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Studies on Physico-Chemical Properties and Bioactive Compounds of Six Pomegranate Cultivars Grown in Iran

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Abstract: Pomegranate is an important source of bioactive compounds and has been used extensively in the folk medicine of many centuries. The objective of this study was to analyse and compare the physico-chemical characteristics and bioactive compounds of pomegranate juices obtained from six pomegranate cultivars grown in Iran. This study showed that there were significant differences among the cultivars in all measured factors. The total soluble solids content (°Brix) varied from 15.77 (Shirin-e-Bihaste) to 19.56 (Rabbab-e-Fars), pH values from 3.06 (Faroogh) to 3.74 (Shirin-e-Mohali), titrable acidity concentration (g/100 g) from 0.51 (Shirin-e-Bihaste) to 1.35 (Rabbab-e-Fars) and total sugars content (g/100 g) from 16.88 (Rabbab-e-Fars) to 22.76 (Faroogh). The highest and lowest level of total anthocyanins (mg/100 g) were recorded in Aghaye and Shahvar (27.73 and 7.93), respectively. The concentration of ascorbic acid (mg/100 g) was between 8.68 (Aghaye) and 15.07 (Shirin-e-Bihaste). The total phenolics content (mg tannic acid/100 g) ranged from 526.40 (Shahvar) to 797.49 (Aghaye). The total tannins level (mg tannic acid/100 g) was observed in the pomegranate cultivars between 18.77 (Shahvar) and 38.21 (Aghaye). The values of the condensed tannins (mg catechin/100 g) ranged from 12.14 (Shirin-e-Bihaste) to 12.57 (Aghaye). The antioxidant activity varied from 46.51 (Shahvar) to 52.71% (Aghaye). The results also showed that antioxidant activity was positively correlated with total phenolics (r = 0.912), total tannins (r = 0.838), condensed tannins (r = 0.859) and total anthocyanins (r = 0.928). These data suggested that the cultivar was the main parameter which influences the composition of bioactive compounds in pomegranates.

Key words: Pomegranate, physicochemical, total phenolics, total tannins, antioxidant activity, Iran

INTRODUCTION

Punica granatum L., commonly known as pomegranate belongs to the family Punicacea. It is one of the important and the oldest edible fruit, cultivated in Mediterranean countries, Iran, India, Afghanistan, China, Japan, Russia and the United States (Patil and Karade, 1996). Iran is one of the native lands of the pomegranate which is grown in every area, both coastal and mountainous areas. This fruit is one of the most important commercial fruits in Iran and its total production in 2005 was 670,000 tons (Anonymous, 2005). Fruit is consumed fresh or processed into juice, jams, syrup and sauce (Al-Maiman and Ahmad, 2002).

Pomegranate juice has become more popular because of the attribution of important biological actions (Lansky *et al*., 1998). Recently, the high antioxidant activity of pomegranate juice has been reported (Gil *et al*., 2000). The antioxidant capacity of pomegranate juice is greater than other fruit juice and beverages (Seeram *et al*., 2008). It has been reported that pomegranate juice has potent anti-atherogenic action in atherosclerotic mice and humans (Aviram *et al*., 2000; Kaplan *et al*., 2001; Negi *et al*., 2003). These beneficial effects have been attributed to the high levels of antioxidant activity (Gil *et al*., 2000), seemingly the result of the remarkably high content and unique composition of phenolic compounds (Poyrazoglu *et al*., 2002; Seeram *et al*., 2005). Pomegranate juice is a rich source of polyphenols such as gallic acid, ellagitannins, gallotannins, chlorogenic acid, caffeic acid, ferulic acid, coumaric acids and catechin and anthocyanins (Gil *et al*., 2000; Poyrazoglu *et al*., 2002). The anthocyanins include 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin and pelargonidin (Du *et al*., 1975).

The composition of pomegranate juice depends on cultivar type, growing region, climate, maturity, cultural practice, storage and processing factors (Melgarejo *et al*.,

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2000; Ozkan, 2002). Due to the increasing interest in pomegranate consumption and the knowledge that the nutritional composition varies according to the cultivar, this work aimed at evaluating the total phenolics, total tannins, condensed tannins content, antioxidant activity and physico-chemical properties of six pomegranate juice cultivars grown in Iran. A more detailed data of the variability of the compositions of different cultivars will be of benefit in the future selection of pomegranate genotypes with improved nutritional quality and suitable processing properties for the manufacturing of pomegranate products.

