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Full Length Research Paper

Influence of harvesting date on some physicochemical properties of nectarine leaf and fruit

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The influence of harvesting date were studied on antioxidant activity and some physicochemical properties of nectarine (cultivar, red gold) leaf and fruit including weight, pigments, carbohydrate and total phenolic and flavonoids contents. Leaves and fruit were harvested in four different stages; two weeks after fruit set (AFS), five weeks AFS or pit hardening (PH), three and six weeks after PH. The results showed that the content of carbohydrates increased gradually as harvesting date delayed. The content of sucrose decreased gradually in the next harvests. The highest carbohydrate contents were observed at the last harvest time. It is suggested that the observed changes may be due to the replacement of sucrose by other kind of carbohydrate like glucose during late stage of ripening. The results showed that although the content of total phenol and flavonoid in fruit were reduced significantly by delaying in harvest time, no significant reduction was observed in antioxidant activity. It is concluded that the other compounds such as anthocyanin, carotenoids and vitamin C have more role in the antioxidant activity of ripened nectarine fruit.

Key words: Antioxidant activity, chlorophyll, flavonoids, harvesting time, nectarine, sugar, total phenols, DPPH.

INTRODUCTION

Fruits and vegetables contain significant levels of biologically active components with physiological and biochemical functions which benefit human health. In recent years, food assumed the status of quality called functional food. Fruit is an excellent food characterized by a low content of calories and a high amount of antioxidant substances which are able to prevent a wide range of pathological states, such as cancer, cardio-vascular diseases and degenerative illnesses connected to the aging processes. Among them, stone fruits play an important role in human health due to the range of phenolic compounds and carotenoids. Nectarine, even though having a total antioxidant capacity (TAC) lower than some other fruits, such as strawberry, kiwifruit, apple, orange (Szeto et al., 2002), are 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 antioxidant activity (Gil et al., 2002). The content of phytochemical compounds of fruits is influenced by numerous pre-harvest factors, including

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Abbreviations: AFS, After fruit set; **PH,** pit hardening; **TAC,** total antioxidant capacity; **BHA,** butylated hydroxyanisole; **SAS,** statistical analysis system; **DAF,** days after flowering; **PAL,** phenylalanine ammonia-lyase; **CL,** coa ligase.

genotype, rootstock, climate conditions, agronomical factors and harvest time (Cevallos-Casals et al., 2006). In nectarine, the time of harvest influences total antioxidant activity strongly, as during ripening a large number of biochemical, physiological and structural changes take place. These include changing in skin color, sugar accumulation, decrease in organic acids and development of volatile and aromatic substances, fruit softening increase in nutritional and healthful compounds (Remorini et al., 2008). Most of the parameters mentioned above are directly or indirectly related to the fruit ripeness. Some compounds like phenol and flavonoid as well as vitamin C have direct role in pharmaceutical aspect of product. There is negligible information about the factors influencing their accumulation in leaf and fruit. The present research was done to determine the accumulation of some phytochemicals of nectarine leaf and fruit based on harvesting date.

MATERIALS AND METHODS

Plants material

Eight nectarine red-gold (cultivar) trees were selected randomly from a 6 years old nectarine garden in Gorgan, Iran. Leaves and fruit were harvested four times and from four different positions of tree. Exactly two weeks AFS the first harvest was executed. The second harvest was performed five weeks AFS or PH stage. Third and fourth harvests were done 3 and 6 weeks after PH, respectively.

