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Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates

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ABSTRACT

This study was carried out to detect diversity in phytochemical characteristics among the 76 pomegranate accessions grown in the eastern Mediterranean region of Turkey. Total phenolics (TP), total anthocyanins (TA), total antioxidant capacity (TAC), fructose (FRUC), glucose (GLUC), sucrose (SUC), and variables describing fruit, skin, and aril colors were investigated. These pomegranate accessions exhibited great diversity in values for TA (range of 1.1–63.3 mg cy-3-glucoside/100 g jw), TP (range of 108.0–944.9 mg GAE/100 g jw), and TAC (range of 7.4–17.5 mmol Fe^{2+}/kg jw). The high levels of TP, TA, and TAC contained in fruit from the accessions 'Ekşi 5', 'Tatlı 14', 'Antepnarı', and 'Tatlı 2', with their dark red fruit peel and aril colors, make them particularly suitable for consumption as fresh fruit, aril extracts, and juice. The results demonstrated that the pomegranate accessions analyzed here showed variable TP, TA, and TAC profiles depending on aril color and maturity index group.

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1. Introduction

The pomegranate (*Punica granatum* L.), one of the oldest edible fruits, has a long history of extensive use in the folk medicine of various countries (Holland et al., 2009). Turkey is one of the main European pomegranate producers, with production rapidly increasing from year to year, mainly in its Mediterranean region.

Recently, consumers are becoming more aware of the contribution of diet to their health, therefore they are more interested in buying food rich in bioactive compounds (Gil et al., 2000). In addition, consumer acceptance of healthy products is also related to quality and sensory properties (Borochov-Neori et al., 2009). Pomegranate fruits are commonly used in the food and food processing industries due to their excellent nutritional and health values, and as a raw material for the manufacture of secondary products such as jellies, dyes, and cosmetics (Opara et al., 2009). They are rich in polyphenols, including ellagitannins, gallotannins, ellagic acids, gallagic acids, catechins, anthocyanins, ferulic acids, and quercetins. These polyphenols exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth, and decreasing the risk of cardio- and cerebrovascular diseases and some cancers (Mena et al., 2011). Several researchers have reported that preparations containing pomegranate extract can be used to prevent or cure atherosclerosis, diarrhea, gastric ulcers, venereal disease, and estrogen-related diseases (Holland et al., 2009). Gil et al. (2000) indicated that pomegranate juice possessed antioxidant activity 3-fold higher than that of red wine or green tea and 2-, 6- and 8-fold higher levels than that detected in grape/cranberry, grapefruit, and orange juice, respectively. Anthocyanins are the water-soluble pigments responsible for the bright red color of pomegranate juice (Mena et al., 2011).

The edible portions of pomegranate fruit include the arils, which constitute 45–60% of total fruit weight (Kulkarni and Aradhya, 2005; Tehranifar et al., 2010). The arils can be consumed fresh, but are also used in the preparation of juices, canned beverages, jellies, jams, flavorings, and colorings for drinks (Melgarejo et al., 2000). The arils include the testa and seed coat, and are fleshy or pulpy. The testa is sweet, sweet–sour, or sour; its color is white, pink, or red; and it is rich in organic acids and phenolic compounds. Just as for many other fruit species, pomegranate cultivars differ in sweetness and sourness, which are directly related to the quality and quantity of the organic acids and sugars in the fruit (Holland and Bar-Ya'akov, 2008), and in flavor, which is related to volatiles (Melgarejo et al., 2011).

Several phenolic (Tehranifar et al., 2010; Mena et al., 2011) and flavonoid compounds (Shams Ardekani et al., 2011), fatty acids (Parashar et al., 2010), and sugars and organic acids (Melgarejo et al., 2000; Hasnaoui et al., 2011; Mena et al., 2011) have been characterized in pomegranate fruits. Antioxidant activity and anthocyanin content of juice (Gözlekçi et al., 2011; Hasnaoui et al., 2011; Mena et al., 2011) and peel (Gözlekçi et al., 2011), and the volatiles profile of pomegranates (Melgarejo et al., 2011) have been

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analyzed. However, phytochemical characteristics such as total phenols, total anthocyanins, total antioxidant capacity, and specific sugars have not been compared among numerous pomegranate accessions. In addition, most of the data on the phytochemical properties and the beneficial compounds of pomegranates were obtained using the globally important cultivar 'Wonderful' (Seeram et al., 2008; Adams et al., 2010). Other studies included 40 pomegranate cultivars or accessions from Spain (Melgarejo et al., 2000), 21 from Iran (Tehranifar et al., 2010), eight from Italy (Ferrara et al., 2011), 30 from Tunisia (Hasnaoui et al., 2011), and six from Turkey (Ozgen et al., 2008). To capitalize on the potential benefits of pomegranates, more information on the phytochemical and antioxidant capacities of various pomegranate accessions is needed, but has not been available for the numerous accessions that could potentially be used as parental lines in breeding studies.

The objective of this paper was to study and compare the total anthocyanin, total phenolics, total antioxidant capacity, sugar composition, and color profile of 76 pomegranate accessions selected from Hatay, in the eastern Mediterranean region of Turkey. These descriptive data will be useful to better characterize accessions that may be used for cultivation or for the development of health-promoting pomegranate cultivars in breeding programs.

