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Original Article

The Effect of Mandarin Scions (*Citrus* spp.) on the Peel and Pulp Phenolic Compounds

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Abstract

The purpose of this study was to evaluate the effect of mandarin scions on total flavonoids and individual flavanones as well as antioxidant capacity in peel and pulp of fruits. Total flavonoids and total phenols content was measured using aluminum chloride colorimetric and Folin-Ciocalteau colorimetric method respectively. Assay of DPPH radical scavenging was used to evaluate antioxidant activities. Individual flavanones (narirutin, naringin and hesperidin) detected and analyzed using HPLC-PDA. Among the flavonones, hesperidin was determined in the highest concentration in all investigated samples. Level of flavanones (25.05 mg/g DW) and total flavonoids (10 mg/g DW) in peel of Satsuma mandarin and the amount of phenolic acids (1.17 mg/g DW) in peel of Younesi tangerine was higher than other scions. The results of correlation showed that there was a high positive correlation between the amount of total flavonoids and hesperidin. The results of this study showed useful information on functional and phytochemical compounds in mandarin cultivars that can be used in pharmaceutical industry and provide valuable genetic resources for breeding programs.

Keywords: Mandarin, Citrus reticulata, Citrus unshiu, Scion

Introduction

Mandarin is a tropical and sub-tropical tree belonging to the family Rutaceae [1]. It is one of the most important cops in the world. According to Hodgson's classification, the mandarin group is divided into various natural sub-groups that include common mandarin (*C. reticulata* Blanco), satsuma mandarin (*C. unshiu* Marcovitch), King mandarin (*C. nobilis* Loureiro), Mediterranean mandarin (*C. deliciosa* Tenore), small-fruited mandarin (*C. indica*, *C. tachibana* and *C. reshni*), as well as mandarin hybrids that include tangor (*C. reticulata* × *C. sinensis*, i.e., mandarin× orange hybrid) and tangelo (*C. reticulata* × *C. paradise*, i.e., mandarin × grapefruit hybrid). In addition to the above classification, clementine (*C. clementina* Hort. ex. Tan) also is considered to be a unique and distinct sub-group of mandarins [2].

Mandarins are a good source of phenolic compounds. Phenolic compounds are a large group of plant secondary metabolites which classified as flavonoids and nonflavonoids [3].

The class of flavonoids comprises of at least 6000 molecules, divided into subgroups: flavanones, flavones, flavonols, leucoanthocyanidins, anthocyanins and isoflavonoids. These are abundant in flowers, fruits and leaves and have a diverse set of functions [4].

Among flavonoids, flavanones are the most relevant, while flavones are also present at lower amounts. The most usual flavanones present in *C. reticulata* are hesperidin, narirutin, neoponcirin, didymin, isorhoifolin, and eriocitrin, while others such as naringin, taxifolin, neohesperidin, eridictyol, poncirin, and naringenin are present at lower levels [5]. Hesperidin is also the predominant flavanone in mandarin and narirutin is the second most important flavanone in mandarin. The highest concentration of flavanones is found in the peel as

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compared to the flesh and higher in segment membranes than in the juice vesicles [5].

Flavones are other class of flavonoids present at lower concentration in mandarin. The flavones are diosmin, luteolin, sinensetin and etc [5].

Among nonflavonoids are the phenolic acids, which are frequently bound to sugars or esters in citrus fruits. Chlorogenic acid is the main free phenolic acid in mandarin fruit and detected in both peel and pulp of mandarins. Other phenolic acids are caffeic acid, *p*-coumaric acid, cinnamic acid and etc [5].

Flavonoids play an important role in the prevention of cardiovascular diseases, cancers, and other degenerative diseases [6]. In addition, recent studies have identified antimicrobial and antifungal properties for flavonoids [7]. Flavonoids are important compounds extensively used in food and pharmaceutical industry [8]. Flavonoid constituents in citrus profoundly affect fruit and juice taste and also the human health. For example, naringin, as well as other citrus flavonoids, are of great interest to those in the food and pharmaceutical industries because of their demonstrated antioxidant, anti-inflammatory, antiulcer and cholesterol-lowering effects, as well as their possible beneficial effects on several chronic conditions. Flavonoids may protect plants exposed to biotic or abiotic stresses such as infections, wounding, UV irradiation, ozone, pollutants and other hostile environmental conditions due to their antioxidant and free radical scavenging properties [4].