MATERIALS AND METHODS

Pomegranate cultivars: Pomegranate cultivars were studied: Aghaye, Faroogh, Rabbab-e-Fars, Shahvar, Shirin-e-Bihaste and Shirin-e-Mohali. Commercially ripe fresh fruits were harvested in September 2008 from mature trees (14 years old) randomly selected to represent the population of the plantation from the Agricultural Research Center of the Yazd province, Iran. The fruits were transferred to the laboratory soon after harvest in plastic bags where pomegranates with defects (sunburns, cracks, cuts and bruises in peel) were discarded. Harvested fruits were sorted for size and uniformity of shape and weight. Approximately 4 kg of pomegranate fruit was sampled for each cultivar. Four replicates were maintained for each analysis, each replicate indicating a five pomegranate fruit. All reagents, solvents and standards were of analytical reagent grade.

Physical properties: Fruit fresh weight was determined by weighting the fruits (n = 20) in the air on a precision digital balance with an accuracy of 0.001 g. Following peel and pulp were separated manually, arils per fruit were measured. Fruit juice content was measured by extracting of total arils per fruit using an electric extractor (Toshiba 5020). The juice dry matter was determined by oven drying at 70°C until a constant weight was obtained (AOAC, 1984). Then the juices fresh were analyzed for major chemical compositions and antioxidant activity.

Chemical analysis: The pH measurements were performed using a digital pH meter (Metrohm model 601) at 21°C. Total soluble solids were determined with a digital refractometer (Erma, Tokyo, calibrated using distilled water), results were expressed as degree °Brix at 21°C. Titrable acidity was determined by titration to pH 8.1 with 0.1 M NaOH solution and expressed as g of citric acid/100 g of juice (AOAC, 1984). Maturity index was calculated by dividing total soluble solids to titrable acidity. Total sugars were estimated according to the method described by Ranganna (2001) and results were expressed as g/100 g of juice. Ascorbic acid was determined by employing the method described by Ruck (1963) and results were expressed as mg/100 g of juice. Total anthocyanins were estimated by pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (25 mM) and sodium acetate buffer, pH 4.5 (0.4 M) (Giusti and Wrolstad, 2001). The absorbance was calculated at 510 and 700 nm according to the following equation:

$$A = (A_{510} - A_{700})\, pH_{1.0} - (A_{510} - A_{700})\, pH_{4.5}$$

Results were expressed as mg of cyanidin-3-glucoside equivalents per 100 g of juice using a molar absorptive coefficient of 26,900 and a molecular weight of 449.2.

Total phenolics, total tannins and condensed tannins: Total phenolics were measured using the Folin-Ciocalteau method (Makkar *et al.*, 1993). Total tannins were determined after adding insoluble PVPP and reacting with Folin Ciocalteau reagent (Makkar *et al.*, 1993). Results were expressed as mg of tannic acid equivalents/100 g of juice. Condensed tannins were analyzed according to the method of Porter *et al.* (1986) and results were expressed as mg of catechin equivalents per 100 g of juice.

Antioxidant activity: Antioxidant activity was determined by the DPPH method described by Moon and Terao (1998). Briefly, 0.1 mL of pomegranate juice was mixed with 0.9 mL of 100 mM Tris-HCl buffer (pH = 7.4) to which 1 mL of DPPH (500 μM in ethanol) was added. The control sample was prepared similar way by adding 0.1 mL of water instead of pomegranate juice. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm 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:

$$\text{Antioxidant activity} = \left(1 - \frac{A_{\text{sample 517 nm}}}{A_{\text{sample 517 nm}}}\right) \times 100$$

Statistical analysis: 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 LSD test.

RESULTS AND DISCUSSION

Physical properties: Physical characteristics of the six pomegranate cultivars are shown in Table 1. Significant differences were revealed among the pomegranate cultivars for fruit weight, aril weight, aril percentage, juice weight, juice percentage and juice dry matter. Fruit weight values of pomegranate cultivars ranged between 220.75 (Faroogh) and 346.63 g (Shahvar) (Table 1). Shulman *et al.* (1984) reported that variation of fruit weight depend on the cultivar and ecological condition. As shown in Table 1, aril percentage ranged from 57.86 (Rabbab-e-Fars) to 75.48% (Faroogh) among the cultivars which is consistent with previously reported results for pomegranate cultivars grown in Oman (Al-Said *et al.*, 2009). One of the most important factors from an industrial point of view is the juice content of the aril. The juice percentage (of whole fruit) of the studied pomegranate cultivars varied from 48.02 (Rabbab-e-Fars) to 63.52% (Faroogh) (Table 1). Martinez *et al.* (2006) reported juice percentage between 50.25 and 64.17% in Spain cultivars which is in agreement with the results. The variation in juice dry matter was observed among the pomegranate cultivars (11.05 and 14.41%) (Table 1). These values were lower than values reported by Al-Maiman and Ahmad (2002).

