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ORIGINAL ARTICLE

Flavonoid and Anthocyanin Pigments Characterization of Pistachio Nut *(Pistacia vera)* as a Function of Cultivar

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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

In this work, the concentrations of flavonoid and anthocyanin pigments determined from different pistachio (*Pistacia vera*) cultivars collected from Damghan and Rafsanjan, were investigated. The flavonoid compound was evaluated as aluminum chloride complex and stated as mg of rutin equivalents/100g of the sample weight. To estimate anthocyanin content, colorimetric assays were used. The highest concentrations of flavonoid compounds (25mgRu100g^{-1}fw) were found in the Abbas Ali cultivar from Damghan, followed by Kalehghoochi and Fakhri. The lowest level of anthocyanin was obtained in Khanjari from Damghan (7µmolg^{-1}fu). The amount of anthocyanin and flavonoid pigments in different tissues of pistachio fruits showed that there was no significant difference in pistachio hull and kernel. After harvest, different cultivars indicated various values for phytochemical properties and flavonoid compound of pistachio hull and kernel ranged from 12.31 to 30.3 mgRu100g^{-1}fw and 19.22 to 27.92 mgRu100g^{-1}fw, anthocyanin pigment of pistachio hull and kernel from 6.81 to 11.1 µmol g^{-1}fu and 4 to 18 µmolg^{-1}fu, respectively. The pistachio kernel contained high amounts of flavonoid content, especially in the Fakhri cultivar, and the pistachio hull contained low levels of anthocyanin value, especially in the Akbari cultivar. Among the investigated pistachio kernel cultivars, Abbas Ali indicated higher values of anthocyanin pigment than Kalehghoochi, Akbari, Khanjari, and Fakhri. Our findings could be beneficial for introducing interesting properties to the pistachio nut such as cultivar-rich resources from anthocyanin and flavonoid contents and can be used when selecting a special cultivar for a particular application and pharmaceutical industry.

Introduction

Recently, there have been increment efforts in nutritional ingredients of nuts, such as fatty acids, vitamins, minerals, phenolics, and sterols (Rabadán *et al*., 2019). Also, regarding the positive impact of nuts

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on human health, most studies have focused on the antioxidant and flavonoid compounds (Jahanbani *et al.*, 2018 and 2016; Pakzadeh *et al*., 2021). Due to climate changes, environmental stress, and desert weather, it is emphasized to genotypes breed a species resistant to unfavorable conditions (Chelli-Chaabouni *et al*., 2010; Vahdati *et al.*, 2019).

Pistachio (*Pistacia vera L*.) tree can be cultivated under stress factors such as arid, saline and high temperature (Alipour, 2018; Akbari *et al*., 2020). Choosing suitable species and rootstock, resistant to undesirables soil and weather conditions, is important for changing yield efficiency (Hosseini *et al.*, 2022; Behzadi Rad *et al.*, 2021; Raoufi *et al*., 2021; Vahdati *et al.*, 2021). Rafsanjan, Sarakhs, Feyzabad, and Damghan are the main regions of pistachio breeding in Iran (Heidary-Sharifabad *et al*., 2021). About 70 cultivars of female pistachios and different female genotypes are bred in Iran. The main pistachio cultivars in Iran are Mumtaz, Akbari, Koleghoochi, Badami Zarand, Ahmad Aghaei, Sefid pistachio Nogh, Khanjari Damghan, Ouhadi, Qazvini pistachio, and Shah Pasand Damghan (Mandalari *et al*., 2022). Pistachio is a bold nut and is usually used as a snack because of its nice crispness (Noorafshan *et al*., 2021). Moreover, it has a positive effect on human health due to it regulates blood cholesterol levels (Arjeh *et al*., 2020).

