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Morpho-Pomologica and Chemical Properties of Pomegranate (*Punica granatum* L.) Cultivars in Iran

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ABSTRACT

This study was conducted to investigate the physico-chemical properties and antioxidant activity of five pomegranates fruit (*Punica granatum* L.) cultivars grown in Iran. Significant differences were found among the pomegranate cultivars for many of the properties studied. Results showed that, in particular, fruit diameter ranged from 63.63 mm (Syah) to 79.29 mm (Rabab), fruit volume from 153.3 cm³ (Syah) to 293.3 cm³ (Rabab), fruit density from 0.93 g cm⁻³ (Rabab) to 1.13 g cm⁻³ (Torsh Sefeed). Although Syah showed the lowest fruit weight (144.8 g), fruit yield (8.28 ton ha⁻¹) and fruit skin thickness (1.55 mm), Rabab had the highest fruit yield (27.1 ton ha⁻¹) and fruit skin thickness (2.32 mm). Juice volume was between 61.1 and 67.0 cm³. Percent of aril ranged from 59.64% (Rabab) to 75.3% (Syah) and weight of aril was between 108.9 and 199.8 g. Also, results indicated that titratable acidity content varied from 0.39% (Syah) to 1.13% (Torsh Sefeed). The total soluble solids content varied from 12.67 °Brix (Torsh Sefeed) to 15.67 °Brix (Zardeg Anar), pH values from 3.05 to 3.77, Electrical conductivity values from 2.8 to 3.14 dSm⁻¹ and vitamin C content from 59.25 to 69.52 mg 100g⁻¹

¹. The anthocyanins content was observed between 80.36 (Syah) and 216.97 (Zardeh Anar). The antioxidant activity of pomegranate cultivars ranged from 27.24% (Syah) to 84.04% (Torsh Sefeed). These results demonstrated that the cultivar was the major factor which influences the morpho-pomological and chemical (especially, antioxidant activity), properties in pomegranates.

Keywords

Antioxidant activity, Pomegranate, Juice, Cultivar, Fruit

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family (Harde et al., 1970). Pomegranate is one of the commercial horticultural fruits that is mainly cultivated in the North and South America, Mediterranean basin, southern Asia, and India (Ferrara et al., 2011). Recently, increasing demand for pomegranate products by consumers is specifically supported for its nutritional and medicinal characteristics (Lansky and Newman, 2007), due to the antioxidant characteristics of pomegranate fruit (Seeram et al., 2008) that contains anticarcinogenic (Bell and Hawthorne, 2008), antiviral (Kotwal, 2007), antimicrobial (Reddy et al., 2007), and anti atherosclerotic compounds that is able to reduce LDL oxidation and blood pressure (Aviram et al., 2004). These activities are principally due to the high levels of antioxidant activity and total polyphenols content of pomegranate (Tzulker et al., 2007). According to previous studies, the antioxidant activity in pomegranate juice was about 3-times higher than that of red wine or green tea (Gil et al., 2000), and 2-, 6- and 8-times higher than those in grape/cranberry, grapefruit, and orange juice, respectively (Rosenblat and Aviram, 2006).

Pomegranate fruit, as one of the native fruits in Iran, production has increased due to increasing evidence on its high value of beneficial materials. Iran is one of the most important producers of pomegranate (67×10^4 tons in 2005) and exporters in the world (Anonymous, 2005). In spite of different pomegranate cultivars grown in Iran, only a few published results on the characteristics of the cultivars in the literature are available. Thus, the main objective of this study was to investigate and compare morpho-pomological characteristics and the levels of

antioxidants, total polyphenols, and vitamin C in five cultivars of pomegranate in order to better characterize cultivars that can be utilized for pomegranate cultivation.

MATERIALS AND METHODS

Pomegranate Cultivars and Fruits Collections

The collection of pomegranate fruits was conducted in the years 2014. In the present study, five pomegranate cultivars were studied: Syah Arsanjani, Zardeh Anar Arsanjani, Torsh Sefeed Arsanjan, Kalantari Arsanjan, and Rabab Arsanjan. The pomegranate fruits were collected from adult trees (\approx 25-year-old) located in private small orchards. Commercially ripe fresh fruits were harvested in September 2014 from different mature trees randomly selected to represent the population of the plantation from Fars province, Iran. Fruits were transported to the laboratory soon after harvest, where pomegranates with defects were discarded. About 2 kg of pomegranate fruit was sampled for each cultivar. The fruits were kept at 4 °C until analysis. Three replicates were maintained for each analysis.

