

Changes in Physical Properties, Chemical Composition and Antioxidant Activity of Four Pistachio Cultivars at Ten Maturity Stages

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ABSTRACT

Although data about the importance of pistachio in human nutrition has extensively increased in the last years, the fruit physical and chemical characteristics of some Iranian pistachio cultivars during fruit ripening have not been evaluated in detail, yet. Thus, the investigation of physicochemical properties and antioxidant activity of four pistachio cultivars ('Akbari', 'Kalleghoochi', 'Ohadi' and 'Sephid') at ten different developing stages, from 11 June to 9 September (10 day intervals), was the aim of the present study. The fresh weight, length and diameter of different cultivars fruit increased significantly throughout fruit maturity and reached the maximum values at last harvesting stage. The shell splitting, early splitting and hull cracking were started since the 6th (31 July), 5th (21 July) and 6th (31 July) sampling dates, respectively. The mentioned parameters significantly showed an ascending trend in all cultivars since above times until the end of the harvesting period. The blank percentage decreased significantly from 4th (11 July) sampling date to the last date of harvesting for all cultivars. Significant decreases in total phenolics, total tannins, condensed tannin and antioxidant activity occurred with the advance in fruit development for all cultivars. Our results provide important information on the changes in physical and chemical properties of pistachio during fruit growth and maturity, which is very useful for determination of the fruit quality. Also, statistically significant differences were observed between different cultivars investigated in all parameters measured. Overall, the kind of cultivar and date of harvest are the important factors determining the physical characteristics, chemical composition and antioxidant activity in pistachios.

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INTRODUCTION

Pistachio (*Pistachia vera* L.) belongs to the Anacardiaceae family, and is one of the important tree nuts. It has been cultivated widely in the Middle East, United States and Mediterranean countries [21]. Iran is one of the most important producers and exporters of pistachio and has the highest cultivation area of this crop in the world. The total pistachio production of Iran was 472,097 tons in 2012 [16], and its production is rapidly increasing year by year. It is widely consumed as a snack food, both raw and roasted, confectionery ingredient in cakes, ice cream, bread and sauces.

The pistachio has been of recent interest for its nutritional, antioxidant activity and its consumers increased considerably. In this sense, pistachio kernels are known to contain considerable of sterols, vitamins (A, B1, B2, B6, etc.,), minerals (Ca, Mg, K, P, Cu, etc.,), fatty acids (oleic acids, linoleic acids, etc.,) and phenolic compounds (anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, isoflavons, flavanones, stilbenes and phenolic acids), which are essential for the human diet [8,18,27,36,44,43,45]. In addition, the green hull of pistachio contains substantial amounts of protein, fatty acids, minerals, vitamins, phenolic compounds and essential oil [10,17]. Recently, the antioxidant and antiproliferative activity of pistachio have been reported [46].

The composition of pistachio nuts may vary depending upon the cultivar type, growing region, climate, maturity and cultural practice [3,28,40,41,44]. Various reports have shown significant variations in protein, fatty acids, pigments, amino acids, sugar, mineral elements, flavonoid, phenolic compounds and antioxidant activity of pistachio cultivars during the years [7,12,21,24,28,38]. These parameters may supply important information to the consumer in terms of recognizing a more nutritional fruit.

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According to Panahi and Khezri [30], determining the optimal harvest date of pistachio nut is one of the most important horticultural practices to increase the quality of nut production. However, little research has been done on physical and chemical properties of pistachio nuts during development. Labavitch *et al.* [25] have evaluated physiological and compositional changes associated with maturation of Kerman pistachio nuts. Changes of lipid content in pistachio seed during development were analyzed by Chahed *et al.* [9]. Panahi and Khezri [30] also have studied effect of harvesting time on nut quality of pistachio cultivars.