Mandarin peel and pulp are excellent source of flavonoids. The quantity of flavonoids present in the mandarin fruit is variable and depends upon a number of factors, including: rootstock [9] cultivar [10], and etc.

Several studies have shown that the mandarin cultivars affect the flavonoids in fruits [11- 13].

Aghajanpour *et al*. [11] showed that mandarin cultivars can influence hesperidin and naringin content in peel and pulp. They found the highest of hesperidin in pulp and the highest of naringin in peel and pulp with trees of Satsuma mandarin. Levaj *et al*. [12] showed that mandarin cultivars can influence narirutin and naringin content in pulp. They found that the lowest naringin in pulp of Satsuma mandarin while they found the lowest narirutin in pulp of Clementine mandarin. Bermejo *et al*. [13] showed that Satsuma mandarin (Owari) contained the highest amount of hesperidin and narirutin in the peel, compared with other cultivars. Studies have shown that total flavonoides vary among mandarin species and varieties, and their content is always higher in the peel than in the pulp tissue [5].

It seems that some scions increase the flavonoids in the fruit, so we evaluate the different scions to see if they affect the flavonoids.

Material and Methods

Chemicals and Standards

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

Scions

In 2001, trees 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]. Satsuma mandarin, Mahalli, Younesi, Adib, Clementine and Atabaki were used as scions in this experiment (Table 1).

Table 1 Common and botanical names for citrus taxa used as rootstock and scions

Common name	Botanical name	Parents	Category
Satsuma mandarin (scion)	*C. unshiu cv. Miyagawa*	Unknown	Mandarin
Mahalli (scion)	*C. reticulata cv. Mahalli*	Unknown	Tangerine
Younesi (scion)	*C. reticulata cv. Younesi*	Unknown	Tangerine
Adib (scion)	*C. reticulata cv. Adib*	Unknown	Tangerine
Clementine (scion)	*C. clementina cv. Cadox*	Unknown	Mandarin
Atabaki(scion)	*C. reticulata cv. Atabaki*	Unknown.	Tangerine
Sour orange (Rootstock)	*C. aurantium L.*	Mandarin ×Pomelo	Sour orange

Preparation of Peel and Pulp 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 with 6 treatments including Satsuma mandarin, Mahalli, Younesi, Adib, Clementine and Atabaki and 3 replicates.

Peel and Pulp Extraction Technique

The peel and pulp were extracted according to the method of Chen *et al.* [14] with slight modifications. In order to obtain the phenolic compounds from samples, 0.2 g of dried peel or pulp (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 was performed with an ultrasound cleaning bath-Fisatom Scientific-FS14H (Frequency of 40 KHz, nominal power 90 W and 24 × 14 × 10 cm internal dimension 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.

Determination of Total Flavonoid Content

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 and used to obtain a standard curve at concentrations of 50, 75, 100 and 125 mg/L. 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 total flavonoid content was calculated from calibration curve and the result was expressed as mg rutin equivalent per g dry weight [14].

Determination of Total Phenol Content

The total phenol content was determined by Folin-Ciocalteu's reagent. Standard solutions of gallic acid were prepared by dissolving 6.2 mg gallic acid with 25 ml distilled water and used to obtain a standard curve at concentrations of 0, 62.5, 125 and 250 mg/L. 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 total phenol content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per g dry weight [14].

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.

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.

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.

DPPH Free Radical Scavenging Activity

The free radical scavenging activity was measured according to the method of Umamaheswari and Asokkumar [15] 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.

