The effect of rootstocks on the peel phenolic compounds, cartenoids, chlorophylls and ethylene of Younesi tangerine (*Citrus reticulata*)

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Abstract

Studies have demonstrated that phenolic compounds and carotenoids are essential for prevention of diseases. On the other hand, endogenous ethylene enhances the ripening of fruits and stimulates the biosynthesis of carotenoids as well as chlorophylls destruction. The aim of this study was to evaluate the effect of rootstocks on phenolic compounds and carotenoids. The contents of individual phenolic compounds in peel were evaluated by HPLC. Total flavonoids content was determined using colorimetric assay. Free radical activity was evaluated with DPPH reagent, and total carotenoid and chlorophylls contents were measured using a spectrophotometer. The content of ethylene in fruits was determined by Gas chromatography. HPLC analysis identified seven phenolic compounds in the peel of Younesi tangerine. Hesperidin was the major compound for all rootstocks. Among the six rootstocks studied, flying dragon demonstrated the highest level of phenolic compounds, carotenoids, and ethylene. Findings suggest that the rootstocks can affect the amount of phenolic compounds, carotenoids, and ethylene of the fruit.

Keywords: carotenoids; citrus rootstocks; ethylene; phenolic compounds; Younesi tangerine

Abbreviations: HPLC:High performance liquid chromatography; DPPH: 2, 2-diphenyl-1-picryhydrazyl radical.

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Introduction

In 1968, Younesi tangerine (*Citrus reticulata*) was created from nuclear tissue of Ponkan tangerine by Ramsar Research Institute. It is one of the local tangerines that are widely developed in northern Iran. Although it is an important crop, no study has been done on phenolic compounds, carotenoids, and ethylene of Younesi tangerine.

Phenolic compounds have been classified into two major categories: phenolic acids and flavonoids. All flavonoids can be classified into flavanones, flavones, and flavonols (Malik et al., 2014). Flavanones are known as the dominant flavonoid in citrus fruit and are the most

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

Common name	Botanical name	Parents	category
Younesi (scion)	*Citrus reticulata cv. Younesi*	Unknown	Tangerine
Sour orange (Rootstock)	*Citrus aurantium L.*	Mandarin ×Pomelo	Sour orange
Swingle citrumelo(Rootstock)	*Swingle citrumelo*	*C.paradisi cv. Duncan × P.trifoliata (L.) Raf*	Poncirus hybrids
Trifoliate orange(Rootstock)	*Poncirus trifoliata (L.) Raf*	Unknown	Poncirus
Flying dragon(Rootstock)	*Poncirus trifoliata (L.) Raf*	Unknown	Poncirus
Orlando tangelo(Rootstock)	*Citrus sp. cv. Orlando*	*Citrus reticulata cv. Dancy × Citrus paradisi cv. Duncan*	Tangelo
Murcott(Rootstock)	*Citrus sp. cv. Murcot*	*C.reticulata × C.sinensis*	Tangor

abundant. Hesperetin and naringenin are the most important flavanones in citrus fruit (Bartosz, 2013). Flavonoids exhibit a key function in the reduction of cardiovascular diseases, cancers and other degenerative diseases (Paliyath et al., 2011). Carotenoids are also known to reduce cancers, cataracts, and heart disease (Preedy et al., 2011). Moreover, there are reports on antibacterial and antifungal benefits of flavonoids (Brahmachari, 2012) and carotenoids (Neng-Guo et al., 2010). Flavonoids are extensively applied in the foodstuff and medicine products (Nagy et al., 1977). Carotenoids are also widely used in the foodstuff, cosmetic, and medicine products as natural coloring agent (Rostagno and Prado, 2013).

In citrus fruits, ethylene stimulates ripening by increasing the biosynthesis of carotenoids and chlorophylls destruction (Paliyath et al., 2009). Citrus peel is a fantastic resource of flavonoids and carotenoids. The amount of citrus flavonoids is changeable and is dependent on the rootstock (Legua et al., 2014) cultivar (Milella et al., 2011). A number of research studies have indicated that the rootstocks can influence the flavonoid compounds in citrus (Mashayekhi et al., 2013; Aghajanpour et al., 2015). The aim of this research was to identify rootstock that can synthesize the maximum level of phenolic compounds and carotenoids.

