid content was determined by the aluminum chloride colorimetric method. Standard solutions of rutin were prepared by dissolving 16.2 mg rutin with 70% ethanol into 100 ml after shaking evenly. Standard solutions of rutin at concentrations of 50, 75, 100 and 125 mg/L used to obtain a standard curve. Standard solutions of rutin were pipetted into four flasks of 10 ml, respectively, and diluted to 5 ml with 70% ethanol solution. Sodium nitrite solution (5%, 0.5 ml) was added to the standards and maintained for 5 min. Then, 0.5 ml of aluminium chloride (10%) was added. It remained at room temperature for 6 min. Finally, 5 ml of sodium hydroxide (1 M) was added. The mixture was diluted to 10 ml with distilled water.

The absorbance of all the samples was measured using a spectrophotometer (UV 1600 PC,Shimadzu, Tokyo, Japan) at 415 nm. The regression equation of rutin density and absorption value was obtained using rutin density (X) as the abscissa axis and absorption value (Y) as the vertical axis (Fig. 8). The total flavonoid content was calculated from calibration curve and the result was expressed as mg rutin equivalent per g dry weight **[**13**]**.

Determination of Total Phenol Content

Folin-Ciocalteu method is used to measure total phenols. This assay is based on oxidation of phenolic analyte and reduction of the reagent to form a chromophore. This method is an electron transfer-based assay which measures the reducing capacity of a solution, and has been correlated with phenolic content. This method provides neither a means to distinguish tannins (phenolics which precipitate proteins) from non tannin phenolics, nor a means to identify specific types of tannins in a mixture. Using Folin-Ciocalteu, the results are generally expressed as tannic or gallic acid equivalent. Among of methods, we found the Folin-Ciocalteu method to be highly reproducible. This method is also the most sensitive and the Folin-Ciocalteu reagent is commercially available **[**15**]**.

The total phenol content was determined by Folin-Ciocalteu's reagent. Standard compound of gallic acid (6.2 mg) was weighed accurately and dissolved with distilled water (25 ml). Standard solutions of

gallic acid at concentrations of 0, 62.5, 125 and 250 mg/L used to obtain a standard curve. Standard solutions of gallic acid were pipetted and diluted to 5 ml with distilled water. Then Folin- Ciocalteau reagent (0.5 ml) was added. It remained at room temperature for 2 min. Finally, sodium carbonate (5%, 0.5 ml) was added. It remained at room temperature for 3 h.

Absorbance was measured using a spectrophotometer (UV 1600 PC,Shimadzu, Tokyo, Japan) at 760 nm. The regression equation of gallic acid (X) and absorption value (Y) was obtained by using gallic acid density (X) as the abscissa axis and absorption value (Y) as the vertical axis (Fig. 9).The total phenol content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per g dry weight **[**13**]**.

DPPH Free Radical Scavenging Activity

The DPPH methodology was developed and makes use of the stable free radical DPPH, which has a strong purple color that can be measured spectrophotometrically. In the presence of compounds that are capable of either transferring an electron or donating hydrogen, the DPPH will become discolored. In the literature, the change in DPPH absorbance after the addition of a test material is often used as an index of the antioxidant capacity of the material. Although the DPPH method is widely used, it does have some limitations. The radical portion of the molecule is a nitrogen atom located at the center of the structure. While this centralized location is freely accessible to small molecules, larger molecules may have limited access to the radical portion due to steric hindrances [16].

The free radical scavenging activity was measured according to the method of Umamaheswari and Asokkumar [17] with slight modification. Briefly, 0.2 ml of extract was mixed with 2 ml DPPH (2, 2-diphenyl-1-picryl-hydrazyl). It remained at room temperature for 30 min. Absorbance was measured at 517 nm. DPPH expressed as (%).

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 7 phenolic compounds. 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.

Results

Peel Compounds of the Satsuma Mandarin

HPLC analysis of the peel compounds extracted from Satsuma mandarin allowed identification of 7 phenolic components (Table 2, Fig. 10): 3 flavanones, 1 flavone and 3 phenolic acids.