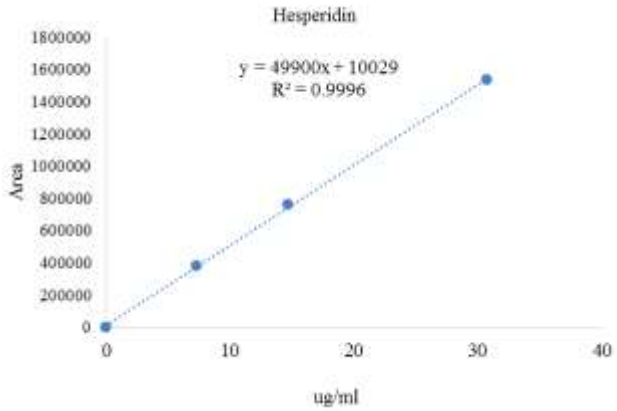

Fig. 1 The standard curve of hesperidin

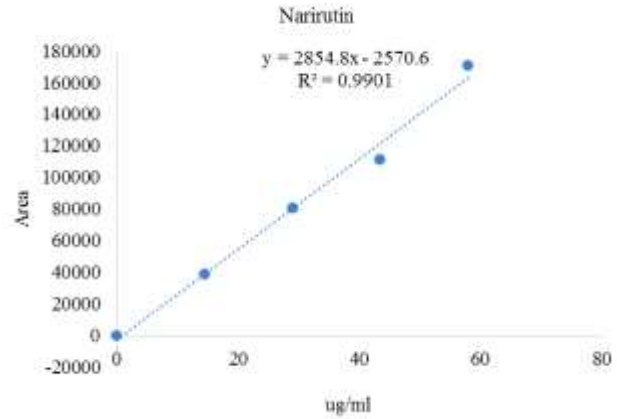

Fig. 2 The standard curve of narirutin

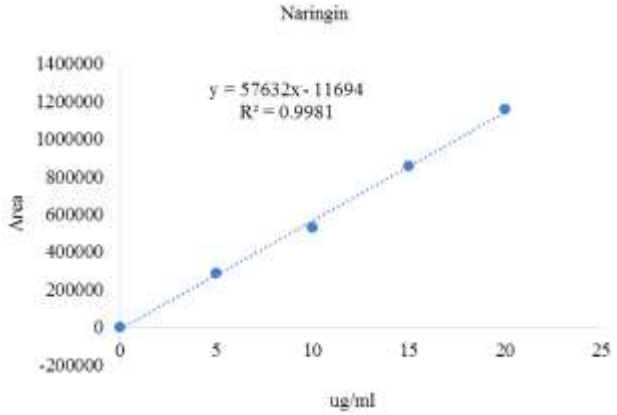

Fig. 3 The standard curve of naringin

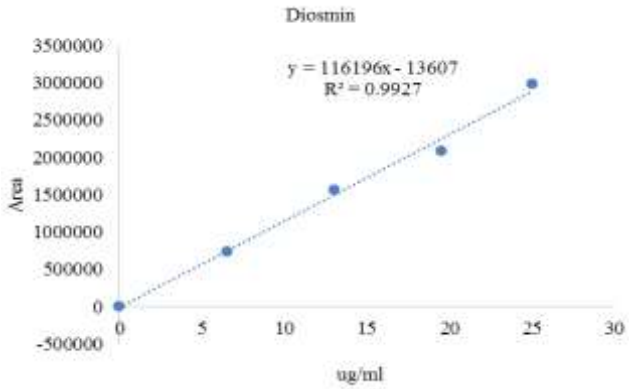

Fig. 4 The standard curve of diosmin

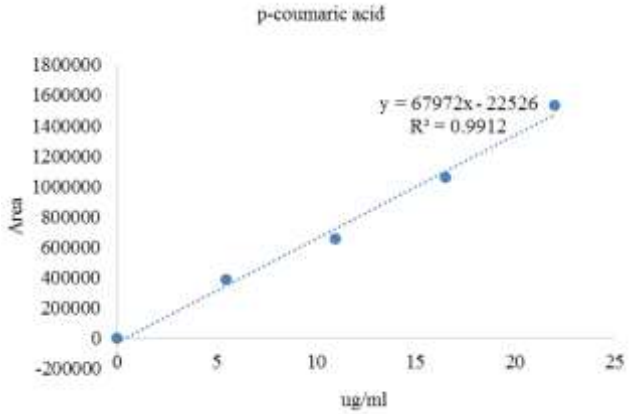

Fig. 5 The standard curve of p-coumaric acid

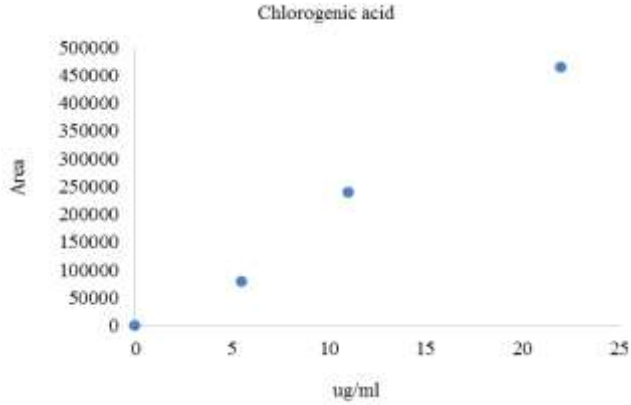

Fig. 6 The standard curve of chlorogenic acid

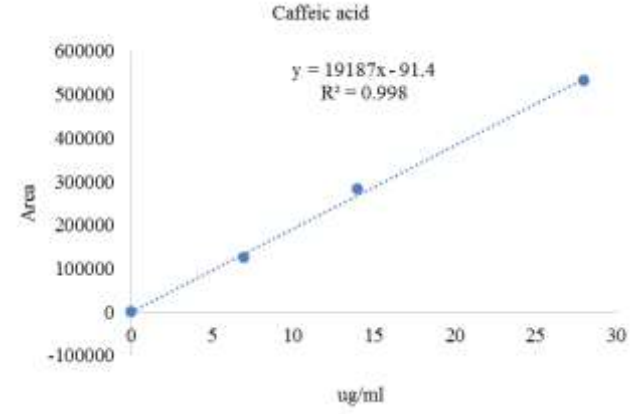

Fig. 7 The standard curve of caffeic acid

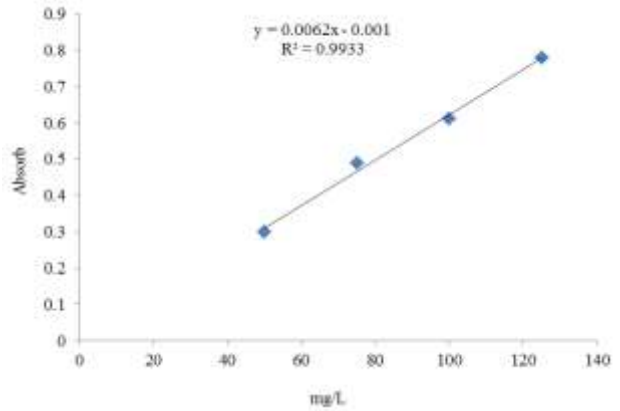

Fig. 8 The standard curve of rutin

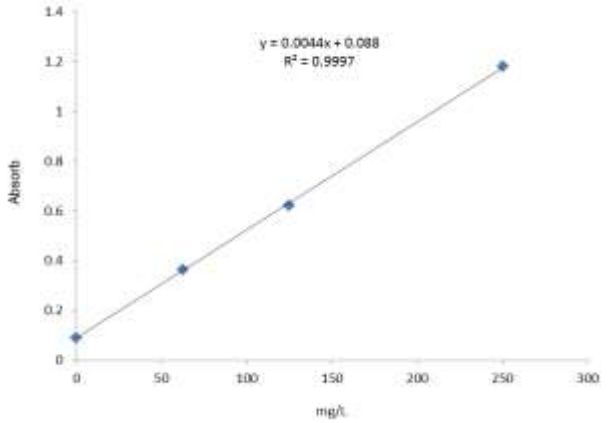

Fig. 9 The standard curve of gallic acid

Results

Peel Compounds of the Mandarin Scions

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

Flavanones

Narirutin, naringin and hesperidin were three flavanones that recognized in this study. The amount of total flavanones ranged from 16.61 to 25.05 mg/g DW for peel. Moreover, the amount of total flavanones ranged from 1.95 to 3.59 mg/g DW for pulp. Hesperidin was the dominant flavanone in this study. For all the flavanones, the differences among mandarin scions were found significant on the 1% level. Fruits of Satsuma mandarin showed significantly increase in hesperidin and naringin but decrease of narirutin. Among six scions evaluated, peel and pulp of Satsuma mandarin indicated the maximum level of flavanones (Table 2 and 3).

Flavones

One flavone identified in peel was diosmin. There was statistically significant difference on the 1% level in diosmin. The total amount of flavones ranged from 0.02 to 0.10 mg/g DW for peel. Among six scions examined, Clementine showed the highest content of flavones (Table 2).