Material and Methods

Chemicals and standards

Hesperidin, naringenin, narirutin, diosmin, caffeic acid, *p*-coumaric acid, Chlorogenic acid, gallic acid standards, 1,1-diphenyl-2-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 plots with three replication at Ramsar Research Station (Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate, average rainfall and temperature 970 mm and 16.25°C per year, respectively and the soil classified as loam-clay with pH ranging from 6.9 to 7). Sour orange, Swingle citrumelo, Trifoliate orange, Flying dragon, Orlando tangelo, and Murcott 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. (2010) with slight modifications. In order to obtain the phenolic compounds from the Peel, 0.2 g of dried peel

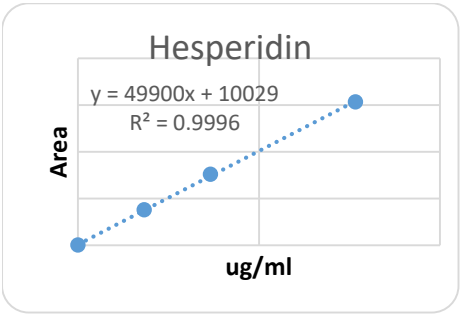

Fig I. The standard curve of hesperidin

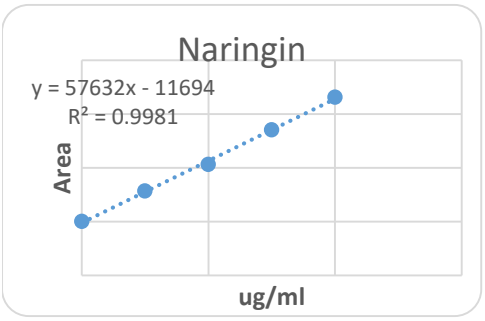

Fig II. The standard curve of naringin

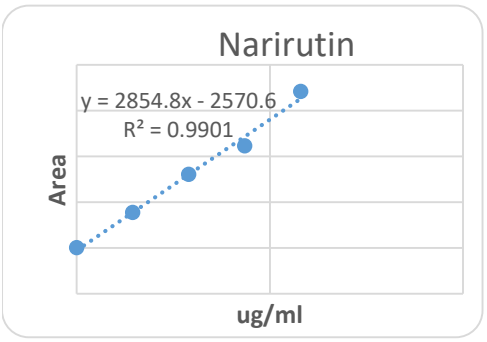

Fig III. The standard curve of narirutin

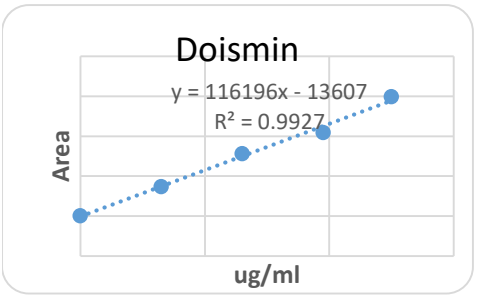

Fig. IV The standard curve of doismin

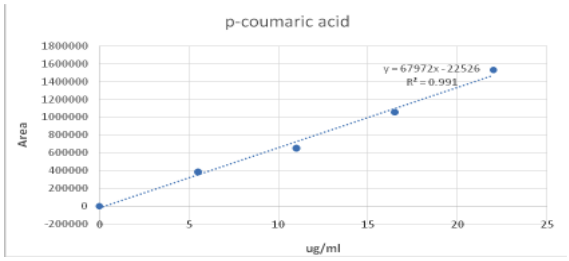

Fig. V. The standard curve of p-coumaric acid

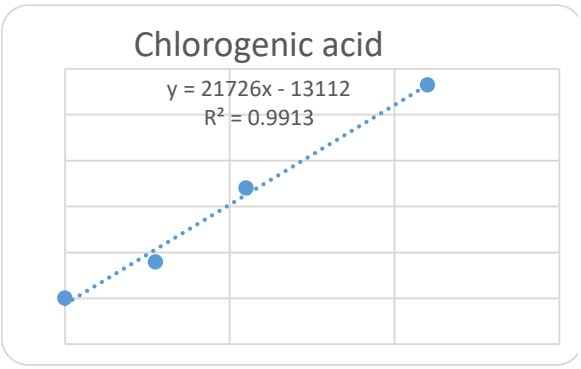

Fig. VI The standard curve of chlorogenic acid

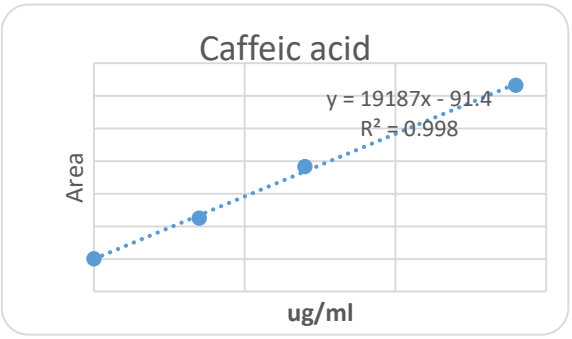

Fig. VII. The standard curve of caffeic acid

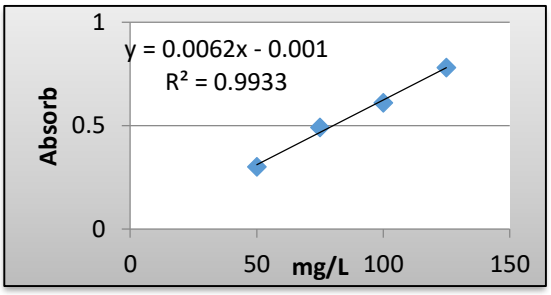

Fig. VIII. The standard curve of rutin

(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 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 sealed vial which was then kept in a 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 a 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. The column temperature was maintained at 25 °C, and the injection volume for all samples was 10 µL. Elution was performed isocratically with the mobile phase consisting of 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, and chlorogenic acid. Chromatograms were also recorded at 283 nm for narirutin, naringin, and hesperidin. Identification of phenolic acids and flavanone glycosides was based on the 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, narirutin, diosmin, caffeic acid, and *p*-coumaric acid in HPLC grade methanol and stored at -20 °C for analyses. Calibration was performed by injecting the standard three times at five different concentrations. Standard solution of hesperidin diluted in ethanol at concentrations of 0, 7.33, 14.67, 22 and 30.69 ug/mL was used to obtain a standard curve.