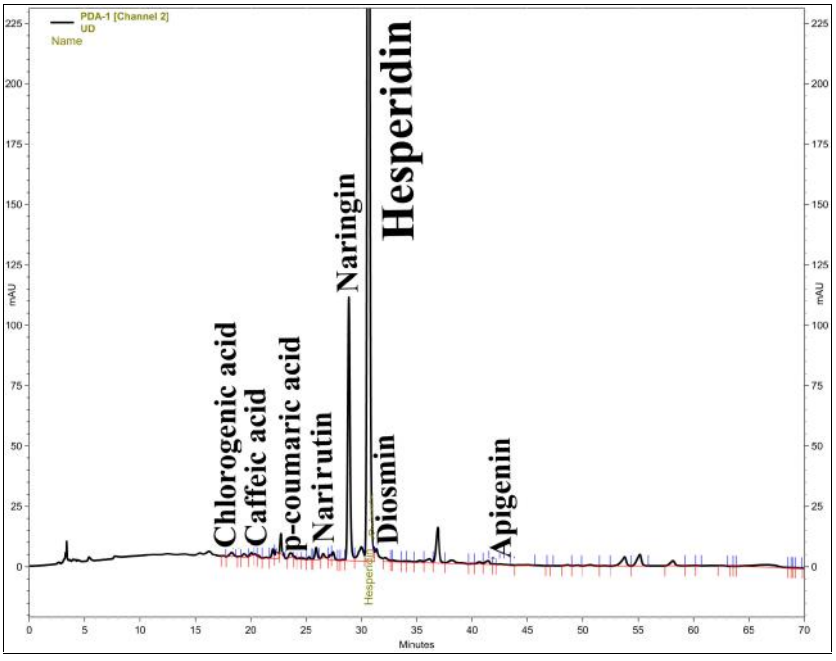

Fig. 10 HPLC chromatogram of phenolic components of Satsuma mandarin

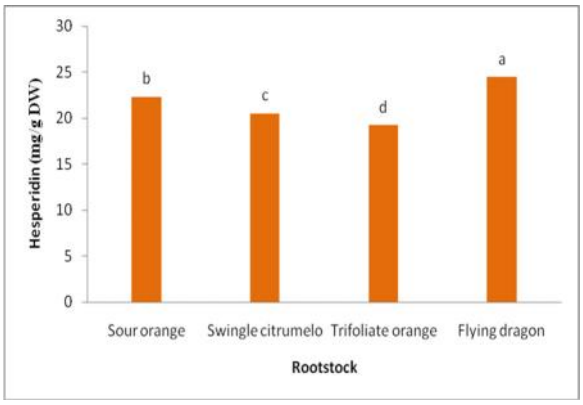

Fig. 11 The effect of rootstocks on hesperidin

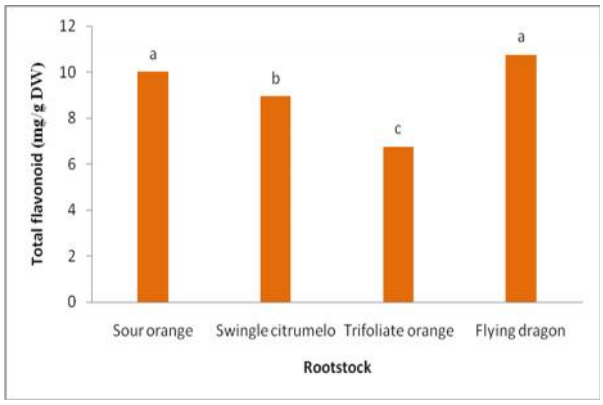

Fig. 12 The effect of rootstocks on total flavonoid

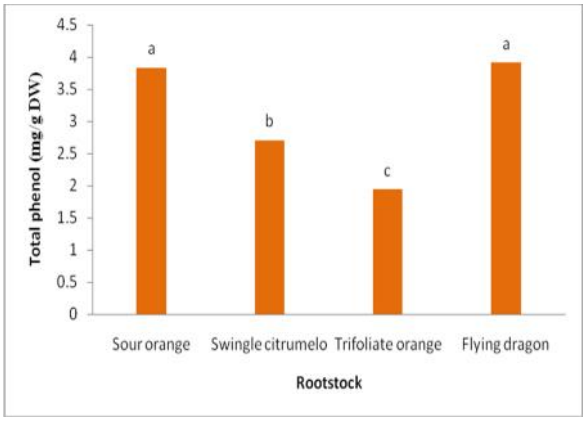

Fig. 13 The effect of rootstocks on total phenol

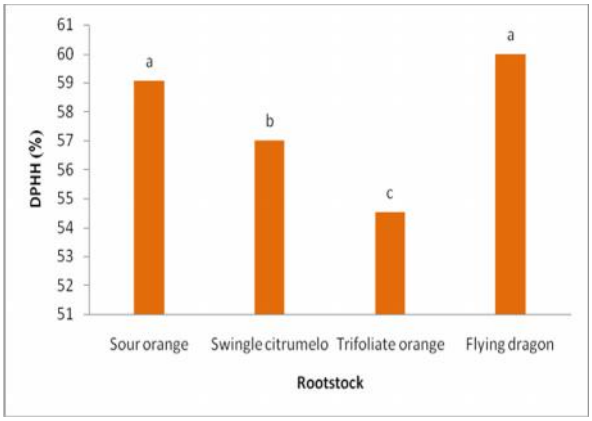

Fig. 14 The effect of rootstocks on DPPH

Flavanones

Three flavanones that identified in this analysis were narirutin, naringin, and hesperidin. In addition they were quantified from 22.01 to 27.73 mg/g DW. The concentration of hesperidin was higher in our samples. Among four rootstocks examined, Flying dragon showed the highest content of flavanones (Table 2, Fig. 11).

Flavones

One compound identified in this analysis was diosmin. The total amount of flavones ranged from 0.02 to 0.04 mg/g DW. Among four rootstocks examined, Flying dragon and Sour orange showed the highest content of flavones (Table 2).

Phenolic Acids

Three phenolic acids identified in this analysis were chlorogenic acid, caffeic acid and p-coumaric acid. The total amount of phenolic acids ranged from 0.22 to 0.36 mg/g DW. Chlorogenic acid was identified as the major component in this study and was the most abundant. Among four rootstocks examined, Trifoliate orange showed the highest content of phenolic acids (Table 2).

Results of Total Flavonoid Content

The amount of total flavonoid ranged from 6.76 to 10.74 mg/g DW. Among four rootstocks examined, Flying dragon showed the highest content of total flavonoid (Table 2, Fig. 12).

