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Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices

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ABSTRACT

Pomegranate fruit and pomegranate juices (PJs) have taken great attention for their health benefits in the last years. The purpose of this study is to analyse the antioxidant activities, along with the organic acid and sugar contents of pomegranate juices sold in the Turkish markets. In the present study, we evaluated total phenolics (TPs), free radical scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing capacity of seven commercial PJs. Organic acid and sugar contents of juices were determined by capillary zone electrophoresis. The results showed that commercial pomegranate juices had markedly high total phenolic contents and antioxidant capacity. Fructose (F) and glucose (G) were found as the major sugars. The major acids were citric and malic. From the F/G ratio, organic acid profiles, TPs, and antioxidant capacity values, a possible adulteration was detected in one of the juices.

1. Introduction

Pomegranate fruit (*Punica granatum*) has taken great attention for its health benefits in the last years. In the past decade, numerous studies on the antioxidant activity have shown that pomegranate juice contains high levels of antioxidants - higher than most other fruit juices and beverages (Gil, Tomas-Berberan, Hess-Pierce, Holcroft, & Kader, 2000; Seeram et al. 2008). Besides these findings, some clinical research studies suggest that pomegranate juice changes the blood parameters such as LDL, HDL, and cholesterol (Aviram & Dornfeld, 2001; Aviram et al., 2000, 2004; Kaplan et al. 2001), increase the prostate specific antigen (PSA) (Pantuck et al., 2006), and may be helpful against heart disease (Sumner et al., 2005), Alzheimer's disease (Singh, Arseneault, Sanderson, Morthy, & Ramassamy, 2008), cancer (Khan, Afaq, Kweon, Kim, & Mukhtar, 2007; Malik & Mukhtar, 2006; Seeram et al., 2007), improvement of sperm quality (Türk et al., 2008) and erectile dysfunction in male patients (Forest, Padma-Nathan, & Liker, 2007).

Pomegranate arils have been consumed in Turkey as fresh fruit and juice, and also in salads and desserts. Turkey contributes 150,000 tons of the world annual production of one million tons. Following its health benefits popularity, recently many commercial pomegranate juices appeared in markets. The major contribution to the total antioxidant capacity of pomegranate juice is attributed to punicalagin originating from the peels (Gil et al., 2000; Tzulker et al., 2007). Since the whole fruit is pressed to prepare commercial juices, it would be expected a large amount of bioactive compounds from the peels are extracted and consequently commercial juices would have high antioxidant activity.

Although there is an important body of works in the literature on the antioxidant capacities of pomegranate from different countries, including Turkey (Çam, Hışıl, & Durmaz, 2009; Poyrazoğlu, Gökmen, & Artık, 2002; Özgen, Durgaç, Serçe, & Kaya, 2008), studies on commercial pomegranate juices are almost nonexistent. Gil et al. (2000) gave the comparison of the antioxidant capacity of only one brand of commercial pomegranate juice with fresh aril juice. In an article that compares the antioxidant capacities of beverages in the USA market, the value for only one brand of pomegranate juice is reported (Seeram et al. 2008).

The purpose of this study is to analyse the antioxidant activities, along with the organic acid and sugar contents, of pomegranate juices sold in the Turkish markets. Organic acid profiles in juices characterise flavours, freshness, or spoilage of juices. Because organic acid profiles are distinct to each type of fruit juice, their profiles also give important evidences about adulteration of a juice with cheaper juices. Similarly, sugar profiles (glucose and fructose) are important markers to detect adulteration in fruit juices.

In pomegranates, in comparison with the determination of antioxidant capacity, very few works exist on the determination of

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organic acid and sugar (Melgarejo, Salazar, & Artes, 2000; Özgen et al., 2008; Poyrazoğlu et al., 2002). In the present study, organic acid and carbohydrate contents of commercial pomegranate juices were determined by an efficient and rapid capillary electrophoresis technique.

2. Materials and methods

2.1. Chemicals

2,6-pyridinedicarboxylic acid, citric acid monohydrate, malic acid, and N-Cetyl-N,N,N-trimethylammonium bromide (CTAB) were purchased from Merck (Darmstadt, Germany), glycyl-glycine from Fluka (Buchs, Switzerland) D(+)-Glucose and D(−) Fructose, Folin–Ciocalteu reagent, gallic acid, 2,2 diphenyl-1-picrylhydrazyl, 2,4,6-tripyridyl-s-triazine, and $FeSO_4 \cdot 7H_2O$ were from Sigma Chemical Co (Steinheim, Germany).

