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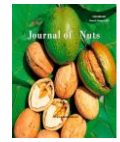

Effects of Location in the Tree Canopy on Some Quality Characteristics of Fresh Pistachio Fruit

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A R T I C L E I N F O

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A B S T R A C T

Fresh pistachio fruit cv. Kalleghochi was harvested from the exterior and interior parts of the tree canopy in four geographical directions. The fruit position in exterior and interior parts of the tree canopy has a significant influence on the number of nuts per ounce, pistachio splitting, hull weight, shell weight, kernel weight, colour indices and total anthocyanin content. Results indicated that the number of nuts per ounce, pistachio splitting, kernel weight, red skin colour (a^*) and total anthocyanin content of outer-canopy fruits were higher than the fruits from inner-canopy fruits. Pistachio splitting was highest (98.6%) in outer-canopy in comparison to inner-canopy (50.3%) fruits. Results also indicated that the sunlight exposure increased red colour development and accumulation of anthocyanins. The total anthocyanin contents of hull in outer-canopy were 91% higher than those in inner-canopy fruits. Therefore, phenolic accumulation in pistachio hull may be taken into consideration as an important source of functional compounds in food systems with health promoting effects and antimicrobial activity. Results of this study suggest that the fruit position within the canopy is an important factor in determining physicochemical characteristics of pistachio fruit.

Introduction

Iran is one of the important pistachio (*Pistacia vera* L.) producers in the world (about 478600 tons in 2013). Production is mainly done in the provinces of Kerman, Khorasan, Fars, Semnan, Markazi, Sistan and Baluchestan, Qazvin, Isfahan, Yazd, and Qom. Kerman Province provides 67% of total Iranian pistachio (Rafiee *et al.*, 2017; Taghizadeh-Alisaraei *et al.*, 2017).

Pistachio nut as a unique functional food is a rich source of unsaturated fatty acids and phenolic compounds (Tomaino *et al.*, 2010). In addition,pistachio hull contains substantial amounts of protein, fatty acids, minerals, vitamins, and phenolic compounds (Barreca *et al.*, 2016; Goli *et al.*, 2005; Rajaei *et al.*, 2010).

Phenolic compounds have shown high antioxidant activity, as well as chemopreventive, cardioprotective and vasoprotective properties (Tomaino *et al.*, 2010). Pistachio hull has been included in 35-40% of waste product of the pistachio industry, having scarce use (Barreca *et al.*, 2016; Rajaei *et al.*, 2010).

Pistachio tree is a highly tolerant plant to saline soils and grows in hot and arid conditions (Tsantili *et al.*, 2011). Physical and chemical changes of fruits dur-

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ing the ripening period are affected by variety or genetic make-up, growing region, environmental parameters (light, temperature, relative humidity), agronomic practices and ripening stage of fruit (Akhavan *et al.*, 2015; Arena *et al.*, 2017; Nuncio-Jáuregui *et al.*, 2014).

The location within tree canopy influences microclimate of individual fruit as well as their external and internal qualities (Feng *et al.*, 2014). Sun exposure affects the photosynthetic efficiency of leaves (Arena *et al.*, 2017), along with fruit quality parameters including fruit ripening, fresh weight, pH, total titra acidity, total soluble solids, aroma and flavor (Arena *et al.*, 2017; Feng *et al.*, 2014; Nuncio-Jáuregui *et al.*, 2014), external or internal colour (Arena *et al.*, 2017; Nuncio-Jáuregui *et al.*, 2014; Unuk *et al.*, 2012), phenolic compounds and antioxidant activity (Arena *et al.*, 2017; Drogoudi and Pantelidis, 2011; Feng *et al.*, 2014; Nuncio-Jáuregui *et al.*, 2014), carbohydrates, amino acids (Feng *et al.*, 2014); organic acids (Arena *et al.*, 2017; Feng *et al.*, 2014; Nuncio-Jáuregui *et al.*, 2014), and lipid composition (Pannico *et al.*, 2017). In this regard, the synthesis of phenolic compounds is influenced by genetic background, development stage of fruit and environmental factors such as nutrient availability, temperature and light in particular (Hagen *et al.*, 2007).