Standard solutions of naringenin at concentrations of 0, 5, 10, 15, and 20 ug/mL were used to obtain a standard curve. Standard solutions of narirutin at concentrations of 0, 14.5, 29, 43.5, and 58 ug/mL were used to obtain a standard curve. Standard solutions of diosmin at concentrations of 0, 6.5, 13, 19.5, and 25 ug/mL were 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 were used to obtain a standard curve. Standard solutions of caffeic acid at concentrations of 0, 7, 14, and 28 ug/mL were used to obtain a standard curve. Finally, standard solutions of Chlorogenic acid at concentrations of 0, 5.5, 11, and 22 ug/mL were used to obtain a standard curve (Figs. I to VII). 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 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 were used to obtain a standard curve. These solutions 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 solutions and maintained for 5 min. Then, 0.5 ml of aluminum chloride (10%) was added. The solution was kept 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 absorbances of all samples were 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. VIII). The total flavonoid content was calculated from calibration curve and the result was expressed as mg rutin equivalent per g dry weight (Chen et al., 2010).

Determination of total phenol content

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 150 mg/L were used to obtain a standard curve. These solutions were pipetted and diluted to 5 ml with distilled water. Then Folin- Ciocalteau reagent (0.5 ml) was added and the mixture was kept at room temperature for 2 min. Finally, sodium carbonate (5%, 0.5 ml) was added and the mixture was kept 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. IX.). 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 (Chen et al., 2010).

DPPH free radical scavenging activity

The free radical scavenging activity was measured according to the method of Umamaheswari and Asokkumar (2012) with slight modification. Briefly, 0.2 ml of extract was mixed with 2 ml DPPH (2, 2-diphenyl-1-picryl-hydrazyl). The mixture was kept at room temperature for 30 min and the absorbance was measured at 517 nm. DPPH expressed as (%).