Data about the importance of pistachios in human nutrition has increased extensively in the last years. Regarding to this fact that various pistachio cultivars have been grown in different regions of Iran, the physical and chemical characteristics of some pistachio cultivars during fruit ripening have not been evaluated in detail, yet. As far as we know, there are no data in the literature about changes in total tannins, condensed tannin and antioxidant activity during growth and development of pistachio in Iran. Therefore, the objective of our present research was to investigate changes in physicochemical properties and antioxidant activity of four pistachio cultivars at ten different maturity stages.

MATERIALS AND METHODS

The pistachio cultivars studies including: 'Akbari', 'Kalleghoochi', 'Ohadi' and 'Sephid'. In order to conduct this research, four of tree same age (15 years) for each cultivar (total of 16 trees) were randomly selected to represent the population of the plantation from Feyzabad of the Khorasan-e-Razavi province, Iran. Feyzabad is located on the north east part of Iran at $35^{\circ}01$ 'N latitude and $58^{\circ}78$ 'E longitude. The average temperature, the amount of rainfall and relative humidity in growing season of 2013 were 28.65°C, 20 mm and 26%, respectively. Soil characteristics were texture being sandy-loam, EC = 4.12 (ds/m) and soil pH = 8.2. The cultivars grafted on 'Sephid' seedling and were planted in 3*6 m layout. Trees were grown under traditional irrigation and routine cultural practices suitable for commercial fruit production. All cultivars were grown under the same geographical conditions and with the same applied agronomic practices.

The samples were harvested at different developing stages from 11 June to 9 September (10 day intervals). At each sampling time, four clusters were randomly collected from four geographical orientations of each tree. Samples were transferred soon after harvest in plastic bags to the laboratory, where combined and they were used for analysis.

Physical Properties:

The procedure of Panahi and Khezri [30] was adapted for measurements of early splitting, shell splitting, hull cracking and blank percentage. Fruits were weighted individually in the air on a balance of accuracy of 0.001 g. The length and diameter of the fruits were measured with a vernier caliper. The measurement of the fruit length was made on the polar axis, i.e., between the apex and the end of the stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. After measuring the whole fruit size, hulls and shells were manually separated from the fruits, and then the samples of kernel were dried to a constant weight in a vacuum oven at 70 °C. These samples were subsequently used for the chemical analyses.

Total phenolics, total tannins and condensed tannin:

Total phenolics and total tannins were measured using the Folin-Ciocalteu reagent according to the method of Makkar *et al.* [26]. Briefly, 200 mg samples were extracted in 10 ml of 50% methanol for 24 h at room temperature. Then contents were centrifuged $(3000 \times \text{g at 4}^\circ\text{C})$ for 10 min and the supernatant collected and kept in refrigerator (4°C). Non-tannin phenolics were determined following absorption of tannins in total phenolic extract to insoluble polyvinylpyrrolidone (PVPP). After addition of 100 mg PVPP to 100 mm × 12 mm test tubes, 1ml distilled water and 1 ml tannin containing extract were added and vortexed. The tubes were kept at 4°C for 15 min, vortexed again. After centrifugation (3000 × g at 4°C for 10 min), supernatant collected and the phenolic content of this supernatant was defined as the non-tannin phenolics. Total tannins were calculated as the difference between total phenolics and non-tannin phenolics. The results were expressed as mg tannic acid equivalents per 1 g of dry matter. Condensed tannin were analysed according to the method of Porter *et al.* [33] and results were expressed as mg catechin equivalents per 1 g of dry matter.

Antioxidant activity:

Antioxidant activity was assessed according to the method of Ismail *et al.* [20]. Briefly, 1 g of dry matter sample was extracted with 10 ml methanol (85%). 1 ml of this extracts were mixed with 2 ml of 0.15 mM DPPH in methanol. The mixtures were shaken vigorously and left to stand for 30 min (under dark condition). The control was prepared by adding 2 ml of DPPH to 1 ml methanol. Absorbance of the resulting solution was measured at 517 nm by a Cecil 2010 UV–visible spectrophotometer. The reaction mixture without DPPH was

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used for the background correction. The antioxidant activity is expressed in the form of the percentage of free radical scavenging.