Factors affecting nutritional value, chemical compositions and quality characteristics of pistachio nut and hull have not yet been fully established. Many studies have focused on studying physicochemical characteristics of hull (Barreca *et al.*, 2016; Goli *et al.*, 2005; Rajaei *et al.*, 2010; Tomaino *et al.*, 2010) and nuts (Arcan and Yemenicioğlu, 2009; Kashaninejad *et al.*, 2006; Tomaino *et al.*, 2010; Tsantili *et al.*, 2011), comparing different cultivars or growing areas (Bellomo and Fallico, 2007), and agronomic practices (Salarizadeh *et al.*, 2016). It should be noted that in different plant species, physicochemical properties are affected by a number of environmental factors (sunlight exposure, UV radiation, and temperature and water availability) and agronomic practices (Arena *et al.*, 2017).

Although pistachio hull contains substantial amounts of phenolic compounds with antioxidant properties, it is considered as a waste product. In recent years, the potential of hull extract has become a major focus of research for use in pharmaceutical and food applications (Goli *et al.*, 2005; Rafiee *et al.*, 2017; Rajaei *et al.*, 2010). Scientific studies suggest that phytonutrient-rich products can be beneficial to health.

The location of fruit in the tree canopy or the intensity of received light has an important role in the qualitative characteristics (colour, size, sugar content and etc.) of fruit (Unuk *et al.*, 2012). The positive relationship between the fruit location in the tree canopy with temperature and light intensity has been reported. The accumulation of these small variations during fruit development can cause significant changes in fruit quality characteristics, particularly their colour and phenolic compounds (Unuk *et al.*, 2012). Therefore, the aim of this study was to find how the fruit location in the tree canopy affects some quality characteristic of pistachio at the commercial harvest of tested cultivar.

Materials and Methods

Plant material and experiment

Commercially matured fresh pistachio fruits were harvested in September 2016 from different mature trees (14-year-old) randomly from the Agricultural Research Center of the Shahid Bahonar University of Kerman. The distance between the trees was 3 × 6 m. Trees were grown under the same geographical conditions, traditional irrigation and the same agronomic practices. Fruits were collected from the interior (inner branches as shaded regions) and exterior (outer branches as sun-exposed regions) portions of tree canopy in four geographical directions (north, south, west and east). Five replicates of 100 fruits each from five trees (20 fruits per tree) were collected from each canopy position. Approximately, 5 kg of pistachio fruit was sampled, and immediately transported by a ventilated car to the laboratory. The pistachio samples were cleaned to remove damaged and sunburned pistachio and kept at 4 °C until analysis.

Physical properties

Fresh fruits were weighed using a digital analytical balance (Adam equipment, PGW 4502i, USA, accuracy ±0.001 g) in 5 replicates for fresh unhulled pistachio, soft hull, hard shell and kernel. The percentage of closed- and open-shell pistachio was determined. In addition, nuts without soft hulls were dried in a hot-air oven (Memmert, Germany) at 70 °C for 48 h and weighted for calculating the number of nuts per ounce.

Moreover, surface colour of the fresh unhulled pistachio was measured using a colourimeter (TES 135A, TES Co., Taiwan) to determine L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) indices (Radi *et al.*, 2017).

Total anthocyanin content

Total anthocyanin content of hull was estimated using the method of Wagner (1979). The frozen pistachio hull samples (0.1 g) were soaked immediately in 10 ml of acidified methanol (methanol: HCl 99:1 v/v); then crushed using a glass pestle and kept at 25°C for 24 h in the dark. After centrifugation (4000 g for 5 min), the absorption at 550 nm of the supernatant was measured by a UV-VIS spectrophotometer. Anthocyanin contents were calculated using an extinction coefficient of 33000 M^{-1} cm^{-1}; and the results were expressed as mg g^{-1} fresh weight.