Physical Properties

Four fruits of each cultivar were individually investigated for physical properties.

The weight of the fruit and aril (fresh weight) were determined by weighting theses in the air on a precision digital balance with an accuracy of 0.001 g. Fruit volume was determined by a liquid displacement method. The density of the fruit was obtained by the ratio of weight to volume. The fruit diameter and fruit skin thickness were measured with a digital vernier caliper (Tehranifar et al., 2010).

Chemical Properties

The juices were analyzed for electrical conductivity (EC), pH, titratable acidity (TA), total soluble solids (TSS), vitamin C content, total anthocyanins, and total antioxidant activity. For electrical conductivity determinations, the juice samples were homogenized and measured with a Conductometer (Metrohm). The pH measurements were performed by a digital pH meter (Metrohm). The titratable acidity (TA) was determined using titration to pH 8.1 with 0.1M sodium hydroxide (NaOH) solution and expressed as g of citric acid per 100 g of juice (AOAC, 1984). The total soluble solids (TSS) were measured with a digital refractometer (Erma, Tokyo, calibrated using distilled water). The data were reported as °Brix. The concentration of vitamin C was measured by applying the method described by Ruck (1963). Results were expressed as mg/100 g of juice.

Measurement of total anthocyanin was determined according to modified Wagner (1979) method using acidified ethanol. Briefly, 0.1 g of the arils was homogenized in 2.5 ml acidified ethanol and then kept at 25°C for 24 h in the dark. The extract was centrifuged at 4000 g for 10 min at room temperature. The absorbance was measured spectrophotometrically at 550 nm. The extinction coefficient 33000 ($\text{mol}^{-1} \text{cm}^{-1}$) was applied to calculate the content of total anthocyanin.

Antioxidant activity was determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Brand-Williams et al. (1995). Briefly, 100 μl of pomegranate juice diluted in the ratio of 1:100 with methanol: water (6:4) was mixed with 2 ml of 0.1mM DPPH in methanol. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517nm by a Cecil 2010 UV–visible spectrophotometer. The

reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation (Equation 1):

$$\text{Antioxidant activity (\%)} = [1 - (\text{sample 517 nm}/\text{control 517 nm})] \times 100 \quad (1)$$

Antioxidant activity was determined according to the method described by Brand-Williams et al. (1995). Briefly, 0.1 ml of pomegranate juice diluted in the ratio of 1:100 with methanol: water was mixed with 2ml of 0.1mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixtures were shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation (Equation 2):

$$\text{Antioxidant activity (\%)} = [1 - (\text{sample 517 nm}/\text{control 517 nm})] \times 100. \quad (2)$$

Statistical Analyses

Experimental data were analyzed by SAS software Version 9.1 using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Duncan Multiple Range test.

RESULTS AND DISCUSSION

Physical Properties

The physical characteristics of five pomegranate cultivars analyzed are listed in Table 1. Results indicated that significant differences ($p < 0.05$) were detected in all analyzed properties. In particular, fruit diameters ranged from 63.63 mm (Syah) to 79.29 mm (Rabab). These values were lower than those reported by other studies. It was previously reported demonstrated that the fruit diameter of pomegranates grown in Iran are between 68–86.9mm (Sarkhosh et al., 2009)

and 64.9–86.88mm (Tehranifar et al., 2010). Celik and Erasl (2009) investigated physical characteristics of pomegranate cv. ‘Eksinar’ and indicated that fruit weight and volume were between 154.4 to 289.5 g and from 150.9 to 295.8 cm³, respectively. Also, fruit dimensions varied from 52.9 to 75.0 mm for length, 60.6 to 85.9 mm for width and 63.4 to 81.4 mm for thickness.

Results indicated that ‘Syah’ cultivar had the lowest fruit volume from 153.3 cm³ and ‘Rabab’ (293.3 cm³) had the highest fruit volume (Table 1). These values were lower than those reported by Tehranifar et al. (2010) who found the lowest (204.24cm³) and the highest (341.35cm³) fruit volume were observed in Iranian pomegranate cultivars.

As shown in Table 1, variations in fruit density were observed among the studied cultivars (0.93 g cm⁻³ (Rabab) to 1.13 g cm⁻³ (Torsh Sefeed)). However, Akbarpour et al. (2009) observed that the fruit density of pomegranate cultivars ranged between 0.91 g cm⁻³ and 1.04 g cm⁻³.