The results for the physical properties of the pomegranate cultivars in this study demonstrated that the six cultivars are different in all measured parameters. The Faroogh cultivar seems the most promising combined more percentage of aril and juice which is a highly desirable property in the food processing and beverage industry. The other promising cultivars are Shahvar for its bigger fruits. Both of the cultivars may be useful especially in developing cultivars with the greater agronomic potential.

Chemical analysis: The chemical analysis results of the pomegranate cultivars investigated are shown in Table 2. Significant differences were detected in all measured parameters. The pH values varied from 3.06-3.74 which Shirin-e-Mohali showed the highest pH (Table 2). Similar findings have been published for pomegranate of different cultivars grown in Turkey, with pH values between 2.82 and 3.81 (Cam *et al.*, 2009a). The total soluble solids content were between 15.77 (Shirin-e-Bihaste) and 19.56 °Brix (Rabba-e-Fars) (Table 2). Poyrazoglu *et al.* (2002) reported total soluble solids levels of some pomegranate cultivars Turkey between 16-19 °Brix which is in agreement with the results. As shown in Table 2, the highest (1.35 mg/100 g) and the lowest (0.51 mg/100 g) content of titrable acidity were observed in Rabbab-e-Fars and Shirin-e-Bihaste, respectively. Poyrazoglu *et al.* (2002) also reported similar results in their study. The total sugars content of studied cultivars ranged from 16.88-22.76 (g/100 g) (Table 2). The highest amount of total sugars was observed for Faroogh followed by Shahvar while the lowest was in Rabbab-e-Fars. The results were higher than values (13.9-16.06 g/100 g) observed by Poyrazoglu *et al.* (2002).

Anthocyanins are a member of phenolics compounds that contributes to the red, blue or purple color of many fruits including pomegranate juice and they are well known for their antioxidant activity. The level of total anthocyanins of six pomegranate cultivars was within 7.93-27.73 (mg /100 g) that Aghaye having the highest

Table 1: Physical characteristics of six Iranian pomegranate cultivars

	Cultivars					
Parameters	Aghaye	Faroogh	Rabbab-e-Fars	Shahvar	Shirin-e-Bihaste	Shirin-e-Mohali
Fruit weight (g)	277.54±10.14^{b}	220.75±8.03^{c}	235.10±9.87^{c}	346.63±11.90^{a}	228.65±20.34^{c}	292.54±6.400^{b}
Aril weight (g)	194.87±8.70^{b}	166.67±7.16^{c}	136.13±9.46^{ds}	228.92±11.85^{a}	151.79±14.60^{cd}	210.14±4.300^{ab}
Aril percentage	70.19±0.92^{b}	75.48±0.58^{a}	57.86±2.09^{d}	66.01±1.160^{c}	66.35±1.610^{c}	71.85±1.530^{b}
Juice weight (g)	147.65±17.26^{c}	140.24±5.57^{c}	112.89±5.31^{d}	209.44±3.730^{a}	132.38±8.680^{cd}	174.87±7.610^{b}
Juice percentage (of whole fruit)	53.08±4.28^{bc}	63.52±0.65^{a}	48.02±1.02^{c}	60.49±2.880^{a}	57.99±1.630^{ab}	59.78±2.340^{a}
Juice dry matter (%)	14.41±0.86^{a}	11.09±1.56^{c}	12.53±0.17^{bc}	11.60±0.120^{bc}	11.05±0.480^{c}	13.31±1.01s^{ab}

Means of 20 fruits in each row followed by different letters are significantly different ($p<0.05$); ±Standard Deviation