Pistachio kernel is a rich resource of natural antioxidants, vitamins, sterols, fatty acids, minerals, and phenolic compounds (Roozban *et al.*, 2005; Taghizadeh *et al*., 2018). Furthermore, pistachio is also a rich resource of xanthophyll, lutein, carotenoids, and a large number of phenolic contents (Terzo *et al*., 2020). The main type of pistachio phenolic contents contains the pigment flavonoid. Flavonoid compounds have the basic composition of atoms carbon bridge and are affected by their oxidation level (Cheniany *et al.*, 2013) and substitution on the 3-position can be categorized as flavones, flavanols flavonoids, flavanones, isoflavones, and anthocyanidins (Babashpour-Asl *et al.*, 2021; Nutrient Data Laboratory, 2018). Pistachio skin is characterized by different flavonoid compounds such as catechin, genistein-7-O-glucoside, eriodictyol-7-O-glucoside, rutin, naringenin-7-O-neohesperidoside, eriodictyol, isoquercetin, daidzein and anthocyanidins (cyanidin-3-O-glucoside and cyanidin-3-O-galactoside) (Grace *et al*., 2016).

Anthocyanin colorants are sensitive to light, pH and, temperature (Eshghi *et al.*, 2014). The color of anthocyanin pigment changes from yellow to colorless products under unfavorable conditions (Alappat and Alappat, 2020). Anthocyanin's multifunctional properties contain water-soluble pigment, various colors at different pHs, and are effective on human health proposing a fine chance for their modern packaging in food industries. Pistachio anthocyanin can be used in smart packaging to identify the microbial spoilage of food (Marvizadeh *et al*., 2014, Marvizadeh *et al*., 2016; Fallah *et al*., 2022).

The objectives of the present work were (1) to determine anthocyanin and flavonoid compounds in five chosen pistachio cultivars from Damghan and Rafsanjan and (2) to evaluate and compare five pistachio cultivars concerning pigments amount of pistachio kernel and hull.

Materials and Methods

Materials and geographical indices

Different fruits of pistachio cultivars including Kalehghoochi and Akbari from Rafsanjan region and Abbas Ali, Khanjari, and Fakhri from Damghan region were collected in September 2021. The kernels and hull of the pistachio samples were separated and then the pistachio kernel was air-dried at 25°C for 48h. At last, kernels were placed in a plastic bag at -18°C until experiments. All chemical materials and solvents applied in this search were purchased from Merck Co. (Darmstadt, Germany).

Determination of total flavonoid content

About 100 mg of specimens were mixed with 10 ml of 80% methanol. The suspension was centrifuged for 12 min at 2000g. Supernatants were applied for subsequent tests. The flavonoid compounds were estimated using the colorimetric assay explained by Nadernejad *et al*. (2013). The light transmittance and absorbance of the samples were determined at 510 nm using a UV-visible spectrophotometer (UV-1650PC, Shimadzu, Tokyo, Japan). The total flavonoid content of samples was stated as mg of rutin equivalents /100g of the sample weight.

Total anthocyanin content analysis

Anthocyanin content of samples was measured according to Wagner (1979). The spicmences (0.1 g) were placed in 10 ml of acidified methanol [HCl/methanol: 1:99 v/v]. The samples were crushed and stored for 1d, at 25°C in the dark. The extract samples were centrifuged at 4,000*g* and 25°C for 5min. The absorbance of the supernatant was determined at 550 nm.

To compute the anthocyanin content, the extinction coefficient (ε) 33,000/ cm mol was applied and the anthocyanin of each sample was stated as μmol g^{-1} fw.

Statistical analysis

Independent sample T-test and one-way ANOVA test were conducted to compare the means of different pigments content of pistachio at $p<0.05$. Statistical analyses were applied using SPSS version 26.0.0.1 (IBM Co. Armonk, New York, U.S.A).

Results

Effect of cultivar on flavonoid content in the different regions

Fig. 1. illustrated flavonoid level in pistachio. Statistical analysis of all values for the flavonoid content of pistachios indicated that the impact of cultivar on pigment content was significant ($p<0.05$). Findings of the cultivar's influence on flavonoid, in specimens collected from the different regions, indicated that among the chosen cultivars, the Abbas Ali pistachio cultivar from Damghan, with 25 mgRu $100g^{-1}$fw flavonoid, was the richest kind in terms of chemical pigment. The flavonoid compound of the other cultivars was reduced according to the following order.

Kalehghoochi>Fakhri> Khanjari >Akbari.

In this experiment, there was a significant difference ($P<0.05$) in flavonoid content between Abbas Ali and Akbari pistachio cultivars.