2.2. Pomegranate juices

Pomegranate juices (PJs) belonging to seven different commercial trade marks were purchased from local markets. All the companies claimed on the packages that they are 100% pomegranate juices (PJs) and that none of them contain extra added ingredients.

2.3. Determination of individual organic acids

Capillary electrophoretic separations were performed with an Agilent capillary electrophoresis system equipped with a diode-array detector. The data processing was carried out with the Agilent ChemStation software. The fused silica capillaries used for separation experiments were 50 μm i.d. and were obtained from Agilent. The total length of the capillary was 64 cm and the length to the detector was 56.5 cm. The new fused silica capillary was conditioned prior to use by rinsing with 1 mol/L NaOH for 30 min and with water for 10 min. The capillary was flushed by 0.1 mmol/L NaOH and water for 2 min each in the beginning of every working day, then the capillary was washed with buffer for 10 min, and 2 min of flushing with buffer was performed between runs.

The capillary zone electrophoresis method developed by Soga and Ross (1997) was slightly modified to the analysis of organic acids in PJs. The method was based on using a background electrolyte, 2,6-pyridinedicarboxylic acid (PDC), for the indirect detection of organic acids and was applied by many authors to the analysis of organic acids in juices and beverages (Esteves, Lima, Lima, & Duarte, 2004; Nutku & Erim, 1999; Öztekin & Erim, 2001). The optimal separation buffer at this application was selected as 5 mmol/L PDC, 0.1 mmol/L CTAB at pH 5.28. Samples were injected at 50 mbar for 5 s. from cathodic side. Separation voltage was set at 25 kV. The signal wavelength was set at 350 nm with a reference at 200 nm. All solutions were prepared with deionized water purified in an Elgacan C114 (Elga, England) filtration system.

2.4. Determination of individual sugars

A capillary electrophoretic method developed by our group was applied to the analysis of sugars in the PJs (Gürel, Hızal, Öztekin, & Erim, 2006). The method was based on using a dipeptide, glycylglycine, as the background electrolyte. This electrolyte, without any additive, improves the resolution of sugars as well as providing their indirect detection. Optimal separation conditions were selected as 75 mmol/L glycylglycine at pH 12.85. Samples were injected at 50 mbar for 5 s. from the anodic side and the voltage was set at 25 kV. The signal wavelength was set at 350 nm with a reference at 207 nm.

2.5. Determination of total phenolics (TPs)

Total phenolics were determined by using Folin-Ciocalteu method (Singleton & Rossi, 1965). 300 μl of diluted PJ in the ratio of 1:100 with methanol:water (6:4) was mixed with 1.5 ml of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 ml of 7.5% of sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature before the absorbance was measured by a Shimadzu UV-1700 spectrophotometer at 760 nm. Results were expressed as gallic acid equivalents (GAE).

2.6. Determination of antioxidant activities

The antioxidant activities of PJs were determined by two methods as previously recommended by Gil et al. (2000) and Tzulker et al. (2007) as the most trustable methods specifically for PJs. The first method is based on the evaluation of the free–radical scavenging capacity. In this method, the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the antioxidant activity of juices (Brand-Williams, Cuvelier, & Berset, 1995). 100 μl of PJ diluted in the ratio of 1:100 with methanol:water (6:4) was mixed with 2 ml of 0.1 mol/L DPPH in methanol. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage.

The second method applied here is based on the measurement of the iron-reducing capacity of juices. The FRAP reagent (Benzie & Strain, 1996), containing 2.5 ml of 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mol/L HCl plus 2.5 ml of 20 mol/L $FeCl_3$ and 25 ml of 0.3 mol/L acetate buffer at pH 3.6, was freshly prepared and warmed to 37 °C prior to use. 40 μl of diluted juice in the ratio of 1:100 with methanol:water (6:4) sample was mixed with 0.2 ml of distilled water and 1.8 ml of FRAP reagent. After incubation at 37 °C for 10 min, the absorbance of the mixture was measured by a UV–Vis spectrophotometer at 593 nm. The results were calculated from the standard curve prepared using different concentrations (300–1100 μmol/L) of $FeSO_4 \cdot 7H_2O$ and after correction for dilution, expressed in mmol Fe^{2+}/L.

