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Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey

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ABSTRACT

Arils from six pomegranate (*Punica granatum* L.) cultivars obtained from various sites from the Mediterranean region of Turkey were evaluated for their chemical and antioxidant properties. These properties included total phenolics (TP), total monomeric anthocyanins (TMA), soluble solids (TSS), titratable acidity (TA), individual sugars and organic acids. Antioxidant capacities of arils were determined by both the ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays. The antioxidant capacities averaged 5.60 and 7.35 mmol TE/l by the TEAC and FRAP methods. Variability among cultivars was greatest for TMA content (CV 132%); individuals ranged from 6.1 to 219 mg cy3-Gluc l^{-1}. TP means averaged 1507 mg GAE/l. Levels of FRAP, TEAC, TP, and TMA were strongly correlated ($r = 0.82$–0.96). The major sugars were fructose (6.4 g/100 ml) and glucose (6.8 g/100 ml), the major acids were citric (1.78 g/100 ml) and malic (0.12 g/100 ml).

1. Introduction

In recent years, there has been an increasing interest in determining antioxidant properties of red fruits, because they are rich dietary sources of antioxidant phenolics and anthocyanins (Kaur & Kapoor, 2001; Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Ozgen et al., 2007; Velioglu, Mazza, Gao, & Oomah, 1998; Wang, Cao, & Prior, 1996). Epidemiological studies have suggested that consumption of red fruit juices, such as grape, berry juices and pomegranate, correlates with reduced risk of coronary heart disease, stroke, certain types of cancers and aging (Malik & Mukhtar, 2006; Prior, 2003). It has been reported that pomegranate juice is one of the important sources of anthocyanins (cyanidin, delphinidin, and pelargonidin), which give the fruit and aril its red colour, and some of the phenolics and tannins (such as punicalin, pedunculagin, punicalagin and ellagic acid) (Kulkarni & Aradhya, 2005). Thus, pomegranate has become more popular because of its health-promoting phytonutritional content (Adams et al., 2006; Faria, Monteiro, Mateus, Azevedo, & Calhau, 2007; Khan & Mukhtar, 2007; Malik et al., 2005).

The pomegranate fruit is round, with leathery skin or rind, typically yellow, overlaid with light or deep pink or rich red. The edible part of the fruit, the arils, is yellow to deep red in colour and are comprised of approximately 80% juice and 20% seed by weight.

The cultivation of the pomegranate (*Punica granatum* L.) is mainly confined to semi-arid mild-temperate to subtropical climates and pomegranates are naturally adapted to regions with hot summers and cool winters, such as Mediterranean countries, Afghanistan, Iran, India, China, Japan, and The United States (California) (Stover & Mercure, 2007). Turkey is one of the native lands of the pomegranate and it is widely grown in the Mediterranean, Aegean, south-east and some of the microclimates of Turkey (Ercisli, Agar, Orhan, Yildirim, & Hizarci, 2007; Ozguven, Tatli, Coskun, & Dasgan, 1996). The total pomegranate production of Turkey is expected to exceed 100,000 tons in 2007. Most of this production takes place in the Mediterranean area; local cultivars are numerous, displaying a variety of attractive aril colours and distinct flavour profiles. For instance, 'Kan' arils exhibit a very deep red colour, whereas those of 'Tatli' are bright cream. The objective of our study was to determine antioxidant activities and total phenolic and anthocyanin contents of these distinct pomegranate cultivars grown in the Mediterranean region of Turkey.

2. Materials and methods

2.1. Plant material

Commercially ripe fruits from six pomegranate cultivars ('Dikenli incekabuk', 'Eksi', 'Kan', 'Katirbasi', 'Serife', and 'Tatli') were harvested from various sites of southern Turkey. Arils of fruits were hand-separated (about 100 g lots) and frozen at −20 °C. Three

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replicates were maintained for each analysis, each replicate indicating three pomegranate fruits.