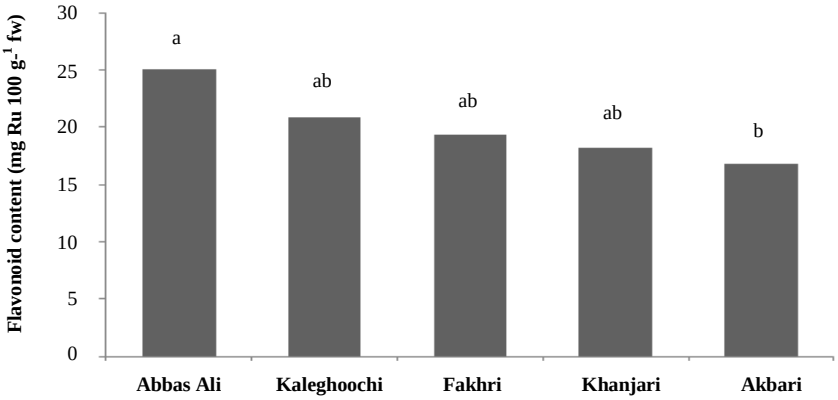

Fig. 1. Flavonoid content of five pistachio cultivars. Vertical bars show the mean±SD at $p< 0.05$

Flavonoid content in kernel and hull of different pistachio cultivars

As illustrated in Fig. 2. there is no significant difference in flavonoid content of hull and kernel pistachio among cultivars collected from various regions. Findings indicated that the flavonoid value reduced from 21.82 mgRu $100g^{-1}$fw for pistachio kernel to 18.23 mgRu $100g^{-1}$fw for pistachio hull.

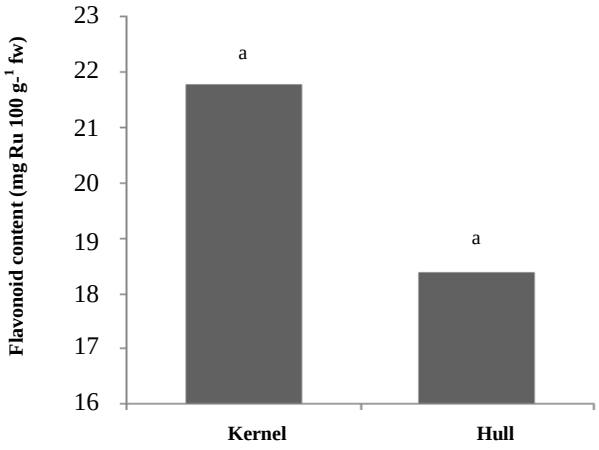

Fig. 2. Flavonoid content in kernel and hull of different cultivars pistachio. Vertical bars show the mean±SD at p< 0.05.

The results of the flavonoid content of the pistachio kernel are shown in Fig. 3. The highest level of total flavonoid was observed in the Fakhri pistachio kernel cultivar with 24.92 mgRu $100g^{-1}$fw, followed by Kalehghochi pistachio kernel cultivar with 22.53 mgRu $100g^{-1}$fw. At last, the lowest flavonoid content was observed in the Khanjari and Abbas Ali pistachio kernels with 19.91 mgRu $100g^{-1}$fw. In this section, there was no significant difference (p>0.05) in flavonoid content between different pistachio kernel cultivars.

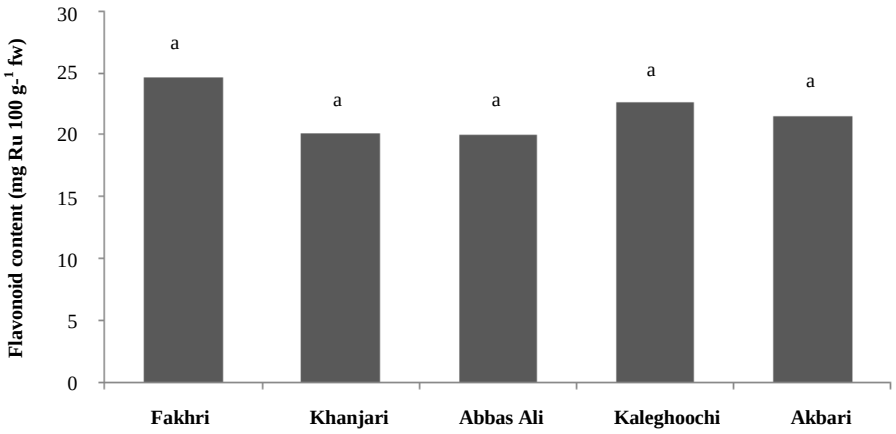

Fig. 3. Flavonoid content in kernel pistachio of different cultivars. Vertical bars show the mean±SD at p< 0.05.