Statistical analysis

Results were statistically assessed with the one-way analysis of variance (ANOVA) using Duncan multiple range test ($p < 0.05$) as post-hoc test for mean separation. All analyses were performed using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA). Data were presented as the means ± SD of five replicates.

Results

Number of nuts per ounce

Fig. 1 shows that the simple and interaction effects of light exposure and geographical direction are significant in the case of number of nuts per ounce ($p < 0.05$). Number of nuts in the outer-canopy (sun-exposed) and southern side was 21.9 per ounce (the largest fruit size); while number of nuts in the outer-canopy and northern and western sides were 25.3 and 25.1 per ounce (the smallest fruit), respectively.

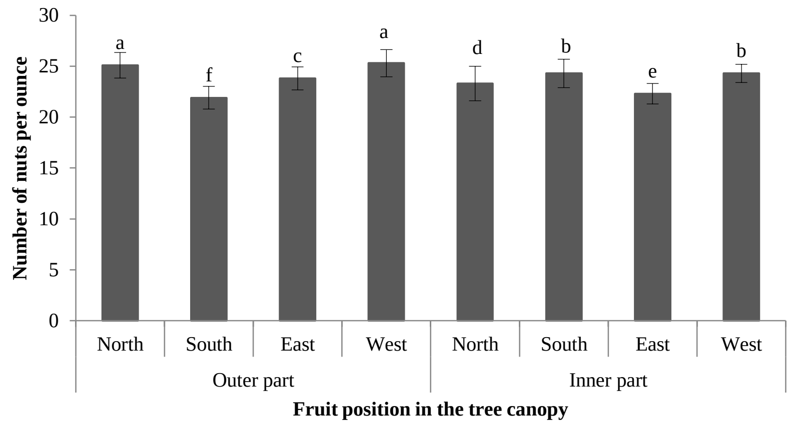

Fig. 1. Effect of fruit location in tree canopy on the number of nuts per ounce of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

Percentage of open-shell pistachio (pistachio splitting)

The opened shell is a qualitative factor of interest in world trade of pistachio nuts. Based on the results, the percentage of open-shell fruit (pistachio splitting) is generally higher in outer-canopy fruit than in the inner-canopy fruit (Fig. 2). The highest percentage of open-shell pistachio (98.6%) was observed in outer-canopy and the northern side of the tree canopy; while the lowest percentage (50.3%) was obtained in inner-canopy and the eastern side of the tree canopy. In terms of geographical directions, it was found that the eastern side of tree canopy had the lowest amount of open-shell pistachio nuts.

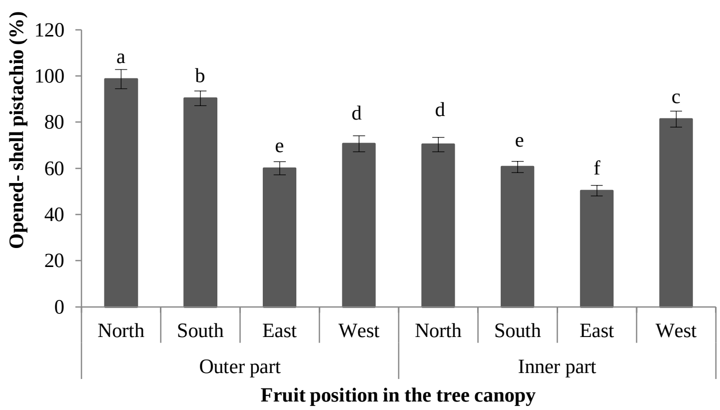

Fig. 2. Effect of fruit location in tree canopy on the pistachio nut splitting of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

The amount of fresh pistachio hull (soft shell)

Based on the results (Fig. 3), light exposure and geographical direction significantly affected the hull weight of raw pistachio fruits ($p < 0.05$). Comparison of the simple main effects (light exposure and geographical direction) showed that the weight of hull was significantly higher in the inner-canopy than in the outer-canopy of pistachio trees. Interaction effect of light exposure by geographical direction revealed that the fruits which were located in the inner-canopy and eastern side had the highest hull weight (1.7 g); while the fruits in outer-canopy and southern side had the smallest hull weight (1.3 g).