2.2. Extraction

Arils from each cultivar were thawed at room temperature and then homogenised in a food processor. Slurries were used to determine total soluble solid (TSS) contents by digital refractometry (Pal-1, Atago) and for levels of titratable acidity (TA), using standard methodology. Chemical analysis was completed within 10 days of storage. Aril colour was analysed using a Minolta portable chromameter (model CR-400; Minolta) which provided CIE L^*, a^*, and b^* values.

2.3. Determination of total phenolics (TP)

TP content was measured according to Singleton and Rossi (1965) procedure. An aliquot of aril slurry was extracted with buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for 1 h in the dark. Three parallel extracts were obtained from each cultivar. Then, extract, Folin–Ciocalteu's reagent and water were incubated for 8 min, followed by adding 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV–VIS spectrophotometer at 750 nm. Gallic acid was used as a standard. The results were expressed as mg gallic acid equivalent in l of fruit juice (GAE/l juice).

2.4. Total monomeric anthocyanins (TMA)

TMA were estimated by a pH differential method (Giusti, Rodriguez-Saona, & Wrolstad, 1999; Giusti & Wrolstad, 2005), using a UV–VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{533} - A_{700})_{\text{pH 1.0}} - (A_{533} - A_{700})_{\text{pH 4.5}}$ with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalents per l of juice.

2.5. Determination of total antioxidant activity

Total antioxidant activity was estimated by two standard procedures, FRAP and TEAC assays, as suggested by Ozgen, Reese, Tulio, Miller, and Scheerens (2006). Ferric reducing ability of plasma (FRAP) was determined according to the method of Benzie and Strain (1996). Assay was conducted using three aqueous stock solutions containing 0.1 M acetate buffer (pH 3.6) and 10 mM TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid (1000:3.3 v/v), and 20 mM ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.97 ml of FRAP reagent and 30 μl of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm in a spectrophotometer.

For the standard trolox equivalent antioxidant capacity (TEAC) assay, ABTS was dissolved in acetate buffer and prepared with potassium persulfate, as described by Rice-Evans, Miller, Bolweel, Bramley, and Pridham (1995) and Ozgen et al. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Ozgen et al., 2006). For the spectrophotometric assay, 3 ml of the $ABTS^{\cdot+}$ solution and 10 μl of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm.

2.6. Extraction of individual sugars and organic acids

Aril slurries (5 g) were diluted with purified water or metaphosphoric acid (2.5%) solution for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45-μm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use. All the samples and standards were injected three times each and mean values were used.

2.7. Chromatographic conditions

The HPLC analyses were carried out using a Perkin Elmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (Perkin Elmer series-200) (Waltham, MA, USA). Separation and determination of organic acids were done by a modified method of Shui and Leong (2002). The separation was carried out on a SGE wakosil C18RS 5 μm column (250 × 4.6 mm ID). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using pH 2.5 sulfuric acid solution (solvent A), and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 μl; column temperature, 30 °C; detection wavelength, 215 nm.

Analysis of sugars was performed according to the method described by Bartolome, Ruperez, and Fuster (1995), using a refractive index (RI) detector (Perkin Elmer). The separation was carried out on a SGE SS Exsil amino column (250 × 4.6 mm ID). The elution solvent used was 80% acetonitrile and 20% deionised water. The column was operated at 30 °C with 0.9 ml/min flow rate. Sample injection volume was 20 μl.

2.8. Statistical analysis

Data were analysed using SAS procedures and software (SAS, Cary, NC, USA). Means and standard deviations were obtained using PROC TABULATE. Coefficients of variation (CV) were calculated, dividing relevant standard deviations by means and multiplying by 100.