2. Materials and methods

2.1. Plant material

Fruit samples of 76 local pomegranate accessions were collected during 2010 and 2011 from nine traditional pomegranate-growing locations in Hatay province (Antakya, Altınözü, Belen, Dörtyol, Hassa, İskenderun, Kırıkhan, Samandağ, and Yayladağı), which is located in the eastern Mediterranean region of Turkey. The names of these pomegranates are mainly given based on local geographic origin (e.g. 'Antepnarı', 'Büğleknarı'), flavor (e.g. 'Ekşi', 'Mayhoş', 'Tatlı'), skin color (e.g. 'Kırmızı Kabuk', 'Kızılgöbek'), or the name of orchard owner (e.g. 'Kara Mehmet', 'Kara Ahmet'). We sampled from pomegranate accessions with different name and fruit quality characteristics such as peel and aril color and fruit taste.

The fruits of these accessions were harvested at the fully mature stage, as indicated by their external and internal color, and peak flavor. Phytochemical identifications of the pomegranate fruits were conducted on samples of 15 fruits per accession in three replicates, each consisting of five fruits. Maturity index (total soluble solids/acidity) values were classified as MI (TSS/acidity) = 5–7 for 'sour', 17–24 for 'sour–sweet', and 31–98 for 'sweet' based on method described by Martínez et al. (2006). According to the MI classification, the most important group was 'sour–sweet', which included 43 accessions, followed by the sweet group consisting of 18 accessions, and the sour group consisting of 15 accessions) (Table 1).

2.2. Fruit extraction

The sampled fruits were cut into halves and arils were hand-separated from the mesocarp (known as the albedo) and the septal membranes of the arils were extracted. Juices from fruits of each accession were obtained by squeezing arils inside a nylon mesh bag by hand. The samples of freshly prepared juice were frozen and stored at −20 °C until analyzed. All triplicates were screened for total anthocyanins, total phenolic contents, and antioxidant capacity following a single extraction procedure (Beccaro et al., 2006). For this procedure, 10 g aliquots of each sample were transferred to polypropylene tubes and extracted with 25 mL of extraction buffer containing methanol, deionized water, and hydrochloric acid for 1 h at room temperature.

2.3. Total phenolics (TP)

Total phenolics (TP) were determined using the Folin–Ciocalteu method (Slinkard and Singleton, 1977). To determine TP, 0.5 g of each extract was combined with Folin–Ciocalteu's phenol reagent and water 1: 2 (v/v) and incubated for 8 min at room temperature, followed by the addition of 10 mL of 15% (w/v) sodium carbonate. The mixture was allowed to stand for 2 h at room temperature. The absorbance of each sample was measured at 750 nm using a spectrophotometer (Shimadzu UV-1208, Japan). Values of TP were estimated by comparing the absorbance of each sample with a standard response curve generated using gallic acid. Data were expressed as mg gallic acid equivalents (GAE) on a juice weight (jw) basis (mg GAE/100 g jw).

2.4. Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC), was measured by FRAP, the ferric reducing antioxidant power assay, which was conducted according to Pellegrini et al. (2003). To conduct the assay, a 9 mL aliquot of FRAP reagent (a mixture of 25 mL acetate buffer, 2.5 mL TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine], and 2 mL ferric chloride were mixed with 9 mL of methanolic fruit extract prepared by the protocol above. The samples were incubated at 37 °C for 30 min, and the absorbance of the reaction mixture at 593 nm was then determined in a spectrophotometer (Shimadzu UV-1208, Japan). To determine the antioxidant capacity of samples, absorbance values were compared with those obtained from standard curves of $FeSO_4 \cdot 7H_2O$ (10–100 μM). Antioxidant capacity values were expressed as Fe^{2+} equivalents mmol/kg juice weight (jw).

2.5. Total anthocyanins (TA)

Total anthocyanins (TA) were estimated by the pH differential method (Cheng and Breen, 1991) using a two buffer system. Absorbance was measured at 520 and 700 nm in buffers at pH 1.0 and pH 4.5, where $A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$. Results were expressed as mg cyanidin-3-glucoside (cy-3-glucoside, molar extinction coefficient of 29.6 and molecular weight of 449.2) equivalents per 100 g fresh weight of juice.

2.6. Sugar composition

Fruit juice (10 g) was diluted with purified water (40 mL) to prepare solutions for detection of individual sugars. The homogenate was centrifuged at 10,000 rpm for 5 min. Supernatants were then filtered through 0.45 μm membrane filters (Millipore, USA) prior to high-performance liquid chromatography (HPLC) analysis. Mobile-phase solvents were degassed before use. All samples and standards were injected three times each, and the mean values for all chromatography runs were evaluated. The HPLC analyses were conducted using a Shimadzu HPLC system, with an LC-10AT pump and RID-10 A detector (Shimadzu, Japan). The separation was carried out on an EC 250/4 Nucleosil C18 carbohydrate column (250 mm–4.0 mm i.d.) (Macherey–Nagel, USA). The elution solvent contained 80% acetonitrile and 20% deionized water. The column was operated at 25 °C with a flow rate of 2.0 mL/min.

2.7. Color measurement

Fruit peel and aril colors were measured using a colorimeter (Chroma Meter CR-300, Minolta Co., Osaka, Japan), standardized with calibration plate set CR-A47 against a white background. Color parameters were expressed as tristimulus colorimetric measurements, that is, L^*, a^*, Chroma (C), and hue° ($h°$). The L values indicate darkness and high L^* values indicate lightness. Negative a^* values