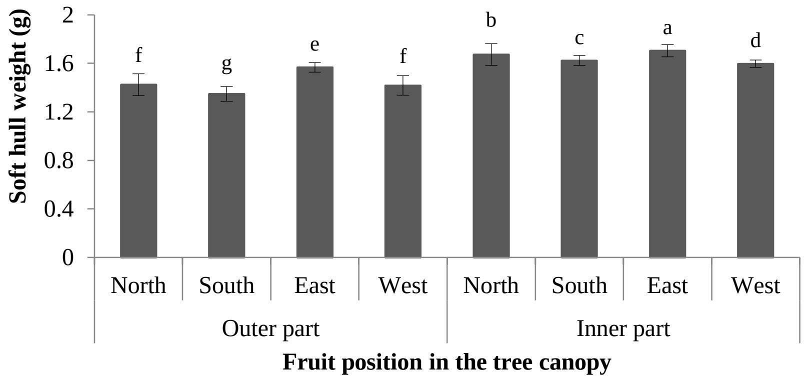

Fig. 3. Effect of fruit location in tree canopy on the hull weight of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

The amount of fresh pistachio hard shell

In terms of pistachio hard shell weights, simple main and interaction effects were found to be significant at $p < 0.05$. The hard shell weights of inner canopy fruits were 2-times higher than the outer-canopy fruits (Fig. 4). In terms of geographical direction, the hard shell weights of the eastern side of the tree canopy were higher than the other directions. Based on the results, the lowest pistachio hard shell weight (0.621 g) was observed in the sun-exposed and southern side of tree canopy. On the other hand, the pistachio fruit located in shaded regions and eastern side of tree canopy had the highest pistachio hard shell weight (0.742 g).

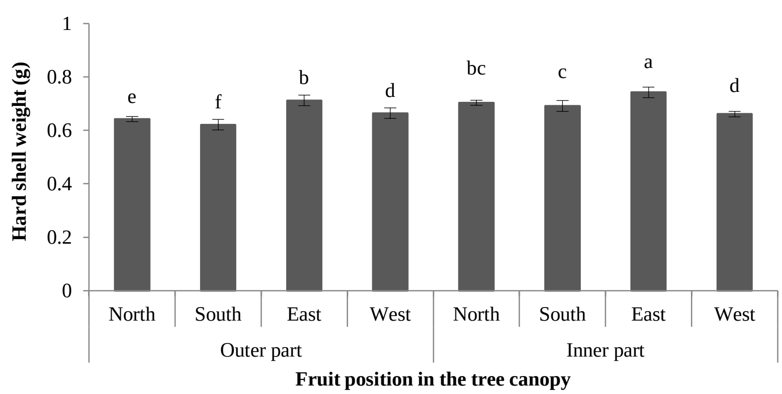

Fig. 4. Effect of fruit location in tree canopy on the hard shell weight of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

The amount of pistachio kernel

Comparison of kernel weight showed that the interaction effect of light exposure by geographical direction is not significant ($p > 0.05$), but their main effects are significant ($p < 0.05$). Weight of pistachio kernels in outer-canopy was significantly higher (6.6%) than the kernels located in the inner parts of the tree canopy ($p < 0.05$). In terms of geographical directions effect, the highest kernel weight (0.861 g) and lowest kernel weight (0.820 g) were observed in the eastern and western sides of the tree canopy, respectively (Fig. 5).