3. 3.Result and discussion

3.1. Organic acids

Malic acid and citric acid were detected as the main organic acids in the commercial PJs, as reported before for PJs (Melgarejo et al., 2000; Poyrazoğlu et al., 2002; Özgen et al., 2008). Fig. 1 shows the electropherogram of one of the PJs (PJ-C). As seen from the figure, both acids were completely separated from each other at the selected separation conditions. A baseline peak separation was also achieved, due to the high separation capacity of capillary electrophoresis, in the sample PJ-A containing a high amount of citric acid in comparison with malic acid.

The calibration curves were linear between 0.0134 and 0.134 mg/mL with 0.996 regression and between 0.021 and 0.21 mg/mL with 0.995 regression for malic and citric acid, respectively. All juices were diluted in the 1:50 ratio with water and injected three times. The levels of malic and citric acid in seven commercial PJs were given as averages of three injections with their standard deviations in Table 1. As seen from the Table 1, citric acid concentrations of PJs varied from 0.393 to 1.306 g/100 mL. Citric acid levels of 40 Spanish pomegranate cultivars were reported by Melgarejo et al. (2000) as between 0.142–2.317 g/100 g; by Poyrazoğlu et al. (2002), for 13 pomegranates from four different region of Turkey, as between 0.033–0.896 g/100 ml, and by Özgen

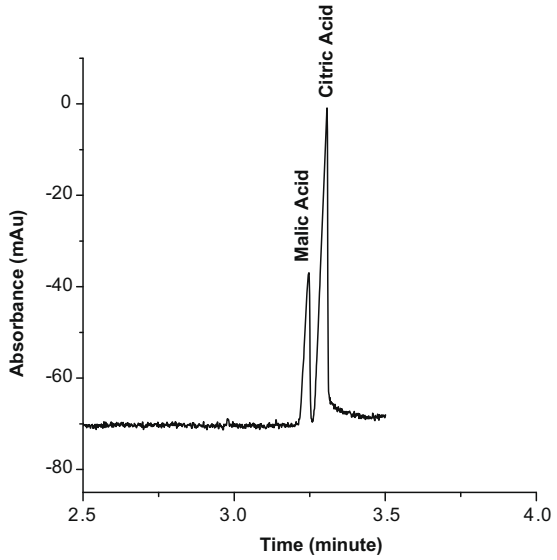

Fig. 1. Electropherogram of the PJ-C. Conditions: capillary 56.5 cm effective length ×50 μm I.D; separation electrolyte 5 mmol/L PDC, 0.1 mmol/L CTAB; pH 5.28; voltage −25 kV.

Table 1
Organic acid and sugar contents of PJs.

Commercial PJs	Malic acid (mg/mL)	Citric acid (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)
A	0.397 ± 0.01	13.06 ± 0.20	65.42 ± 1.47	69.90 ± 1.52
B	1.29 ± 0.01	6.20 ± 0.24	45.40 ± 0.12	58.18 ± 2.14
C	2.36 ± 0.12	7.87 ± 0.11	39.78 ± 0.11	55.13 ± 0.93
D	0.524 ± 0.010	8.09 ± 0.16	69.14 ± 0.09	63.24 ± 1.66
E	4.11 ± 0.04	3.93 ± 0.02	42.99 ± 1.22	93.63 ± 3.63
F	0.285 ± 0.003	7.36 ± 0.30	46.01 ± 0.05	45.49 ± 0.03
G	2.15 ± 0.02	5.65 ± 0.26	50.33 ± 1.31	63.87 ± 2.16

et al. (2008), for six pomegranates from Mediterranean region of Turkey, as between 0.20 and 3.20 g/100 mL.

