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Variations in antioxidant capacity of nectarine fruits (*Prunus persica* cv. red-gold) affected by harvest date

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Abstract

The influence of harvesting date on some physicochemical properties of nectarine (*Prunus persica* var. Red gold) fruit including fruit weight, pigment content, total carbohydrate, total phenol and antioxidant activity were studied. Fruits were harvested in four different stages; a) two weeks after fruit set, b) pit hardening, c) three weeks after pit hardening and 6 weeks after pit hardening. Results showed that the heaviest fruit either wet or dry was observed in the last harvesting date. The reduction in sucrose at the final harvest might be due to conversion to monosaccharides like fructose or glucose. On the other hand, although the content of total phenolic and flavonoid content of fruits were significantly reduced in the last harvest, no significant reduction was observed in their antioxidant activity. Generally, it can be concluded that compounds like anthocyanin, carotenoids and vitamin C play important role in antioxidant activity in ripened nectarine fruit and their content differ in different harvest times.

Keywords: Nectarine; Harvesting time; Total phenol; Flavonoids; Antioxidant activity.

Introduction

Fruits and vegetables contain significant levels of biologically active components with physiological and biochemical functions which benefit human health. Fruits are generally characterized by their low content of calories and a high amount of antioxidant substances (Nikkhah et al., 2009) which are able to prevent a wide range of pathogenic disorders, such as cancer, cardio-vascular diseases, and degenerative illnesses related to the aging processes. Stone fruits among others play an important role in human health due to their carotenoids and phenolic compounds. Nectarine, even though having a total antioxidant capacity (TAC) lower than some other fruits, such as strawberry, kiwifruit, apple, orange (Szeto et al., 2002), is economically and nutritionally important because they can form a significant component of the diet. Phenolic compounds represent the major sources of

antioxidant capacity in peach and nectarine (Chang et al., 2000). Vitamin C and carotenoids also contribute to their antioxidant activity (Gil et al., 2002; Dalla Valle et al., 2007; Celia et al., 2009). The content of phytochemicals of fruits is influenced by numerous pre-harvest factors, including genotype, rootstock, climate conditions, agronomical factors and harvest time (Cevallos-Casals et al., 2006). During ripening, a large number of biochemical, physiological and structural changes such as changes in skin color, sugar accumulation, contents of organic acids, and development of volatile and aromatic substances, fruit softening take place (Remorini, et al., 2008). The present experiment was carried out to study the variation of antioxidant compounds of nectarine fruits in different developing stages of ripening.

Material and Methods

Eight nectarine (*Prunus persica* cv. red-gold) trees were randomly selected from a 6 years old nectarine garden located in gorgan, northern part of Iran. To have four replications, fruits of every two trees were randomly mixed. Sampling was carried out at four stages. The first stage was conducted exactly two weeks after fruit set (2009/04/21). The second harvest was performed five weeks after fruit set or at pit hardening stage (2009/05/12). Third and fourth harvests were done three (and six weeks after pit hardening stage (2009/06/02), respectively. Fruits were harvested from four different parts on a tree.

Chlorophyll and carotenoid content were measured as follows; 1 gram of finely chopped sample was mixed with 80% acetone and centrifuged at 5000 rpm for 5 min. Then the absorbance of filtered extract was measured at 480, 510, 645 and 663 nm using a UV/Visible spectrophotometer and 80% acetone was used as blank. For measuring of anthocyanin one gram of fresh fruit was extracted with 10 ml acidic methanol at 4 $^{\circ}$C in dark for 24 h. Then the sample was centrifuged at 4000 rpm for 10 min and the absorbance was measured at 520 nm. Soluble sugar was determined based on the method of McCready et al. (1950): 40 mg of sample was added to 5 ml of 80% ethanol and kept in a 70 $^{\circ}$C water bath for 10 min. Then the alcoholic extract was centrifuged at 1000 rpm for 15 min and the supernatant was concentrated to one fifth of initial volume and was kept in refrigerator for sugar calculation. The content of total sugar of samples was calculated according to the method of McCready et al. (1950); 0.2 ml of concentrated extract was mixed with 3 ml of Antron indicator and incubated in a water bath at 100 $^{\circ}$C for 20 min and was measured at 620 nm. The standard curve was drawn using different concentrations of glucose with a glucose free solution as blank. The content of non-reducing sugars including sucrose and glucose were calculated based on the method of Handle (1968). 0.1 ml of concentrated extract was mixed with 30% Potassium hydroxide and incubated in a 100 $^{\circ}$C water bath. After cooling, 3 ml of Antron indicator was added to the sample and the sample was incubated in 40 $^{\circ}$C for 20 min. The absorbance was measured at 620 nm. The standard curve was drawn by preparing different concentrations of sucrose according their absorbance.