Determination of chlorophyll, sugar and carotenoids contents

One gram of well scattered sample was mixed with 30 ml of 80% acetone until lost of its color. Colored solution was filtered through Whatman No. 2 filter paper and then centrifuged at 5000 rpm for 5 min. The filtered solution volume was adjusted to 50 ml with 80% acetone. The absorbance of extract was measured spectrophotometrically at 480, 510, 645 and 663 nm. Acetone 80% was used as blank. Chlorophyll and carotenoid contents were measured according to Mencarelli and Saltveit (1988). Anthocyanin: One gram of fresh fruit was extracted by 10 ml of acidic methanol. It was incubated in a refrigerator for 24 h. Sample was centrifuged in 4000 rpm for 10 min and its absorbance was measured at 520 nm (Warnger, 1979). Soluble sugar was extracted based on the method of McCready et al. (1950). 40 mg of sample was added to 5 ml of 80% ethanol and was transferred to water bath at 70°C for 10 min. The alcoholic extract was centrifuged at 1000 rpm for 15 min and the surfactant was concentrated to one fifth and was used for sugar calculation. The content of total sugar was calculated using method of McCready et al. (1950); 0.2 ml of concentrated extract was mixed with 3 ml of Antron indicator and incubated in water bath at 100°C for 20 min. The light adsorption of samples was measured at 620 nm. The standard curve of spectrophotometer was obtained with different concentrations of glucose. A glucose free sample was used as blank. The content of unreduced sugars (sucrose) of sample was calculated based on the method of Van Handel (1968). 0.1 ml of concentrated extract was mixed with hypochlorite 30% and incubated in water bath at 100°C. After cooling, 3 ml of Antron indicator was added to the sample and the sample was incubated in 40°C for 20 min. The standard curve was drawn with different concentrations of sucrose and light adsorption was recorded at 620 nm. Sucrose free sample was used as blank.

Determination of total flavonoid contents

Samples were dried at room temperature and coarsely ground before extraction. A known amount of samples (50 g) were extracted at room temperature by percolation method using absolute methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained. Colorimetric aluminum chloride method was used for determination of total flavonoids (Ebrahimzadeh et al., 2008). Briefly, 0.5 ml solution of extract in methanol were 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 reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 $\mu g\ ml^{-1}$ in methanol.

Determination of total phenolic contents

Total phenolic content was determined by the Folin-Ciocalteau method (Ebrahimzadeh et al., 2008). The sample (0.5 ml) was mixed with Folin-Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1M) was then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. The results were expressed as gallic acid equivalents. The standard curve was prepared by 0, 50, 100, 150, 200 and 250 mg ml^{-1} solutions of gallic acid in methanol: water (50:50, v/v).

1,1-Diphenyl-2-picryl hydrazyl radical (DPPH) radical-scavenging activity

The stable DPPH was used for determination of free radical-scavenging activity of the extracts (Ebrahimzadeh et al., 2008). Different concentrations of each extracts were added to equal volume of methanol solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, butylated hydroxyanisole (BHA) and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Statistical analysis

The study was performed in complete randomized manner. The experiment was repeated three times. The data were analyzed using statistical analysis system (SAS) software. Averages comparison was done through Duncan test at 5% probability.

RESULTS and DISCUSSION

The results show that the harvest time influenced significantly all measured parameters. As presented in Table 1, the wet and dry weights of fruit were increased dramatically by fruit maturity progressing. The highest wet (596 g) and dry weights (74 g) were observed at the last harvesting time. The rate of wet and dry weights of

Table 1. The effect of harvest time on some physiochemical properties of nectarine leaf and fruit.

Parameter	Fruit				Leaves			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
F.W (g)	42	194	361	596	4.5	9	8.9	9.3
D.W (g)	5.4	26	57	74	1.3	3	2.8	3.3
FW/DW	3.3	7.6	6.3	7.3	3.3	3.2	3.3	2.8
Chl a[1]	0.15	0.02	0.1	0.1	0.07	0.15	0.08	0.5
Chl b[1]	0.28	0.35	0.15	0.1	0.02	0.2	0.17	0.6
Chl a+b[1]	0.43	0.37	0.25	0.2	0.09	0.35	0.25	1.1
Carotenoid[1]	0.04	0.07	0.08	0.18	0.07	0.66	0.67	0.24
Anthocyanin[2]	0.27	0.19	0.11	0.37	-	-	-	-
Total sugar[2]	85	86	51	56	114.7	116	56.7	44.2
Sucrose[3]	52	57	41	30	44.7	71.7	39	26

T; harvest time, FW; fresh weight, DW; dry weight, Chl; Chlorophyll; [1] mg/g FW, [2] μM /g FW, [3] μg.