Malic acid concentrations of commercial juices found ranging between 0.0285 and 0.411 g/100 mL for PJs. The reported levels of this acid in literature are 0.135–0.176 g/100 g by Melgarejo et al. (2000); 0.056 and 0.686 g/100 mL by Poyrazoğlu et al. (2002), and 0.09 and 0.15 g/100 mL by Özgen et al. (2008). Malic acid content for Juice-E is seen comparatively higher than the other juices and reported values for PJs, except for the high reported level by Poyrazoğlu et al. (2002). As it will be discussed later, the other analytical data of this juice are also exceptional compared to others.

3.2. Sugars

Glucose (G) and fructose (F) were the only sugar types detected in PJs. Fig. 2 shows the electropherogram of a PJ-D. G and F contents of juices were calculated from calibration curves drawn between 0.36 and 3.6 mg/mL for both sugar types with 0.997 and 0.982 regression for glucose and fructose, respectively. All juices were diluted in the 1:50 ratio with water and injected two times. Sugar contents and standard deviations of seven PJs were given in Table 1. G contents changes between 3.98 and 6.91 g/100 mL and F between 4.55 and 9.36 g/100 mL. Individual sugar contents reported in the literature are 5.66–6.45 g /100 g for G and 5.96–7.04 g/100 g for F for 40 Spanish cultivars (Melgarejo et al., 2000) and 5.80–7.62 g/100 ml for G and 5.80–7.06 g/100 ml for F for six pomegranate arils from Turkey (Özgen et al., 2008). Again here, the fructose content of juice E is higher than those of other juices.

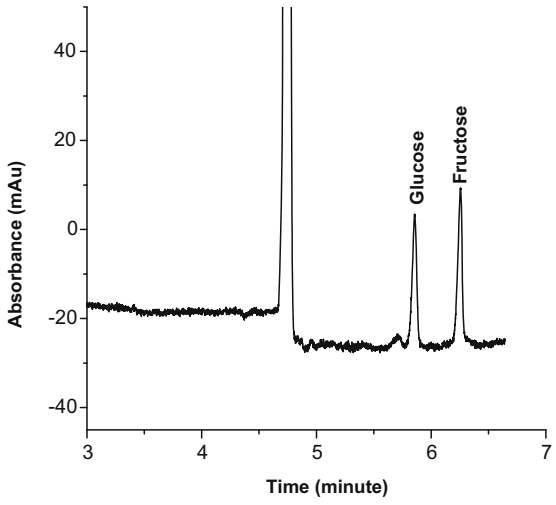

Fig. 2. Electropherogram of the PJ-D. Conditions: capillary 56.5 cm effective length ×50 μm I.D; separation electrolyte 75 mmol/L glycylglycine; pH 12.85; voltage 25 kV.

Table 2
Total phenolic contents and antioxidant activities of commercial pomegranate juices.

Sample	Total phenolics (mg GAE/L)	DPPH (inhibition%)	FRAP (mmol/l Fe^{+2})
A	6087 ± 207	43.89 ± 8.16	87.89 ± 12.76
B	2602 ± 16	25.19 ± 0.47	40.14 ± 6.06
C	4993 ± 239	30.80 ± 9.52	82.87 ± 10.53
D	7846 ± 191	48.49 ± 4.95	109.9 ± 13.70
E	144 ± 80	10.37 ± 2.69	18.34 ± 9.21
F	10086 ± 85	67.46 ± 2.54	121.8 ± 8.30
G	4335 ± 85	32.35 ± 0.52	72.66 ± 9.25

3.3. Total phenolics

The PJs exhibited high amount of TP as seen from Table 2. TPs range from 2602 to 10086 mg/L, except juice E. This juice showed very low TPs level. Gil et al. reported the TPs of PJ from fresh arils as 2117 ± 95 mg/L and for a commercial PJ as 2566 ± 131 mg/L. Tzulker et al. (2007) reported that polyphenol content of juices prepared from arils and whole fruit of 29 pomegranate accessions. TPs from whole fruits are reported as around 1875 and 11250 mg/L, which are higher by about 6.5-fold in comparison to the juices prepared from the arils. TPs of six pomegranate arils from Mediterranean region of Turkey are reported between 1245 and 2076 mg/L (Özgen et al., 2008), and of eight pomegranate arils widely grown in Turkey are between 2083–3436 mg/L (Çam et al., 2009).