Results of Total Phenol Content

The amount of total phenol ranged from 1.94 to 3.91mg/g DW. Among four rootstocks examined, Flying dragon showed the highest content of total phenol (Table 2, Fig. 13).

Results of DPPH Free Radical

The amount of total DPPH ranged from 54.53 to 60.00%. Among four rootstocks examined, Flying dragon showed the highest content of DPPH free radical (Table 2, Fig. 14).

Table 2 Statistical analysis of variation in peel phenolic compounds of Satsuma mandarin on four different rootstocks.

	Sour orange		Swingle citrumelo		Trifoliate orange		Flying dragon		
Compounds (mg/g DW)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F value
a) Flavanones									
1) Narirutin (mg/gr DW)	1.25 ab	0.08	1.08 bc	0.06	1.04 c	0.05	1.30 a	0.08	F**
2) Naringin (mg/gr DW)	1.53 c	0.09	1.80 ab	0.09	1.75 ab	0.1	1.99 a	0.12	F**
3) Hesperidin (mg/gr DW)	22.27 b	0.65	20.49 c	0.51	19.22 d	0.58	24.44 a	0.51	F**
total	25.05	0.82	23.37	0.66	22.01	0.73	27.73	0.71	
b) Flavones									
1) Diosmin (mg/gr DW)	0.04 a	0.006	0.02 b	0.00	0.03 b	0.00	0.04a	0.006	F**
c) Phenolic acids									
1) Chlorogenic acid (mg/gr DW)	0.16 b	0.006	0.22 a	0.01	0.24 a	0.01	0.18 b	0.01	F**
2) Caffeic acid (mg/gr DW)	0.01 b	0.00	0.02 a	0.006	0.03 a	0.006	0.01 b	0.00	F**
3) p-coumaric acid (mg/gr DW)	0.05 b	0.00	0.09 a	0.006	0.09 a	0.006	0.06 b	0.006	F**
total	0.22	0.006	0.33	0.02	0.36	0.02	0.25	0.01	
total flavonoid (mg/gr DW)	10 a	0.49	8.94 b	0.51	6.76 c	0.39	10.74 a	0.48	F**
total phenol(mg/gr DW)	3.83 a	0.29	2.70 b	0.21	1.94 c	0.24	3.91 a	0.27	F**
DPPH %	59.06 a	1.00	57.00 b	0.93	54.53 c	1.00	60.00 a	1.10	F**