To determine the content of total flavonoid, dried powdered samples were extracted at room temperature by methanol percolation. All extracts were concentrated using rotary vacuum evaporator. The obtained extract was freeze-dried for further applications. Colorimetric aluminum chloride method was used for flavonoid calculation (Ebrahimzadeh et al., 2008). Briefly, 0.5 ml of each of the methanolic extract was separately mixed with

1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the solution was measured at 415 nm with a double beam UV/Visible spectrophotometer (Perkin Elmer). Quercetin ranging from 12.5 to 100 mg ml^{-1} in methanol was used for standard curve preparing. Total flavonoid was calculated as milligram equivalent quercetin per gram sample based on the calibration curve.

The content of total phenolic compounds was determined using Folin-Ciocalteau method (Ebrahimzadeh et al., 2008). 0.5 ml of the sample extracts was mixed with Folin-Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min followed by addition of aqueous 4 ml 1 M Na_2CO_3. The mixture was allowed to stand for 15 min and the total phenol content was determined spectrophotometrically at 765 nm. The standard curve was made by dissolving gallic acid to obtain 0, 50, 100, 150, 200, and 250 mg ml^{-1} gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), a widely accepted reference compound.

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used to determine free radical-scavenging activity of the extracts (Ebrahimzadeh et al., 2008). Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 μM). The absorbance of the solution was recorded at 517 nm, after 15 min at room temperature. The experiment was repeated three times. Vitamin C, BHA and quercetin were used as control standards. The IC_{50} values which denote a specific concentration which is required to scavenge 50% of DPPH free radicals, were also calculate.

Completely randomized design with four replications performed in present experiment and the data were analyzed using SAS software. Mean value comparison of data was done through Duncan test at 5% probability.

Results and Discussion

The results showed that, all measured parameters were significantly influenced by harvest date. As presented in table 1, the wet and dry weights of the fruits were dramatically increased by progress in fruit maturity. The highest wet (536 g) and dry weight (74 g) was observed at the last harvesting time. The wet and dry weights of fruits harvested at the first harvest time (two weeks after fruit set) were lower than those of other. The chlorophyll content of fruit was decreased with progress in fruit maturity. During maturity, the increase in the activity of chlorophyll-decomposing enzymes, results in the reduction of chlorophyll content quickly. Contrary to chlorophyll, the carotenoid and anthocyanin contents were at their highest levels in mature fruits. The surprising observation was that no significant difference was between the anthocyanin content of fruits harvested at first and last harvests. Based on our observations, it can be concluded that higher content of anthocyanin at early fruit developing stage could be related to the higher contents of carbohydrate as precursors on anthocyanin. Reduction in anthocyanin content was seen later during pit harvesting stage in which a diminish carbohydrate content in the fruit was obvious. Also the observed reduction in anthocyanin could be related to the dilution effect came from bigger fruit size. Since anthocyanin is structurally sugar in nature, thus fluctuations in carbohydrate contents directly influence its concentration (Hapkines,

1999). This conclusion is in agreement with the findings of Sulfonaly and his co-workers (2006), who suggested that carbohydrates can influence anthocyanin in Arabidopsis directly as a structural constituent and indirectly as a gene activator. Our results showed that the content of sucrose reduced during fruit maturity. It can be concluded that, sucrose converts to monosaccharides such as glucose and fructose with progress in fruit maturity.

Table 1. Effect of harvest date (4 stages) on some physio-chemical properties of nectarine fruit.