Phenolic Acids

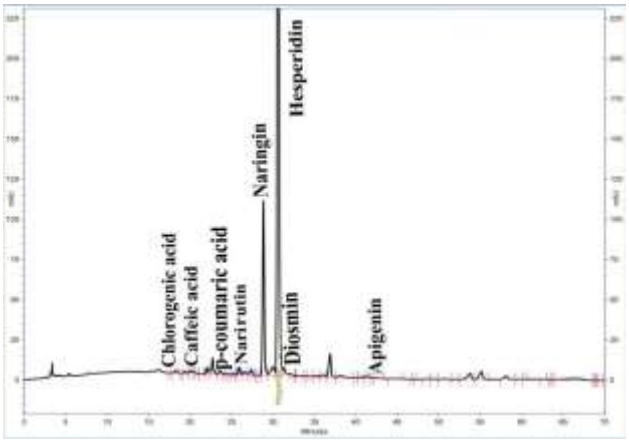

Fig. 10 HPLC chromatogram of peel phenolic components of Satsuma mandarin

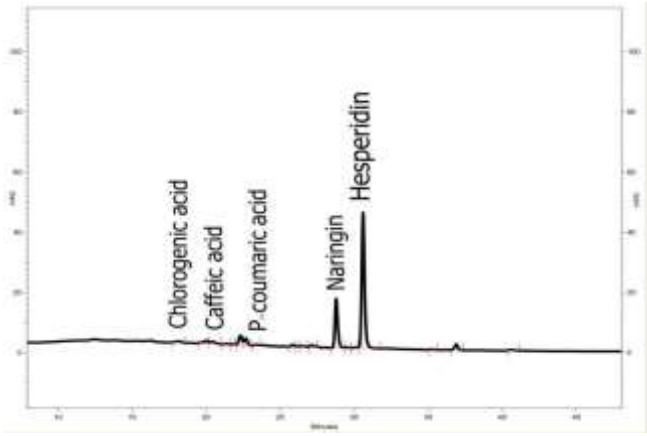

Fig. 11 HPLC chromatogram of pulp phenolic components of Satsuma mandarin

Pulp Compounds of the Mandarin Scions

HPLC analysis of the pulp compounds extracted from mandarin scions allowed identification of 5 phenolic components (Table 3, Fig. 11): 2 flavanones and 3 phenolic acids.

Chlorogenic acid, caffeic acid and p-coumaric acid were three phenolic acids that recognized in this study. The amount of phenolic acids ranged from 0.13 to 1.17 mg/g DW for peel. Moreover, the amount of phenolic acids ranged from 0.04 to 0.34 mg/g DW for pulp. There was statistically significant difference on the 1% level in chlorogenic acid, caffeic acid and p-coumaric acid. The highest chlorogenic acid content was found in peel of Younesi tangerine (1.02 mg/g DW) while the lowest was found in peel of Adib (0.07 mg/g DW) (Table 2 and 3).

Results of Total Flavonoid Content

There was significant difference on the 1% level in the content of total flavonoid. Not only Satsuma mandarin indicated the maximum level of total flavonoid (10.00

mg/g DW) for peel but also indicated the maximum level of total flavonoid (3.73 mg/g DW) for pulp. Moreover, content of total flavonoid in peel was higher than pulp for all scions (Table 2 and 3).

Results of Total Phenol Content

There was significant difference on the 1% level in the content of total phenol. Not only Younesi tangerine showed the highest content of total phenol for peel (4.64 mg/g DW) but also it showed the highest content of total phenol for pulp (2.03 mg/g DW). Moreover, content of total phenol in peel was higher than pulp for all scions (Table 2 and 3).

Results of DPPH Free Radical

There was significant difference on the 1% level in the content of DPPH. The amount of total DPPH ranged from 50.63 to 71.87% for peel. Moreover, the amount of DPPH ranged from 21.12 to 29.43 mg/g DW for pulp. Among six scions examined, Satsuma mandarin showed the highest content of DPPH free radical for peel. Moreover, Mahalli showed the highest content of DPPH free radical for pulp. According to obtained results, content of DPPH in peel was higher than pulp for all scions (Table 2 and 3).

Results of Correlation

Naringin and total flavonoid showed a high positive correlation with hesperidin at about 0.85 and 0.95, respectively. Narirutin showed a negative correlation with hesperidin at about 0.73 (Table 4).