3. Results and discussion

Some of the fruit characteristics such as flavour-type, aril color, TSS, and acidity, are given in Table 1. TSS displayed the lowest variation of all parameters (CV 4.9%). 'Kan', 'Dikenli incekabuk', and 'Katirbasi' had more than 17% TSS content. Cultivars exhibited a range of flavour (from sour to sweet) and acidity (from 0.5 to 3.8%). 'Serife' had the highest acidity while 'Tatli' had the lowest. The variation was much higher than those reported by Drogoudi, Tsipouiridis, and Michailidis (2005) who studied 20 different pomegranate accessions in Greece. The highest acidity in that study was 2.4%, compared to 3.8% in our work. Aril colours as light as cream ('Tatli') or deep red ('Kan') were indicated by chroma and hue values. 'Kan' with a dark red colour, high TSS and relatively high acidity, might be good choice for both fresh fruit and juice markets. In fact, this is the most popular local cultivar among the consumers in the area. Attractive aril colour is one of the most important sensory attributes and, perhaps, nutritional advantages of pomegranate, with this characteristic might open opportunities for 'Kan' to be an important pomegranate cultivar for consumers.

Pomegranate exhibits good antioxidant capacity and is an effective scavenger of several reactive oxygen species, primarily due to its high levels of phenolic acids, flavonoids and other polyphenolic compounds (Aviram et al. 2002; Kulkarni & Aradhya, 2005). Our study also showed that selected cultivars had high amounts of TP and anthocyanins. The amount of TP varied between 1245 and 2076 mg gallic acid equivalent (GAE)/l of fruit juice and TMA between 6.12 and 219 mg cyanidin 3-glucoside equivalents/l of fruit juice (Table 2). TP means averaged 1507 mg GAE/l. Variability among cultivars was greatest for TMA content (CV 132%); 'Kan'

Table 1
Several characteristics of the pomegranate cultivars studied

Cultivar	Type	Aril colour	Chroma	Hue	TSS (°Brix)	Acidity (%)	pH
'Dikenli incekabuk'	Sour–sweet	Pink	18.5 ± 4.6	25.2 ± 3.6	17.4 ± 0.1	2.4 ± 0.1	2.98 ± 0.01
'Eksi'	Sour	Pink	19.9 ± 7.3	28.9 ± 13.3	16.8 ± 0.5	2.2 ± 0.1	3.24 ± 0.01
'Kan'	Sour–sweet	Dark red	19.6 ± 5.5	17.7 ± 1.5	17.9 ± 0.3	2.4 ± 0.2	3.32 ± 0.01
'Katirbasi'	Sour–sweet	Light pink	14.0 ± 1.5	25.5 ± 4.4	17.3 ± 0.3	1.4 ± 0.1	3.68 ± 0.02
'Serife'	Sour	Light pink	16.7 ± 2.4	32.8 ± 7.0	16.3 ± 0.2	3.8 ± 0.1	3.17 ± 0.02
'Tatli'	Sweet	Cream	9.2 ± 0.8	70.1 ± 9.6	14.7 ± 0.2	0.5 ± 0.1	3.63 ± 0.02
Mean			16.3 ± 2.5	33.3 ± 4.3	16.7 ± 0.1	2.1 ± 0.1	3.34 ± 0.01
CV (%)			7.3	23.7	4.9	48.2	29.9

Values represent means ± standard deviations calculated from three replicates.

Table 2
Total phenolic content (TP), total monomeic anthocyanins (TMA), antioxidant capacity (TEAC and FRAP), titratable acidity (TA) and total soluble solids (TSS) of pomegranate cultivars grown in southern Turkey

Cultivar	TP[a] (mg GAE/l)	TMA[b] (mg Cy-3Gluc/l)	TEAC[c] (mmol TE/l)	FRAP[d] (mmol TE/l)
'Dikenli incekabuk'	1395 ± 12	38.2 ± 0.79	5.84 ± 0.07	7.84 ± 0.17
'Eksi'	1465 ± 21	37.5 ± 1.21	5.33 ± 0.11	7.52 ± 0.35
'Kan'	2076 ± 54	219.0 ± 7.18	7.70 ± 0.06	10.9 ± 0.72
'Katirbasi'	1326 ± 105	41.2 ± 0.88	4.38 ± 0.05	5.37 ± 0.49
'Serife'	1532 ± 16	18.0 ± 0.09	5.64 ± 0.04	7.80 ± 0.20
'Tatli'	1245 ± 36	6.1 ± 0.35	4.73 ± 0.03	4.63 ± 0.44
Grand mean	1507 ± 35	60.0 ± 2.69	5.60 ± 0.03	7.35 ± 0.21
CV (%)	19.7	131.8	20.8	30.3