	T_1	T_2	T_3	T_4
Fresh weight (g)	42^{d}	194^{c}	361^{b}	596^{a}
Dry weight (g)	5.4^{d}	26^{c}	57^{b}	74^{a}
Fresh weight/ dry weight	3.3^{b}	7.6^{a}	6.3^{a}	7.3^{a}
Chlorophyll^{a*}	0.15^{a}	0.02^{c}	0.1^{b}	0.1^{b}
Chlorophyll^{b*}	0.28^{a}	0.35^{a}	0.15^{b}	0.1^{b}
Chlorophyll^{a+b*}	0.45^{a}	0.02^{d}	0.35^{b}	0.2^{c}
Carotenoid*	0.04^{b}	0.07^{b}	0.08^{b}	0.18^{a}
Anthocyanin**	0.27^{a}	0.19^{b}	0.11^{b}	0.37^{a}
Total sugar***	85^{a}	86^{a}	51^{b}	56^{b}
Sucrose***	52^{a}	57^{a}	41^{b}	30^{c}

T: harvest time, * mg/g fw, ** micro mol/g fw, *** microgram/g fw.

Effect of harvest date on total phenol and flavonoid content of nectarine

The total phenol content of fruit at first harvest was higher (37 equivalent gallic acid/gr dried material) than those of next harvests. It seemed the content of phenolics became lower with delay in harvesting (Table 2). Our results were in accordance with those of Romriny et al. (2008). They reported the more maturity the fruits, the lower the total phenols and vice versa. The reason of this conversion could be due to series of chemical and enzymatic changes like glycoside hydrolysis by glycosidase, oxidation of phenolic compounds by phenol oxidase and polymerization of free phenolic compounds (Romriny et al., 2008). It was proven that the content of phenolic compounds like flavonol and cyanidin-3-glucoside in nectarine gold cultivar, decrease during fruit maturity (Andreoti et al., 2008). They also showed that the content of flavan-3-ols increases at the stage of 40 to 70 days after flowering (DAF) or the end of pit hardening, and gradually decreases during ripening (120 DAF). Reduction in the content of phenolic compounds is a symptom of ripening in most fruits. It seems that the role of phenolic compounds in immature fruits refers to its defense mechanism. The presence of these compounds decreases the edible quality of the fruit and phenolic compounds gradually decreases during ripeness process with the activity of enzymes like phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (CL), and hydroxycinnamoyl CoA: quinate hydroxycinnamoyl transferase (Ding et al., 2001).

The results also indicated that, the total flavonoids gradually decreased as maturity proceeded. The highest content of total flavonoid (14.6%) was recorded at first harvest and the lowest percentage (4.2%) was detected at the last harvest time (Table 2). It can be concluded that there was negative proportion between polyphenolic compounds accumulation and higher temperatures. During early harvests (early spring) a lower daily temperature induces these compounds to accumulate. A similar result was observed in walnut fruit (our unpublished data). In this experiment it has been observed that phenol and

flavonoid contents of walnut green husk reduced in lower temperature. Our conclusion has been confirmed by Keshavakant and Naithani (2007) who showed that low temperature induces the polyphenol accumulation of aerial parts of Sal (*Shorea robusta*) seedling.

Antioxidant activity of nectarine fruits

Since the phenolic content of the fruits reduced by delayed harvest, a reduction in antioxidant activity of the fruit could be expected. On the other hand, increase in the content of compounds like anthocyanins, carotenoids as well as vitamin C could be explained why no reduction in antioxidant activity was observed. It was confirmed that during ripening process the carotenoid as well as vitamin C content of nectarine dramatically increased (Romerini et al., 2008), and same event was confirmed in strawberry by Maas et al. (1995). It can be concluded that, the best explanation for increased antioxidant activity was the presence of pigments and vitamin C in the fruits. However, phenolic and flavonoid compounds did not have an important role as it can be seen from the table 2.

Table 2. Effect of harvest date (4 stages) on the antioxidant activity and the content of phenolic and flavonoids of nectarine fruits.