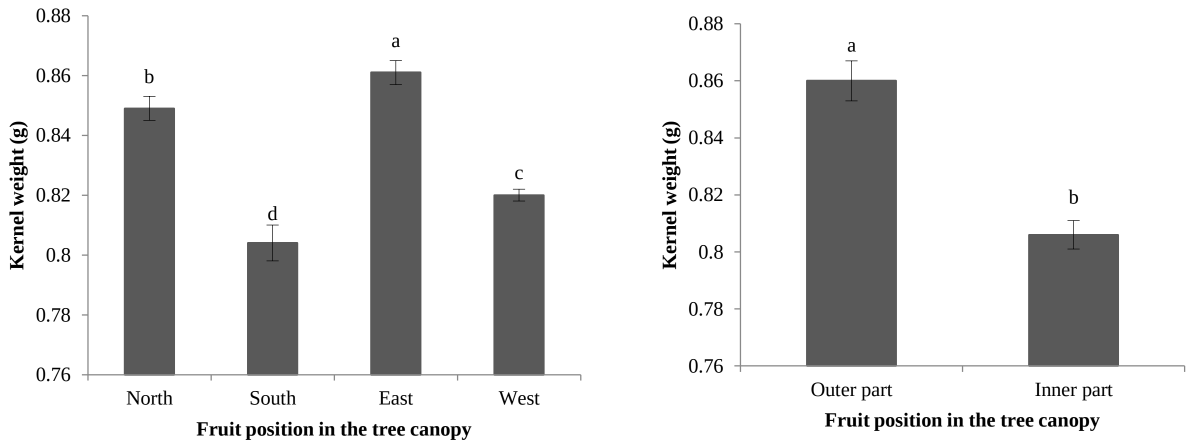

Fig. 5. Effect of fruit location in tree canopy on the kernel weight of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

Hunterlab indices

Colour is an important factor in the perception of quality characteristics of fruit. Fig. 6 demonstrates the changes in surface colour of the fresh pistachio fruits located in different parts of tree canopy, as given by L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). The results revealed that the fruit position on the tree canopy significantly affected colour indices ($p < 0.05$). The simple main effects and interaction of light exposure by geographical directions showed significant difference in the L* values ($p < 0.05$). Although the fruit location in interior and exterior parts of the canopy significantly affected the colour indices of a* and b*, the effect of geographical directions and their interaction (light exposure × geographical direction) was not significant ($p > 0.05$). The fruit located in outer-canopy had the higher a* and lower L* and b* values compared to inner-canopy, that indicate higher red hull colour of exterior fruits.