Table 2 Statistical analysis of variation in peel phenolic compounds as affected by different scions

Compounds (mg/g DW)	Satsuma mandarin	Mahalli	Younesi	Adib	Clementine	Atabaki	F value
a) Flavanones							
1) Narirutin (mg/gr DW)	1.25±0.14 c	1.46±0.10 bc	1.88±0.17 a	1.70±0.14 ab	1.86±0.17 a	1.94±0.12 a	F**
2) Naringin (mg/gr DW)	1.53±0.16 a	0.35±0.14 b	0.10±0.02 b	0.20±0.12 b	0.09±0.02 b	0.22±0.10 b	F**
3) Hesperidin (mg/gr DW)	22.27±1.12 a	19.44±0.87 b	16.57±0.78 cd	16.49±0.92 cd	16.41±0.76 cd	14.45±0.81 d	F**
total	25.05±0.42	21.25±1.11	18.55±0.97	18.39±1.18	18.36±0.95	16.61±1.03	
b) Flavones							
1) Diosmin (mg/gr DW)	0.04±0.006 bc	0.02±0.00 c	0.06±0.01 b	0.03±0.00 c	0.10±0.01 a	0.05±0.006 b	F**
c) Phenolic acids							
1) Chlorogenic acid (mg/gr DW)	0.16±0.01 b	0.09±0.02 b	1.02±0.16 a	0.07±0.05 b	0.12±0.03 b	0.15±0.05 b	F**
2) Caffeic acid (mg/gr DW)	0.01±0.00 d	0.05±0.01 b	0.08±0.01 a	0.04±0.01 b	0.00	0.02±0.00 c	F**
3) p-coumaric acid (mg/gr DW)	0.05±0.00 b	0.03±0.00 c	0.07±0.01 ab	0.02±0.00 cd	0.08±0.01 a	0.01±0.00 d	F**
total	0.22±0.01	0.17±0.03	1.17±0.18	0.13±0.06	0.20±0.04	0.18±0.05	
total flavonoid (mg/gr DW)	10±0.85 a	9.01±0.71 ab	7.79±0.71 bc	8.06±0.74 bc	7.32±0.47 bc	7.01±0.67 c	F**
total phenol (mg/gr DW)	3.83±0.50 ab	3.75±0.36 ab	4.64±0.54 a	2.90±0.22 b	3.52±0.47 b	3.58±0.22 b	F**
DPPH %	59.06±1.73 b	71.87±2.61 a	60.31±3.81 b	50.63±2.99 c	53.43±0.54 bc	57.62±2.87 b	F**

Mean is average composition (mg/g DW) in six different scions 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 $P \leq 0.01$.

Table 3 Statistical analysis of variation in pulp phenolic compounds as affected by different scions

Compounds (mg/g DW)	Satsuma mandarin	Mahalli	Younesi	Adib	Clementine	Atabaki	F value
a) Flavanones							
1) Naringin (mg/gr DW)	0.59±0.05 a	0.14±0.02 bc	0.07±0.02 cd	0.11±0.03 cd	0.04±0.02 d	0.10±0.03 cd	F**
2) Hesperidin (mg/gr DW)	3.00±0.16 a	2.49±0.19 ab	2.37±0.26 bc	1.84±0.22 d	2.08±0.28 cd	2.23±0.24b c	F**
total	3.59±0.21	2.63±0,21	2.44±0.28	1.95±0.25	2.12±0.30	2.33±0.27	
b) Phenolic acids							
1) Chlorogenic acid (mg/gr DW)	0.13±0.01 b	0.07±0.01 c	0.25±0.02 a	0.05±0.01 d	0.02±0.01 e	0.04±0.01 d	F**
2) Caffeic acid (mg/gr DW)	0.005±0.00 d	0.04±0.01 ab	0.05±0.01 a	0.02±0.00 b	0.00	0.01±0.00 c	F**
3) p-coumaric acid (mg/gr DW)	0.02±0.00 b	0.01±0.00 c	0.04±0.01 a	0.01±0.00 c	0.02±0.00 b	0.004±0.00 d	F**
total	0.15±0.01	0.12±0.02	0.34±0.04	0.08±0.01	0.04±0.01	0.05±0.01	
total flavonoid (mg/gr DW)	3.73±0.33 a	3.19±0.36 a	2.26±0.26 b	1.92±0.22 b	2.00±0.28 b	2.13±0.29 b	F**
total phenol (mg/gr DW)	1.63±0.16 ab	1.66±0.17 ab	2.03±0.19 a	1.33±0.12 bc	1.22±0.17 cd	1.13±0.19 d	F**
DPPH %	25.86±1.89 ab	29.43±1.92 a	25.76±2.16 ab	24.80±2.30 ab	23.10±2.18 b	21.12±2.02 b	F**

Mean is average composition (mg/g DW) in six different scions 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 $P \leq 0.01$.