3.4. Antioxidant activity

Antioxidant activities of PJs were given in Table 2. There are good correlations between the results of two methods ($r = 0.95$). The antioxidant and TP levels are also positively and significantly correlated ($r > 0.98$). TPs and antioxidant capacities of juices are graphically shown in Fig. 3.

PJ contains high amounts of hydrolyzable tannins and anthocyanines. However, the results of exhaustive work of two groups (Gil et al., 2000; Tzulker et al., 2007) on antioxidant activity of PJs suggested that punicalagin originating from the peels is one of the major phytochemicals contributing to the total antioxidant capacity of pomegranate juice, whilst anthocyanins play only a minor role in this activity. Gil et al. (2000) reported for the first time that the

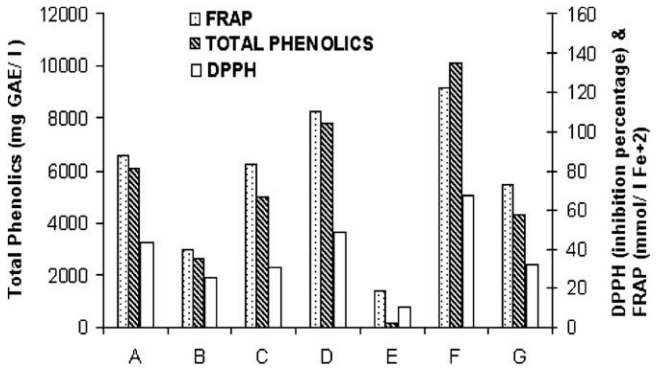

Fig. 3. Total phenolics and antioxidant activities of commercial pomegranate juices by the DPPH and FRAP methods.

activity was higher in commercial juices than in the experimental ones obtained in the laboratory by hand pressing the arils. The Tzulker et al. (2007) reported that polyphenol content increased by about 6.5-fold and the antioxidant activity by about 20-fold, for juices prepared from the whole fruit in comparison to the juices prepared from the arils alone. TPs of peel extracts found by Li et al. (2006) were nearly 10-fold as high as that of pulp extract.

Commercial PJs prepared from pressing the whole fruits would be expected to contain a large amount of phenolics from both arils and peels and to have enhanced antioxidant capacity. This expectation was fulfilled in the juices studied in the present work. Six of the seven PJs studied here exhibited high TP contents and antioxidant capacities compared to the reported values from aril juices. Both TP and antioxidant level for PJ-E is comparatively low compared to the other six.

After considering all analytical results for this PJ, a suspicion of adulteration arises. The malic acid level of PJ-E is found higher compared with the other PJs. As is well known, high malic acid is specific to apple. Again in PJ-E, the amount of fructose was found to be higher than the other PJs. Although the amounts in G and F are found different based on being sour and sweet pomegranate, in general in the reported values for PJs, these two sugar amounts are close to each other. In this study the F/G ratios are between 0.91 and 1.39 (mean: 1.15 ± 0.19) for the other 6 PJs, whereas this ratio is 2.18 for PJ-E. Eisele and Drake (2005), in their study on 175 apples found the specific ratio of 2.5 for apple. As seen in Table 2, the TP and antioxidant activity in PJ- E are incomparably lower than those of the other PJs. In the study on fruit juices in the USA (Seeram et al., 2008), the TP of one commercial PJ was reported to be 9.5 times larger than the average TPs of three commercial apple juices. Similarly, the antioxidant activity (DPPH) was approximately 4 times larger. All of these facts point to apple juice adulteration in PJ-E. In many countries, apple is less expensive fruit than pomegranate. In one research on the elderly people, Guo et al. (2008) concluded that daily consumption of pomegranate juice is potentially better than apple juice in improving the antioxidant function in the elderly. The mixing of two or more fruit juices, in order to obtain different tastes, is generally acceptable, but the mixing of another fruit juice (even if a taste advantage is obtained) to a fruit juice that is claimed to be 100% is adulteration.

4. Conclusions

In the six commercial PJs studied in this work, in comparison to aril PJs and other fruit juices reported in the literature, much higher TPs and antioxidant capacities were observed, with increased health benefits for the consumers. From these respects, PJ-C shows an exceptional value. Organic acid and sugar profiles of the PJs give important information on the purity and adulteration of juices. For the fast and simple analysis of these components in PJs, capillary electrophoresis method is recommended.

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