Table 2. Effect of harvest time on antioxidant activity and the content of phenolic and flavonoids of nectarine leaf and fruit.

Parameter	Fruit				Leaves			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Antioxidant activity [1]	2.3	2	2	1	3.5	3	3.7	6.1
Total phenol [2]	37 ± 1.2	22.8 ± 0.9	23.5 ± 1.4	17.9 ± 0.8	64 ± 2.6	57.2 ± 2.3	70.2 ± 4.1	56.5 ± 2.7
Flavonoids [3]	14.6 ± 0.8	11.6 ± 0.5	6.2 ± 0.2	4.2 ± 0.1	65 ± 2.3	76.2 ± 3.1	79.7 ± 3.6	66.5 ± 1.9

[1] IC_{50} of BHA was 29.3 ± 5.9, vitamin C, 3.7 ± 0.1 and quercetin 3.9 ± 0.2 μg ml^{-1}, respectively; [2] mg gallic acid equivalent/g of extract; [3] mg quercetin equivalent/g of extract.

fruit harvested at first harvest time (two weeks after fruit set) was lower than those of other. As the fruit maturity increased, the chlorophyll content decreased. Generally during maturity, the activity of chlorophyll decomposer enzymes increases and reduces the chlorophyll content fast. Contrary to chlorophyll, the carotenoids and anthocyanin contents of fruit were at its highest level during maturity. There was no significant difference between anthocyanin content at the first and the last harvested dates. High content of anthocyanin at the early time could be related to the fruit size. Fruit at its early stage encounters with a higher content of carbohydrate accumulation and the extra carbohydrate is used as substrate in anthocyanin synthesis pathway.

Reduction in the content of carbohydrate during PH could be the reason of observed low anthocyanin content. Also the observed reduction could be related to the dilution effect induces by increasing fruit size. Since anthocyanin is structurally related to sugar, thus fluctuation in carbohydrate content influences directly its concentration (Hapkins, 1999). This conclusion is in agreement with the finding of (Solfenalli et al., 2006), who indicated that carbohydrates directly as a structural constituent and indirectly as gene activator influence anthocyanin accumulation in Aradobsis. Our results show that the content of sucrose reduces during fruit maturity. It can be assign that, during maturity sucrose converts to other mono saccharides like glucose and fructose. The wet and dry weights of leaves also increased dramatically during fruit maturity. The highest wet (9.3 g) and dry weights (3.3 g) were observed at the last harvesting time. The rate of wet and dry weights of leaves harvested at last harvest time was lower than those of other times. At this time, total chlorophyll (a+b) increased dramatically. During the maturity of fruit, carotenoids contents of leaves were increased and at the end stage decreased dramatically. Leaves at early stage of harvesting time, contained higher content of carbohydrate accumulation. Reduction in the content of carbohydrate occurred during PH. Content of sucrose was reduced in leaves during fruit maturity.

Effect of harvest time on total phenol and flavonoid of nectarine leaf and fruit

The total phenol content of fruit was higher (37 mg gallic acid equivalent /g dried material) at the first harvest than those of next harvests, as its content decreased directly by delayed harvest (Table 2). Our result is in accordance with Remorini et al. (2008). They reported that the higher maturity of fruit cause the lower content of total phenol.

This may be attributed to the series of chemical and enzymatic changes like glycoside hydrolysis by glycosidase, phenolic compounds oxidation by phenol oxidizes and polymerization of free phenolic compounds (Remorini et al., 2008). It has been showed that the content of phenolic compounds such as flavanol and cyaniding-3-glucoside of nectarine gold cultivar decrease during fruit maturity (Andreotti et al., 2008). They also showed that the content of flavan-3-ols increase from 40 to 70 days after flowering (DAF) or end of PH and decreased gradually 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 defense mechanism.