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 °C for 4 days to lose all their moisture and then were powdered by a mill. Samples were frozen at –80 °C until analyzed. All extractions were carried out under low light conditions to decrease photo destruction. Briefly, 0.2 g freeze-dried sample was mixed with 10 mL of ethanol solvent (95% v/v), butylated hydroxytoluene (BHT) (100 mgL^{-1}) and diethyldithiocarbamate (DDC) (200 mgL^{-1}). The samples were inverted for two min and kept at 4 °C before they were passed through an ashless filter paper. The filtrates were put in a spectrophotometer and their absorbance was determined at 470 nm, 649 nm, and 664 nm. The concentration of chlorophylls and total carotenoid

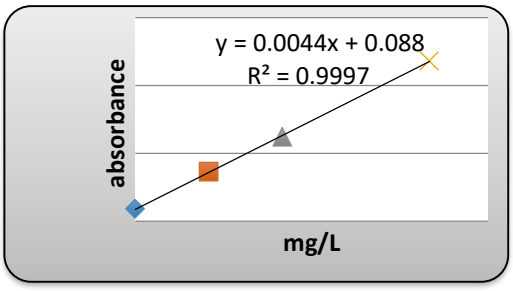

Fig. IX. The standard curve of gallic acid

were calculated by the following formula and 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 placed in a jar. The jar was covered and placed at room temperature for 1h. The temperature was kept constant at 25 °C. The volume of headspace around the fruits was measured. Ethylene was extracted with a 50 mL plastic syringe through the septum of jar. Injection volume was 1 mL.

Analysis of ethylene by GC

An Agilent 7890A gas chromatograph (USA) was used in this study. It was fitted with a HP-5 column. The column temperature was set on 70 °C. The injector temperature was set on 160 °C. The detector temperatures was set at 135 °C. Helium was applied as the carrier gas at a flow rate of 37 ml/min. Ethylene concentration was estimated from calibration curve and the result was displayed as nanoliter per kilogram fresh weight of fruit per hour (nL kg^{-1} h^{-1}).

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variance 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 Younesi tangerine

HPLC analysis of the peel compounds extracted from Younesi tangerine allowed identification of 7 phenolic components (Table 2, Fig. X), including 3 flavanones, 1 flavones, and 3 phenolic acid**.**

Flavanones

Three flavanones identified in this analysis were narirutin, naringin, and hesperidin. In addition, they were quantified from 13.78 to 21.21 mg/g DW. The concentration of hesperidin was higher in our samples. Among six rootstocks examined, flying dragon showed the highest content of flavanones (Table 2).

Flavones

One compound identified in this analysis was doismin. The total amount of flavones ranged from 0.04 to 0.08 mg/g DW. Among six rootstocks examined, flying dragon 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 0.67 to 1.23 mg/g DW. Chlorogenic acid was identified as the major component in this study and was the most abundant. Among six rootstocks examined, Trifoliate orange showed the highest content of phenolic acids (Table 2).

Total flavonoid content

The amount of total flavonoid ranged from 7.60 to 10.58 mg/g DW. Among six rootstocks examined, flying dragon showed the highest content of flavonoid (Table 2).

Total phenol content

The amount of total phenol ranged from 2.11 to 5.01 mg/g DW. Among six rootstocks examined, flying dragon showed the highest content of phenol (Table 2).

DPPH free radical

The amount of total DPPH ranged from 50.63to 65.63%. Among six rootstocks examined, flying dragon showed the highest content of DPPH free radical (Table 2).

Total carotenoid and chlorophylls

The amount of total carotenoid and chlorophylls are given in Table 1. Among six rootstocks evaluated, flying dragon indicated the maximum level of total carotenoid.

Result of the ethylene analysis

GC analyses of fruits allowed for detect ethylene in retention time of 9.43 minutes (Fig. II). Among six rootstocks evaluated, flying dragon indicated the maximum level of ethylene (Table 1).

Results of statistical analyses

Differences in narirutin, naringin, hesperidin, doismin, chlorogenic acid, caffeic acid, p-coumaric acid, total flavonoid, total phenol, DPPH free radical, carotenoids, chlorophyll A, chlorophyll B and ethylene contents were significant at 0.01 probability level (Table 2).