Mean is average composition (mg/g DW) in four different rootstocks used with three replicates. SD = standard deviation. F value is accompanied by its significance, indicated by: NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01. Any two means within a row not followed by the same letter are significantly different at P ≤ 0.01.

Table 3 Correlation matrix (numbers in this table correspond with components mentioned in Table 2).

	narirutin	naringin	hesperidin	diosmin	chlorogenic acid	caffeic acid	p-coumaric acid	total flavonoid	total phenol
naringin	0.29	-	-	-	-	-	-	-	-
hesperidin	0.90**	0.37		-	-	-	-	-	-
diosmin	0.83**	0.01	0.73**	-	-	-	-	-	-
chlorogenic acid	-0.70*	0.29	-0.76**	-0.70*	-	-	-	-	-
caffeic acid	-0.72**	0.09	-0.83**	-0.66*	0.94**	-	-	-	-
p-coumaric acid	-0.72**	0.34	-0.72**	-0.80**	0.96**	0.89**		-	-
total flavonoid	0.86**	0.20	0.93**	0.59*	-0.82**	-0.88**	-0.73**	-	-
total phenol	0.94**	0.12	0.92**	0.75**	-0.86**	-0.87**	-0.82**	0.96**	-
DPPH	0.90**	0.20	0.95**	0.67*	-0.84**	-0.89**	-0.77**	0.99**	0.98**

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

Results of Statistical Analyses

Differences were considered to be significant at P < 0.01. These differences on the 1% level occurred in narirutin, naringin, hesperidin, diosmin, chlorogenic acid, caffeic acid, total flavonoid, total phenol, DPPH free radical and p-coumaric acid (Table 2).

Results of Correlation

Simple intercorrellations between 10 components were presented in a correlation matrix (Table 3). Results showed that there were high positive correlations between total phenol and total flavonoids as well as some individual flavanones. There were also high positive correlations between DPPH scavenging activity and total flavonoid as well as total phenol and some individual flavanones. Total flavonoids demonstrated a significant positive correlation with narirutin and hesperidin (Table3).

Discussion

The flavonoids compounds are powerful antioxidant against free radicals, because they act as “radical-scavengers”. This activity is due to their hydrogen-donating ability. The phenol groups of flavonoids serve as a source of a readily available “H” atom such that the subsequent radicals produced can be delocalized over the flavonoid structure. Phenolic

compounds are known to act as antioxidants not only due to their ability to donate hydrogen or electron but also attributed to their stable radical intermediates, which prevent the oxidation of various food ingredients particularly fatty acids and oil [18].

Based on the results, the highest hesperidin, naringin total flavonoid, total phenol and DPPH were detected in the fruits from the trees on Flying dragon rootstock, followed by Sour orange. These findings are in contrast with those of Mashayekhi *et al*. [9] who found that the highest total flavonoid and total phenol was with trees of Parson Brown and Mars Oranges grafted on Rough lemon rootstock. In addition, Ghasemnezhad *et al.* [10] mentioned that content of hesperidin and total flavonoid of Page mandarin and Thompson novel orange grafted on Swingle citrumelo was higher than those on sour orange rootstock. Dissimilar results were reported by Hemmati *et at*. [11] who found that the fruits from trees of Italian orange grafted on Shelmahalleh rootstock gave total phenol significantly higher than other rootstocks. However, the highest of total flavonoid was found in Salustiana orange grafted on Yuzu . Gil-Izquierdo *et at*. [12] found that lemon trees 'Verna' grafted on sour orange (C. aurantium L.) rootstock produced fruits with higher levels of flavonoids in the juice, compared with trees grafted on Citrus macrophyla L. Legua et at [7] found that juice of 'Clemenules' mandarin grafted on 'Volkameriana' had a much higher content of total phenolics than the others rootstocks. On the other hand, Aghajanpour *et al*. [19] concluded that hesperidin and naringin of five different mandarins was not affected by the rootstocks.