Statistical analysis:

This experiment was conducted according to factorial based on completely randomized design with 4 replicates. Data were analyzed by Statistical Analysis System (SAS) software Version 9.1 using analysis of variance (ANOVA) and differences among means were determined for significance at P < 0.05 using Tukey's test.

RESULTS AND DISCUSSION

Fresh weight and dimensions of the fruit:

The changes in fresh weight and dimensions of pistachio cultivars fruit at different maturity stages analyzed are described in Fig. 1, 2 and 3. For all cultivars, the fruit fresh weight increased significantly throughout the sampling dates. The length and diameter of the fruit increased significantly during early 4 sampling dates (from 11 June to 11 July), while the mentioned parameters did not showed significant differences since that time until the end of the harvesting period for all cultivars. The results showed that the significant differences were revealed among the different cultivars for fresh weight, length and diameter of the fruit. The maximum fresh weight and length of the fruit were found in 'Akbari' and the highest diameter of the fruit recorded in 'Kallegoochi'.

The seasonal increase in fresh weight and dimensions of the fruit are typical of the pattern of fruit growth established for many other fruiting crops. A significant increase in fruit fresh weight throughout maturation was also reported for other pistachio cultivars [13,25]; which is in agreement with our data. In this study, the effect of cultivar was significant on the fresh weight, length and diameter of the fruit, as previously observed in other studies [19,29,34,44]. The fruit size (weight and lineal dimensions) is one of the important characteristics that influence consumer preference in pistachio and other types of fruit. According to the current study, 'Akbari' and 'Kalleghoochi' cultivars seems to be the most promising ones, owing to their bigger fruits. Both of the cultivars may be useful especially in developing cultivars with the greater agronomic potential. Kashaninejad *et al.* [21] stated that the designing the equipment for processing, transportation, sorting, separation and storing of pistachio fruits requires information about their physical properties. Therefore, knowledge of this research is particularly relevant in the design or selection of appropriate packaging for pistachio fruit handling and storage.

Early splitting and shell splitting:

The early splitting and shell splitting were started since the 5th (21 July) and 6th (31 July) sampling dates, respectively. The mentioned parameters significantly showed an ascending trend in all cultivars since above times until the end of the harvesting period. Also, a variation in terms of early splitting and shell splitting percentage was observed among the pistachio cultivars and the differences were statistically significant. The maximum and minimum the percentage of early splitting was detected in 'Kalleghoochi' and 'Akbari', respectively. The highest percentage of shell splitting was detected in 'Kalleghoochi' (Fig. 4 and 5).

The shell splitting is an important quality parameter in the marketability of pistachio fruits [2,30,44]. The shells of most pistachio fruits split naturally on the tree before harvest, which are surrounded by a green hull. This green hull usually remains intact through harvest to protect the kernels against pets and pollutants [14,32]. The pistachio fruits that have both split hulls and shells, known as early splits, their kernels are frequently moldy and damaged by insects [13]. Doster and Michailides [13] and Pearson *et al.* [32] also reported that the early split occurred on the tree before harvest. Their findings were in agreement with current research. Our data agree with those reported by Panahi and Khezri [30] who stated that the percentage of early splitting and shell splitting increased significantly during fruit maturity in different pistachio cultivars. According to results, percentage of early splitting and shell splitting were significantly affected by cultivars. This dependence on cultivar agrees well with other reports [1,30,38,39,44].

Hull cracking:

For all cultivars, the hull cracking begun since the 6th (31 July) sampling date; while significantly showed an ascending trend from this time until the end of the harvesting period. Regarding to data observed in the study, there was a significant difference between cultivars in terms of their effects on percentage of hull cracking. The highest percentage of hull cracking was found in 'Kallegoochi', while 'Akbari' had the lowest of hull cracking percentage among the examined cultivars (Fig. 6).