Table 4 Correlation matrix (numbers in this table correspond with components mentioned in Table 2)

Traits No	1	2	3	4	5	6	7
1	1.00	-	-	-	-	-	-
2	-0.73**	1.00	-	-	-	-	-
3	0.85**	-0.71**	1.00	-	-	-	-
4	-0.15	0.38	-0.24	1.00	-	-	-
5	0.95**	-0.58**	0.79**	-0.09	1.00	-	-
6	0.26	0.26	0.11	0.70**	0.31	1.00	-
7	0.45	-0.23	0.15	0.06	0.49*	0.51*	1.00

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

1: hesperidin, 2: narirutin, 3: naringin, 4: chlorogenic acid, 5: total flavonoid, 6: total phenol, 7: DPPH

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 [16].

As noted in the present study, the highest hesperidin and naringin were detected in the fruits from the trees of Satsuma mandarin. These Findings were in agree with those of Aghajanpour *et al*. [11] who found the highest of hesperidin in pulp and the highest of naringin in peel and pulp with trees of Satsuma mandarin. In addition, Bermejo *et al*. [13] found the highest hesperidin in peel with trees of Satsuma mandarin compared with other cultivars. These findings supported the results of this study.

According to the results obtained in the present study, content of hesperidin in Satsuma's peel was 22.27 mg/g DW and content of naringin in Satsuma's peel was 1.53 mg/g DW. These results disagreed with the findings of Cano and Bermejo [17], 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.* [12] reported that content of hesperidin and naringin of Satsuma's peel were 42.33 and 28.74 mg/100g DM respectively. Ma et al. [18] reported a level of 821.54 to 1446.05 microgram/g DW for hesperidin and 237.80 to 563.93 microgram/g DW for narirutin in Satsuma's peel. Bermejo *et al*. [13] reported that content of hesperidin of Satsuma's peel was 55.82 mg/g DM. Nogata *et al*. [19] reported that content of hesperidin of Satsuma's peel was 1540 mg/g Fw. These findings disagreed with the results of this study.

As stated in the present study, content of hesperidin and naringin of Satsuma's pulp were 3.00 mg/g DW and 0.59 mg/g DW respectively. These results disagreed with the findings of Xi *et al.* [20], who reported a level of 7.52 mg/g DM for hesperidin in Satsuma's pulp. Aghajanpour *et al*. [11] reported content of hesperidin of Satsuma's pulp 1.80 mg/100g FW**.** Cano *et al*. [21] reported level of hesperidin of Satsuma's pulp from 37.40 to 58.50 mg/100g FW**.** These findings disagreed with the results of this study.

Based on the results obtained in the present study, total flavonoids content in Satsuma's peel was 10.00 mg/g DW. These results disagreed with the findings of Levaj *et al.* [12], 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 lower than previously published data [12]. It might be related to rootstock and environmental factors that could influence the compositions. However, it should be noted that the extraction method might also affected the results.

The presence and concentrations of flavonoids can be affected by the fruit development stage [22]. On other hand, the rootstock plays an important role in the ripening process and the final degree of ripeness [23].

It was observed that the application of organic fertilizer affected the content of flavonoids present in Kacip Fatimah (*Labisia pumila Benth*) [24]. 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 [25]. An increase in the amount of flavonoids, when Satsuma mandarin used as the scion, showed that either the synthesis of naringenin

chalcone was enhanced or activities of both enzymes increased.

According to obtained results it was obvious that there were high positive correlations between total flavonoids and hesperidin. Levaj *et al.* [12] also reported similar results.

High positive correlations between pairs of phenolic compounds indicated a genetic control [26]. Non-significant negative and positive correlation indicated genetic independence. 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 Satsuma mandarin.

Conclusion

The results of this study showed useful information on functional and phytochemical compounds in mandarin cultivars that can be used in pharmaceutical industry and provide valuable genetic resources for breeding programs. In addition, the obtained results may be useful in the evaluation of new dietary supplements and food products. These results are keys to designing future diet studies that examine the role of mandarins in reducing the risk of disease. Finlay, this study increases our knowledge about variation of phytochemical properties including antioxidant activity and phenolic compounds in mandarin fruits among different cultivars and may be useful to producers, breeders and processors. Further research on the relationship between mandarin scions and flavonoids is necessary.

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