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 [20] and inability to produce, conduct or utilize some endogenous growth promoters such as auxins and giberlins [21].

Based on the results, content of hesperidin in Satsuma's peel was from 19.22 to 24.44 mg/g DW and content of naringin in Satsuma's peel was from 1.53 to 1.99 mg/g DW. These results disagreed with the findings of Cano and Bermejo [22], who reported a level of 81.39 to 86.31 mg/g DW for hesperidin of albedo tissue, and 35.76 to. 42.22 mg/g DW for hesperidin of flavedo tissue in Satsuma's peel. Levaj *et al.* [23] reported content of hesperidin and naringin of Satsuma's peel 42.33 and 28.74 mg/100g DM respectively. Ma *et al*. [24] reported a level of 821.54 to 1446.05 microgram/g DW for hesperidin and 237.80 to 563.93 microgram/g DW for naringin in Satsuma's peel.

Based on the results, total flavonoids content in Satsuma's peel was from 6.76 to 10.74 mg/g DW. These results disagreed with the findings of Levaj *et al*. [23], who reported total flavonoids around 31.07 mg/g DM in Satsuma's peel. The amount of total flavonoids in mandarin peels obtained in presented investigation was not in accordance with previously published data [23]. It might be related to rootstock and environmental factors that could influence the compositions. However, it should be noted that the extraction method and HPLC conditions (column and mobile phase) might also affected the results.

The presence and/or concentrations of flavonoids can be affected by the fruit development stage [25]. On other hand, the rootstock plays an important role in the ripening process and the final degree of ripeness [26].

It was observed that the application of organic fertilizer affected the content of flavonoids present in plant [27]. 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 naringenin chalcone, as an intermediate between Malonyl CoA and flavonoids, led to a rapid description of the biosynthetic pathway of flavonoid compounds. The biosynthetic pathway of flavonoid compounds in higher plants is as follows:

Phenylalanine → Malonyl CoA (+4-comaryol CoA) → Naringenin Chalcone → Naringenin → flavonoids

Reaction pathway catalyzed by chalcone synthase and chalcone isomerase respectively **[**28**]**. An increase in the amount of flavonoids, when Flying dragon used as the rootstock, showed that either the synthesis of naringenin chalcone was enhanced or activities of both enzymes increased.

It is suggested that compounds with cytokinin-like activity stimulate the flavonoid biosynthetic pathway in avocado tissue **[**29**]**. It is generally accepted that cytokinins in higher plants are synthesized mainly in the root system and transported to the shoots through the xylem. **[**30**].**

According to obtained results it was obvious that there were high positive correlations between total flavonoids and some individual flavanones. Levaj *et al*. [23] also reported similar results [23]. Results showed that there were high positive correlations between antioxidant capacity (DPPH) and phenolic compounds. These results agreed with the findings of Gorinstein *et al.* [31].

High positive correlations between pairs of phenolic compounds indicated a genetic control **[**32**]**. Non-significant negative and positive correlation indicated genetic independence. However, without extended information into the biosynthetic pathway of each flavonoid compound, the true significance of the observed correlations is not clear.

Considering that naringenin chalcone is necessary for the synthesis of flavonoids, it can be assumed that there is a specialized function for this molecule and it may be better served by Flying dragon.

Conclusion

In the present study we found that the amount of flavonoids was significantly impressed by rootstocks and there was a great variation in most of the measured characters among four rootstocks. The present study demonstrated that the relative concentration of flavonoids was different according to the type of rootstock. Among four rootstocks examined, Flying dragon showed the highest content of flavonoids. The lowest of flavonoids content were produced by trifoliate orange. Further research on the relationship between rootstocks and flavonoids is necessary.

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