The findings indicated significant differences (p <0.05) in flavonoid value in hull among different cultivars (Fig. 4.). The lowest flavonoid value was observed in the hull of Fakhri, and also the highest observed in Abbas Ali.

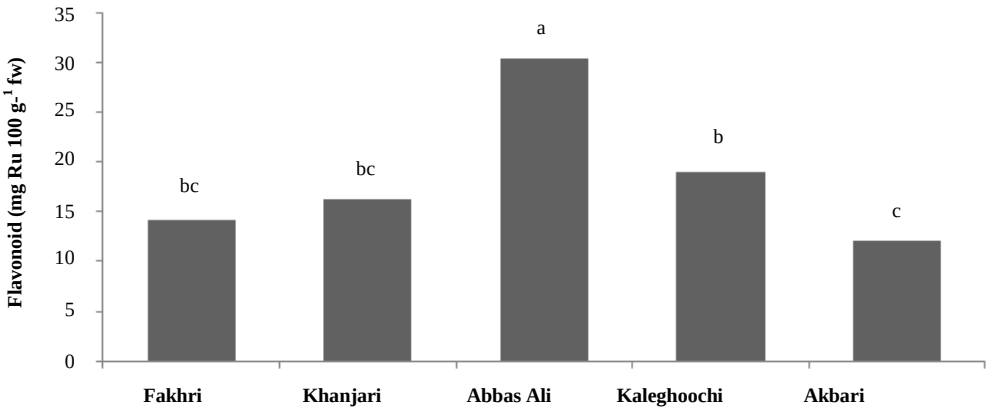

Fig.4. Flavonoid content in hull pistachio of different cultivars. Vertical bars show the mean±SD at p< 0.05.

Effect of cultivar on anthocyanin content in the different regions

Fig. 5. shows anthocyanin concentrations in pistachio. Statistical analysis of all values for anthocyanin content of pistachios indicated that the impact of cultivar on pigment content was significant ($p<0.05$). Findings of the cultivar's influence on anthocyanin, in specimens collected from the different regions, indicated that among the chosen cultivars, the Abbas Ali pistachio cultivar from Damghan, with 12.7 µmolg-1fw anthocyanin content, was the richest kind in term of chemical pigment. The anthocyanin pigment of the other cultivars was reduced according to the following order.

Kalehghoochi > Fakhri > Akbari > Khanjari

In this experiment, there was a significant difference ($P<0.05$) in anthocyanin content between Abbas Ali and Khanjari pistachio cultivars

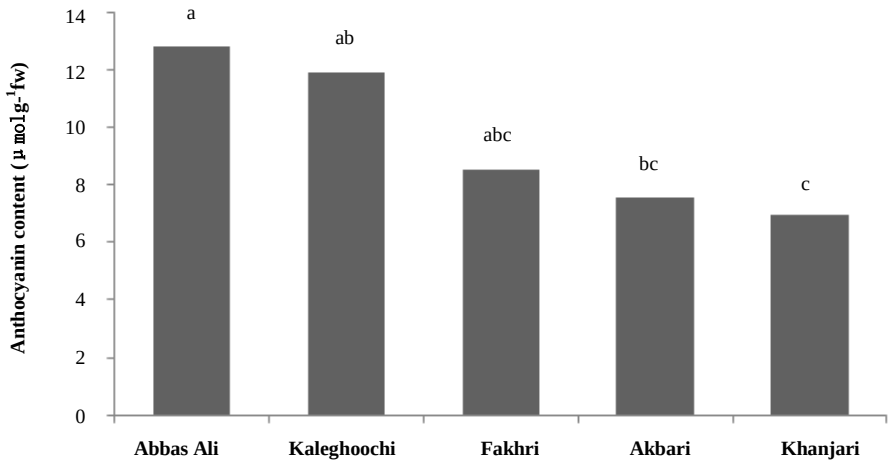

Fig. 5. Anthocyanin content of five pistachio cultivars. Vertical bars show the mean±SD at p< 0.05.