Results of correlation

Simple correllations between 7 components are presented in a correlation matrix (Table 3). Not only hesperidin demonstrated a significant positive correlation with narirutin but also it demonstrated a significant positive correlation with naringin.

Table 2.
Statistical analysis of variation in peel phenolic compounds, carotenoids, chlorophylls, and ethylene of Younesi tangerine on six different rootstocks

	Sour Orange		Swingle Citrumelo		Trifoliate Oange		Flying Dragon		Orlando tangelo		Murcott		
Compounds (mg/g DW)	Mean	St. err	Mean	St. err	Mean	St. err	Mean	St. err	Mean	St. err	Mean	St. err	F value
a) Flavanones													
1) Narirutin	1.88	0.10	1.36	0.07	1.29	0.10	2.89	0.17	2.11	0.15	0.87	0.06	F**
2) Naringin	0.10	0.01	0.09	0.01	0.12	0.01	0.14	0.01	0.19	0.02	0.09	0.01	F**
3) Hesperidin	16.57	0.45	12.65	0.40	16.06	0.46	18.18	0.65	17.25	0.52	12.82	0.41	F**
total	18.55	0.56	14.10	0.48	17.47	0.57	21.21	0.83	19.55	0.69	13.78	0.48	
b) Flavones													
1) Diosmin	0.06	0.01	0.05	0.006	0.05	0.006	0.08	0.006	0.05	0.006	0.04	0.006	F**
c) Phenolic acids													
1)Chlorogenic acid	1.02	0.09	0.59	0.06	0.78	0.07	0.95	0.08	0.73	0.06	0.53	0.06	F**
ffeic acid	0.08	0.006	0.08	0.006	0.39	0.04	0.03	0.00	0.01	0.00	0.03	0.00	F**
3)p-coumaric acid	0.07	0.006	0.10	0.01	0.06	0.006	0.07	0.006	0.08	0.006	0.11	0.01	F**
total	1.17	0.10	0.77	0.07	1.23	0.11	1.05	0.08	0.82	0.06	0.67	0.07	
total flavonoid	7.79	0.41	7.98	0.37	7.63	0.36	10.58	0.40	9.90	0.39	7.60	0.34	F**
total phenol	4.64	0.31	3.86	0.22	4.32	0.25	5.01	0.34	4.85	0.37	2.11	0.15	F**
Cartenoids	0.12	0.01	0.10	0.006	0.09	0.01	0.12	0.01	0.12	0.01	0.06	0.00	F**
Chlorophyl A	0.001	0.00	0.001	0.00	0.03	0.00	0.005	0.00	0.00	0.00	0.04	0.00	F**
Chorophyl B	0.001	0.00	0.002	0.00	0.09	0.01	0.01	0.00	0.00	0.00	0.1	0.01	F**
Total chrophyl	0.002	0.00	0.003	0.00	0.11	0.01	0.01	0.00	0.00	0.00	0.14	0.01	
DPPH free%	60.31	2.20	53.13	1.14	60.00	2.19	65.63	2.27	62.50	2.22	50.63	2.16	F**
Etylen (nL $kg^{-1} h^{-1}$)	23	2.00	11	1.00	14.5	1.00	25	2.00	8	1.00	9.5	1.00	F**

Mean is average composition (mg/g DW) in six different rootstocks used with three replicates. St. err: standard error; F value is accompanied by its significance, indicated by: NS: not significant, *: significant at P≤0.05, **: significant at P≤ 0.01.

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

	Narirutin	Naringin	Hesperidin	Doismin	chlorogenic acid	caffeic acid
naringin	0.58*					
hesperidin	0.84**	0.69**				
doismin	0.42	0.07	0.43			
chlorogenic acid	0.33	-0.33	0.35	0.61**		
caffeic acid	-0.60**	-0.47*	-0.65**	-0.34	0.06	
p-coumaric acid	0.07	0.57*	0.008	0.04	-0.74**	-0.42