The hulls of pistachio fruits sometimes crack while still on the tree. This cracking is distinct from the early splitting; because in early splitting, the hull split is always along the shell split, whereas the hull cracking occurs after the shell split, and the location of the crack is not along the shell split [14]. It was previously showed that

the hull cracking of pistachio fruit significantly increase with the advance in fruit development. Also, hull cracking was found to be dependent on the cultivar [30]. Their results were consistent with our findings.

Blank:

The onset of blank occurred since the 4th (11 July) sampling date. Since this time until the end of the harvesting period the mentioned parameter significantly showed a descending trend in all cultivars. According to data, a significant variation in percentage of blank was found among the studied cultivars. The highest percentage of blank was observed in 'Kalleghoochi', followed by 'Ohadi' and 'Akbari', while the lowest was in 'Sephid' (Fig. 7).

Blank fruits result when there is fruit set and ovary growth, but the embryo fails to grow, leaving the nut shell empty or blank. Panahi *et al.* [31] stated that the blank percentage deceased significantly during fruit development, which is in agreement with our results. Previous studies have also reported variable ranges of blank percentage for different pistachio cultivars [15,31,39].

Total phenolics:

The content of total phenolics is one of the most important parameters for appraising the characterization of pistachio cultivars, with respect to their nutritional value and potential use for different products. As significant decrease in the level of total phenolics were observed during fruit ripening for all cultivars. Also, significant difference in total phenolics concentration was observed among the studied cultivars. With regards to the observations, the maximum amount of total phenolics belongs to 'Kalleghoochi' and the minimum concentration belongs to 'Ohadi' (Fig. 8).

The decrease in the total phenolic content could be due to the oxidation of phenolic level by polyphenoloxidase that characterizes these stages of maturity [4]. Similar to our present finding, decrease in total phenolics concentration with the advance in fruit development has been reported for other pistachio cultivars [28]. Davarynejad *et al.* [12] have been reported that there were significant differences in the total phenolics content of the pistachio cultivars grown in Iran. These results indicated that the level of total phenolics varied among different cultivars of pistachio and there was a high genetic heterogeneity within the studied cultivars. According to the results, as being a good source of total phenolics, pistachio can be considered as an important nutrient for human health. The results were in agreement with the findings reported by Ballistreri *et al.* [6], Nadernejad *et al.* [28] and Tomaino *et al.* [43].

Total tannins and condensed tannin:

Tannins are secondary metabolites which defend plants from herbivores by protein precipitation and increased acidity. It has been reported that tannins play an important role in human health and are implicated with numerous biological properties. The data of this experiment showed that the total tannins content reduced significantly with the advance in fruit development for all cultivars. There was a significant difference between cultivars in terms of their effects on total tannins amount. The highest content of total tannins was observed in 'Kalleghoohi', followed by 'Akbari' and 'Sephid', while the lowest was in 'Ohadi' (Fig. 9).

Condensed tannins are also known as proanthocyanidins, which are polymeric flavonoid molecules that are found in a range of higher plant species. During fruit maturity, concentration of condensed tannin significantly showed a descending trend in all cultivars. In this study, a variation in terms of condensed tannin content was observed among the pistachio cultivars and the differences were statistically significant. Among the investigated cultivars, 'Kalleghoohi' had the highest level of condensed tannin and 'Ohadi' had the lowest condensed tannin amount (Fig. 10).

A decrease in the total tannins reduces the astringency of fruit, which is a desirable sensory attribute in fruits. A significant decline in total tannins content during fruit development was also reported for persimmon [47] and pomegranate [48]. The decrease in condensed tannin amount may be due to increasing enzyme activity such as anthocyanin synthase (AS) and 3-glycosyl transferase (3GT) in the formation of anthocyanins [35]. Zarei *et al.* [48] reported that the amount of condensed tannin decreased significantly during pomegranate fruit ripening. In regard to the chemical composition, since all four pistachio cultivars used in this research were grown in the same location using similar agronomic practices, the differences in phenolic compounds showed that the genetic variability led to the variation in the biosynthesis of phenolic secondary metabolites in these cultivars.