The presence of these compounds is in contrast to edible quality of fruit and gradually decreases during ripeness by the activity of enzymes like phenylalanine ammonia-lyase (PAL), 4-coumarate CoA ligase (CL), hydroxycinnamoyl CoA and quinate hydroxycinnamoyl transferase (Ding et al., 2001). The results of the current study indicated the total flavonoids decreased gradually by progressing of maturity. As the highest content of total flavonoid (14.6 %) was recorded at first harvest and the lowest content (4.2%) was detected at the last harvest time (Table 2). It can be concluded that there is a negative relation between polyphenolic compounds accumulation and high temperature. During early harvests (early spring) a lower daily temperature induces these compounds accumulation. A similar result was observed in walnut fruit. In an experiment it has been demonstrated that the phenol and flavonoid contents of walnut green husk reduced in lower temperature (Ghasemi et al., 2011). 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. In leaves, maximum amount of phenol and flavonoid contents were found in third harvest time (3 weeks after PH stage).

Antioxidant activity of nectarine leaf and fruit

Since the phenolic content of fruit reduced by delayed harvest, a reduction in antioxidant activity of fruit was expected. Increasing the content of compounds like anthocyanin, carotenoids as well as vitamin C could be the main reason why no reduction in antioxidant activity was observed. It has been showed that during ripening the carotenoid as well as vitamin C content of nectarine increased dramatically (Remorini et al., 2008), the event in which confirmed in strawberry by Maas et al. (1995). It can be concluded that the most portion antioxidant activity of matured nectarine related to the presence of pigments and vitamin C. Phenolic and flavonoid compounds do not have important role as it can be seen in Table 2. In leaves, maximum antioxidant activity was in last harvest time (6 weeks after PH stage).

Correlation between antioxidant activity and chemical composition of fruit

Plants contain various classes of phenolic compounds but the functions of most phenolics are obscure. Phenolics appear to be by-products of metabolism in plants. Our results showed that there is a direct correlation (r^2=0.64) between phenolic compounds and antioxidant activity of fruits. In most cases phenolic compounds are the most important antioxidant agents of plant material. In some cases like citrus fruit it has been showed that although the content of phenolic and flavonoids compounds of bark is higher than flashy part, but due to high percentage of vitamin C in flashy part it has higher antioxidant activity than thus of bark (Ghasemi et al., 2009). There are many reports that confirm correlation between phenolic compounds and antioxidant activity of plants (Ghasemi et al., 2009; Ebrahimzadeh et al., 2009). It has been confirmed that anthocyanin and carotenoids also have important role in antioxidant activity of fruits (Maisuthisakul et al., 2007). Phenolic compounds are produced from the shikimic acid pathway, which occurs in plant respiration.

Many other phenolics like cinnamic, p-coumaric, caffeic, ferulic, chlorogenic, protocatechuic and gallic acids also arise from the shikimic acid pathway and subsequent reactions. These are derived from phenyllanine and tyrosine, which are amino acids. Phenolic compounds divided to flavonoid–polyphenols and unflavonoid-polyphenols. Thus, it is expected that the highest flavonoid content is the highest phenolic compounds. There is a direct correlation between total phenol and flavonoid content of fruit (r^2 = 0.71). High amount of phenolic and flavonoid compounds could directly related to antioxidant activity of fruit and is valuable in early harvested fruits which are used as antioxidant source in fruit juice and conserve industry (Awad et al., 2001). Generally, it can be concluded that compounds such as anthocyanin, carotenoids and vitamin C play more important role in antioxidant activity of ripened nectarine fruit and the content of them is strongly depending on harvest date. Thus choosing an appropriate harvesting time could improve the pharmaceutical quality of fruit beside its horticultural quality. There were no correlation between phenol contents of fruit and leaf (r^2 = 0.24). No correlation was found between flavonoid contents of fruit and leaf (r^2 = 0.05). In addition no correlation were found between phenol contents of leaf and its antioxidant activity (r^2 = 0.14) and leaf flavonoid contents and its antioxidant activity (r^2 = 0.23).

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