*: significant at p≤0.05, **: significant at p≤0.01

Discussion

Our observation that rootstocks had effects on the flavonoids was in accordance with previous findings (Aghajanpour et al., 2015). The compositions of the flavonoid obtained from six rootstocks of Younesi tangerine were very similar. However, the relative concentration of compounds was different according to the type of rootstock. Comparison of our data with those in the literatures revealed some consistency with previous studies (Levaj et al., 2009). This might be related to species, varieties, and environmental factors that could influence the compositions. However, it should be noted that the extraction method might also affect the results. Studies showed that fertilizers and irrigation affect the content of flavonoids in plant (Mirshekari and Farahvash, 2011). 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 is catalyzed by chalcone synthase and chalcone isomerase (Frydman et al., 2004). An increase in the amount of flavonoids, when Flying dragon was used as the rootstock, showed that either the synthesis of naringenin chalcone was enhanced or activities of both enzymes increased.

Cytokinins are known to stimulate the biosynthesis of phenolic compounds (Hacskaylo and Mycorrhizae, 1971). On the other hand, Cytokinins are synthesized mainly in the root system of higher plants and are transported to the shoots through the xylem (Gordon et al., 1984).

Research has shown that ethylene can stimulate the biosynthesis of carotenoids and can reduce chlorophylls of citrus peel (Paliyath et al., 2009). On the other hand, the level of ethylene can also be changed by rootstocks (Babalar and Pirmoradian, 1996).

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) (Yang and Guo, 2014).

Considering the fact 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 and carotenoids were significantly influenced by rootstocks and there was a great variation in most of the measured characters among six rootstocks. The present study demonstrated that the relative concentrations of flavonoids and carotenoids varied according to the type of rootstock. Among six rootstocks examined, flying dragon showed the highest content of flavonoids and carotenoids. The lowest concentrations of flavonoids and carotenoids content were produced by Murcott. Further research on the relationship between rootstocks and flavonoids is necessary.

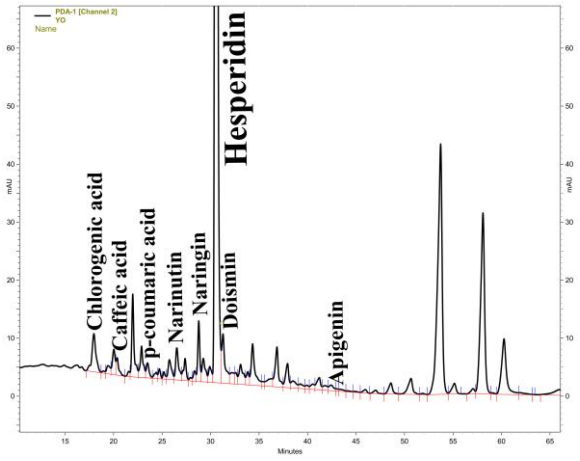

Fig X. HPLC chromatogram of phenolic components of Younesi tangerine

Acknowledgements

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References

Aghajanpour, S.M., A. Ghasemnezhad, M. Faghih-Nasiri and M. Rastegar, 2015. Study the effect of rootstock on the fruit hesperidin and naringin contents of five commercial mandarins. *Plant Products Technology* , (14) 2: 119 -126.

Babalar, M. and M. **Pirmoradian**. 1996. Rootstock effect on ethylene production, fruit size, shape fruit and other qualityparameters during growth period of red delicious apples. *Journal* of *AgriculturalResearch*,27:33-39.

Bartosz, G. 2013. Food oxidants and antioxidants: chemical, biological, and functional properties. CRC Press. Taylor & Francis Publishing Company, Florida.

Brahmachari, G. 2012. Bioactive natural products: Opportunities and challenges in medicinal chemistry. World Scientific, Singapore .

Chen, X., K. Yuan and **L. Hualiang.** 2010. Phenolic contents and antioxidant activities in ethanol extracts of *Citrus reticulata Blanco cv. Ougan* fruit. *Journal of* Food *Agriculture and* Environment, 8 (2): 150-155.

Frydman, A., O. Weisshaus, M. Bar-Peled, D.V. Huhman, L.W.Sumner, F.R. Marin, E. Lewinsohn, R. Fluhr, J. Gressel and **Y. Eyal.** 2004**.** ' Citrus fruit bitter flavors: isolation and functional characterization of the gene Cm1,2 RhaT encoding a rhamnosyltransferase, a key enzyme in the biosynthesis of the bitter flavonoids of citrus' . *The Plant Journal*, 40:88–100.