Although ‘Syah’ showed the lowest fruit weight (144.8 g), fruit yield (8.28 ton ha⁻¹) and fruit skin thickness (1.55 mm), Rabab had the highest fruit yield (27.1 ton ha⁻¹) and fruit skin thickness (2.32 mm) (Table 1). Tehranifar et al. (2010) studied twenty Iranian pomegranate cultivars and found that the average fruit weight of pomegranate cultivars ranged between 196.89 g and 315.28 g. It was previously reported that the fruit weights of pomegranates grown in Iran are between 164.89 g and 375.76 g (Sarkhosh et al., 2009). Ferrara et al. (2011) observed that fruit weight ranged from 168.9 to 574.9 g. However, variation in fruit weight depends on the cultivar and ecological condition (Shulman et al., 1984). In the present study skin thicknesses of

the fruits were lower than values reported by Tehranifar et al. (2010) who found the fruit skin thicknesses were between 3.13 and 5.36 mm.

As shown in Table 1, results indicated that juice volume was between 61.1 and 67.0 cm³. Percent of aril ranged from 59.64% (Rabab) to 75.3% (Syah) and weights of arils were between 108.9 and 199.8 g. However, Ferrara et al. (2011) reported that there were significant differences found in juice volume among genotypes and ranged from 65.2 to 72.2 cm³. Varasteh et al. (2009) evaluated important fruit properties of five pomegranate cultivars in Iran and found that ‘Malas-e-yazdi’ had the highest fruit weight, volume, length, diameter, aril percent and juice content (699.94 g, 715.60 cm³, 103.55 mm, 109.50 mm, 73.88. and 58.31%, respectively). The results for the physical properties of the pomegranate cultivars of the present study confirmed that the five cultivars were different in all investigated properties. ‘Rabab’ cultivar seems the most promising in combined more fruit size, fruit weight and fruit juice.

Chemical Properties

The results of the electrical conductivity, pH, Titratable acidity, total soluble solids, vitamin C, total anthocyanins and antioxidant activity of the five pomegranates from the different cultivars are given in Table 2. Generally, these characteristics were significantly ($P < 0.05$) different among pomegranate cultivars (Table 2).

The electrical conductivity of pomegranate juice ranged from 2.80–3.14 dS/m, the highest was found in ‘Syah’ and the lowest in ‘Zardeg Anar’ (Table 2). Sarkhosh et al. (2009) studied Iranian soft-seed pomegranate and they reported that the electrical conductivities of the pomegranate juice were between 0.3 and 3.4 mmoh/cm.

The pH values ranged between 3.05 and 3.77 (Table 2). The pH values obtained in the current study are greater than those reported by Cam et al. (2009) on pomegranate cultivars grown in Turkey. However, Akbarpour et al. (2009) indicated that the pH values of the pomegranate juice ranged from 2.75 to 4.14.

Also, the results indicated that titratable acidity content varied from 0.39% (Syah) to 1.13% (Torsh Sefeed). Tehranifar et al. (2010) found that titratable acidity content ranged from 0.33 g 100 g⁻¹ to 2.44 g 100 g⁻¹. Akbarpour et al. (2009) also reported that titratable acidities in pomegranate juices were 0.35% and 3.36%.

The total soluble solids content ranged from 12.67–15.67 °Brix, the highest was found in ‘Zardeh Anar’ and the lowest in ‘Torsh Sefeed’. This result is lower than values observed (16–19 °Brix) by Poyrazoglu et al. (2002), while our results were approximately in agreement with values reported by Fadavi et al. (2005) (10–16.5 °Brix), Martinez et al. (2006) (12.36–16.32 °Brix) for five Spanish cultivars and those reported by Tehranifar et al. (2010) for cultivars grown in Iran. Tehranifar et al. (2010) observed that the highest total soluble solids content was found in ‘Torsh Shavar Ferdows’ (15.07 °Brix) and the lowest one was found in ‘Agha Mandali Save’ (11.37 °Brix).

As shown in Table 2 the concentration of vitamin C varied greatly among the genotypes, vitamin C content ranged from 59.25 to 69.52 mg 100g⁻¹. Our results were higher than the range (9.91mg 100 g⁻¹ to 20.92mg 100 g⁻¹) reported by Tehranifar et al. (2010) and Kulkarni and Aradhya (2005). However, Ferrara et al. (2011) observed that there were significant differences found in concentration of vitamin C among the genotypes and ranged from 89.0 to 236.3 mg/L.