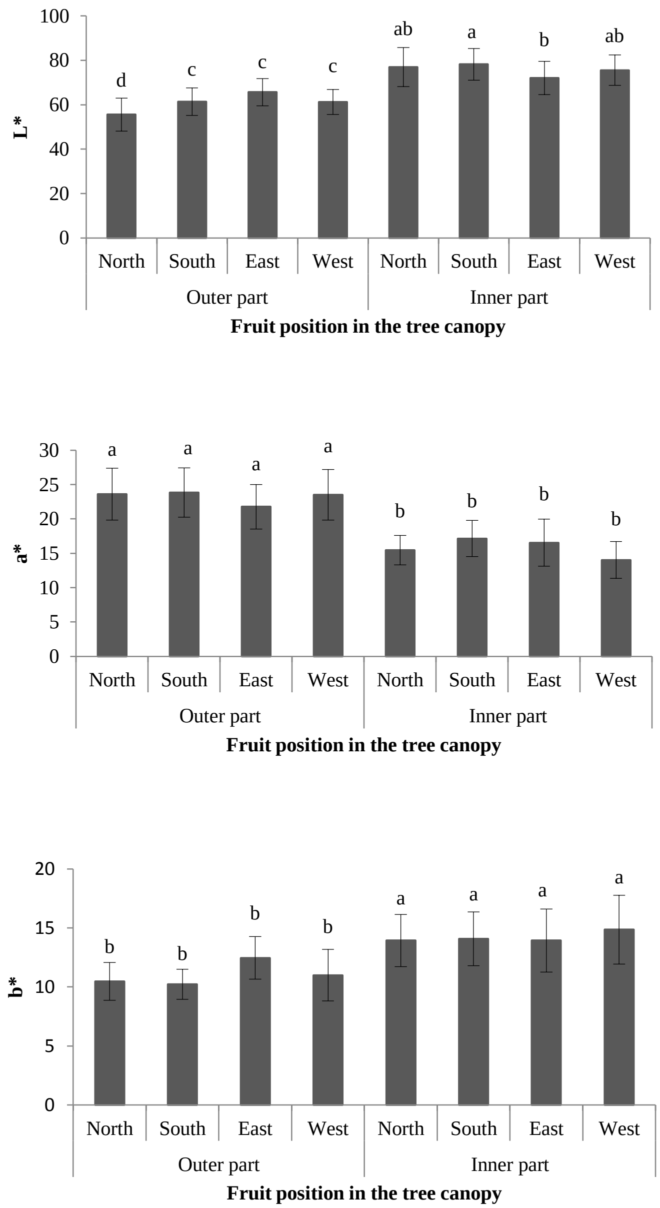

Fig. 6. Effect of fruit location in tree canopy on the colour indices (L*, a* and b*) of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

Total anthocyanin content

Analysis of variance indicates that only the simple main effect of light was significant for anthocyanin concentration in pistachio soft hull. Nevertheless, the simple main effect of geographical positions and the interaction of light exposure by geographical direction was not significant ($p < 0.05$). The mean comparison indicated the significant effects of sunlight on the increase of soft hull anthocyanin content ($p < 0.05$). The total anthocyanin contents of the fruit hull in the outer-canopy parts were 91% higher than the fruit hull located in the inner-canopy parts of the tree canopy (Fig. 7).

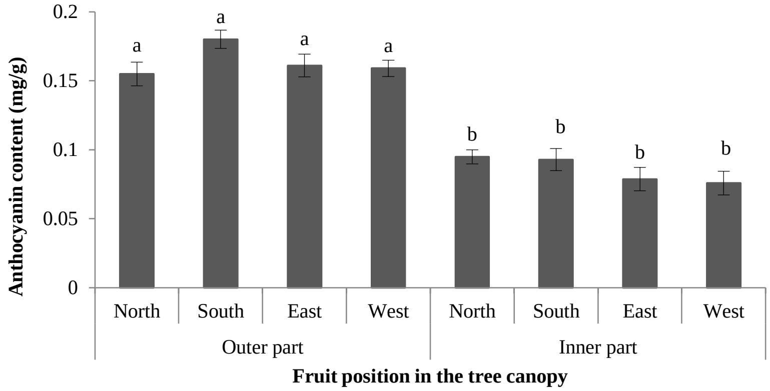

Fig. 7. Effect of fruit location in tree canopy on the total anthocyanin content of Kalleghochi cultivar. Each data point is the mean±S.D (n=5).

Discussion

The simple effect of position in the tree canopy (exterior and interior parts) has a significant influence on the number of nuts per ounce (Fig. 1), pistachio splitting (Fig. 2), hull weight (Fig. 3), hard shell weight (Fig. 4), kernel weight (Fig. 5), colour indices (Fig. 6) and total anthocyanin content (Fig.7). Moreover, the effect of geographical directions and interaction of light exposure by geographical directions on all evaluated parameters except colour indices (a* and b*) and anthocyanin content were significant.

Results indicated that the number of nuts per ounce, pistachio splitting, kernel weight, red skin colour (a*) and total anthocyanin content were all enhanced in outer parts of tree canopy. Sun exposure of leaves in outer portions of the canopy resulted in greater photosynthetic capacity (Feng *et al.*, 2014), while sun exposure of fruits can affect their physicochemical characteristics (Arena *et al.*, 2017).

Fruits from inner-canopy developed at slower rate and consequently reduced fruit size and decreased edible parts (Bartolini *et al.*, 2014). In this regard, reduction of light intensity and light quality via the use of shade cloths significantly delayed fruit ripening in some species (Arena *et al.*, 2017). In other words, the photosynthetically active radiation also affects the microclimate around individual leaves and fruits (temperature and relative humidity), which may lead to morphological, biochemical and physiological changes of leaves and fruit (Bartolini *et al.*, 2014). In this regard, the lower relative humidity and higher temperature at treetop than at lower heights of tree canopy was reported (Sharma and Singh, 2006). The uneven distribution of light inside tree canopy led to considerable changes in the physicochemical characteristics of the fruit (Pannico *et al.*, 2017).