Cultivars represent means ± standard deviations calculated from three replicates; population variability is indicated by the grand mean.
[a] TP contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as mg gallic acid equivalents (GAE)/l of juice.
[b] TMA were determined by the pH-differential method of Giusti et al. (1999). Values are expressed as mg cyanidin 3-glucoside equivalents/l of juice.
[c] TEAC values were determined by the method of Ozgen et al. (2006). Values are expressed as mmol of trolox equivalents/l of juice.
[d] FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as mmol of trolox equivalents (TE)/l of juice.

with dark red aril colour had the highest TMA among all cultivars. Variability of TMA in our study was much higher than those reported by Drogoudi et al. (2005) who studied physical and chemical characteristics of 20 different pomegranate accessions. Also, TP contents of our samples were much higher than those 20 accessions. High correlations between TP and TMA of pomegranate aril have been reported (Drogoudi et al., 2005; Tzulker et al., 2007). We observed similar results in our study ($r = 0.94$). Overall, TMA and TP contents of other fruit crops vary greatly, depending on the phenotype, e.g. cherries and grapes. Also environmental and genetic factors contribute additional variation to the phytonutrient and antioxidant capacity of fruits (Anttonen & Karjalainen, 2005).

Our results showed that the antioxidant capacity among samples averaged 5.60 and 7.35 mM TE by the TEAC and FRAP methods, respectively. Similarly, in other studies (Nilsson et al., 2005; Ozgen et al., 2006), FRAP antioxidant capacity values were proportionally higher than TEAC values, presumably due to differences in reaction kinetics and steady state antioxidant potentials of various reductive substrates as they interact with radicals. 'Kan' had the highest antioxidant capacity, with 7.7 and 10.9 mM TE by the TEAC and FRAP methods, respectively. High antioxidant capacity of arils in our study was comparable to literature report (Tzulker et al., 2007). However, we should emphasize that the homogenates prepared from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than that found in the aril juice in the study where 29 different pomegranate accessions were compared. Most of these differences originated from hydrolyzable tannins and an ellagitannin, known as punicalagin, present in whole pomegranate fruit, as indicated by Tzulker et al. (2007). Levels of FRAP, TEAC, TP, and TMA were strongly correlated $r = 0.82$ to 0.96 (Table 3). The significant positive correlation between antioxidant capacity, TP and TMA supports earlier studies that found a similar relationship, especially with anthocyanin-rich fruits (Moyer et al., 2002; Velioglu et al., 1998; Wang et al., 1996). Phenolic compounds, including

Table 3
Correlation coefficients (r) of total phenolics (TP), total anthocyanin (TMA), antioxidant capacity (FRAP and TEAC), and titratable acidity (TA) as a maturity indicator

Source	TP[a]	TMA[b]	TEAC[c]
TMA	0.94*		
TEAC	0.94*	0.87*	
FRAP[d]	0.93*	0.82*	0.96*

* Significant at $p \leqslant 0.05$.
[a] TP contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as mg gallic acid equivalents (GAE)/l of juice.
[b] TMA were determined by the pH-differential method of Giusti et al. (1999). Values are expressed as mg cyanidin 3-glucoside equivalents/l of juice.
[c] TEAC values were determined by the method of Ozgen et al. (2006). Values are expressed as mmol of trolox equivalents/l of juice.
[d] FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as mmol of trolox equivalents (TE)/l of juice.

anthocyanins, display strong antioxidant activity and they have been shown in many studies to contribute significantly to the antioxidant capacity of fruits (Moyer et al., 2002; Velioglu et al., 1998; Wang et al. 1996).