Anthocyanin content in kernel and hull of different pistachio cultivars

As illustrated in Fig. 6. there is no significant difference in anthocyanin content of hull and kernel pistachio among cultivars collected from various regions. Findings indicated that the anthocyanin value reduced from 10.42 µmolg^{-1}fw for pistachio kernel to 8.23 µmolg^{-1}fw for pistachio hull.

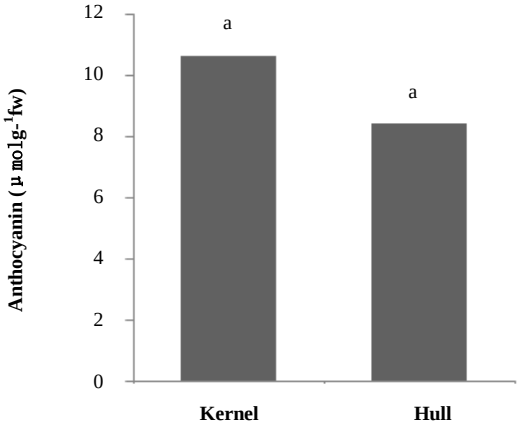

Fig. 6. Anthocyanin content in kernel and hull of different cultivar pistachio. Vertical bars show the mean ± SD at p< 0.05.

The results of anthocyanin content of pistachio kernel are shown in Fig. 7. The highest level of total anthocyanin was observed in the Abbas Ali pistachio kernel cultivar with 18 µmol g^{-1} fw, followed by the Kalehghochi pistachio kernel cultivar with 13.3 µmol g^{-1} fw. At last, the lowest anthocyanin content was observed in the Khanjari pistachio kernel with 4 µmol g^{-1} fw. In this section, there was no significant difference (P>0.05) in anthocyanin content between Fakhri and Akbari pistachio kernel cultivars.

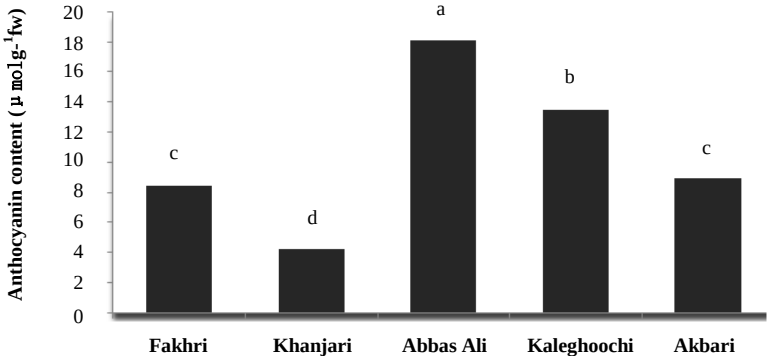

Fig. 7. Anthocyanin content in kernel pistachio of different cultivars. Vertical bars show the mean±SD at p< 0.05.

The findings indicated significant differences (p <0.05) in anthocyanin value in hull among different cultivars (Fig. 8). The lowest anthocyanin value was observed in the hull of Akbari, and also the highest observed in Kalehghoochi.

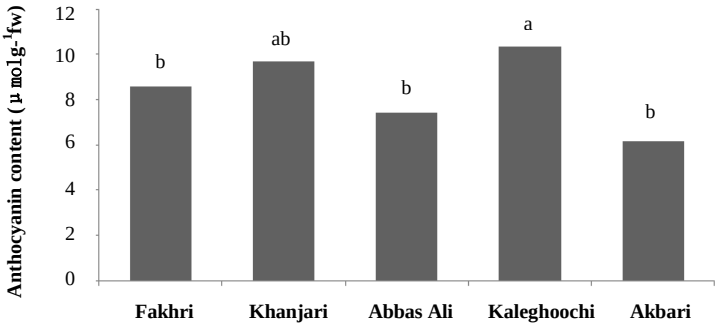

Fig. 8. Anthocyanin content in hull pistachio of different cultivars. Vertical bars show the mean±SD at p< 0.05.