Antioxidant activity:

The determination of antioxidant activity is one of the ways of expressing the nutritional and biological value of fruits. The DPPH radical scavenging assay is commonly employed to evaluate the ability of antioxidant to scavenge free radicals. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. In this research, the amount of antioxidant activity was reduced significantly throughout the sampling dates for all cultivars. On the other hand, the differences in antioxidant activity among the pistachio cultivars

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were significant. The highest and lowest the level of antioxidant activity was observed in 'Kalleghoochi' and 'Ohadi', respectively (Fig. 11).

Tomaino *et al.* [43] have been reported that the phenolic compounds are responsible for the antioxidant activity of pistachio. The data showed that the antioxidant activity decline along with deceases of total phenolics level during fruit development. Thus, it can be concluded that antioxidant activity is closely correlated with the total phenolics content. Previous studies have also found a close correlation between antioxidant capacity and total phenols in plum [11,22], olive [37], nuts such as; walnut, hazelnut and pistachio [5] and pomegranate [42]. Therefore, it seems that the decline in antioxidant activity during pistachio fruit maturity was probably due to a decreased concentration of phenolic compounds. In our data, level of antioxidant activity was significantly affected by cultivars, as previously observed in other studies [12,16]. Regarding to results observed in the study, cultivar type plays an important role in antioxidant activity.



Fig. 1: Effect of different sampling dates on the fruit fresh weight of four pistachio cultivars. The vertical bar is standard error (SE).



Fig. 2: Effect of different sampling dates on the fruit length of four pistachio cultivars. The vertical bar is standard error (SE).



Fig. 3: Effect of different sampling dates on the fruit diameter of four pistachio cultivars. The vertical bar is standard error (SE).

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Fig. 4: Effect of different sampling dates on the early splitting of four pistachio cultivars. The vertical bar is standard error (SE).



Fig. 5: Effect of different sampling dates on the shell splitting of four pistachio cultivars. The vertical bar is standard error (SE).



Fig. 6: Effect of different sampling dates on the hull cracking of four pistachio cultivars. The vertical bar is standard error (SE).

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Fig. 7: Effect of different sampling dates on the blank of four pistachio cultivars. The vertical bar is standard error (SE).



Fig. 8: Effect of different sampling dates on the kernel total phenolics of four pistachio cultivars (The onset of kernel filling occurred between the 3th (1 July) and 4th (11 July) sampling dates). The vertical bar is standard error (SE).



Fig. 9: Effect of different sampling dates on the kernel total tannins of four pistachio cultivars (The onset of kernel filling occurred between the 3th (1 July) and 4th (11 July) sampling dates). The vertical bar is standard error (SE).

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Fig. 10: Effect of different sampling dates on the kernel condensed tannin of four pistachio cultivars (The onset of kernel filling occurred between the 3th (1 July) and 4th (11 July) sampling dates). The vertical bar is standard error (SE).



Fig. 11: Effect of different sampling dates on the kernel antioxidant activity of four pistachio cultivars (The onset of kernel filling occurred between the 3th (1 July) and 4th (11 July) sampling dates). The vertical bar is standard error (SE).

Conclusion:

The changes in the physical and chemical characteristics of pistachio, from fruit set to ripening, clearly explained its growth, development and ripening stages. These results provide important knowledge of changes in total phenolics, total tannins and condensed tannin during fruit growth and maturity, emphasizing that pistachio fruit can be a good source of bioactive compounds. On the other hand, this study showed that there were significant differences among the cultivars in all parameters measured. Thus, the kind of cultivar and date of harvest are the important factors determining the physical characteristics, chemical composition and antioxidant activity in pistachios. Overall, these data can be useful for determination of the fruit quality, developing fruit processing industry and selection of superior desirable pistachio genotypes for bringing to commercial cultivation. However, there are many other cultivars in Iran, more studies of physical and chemical properties are required for them.

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