Although people are generally aware of the interrelationship of diet and health, other quality factors must be present in a health-promoting food, especially superior flavour, before its consumption is widespread (Hilliam, 1995). The combination and the ratio of sugars and organic acids have been related to flavour quality of fruits. The amounts of fructose, glucose, sucrose and total sugars in the pomegranate cultivars are given in Table 4. Fructose and glucose were found to be dominant sugars in all cultivars analysed. The fructose and glucose concentrations averaged 6.4 and 6.8 g/100 ml, respectively. The amount of sucrose found in our samples was almost negligible. Organic acid distribution of pomegranate was dominated by citric acid (mean of 1.78 g/100 ml). Small amounts of malic and ascorbic acids were detected, averaging of 0.12 and 0.03 g/100 ml, respectively (Table 5). Citric acid

Table 4
Mean individual sugar contents (g/100 ml) ± standard deviation of different pomegranate cultivars

Cultivar	Fructose	Glucose	Sucrose	Total
'Dikenli incekabuk'	6.52 ± 0.04	6.51 ± 0.05	0.02 ± 0.01	13.1 ± 0.10
'Eksi'	7.06 ± 0.15	6.99 ± 0.36	0.02 ± 0.01	14.1 ± 0.51
'Kan'	6.60 ± 0.05	7.62 ± 0.05	0.02 ± 0.01	14.3 ± 0.10
'Katirbasi'	6.48 ± 0.03	7.19 ± 0.06	0.04 ± 0.01	13.7 ± 0.08
'Serife'	5.94 ± 0.05	6.66 ± 0.08	0.02 ± 0.01	12.6 ± 0.13
'Tatli'	5.80 ± 0.08	5.80 ± 0.03	0.03 ± 0.01	11.6 ± 0.10
Mean	6.40 ± 0.44	6.80 ± 0.60	0.03 ± 0.01	13.2 ± 0.95
CV (%)	6.9	8.9	28.2	7.2

Table 5
Mean organic acid contents (g/100 ml) ± standard deviation of different pomegranate cultivars

Cultivar	Citric acid	Malic acid	Ascorbic acid	Total
'Dikenli incekabuk'	1.95 ± 0.01	0.10 ± 0.01	0.025 ± 0.001	2.08 ± 0.02
'Eksi'	1.95 ± 0.01	0.09 ± 0.01	0.036 ± 0.001	2.08 ± 0.01
'Kan'	2.16 ± 0.01	0.10 ± 0.01	0.030 ± 0.001	2.28 ± 0.02
'Katirbasi'	1.23 ± 0.01	0.15 ± 0.01	0.069 ± 0.001	1.45 ± 0.03
'Serife'	3.20 ± 0.01	0.12 ± 0.01	0.014 ± 0.001	3.34 ± 0.01
'Tatli'	0.20 ± 0.01	0.13 ± 0.01	0.016 ± 0.001	0.36 ± 0.01
Mean	1.78 ± 0.02	0.12 ± 0.01	0.032 ± 0.019	1.93 ± 0.93
CV (%)	18.0	61.0	60.1	49.1

concentrations of cultivars varied from 0.20 to 3.20 g/100 ml in 'Tatli' and 'Serife', respectively. It is well-known that the sugar/acid ratio in many fruits is a primary driver of flavour quality. In general, there seems to be a modest variation in sugar contents among the cultivars tested, but there was substantial variability in acidity (CV 49.1%).

4. Conclusions

This study showed considerable variation in some of the chemical and antioxidant properties of pomegranate cultivars widely grown in the Mediterranean region of Turkey. The studied local cultivars were only selected from the Mediterranean part of Turkey and probably represent only a portion of the native germplasm. It is important to evaluate and conserve local genetic materials, not only for general consumption, but also for their health advantages. Particularly, the physiological effects of pomegranate juice constituents are remarkable for their preventive potential against heart disease and certain cancers.

Within this limited study of 6 different local cultivars, further evaluations, as well as hybridization within cultivars with favourable traits (from phyto-nutritional and horticultural perspectives) would be necessary for evolving new varieties. Cultivars rich in bioactive molecules offer genes with desired antioxidant properties and fruit chemistry profiles for enhanced health.

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