Discussion

A special focus should be placed on the different flavonoid compounds found in pistachios because of the variety, abundance, and accessibility of these beneficial chemicals. The main flavonoids identified

in pistachios are flavanols, flavonols, flavanones, isoflavones and, anthocyanins, reaching 16–70 mg/100 g depending on the variety (Haytowitz *et al*., 2018). Pistachio kernel is defined by the presence of genistein-7-O-glucoside, catechin, rutin (quercetin-3-O-rutinoside), eriodictyol-7-O-glucoside, naringenin-7-O-neohesperidoside, eriodictyol, isoquercetin, daidzein, genistein, and apigenin (Mandalari *et al*., 2022). High concentrations of total flavonoid content and anthocyanin of pistachio kernel based on Ahmadaghaii, Ohadi and Kallehghoochi cultivars and three rootstocks (Mutica, Ahli, Sarakhs) have been reported by Nadernejad *et al*. (2013). They stated that the flavonoid and anthocyanin content of pistachio kernel ranges is 15.24 mg Ru 100 g^{-1}fw and 7.66 µ mol g^{-1} fw in mutica-Ahmadaghaii and Ahli-Ohadi, respectively.

The chemical properties of various cultivars can be influenced by environmental factors (Taghizadeh *et al*., 2018). Estimation of plant chemistry in several countries has revealed significant variance (Fattahi *et al*., 2016). Also, the chemical factors of pistachio kernel maybe attributed to the horticultural practice, climate, cultivation site, and cultivar (Nadernejad *et al*., 2012). Hence, Taghizadeh *et al*. (2018) the effect of cultivar on the chemical characteristics of Akbari, Kalehghoochi, Ahmadaghaei, Badami-e-sefid and, Ohadi pistachios was investigated. They stated that proanthocyanidins and flavonoid compounds were the highest level in Akbari pistachio from Rafsanjan. The difference among searches on the same variety could be attributed to evaluation conditions and postharvest management before sampling (Ballistreri *et al*., 2009). In the various cultivars of fresh pistachio such as Bronte, Cerasola from Italy, Aegina and Pontikis from Greece, Joley, Kerman and, Momtaz from Iran, Sirora from Syria. Tsantili *et al*. (2011) found that flavonoid compounds ranging from 3.5 mg CE g^{-1} d.w. to 7.2 mg CE g^{-1} d.w. They reported that the impact of the cultivar on pigment compounds was significant and the highest level of flavonoid among the pistachios of Greece, Italy, and Iran is in the Pontikis cultivar (7.2 mg CE g^{-1} d.w.) from Greece. According to cultivar properties, those with the highest proanthocyanidin content and total flavonoid compounds seem to be better able to survive unfavorable environments for example poor water quality, infertile soil, arid climates and, particular geographical characteristics (Amiri *et al*., 2015).

Phenylalanine ammonia-lyase enzyme (PAL) activity plays a positive role in the production of anthocyanin. Recently studies showed the effect of temperature and light on the enzyme activity and the anthocyanin content of pistachio. In addition to the ripening stage, the region and the type of cultivar are also effective in the production of pigments of pistachio (Longo *et al*., 2007). Bellomo and Fallico (2007) have shown that the highest amount of anthocyanin pigment among the pistachios of Greece, Italy, Iran and, Turkey in three different harvest stages is in the ripe Italian samples. Hence, serious changes in antioxidant properties and phenolic value have been reported to be related to the region of bred(Taghizadeh *et al*., 2018). The chemical tests of pistachio cultivars were accepted our findings that total flavonoids and anthocyanin compound different among the pistachio cultivars(Davarynejad *et al*., 2012). Recently, Akbari *et al*. (2018) studied anthocyanin content in Akbari, Kalehghoochi, and Ghazvini cultivars. The authors reported that the highest concentration of the pigment among the different cultivars is in Ghazvini cultivar (0.843 mg g^{-1}).

Our findings represented that different cultivars, affect the chemical properties of the pistachio nut. Evaluation and understanding of phytochemical characterization can be utilized in medicine and pharmaceutical sciences.

Conclusions

The pistachio kernel cultivars are a rich source of anthocyanin and flavonoid compounds. Also, anthocyanin and flavonoid amounts were found

depending on the cultivar type. The pigment contents of pistachio kernel were observed higher than those of pistachio hull samples. The dominating pistachio kernel cultivars in flavonoid and anthocyanin content were Fakhri and Abbas Ali respectively. Finally, the dominating pistachio hull cultivars in flavonoid and anthocyanin content were Abbas Ali and Kalehghoochi respectively.

Conflict of interests

The authors declare that there is no conflict of interest.

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