Gordon, A., J. Stevens and **N.W. Melvin**. 1984.' Fruit set and cytokinin-like activity in the xylem sap of sweet cherry (*Prunus. avium*) as affected by rootstock' , *Physiologia Plantarum*, 61: 464-468.

Hacskaylo, E. 1971. Mycorrhizae: Proceedings, Volume 1. U.S. Forest service: for sale by the supt. of docs. U.S. Govt. Print. Off, Washington.

Legua, P., J.B. Fornerb, F.C.A. Hernándeza and **M.A. Forner-Giner** 2014.' Total phenolics, organic acids, sugars and antioxidant activity of mandarin (*Citrus clementina Hort. ex Tan*.): Variation from rootstock.' Scientia Horticulturae, 174: 60–64.

Levaj, B., V. Dragovic-Uzelac, D.B. Kovacevic and **N. Krasnici**, 2009. ' Determination of flavonoids in pulp and peel of mandarin fruits'. *Agriculturae Conspectus Scientificus*, 74 (3): 221-225.

Malik, A., Z. Erginkaya, S. Ahmad and **H. Erten**. 2014. Food processing: strategies for quality assessment, Springer, New York.

Mashayekhi, K., H. Sadeghi, V. Akbarpour, S. Atashi, S.J.Mousavizadeh, M.Abshaei, and **Z. Nazari**. 2013.' Effect of some citrus rootstocks on the amount of biochemical composition of Parson Brown and Mars Oranges in Jiroft'. *Journal of* Horticultural *Science*,27 (1):9-17.

Milella, L., M.Caruso, F.Galgano, F. Favati, M.C.Padula and **G.Martelli**. 2011.' Role of the cultivar in choosing Clementine fruits with high level of health-promoting compounds.' *Journal of Agricultural and* Food Chemistry, 59(10): 5293–5298.

Muthiah, P.L., M. Umamaheswari and **K. Asokkumar**. 2012.' In vitro antioxidant activities of leaves, fruits and peel extracts of citrus.' *International Journal of* Phytopharmacy, 2 (1):13-20.

Nagy, S., P.E. Shaw and **M.K.S. Veldhui** . 1977.' Citrus science and technology 'Volume 2, Avi Publishing Company, The University of California. Berkeley, California.

Nengguo, T., G. Yumei, L. Yuejin and **G. Fei**. 2010. 'Carotenoids from the peel of Shatian pummelo (*Citrus grandis* Osbeck) and its antimicrobial activity.' *American-Eurasian Journal* of Agricultural & *Environmental* Science, 7(1):110-115.

Paliyath,G., D.P. Murr, A.K. Handa and **S. Lurie**. 2009. 'Postharvest biology and technology of fruits, vegetables and flowers '. John Wiley & Sons, New York.

Paliyath, G., M. Bakovic and **K. Shetty**. 2011. 'Functional foods, nutraceuticals and degenerative disease prevention '. John Wiley & Sons, New York.

Preedy, V.R., R.R. Watson and **V.B. Patel**. 2011. 'Nuts and seeds in health and disease prevention '. Academic Press, London.

Rostagno, M.A. and **J.M. Prado**. 2013. 'Natural product extraction: principles and applications '. Royal Society of Chemistry, London.

Stevens, G. and **M.N. Westwood**, 1984. 'Fruit set and cytokinin-like activity in the xylem sap of sweet cherry (*Prunus. avium*) as affected by rootstock'. *Physiologia Plantarum*, 61(3): 464-468.

Tomala, K., J. Andziak, K. Jeziorek and **R.Dziuban,** 2008. 'Influence of rootstock on the quality of 'Jonagold' apples at harvest and after storage '.*Journal of* Fruit *and Ornamental* Plant Research, 16: 31-38.

Van-Wyka A.A., M. Huysamera and **G.H.Barry**. 2009. '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 Biology and Technology*, 53: 109–116.

Yang, J. and **L. Guo. 2014.** 'Biosynthesis of β-carotene in engineered *E. coli* using the MEP and MVA pathways '. *Microbial Cell Factories*, 13 (160):1-11.