Table 2: Chemical composition of six Iranian pomegranate juice cultivars

	Cultivars					
Parameters	Aghaye	Faroogh	Rabbab-e-Fars	Shahvar	Shirin-e-Bihaste	Shirin-e-Mohali
pH	3.37±0.04^{c}	3.06±0.03^{e}	3.23±0.020^{d}	3.70±0.005^{a}	3.58±0.010^{b}	3.74±0.010^{a}
Total soluble solid (°Brix)	16.27±0.11^{d}	17.62±0.09^{b}	19.56±0.390^{a}	16.67±0.150^{cd}	15.77±0.090^{e}	16.95±0.100^{c}
Titrable acidity (g/100 g)	0.99±0.01^{c}	1.12±0.01^{b}	1.35±0.007^{a}	0.58±0.009^{d}	0.51±0.006^{f}	0.54±0.009^{e}
Total sugars (g/100 g)	20.02±0.16^{c}	22.76±0.08^{a}	16.88±0.080^{f}	22.22±0.050^{b}	18.55±0.030^{e}	19.44±0.150^{d}
Total anthocyanins (mg/100 g)	27.73±0.65^{a}	21.24±0.25^{c}	24.43±0.410^{b}	7.93±0.080^{e}	9.85±0.650^{d}	8.32±0.290^{e}
Ascorbic acid (mg/100 g)	8.68±1.13^{c}	10.18±0.76^{c}	9.78±0.440^{c}	12.21±0.500^{b}	15.07±1.100^{a}	14.85±1.130^{a}

Means of 20 fruits in each row followed by different letters are significantly different ($p<0.05$); ±Standard Deviation

Table 3: Total phenolics (mg tannic acid/100 g), total tannins (mg tannic acid/100 g), condensed tannins (mg catechin/100 g) and antioxidant activity (percentage of inhibition) of six Iranian pomegranate juice cultivars

	Cultivars					
Parameters	Aghaye	Faroogh	Rabbab-e-Fars	Shahvar	Shirin-e-Bihaste	Shirin-e-Mohali
Total phenolics	797.49±0.110[a]	720.53±0.150[b]	786.20±0.200[a]	526.40±0.120[d]	568.63±0.110[c]	565.81±0.110[c]
Total tannins	38.21±0.150[a]	24.40±0.040[b]	32.60±0.240[a]	18.77±0.220[b]	18.81±0.310[b]	21.25±0.490[b]
Condensed tannins	12.57±0.006[a]	12.21±0.002[b]	12.50±0.005[a]	12.51±0.002[a]	12.14±0.002[b]	12.18±0.005[b]
Antioxidant activity	52.71±1.320[a]	49.96±0.430[b]	51.44±1.490[ab]	46.51±0.110[c]	47.03±0.740[c]	47.49±0.560[c]

Means of 20 fruits in each row followed by different letters are significantly different ($p<0.05$); ±Standard Deviation

amount of total antocyanins than the other cultivars (Table 2). The results were lower than values (8.1 and 36.9 mg/100 g) reported by Cam *et al.* (2009b). Ascorbic acid is abundant and has many biological functions in fruits which include roles in many aspects of redox control and antioxidant activity that prevent, for example, the browning of tissues (Kulkarni and Aradhya, 2005). The concentration of ascorbic acid varied from 8.68-15.07 (mg/100 g). The highest content of ascorbic acid was observed in Shirin-e-Bihaste and the lowest one in Aghaye (Table 2). Six pomegranate juices showed higher ascorbic acid values than pomegranate juice from Ganesh variety (>10 mg/100 g) reported by Kulkarni and Aradhya (2005).

Overall, these results indicate that six cultivars are different in terms of their total soluble solids, pH, titrable acidity, total sugars, total anthocyanins and ascorbic acid and also there was a high genetic heterogeneity within the studied cultivars. All the cultivars investigated were suitable for direct consumption and production pomegranate juice because they had high level of soluble solids.

Total phenolics, total tannins and condensed tannins: The results for total phenolics, total tannins and condensed tannins of the pomegranate from the different cultivars are given in Table 3. A variation in terms of total phenolic content was observed among the pomegranate cultivars (526.40-797.49 mg tannic acid/100 g) and the differences were statistically significant. The highest content of total phenolics was observed in Aghaye followed by Rabbab-e-Fars while the lowest one was in the Shahvar (Table 3).