	T_1	T_2	T_3	T_4
Antioxidant activity ($1/IC_{50}$)	2.3^a	2^a	2^a	1^a
phenol Total*	37^a	22.8^b	23.5^b	17.9^c
Flavonoids**	14.6^a	11.6	6.2	4.2

T: harvest time, * mg gallic acid equivalent/g of powder extract, ** mg quercetin equivalent/g of powder extract.

Influence of chemical composition of the fruits on antioxidant activity

Plants carry a wide range of phenolic compounds. The functions of most of which are not fully understood. Phenolics appear to be by-products of metabolic processes in plants. Our results showed that there was a direct correlation between phenolic compounds and antioxidant activity of fruits (Figure 1). In most cases phenolic compounds are the most important antioxidant agents of plant material. It has been suggested that although the content of phenolic and flavonoid compounds of peels in citrus fruits was higher than pulps in citrus fruits. However, due to higher vitamin C content in pulps the antioxidant activity was higher than that of peels (our unpublished data). Maisuthisakul et al. (2007) showed that there was a strong correlation between phenolic compounds and antioxidant activity of plants. It has also been confirmed that anthocyanin and carotenoids have had important role in antioxidant activity of fruits (Maisuthisakul et al., 2007).

From the biochemical point of view phenolic compounds are produced from the shikimic acid pathway, which occurs in plant respiration process. As phenolic compounds are divided to flavonoid-polyphenols and non flavonoid-polyphenols, therefore it is expected that the higher flavonoid content the higher phenolic compounds and vice versa. From Figure 2 it can be revealed that, there is a positive correlation between total phenol and flavonoid content of fruits (r^2=0.7). High amounts of phenolic and flavonoid compounds can obviously boost antioxidant capacity of fruits, especially when are harvested early in the season (Awad et al., 2001).

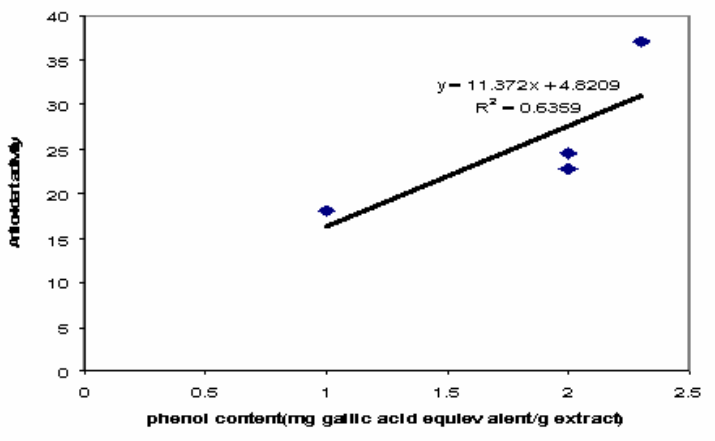

Figure 1. Correlation between total phenol and antioxidant activity in nectarine fruit.

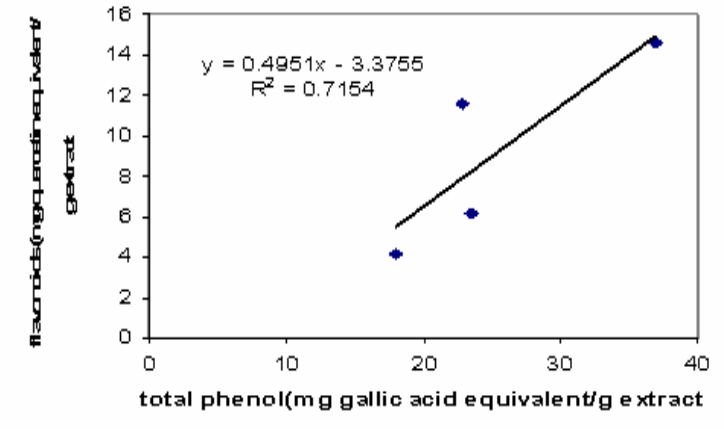

Figure 2. Correlation between phenol and flavonoid contents of nectarine fruit.

Generally, it can be concluded that compounds like anthocyanin, carotenoids and vitamin C play important roles in antioxidant activity of ripened nectarine fruits and their content is strongly depended on harvest date. Thus choosing an appropriate harvesting time can improve the nutritional and functional properties of fruits.

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