The results for total anthocyanins of the pomegranate from different cultivars are displayed in Table 2. A significant variation in total anthocyanins content was observed among the five varieties of pomegranate investigated and the values ranged from 80.36 (Syah) to 216.97 (Zardeg Anar) (Table 2). Anthocyanins are a member of the phenolics compounds that are well-known for their antioxidant activity (Tehraniifar et al., 2010). These results showed that the contents of total anthocyanins varied among different cultivars of pomegranate and there was a high genetic heterogeneity found within the investigated cultivars.

As shown in Table 2, it was observed that 'Syah' cultivar had surprisingly the lowest antioxidant activity (27.24%) and Torsh Sefeed (84.04%) had the highest antioxidant activity. In this study antioxidant activity was higher than values reported by Tehraniifar et al. (2010) who found that the antioxidant activity values ranged from 15.59% to 40.72%. Antioxidant activity has been reported for eight pomegranate juices in Iran. 18.6–42.8% (Mousavinejad et al., 2009) and seven pomegranate juices in Turkey. 10.37–67.46% (Tezcan et al., 2009). There was a significant and positive correlation ($r = 0.54$) between anthocyanins content and antioxidant activity.

Correlation coefficients (r) between the variables investigated are shown in Table 3. Titratable acidity (TA) and anti-oxidant activity were significantly correlated with pH at the 0.01 probability level ($p < 0.01$), ($r=0.93^{**}$ and $r=0.92^{**}$, respectively, Table 3). TA was also significantly correlated with anti-oxidant activity at the 0.01 probability level ($p < 0.01$), ($r=0.914^{**}$, Table 3). However, the correlation between TA and EC was significant only at the 0.05 probability level ($p < 0.05$), ($r=0.56^*$, Table 3).

CONCLUSIONS

The results of the present study indicated that statistically significant differences were observed in morpho-pomological and chemical properties of the various pomegranate cultivars. Therefore, cultivar type plays a significant role in terms of physico-chemical properties of the pomegranate juice. Among the five cultivars studied, Rabab showed the highest content of antioxidant activity, total anthocyanins, and vitamin C which are suitable for fresh consumption and human health benefits.

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TABLE 1. Some physical properties of five Iranian pomegranate cultivars

Cultivars	Fruit diamete	Fruit volum	Fruit densit	Fruit weight	Fruit yield	Juice volum	Fruit skin	Percen t of aril	Weigh t of
Syah	63.63 b	153.27	0.95 c	144.8	8.28	62.40	1.55 c	75.30 a	108.95
Zardeh	69.04 b	189.33	1.04 b	196.7	11.5	66.07 a	1.59 c	71.07	139.58
Torsh	77.59 a	251.33	1.13 a	282.7	12.2	66.80 a	1.92 b	70.52	199.87
Kalantari	66.68 b	180.00	0.96 c	172.6	9.67	61.13	1.60 c	68.34	117.89
Rabab	79.29 a	293.33	0.93 c	271.9	27.0	67.00 a	2.32 a	59.64 c	162.08

In each column, the values followed by at least one common letter character are not statistically different at 5% probability level, according to the Duncan Multiple Range test

TABLE 2. Some chemical properties of five Iranian pomegranate cultivars

Cultivars	Electrical conductivity (dS/m)	pH	Titratable acidity (TA)	Total soluble solids	Vitamin C (mg/100g)	Total anthocyanins	Total antioxidant activity
Syah	3.14 a	3.77	0.39 d	14.27 b	59.55 b	80.36 c	27.24 d
Zardeh	2.80 d	3.42	0.86 c	15.67 a	59.25 b	216.97 a	70.98 c
Torsh	2.92 c	3.05	1.13 a	12.67 c	60.13 b	82.91 c	84.04 a
Kalantari	3.02 b	3.29	0.97 b	14.80 b	69.52 a	212.24 a	77.91 b
Rabab	3.04 b	3.25	0.84 c	13.93 b	66.88 a	183.52 b	83.69 a

In each column, the values followed by at least one common letter character are not statistically different at 5% probability level, according to the Duncan test

TABLE 3. Pearson correlation coefficients (r) between variables investigated.

Properties	pH	EC	TSS	Vitamin C	TA	Antioxidant	Anthocyanins
pH	1						
EC	0.41	1					
TSS	0.43	-0.19	1				
Vitamin C	-0.31	0.27	0.13	1			
TA	-0.93**	-0.56*	-0.31	0.21	1		
Antioxidant	-0.92**	-0.46	-0.21	0.39	0.914**	1	
Anthocyanins	-0.20	-0.34	0.66**	0.51	0.30	0.54*	1

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.