Number of nuts per ounce (28 g) is an important factor in marketing of pistachio (Rahemi and Ramezanian, 2007). Number of nuts per ounce indicates the size and weight of the pistachio nuts, so larger nuts size leads to decrease in the number of nuts per ounce. The average number of nuts per ounce is classified as follows: colossal (less than 18), extra large (18-20), large (21-25), medium (26-30), and small (more than 30) (US International Trade Commission, 2005). Therefore, 'Kalleghochi' cultivar is classified in the large category as reported in previous studies (Panahi and Khezri, 2011). Similar to our results, the larger size of shaded fruits (tanagers, manikins and grape) than sun-exposed fruits has been reported (Levey, 1987; Uhlig, 1998). Similarly, the fruit size, maturity, and oil content of olive fruit are influenced by the amount of photosynthetically active radiation intercepted by the canopy (Bartolini *et al.*, 2014). On the other hand, Feng *et al.* (2014) reported that apple fruit from the outer-canopy was heavier than that from the inner-canopy.

Light penetration has important effects on the fruit sensorial and nutritional quality (Gullo *et al.*, 2014). Skin colour is one of the aspects that determine both optimal harvest and stage of development (Unuk *et al.*, 2012), and also surface colour may provide useful information about the health value of the fruit (Hagen *et al.*, 2007). Even though fruit location in the exterior and interior parts of the tree canopy significantly affected the surface colour of pistachio fruit (Fig. 6), the factor "geographical directions" showed no important effects on this particular quality parameter. Colour differences of exterior and interior fruits of tree canopy have been reported previously (Gullo *et al.*, 2014; Hagen *et al.*, 2007; Nuncio-Jáuregui *et al.*, 2014; Uhlig, 1998). The location in the canopy affects the intensity of red surface colour and red colouring compounds such as anthocyanins (Unuk *et al.*, 2012). The results indicated that the a* (Fig. 6) and total anthocyanin content (Fig. 7) of exterior fruits were significantly higher than interior fruits of tree canopy. The sunlight exposure increased red colour development and accumulation of selected phenols; mainly anthocyanins in apple skin (Hagen *et al.*, 2007). In addition, fruit position within the tree canopy increased external red colour of pomegranate fruit; which implies that external quality attributes are more susceptible to environmental changes than internal attributes (Nuncio-Jáuregui *et al.*, 2014). Moreover, the better colouration of apple fruit is highly dependent on the location in the canopy (Feng *et al.*, 2014). In this regard, better light penetration inside the canopy due to the higher accumulation of polyphenols can promote fruit colour (Gullo *et al.*, 2014). The chlorophyll, carotenoid and anthocyanin pigments determine the dominant colour of fruit. The anthocyanins are compounds that are characterised by having light-dependent metabolism, and sunlight exposure increased their biosynthesis and accumulation (Gullo *et al.*, 2014). Also, temperature variations within tree canopy resulted in the difference in peel colour between outside and inside fruit (Nilsson and Gustavsson, 2007); so that the hull colour of interior fruits was higher than exterior fruit.

Conclusions

This study shows that fruit location on tree canopy, especially light exposure can increase some commercial properties of pistachio fruit such as pistachio splitting and kernel weight. The fruits in inner-canopy (shade parts) developed at slow rate and consequently decreased edible parts. The results indicated that the redness and total anthocyanin content of exterior fruits was significantly higher than interior fruits of tree canopy. Based on literature fruit position on tree canopy not only significantly affected the physical properties but also influenced the concentrations of both primary and secondary metabolites of fruits. These issues need to be studied in more detail than we have done here, and more research is needed to evaluate other physical and chemical composition of pistachio fruit. The results of the current study can be used to provide better strategies for orchard planting system and pruning strategies for improved yield and fruit quality. In addition, sunlight exposure increases the phenolic content of pistachio soft hull; therefore, the results of the current study can be used in the field of waste application.

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