The reported levels of total phenolics in literature are 124.5-207.6 mg/100 g by Ozgen *et al.* (2008), 208.3 and 343.6 mg/100 g by Cam *et al.* (2009b), 14.4 and 1008.6 mg/100 g by Tezcan *et al.* (2009) and 238 and 930.4 mg/100 g by Mousavinejad *et al.* (2009). Tannins are secondary metabolites which defend plants from herbivory by protein precipitation and increased acidity. It has been reported that tannins play an important role in human health and are implicate with numerous biological properties. In this study, the differences in total tannins content among the pomegranate cultivars were statistically significant and the values ranged from 18.77 (Shahvar) to 38.21 (Aghaye) (mg tannic acid/100 g) (Table 3). The results were lower than values (41.7-53.9 mg/100 g) observed by Gil *et al.* (2000) while the results were higher than values (15-32 mg/100 g) reported by Mousavinejad *et al.* (2009). Condensed tannins are also known as proanthocyanidins which are polymeric flavonoid molecules that found in a range of higher plant species. There were significant differences in the condensed tannins content of the pomegranate cultivars and the values varied from 12.14-12.57 (mg catechin/100 g). The highest level of condensed tannins was recorded in Aghaye and the lowest one in Shirin-e-Bihaste (Table 3).

In regard to the chemical composition, since all six pomegranate 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 antioxidant activity of the studied pomegranate cultivars are shown in Table 3. A significant difference in antioxidant activity was found among the six cultivars of pomegranate studied and the values ranged from 46.51-52.71%. The highest and the lowest antioxidant activity were detected in Aghaye and Shirin-e-Bihaste, respectively. Cam *et al.* (2009b) reported antioxidant activity for eight pomegranate cultivar varied from 73-91.8% growth in Turkey which is higher than the result. In another study, antioxidant activity of Ganesh pomegranate cultivar of juice was reported as 69% (Kulkarni and Aradhya, 2005). The variation in comparison with the data of the present research may the result of other factors such as the different pomegranate cultivars and sample extraction method used in the experiments.

Correlation analysis: As shown in Table 4, the antioxidant activity was positively correlated with the total phenolics ($r = 0.912$), total tannins ($r = 0.838$), condensed tannins ($r = 0.859$) and total anthocyanins ($r = 0.928$). Feryal *et al.* (2005) also reported a positive correlation between total antioxidant activity and total phenolics ($r = 0.930$) in fruits growth in Turkey. In

Table 4: Correlation coefficients (r) of pH, Total Soluble Solids (TSS), Titrable Acidity (TA), Total Sugars (TS), Ascorbic Acid (AA), Total Anthocyanins (TAs), Total Phenolics (TPs), Total Tannins (TTs), Condensed Tannins (CTs) and Antioxidant activity (AC) of six Iranian pomegranate juice cultivars

Chemicals	pH	TSS	TA	TS	AA	TAs	TPs	TTs	CTs	AC
pH	1									
TSS	-0.536^{ns}	1								
TA	-0.887**	0.776**	1							
TS	-0.178^{ns}	-0.301^{ns}	-0.015^{ns}	1						
AA	0.750**	-0.420^{ns}	-0.805**	-0.350^{ns}	1					
TAs	-0.811**	0.440^{ns}	0.884**	0.065^{ns}	-0.860**	1				
TPs	-0.812**	0.509^{ns}	0.904**	-0.034^{ns}	-0.807**	0.985**	1			
TTs	-0.520*	0.330^{ns}	0.695*	-0.059^{ns}	-0.754**	0.878**	0.884**	1		
CTs	-0.476^{ns}	0.386^{ns}	0.706*	-0.161^{ns}	-0.733*	0.866**	0.865**	0.941**	1	
AC	-0.679*	0.382^{ns}	0.796**	-0.003^{ns}	-0.810**	0.928**	0.912**	0.838**	0.859**	1

*Correlation coefficients (r) significant at p<0.05, **Correlation coefficients (r) significant at p<0.01, NS = Not Significant

addition, A high and significant correlation between total anthocyanins and total phenolic content was determined (r = 0.985). Similar findings have been reported by Ozgen *et al.* (2008). According to the results of the correlation analysis (Table 4), there was a significant positive correlations between titrable acidity with the total anthocyanins (r = 0.884), total phenolics (r = 0.904), total tannins (r = 0.695), condensed tannins (r = 0.706) and antioxidant activity (r = 0.796) while there was a significant negative correlation between titrable acidity and pH (r = -0.886).

CONCLUSION

The physico-chemical properties and antioxidant activity differ between the cultivars studied indicating that cultivar is the main parameter determining the composition of bioactive compounds in pomegranates. The results provide important information of the physico-chemical characteristics of pomegranate cultivars which can be useful for developing fruit processing industry and selection of superior desirable pomegranate genotypes for bringing to commercial cultivation. Additionally, the correlation analysis indicates that the total phenolics content contributes significantly to the antioxidant activity of pomegranates.

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