Food Chemistry 112 (2009) 721–726

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Classification of eight pomegranate juices based on antioxidant capacity measured by four methods

Mustafa Çam [a,*], Yaşar Hışıl [a], Gökhan Durmaz [b]

[a] Ege University, Department of Food Engineering, 35100 Bornova, Izmir, Turkey
[b] Inonü University, Department of Food Engineering, 44280 Malatya, Turkey

ARTICLE INFO

Article history:
Received 10 December 2007
Received in revised form 29 April 2008
Accepted 9 June 2008

Keywords:
Pomegranate
Antioxidant capacity
DPPH
ABTS
β-Carotene bleaching
Principal component analysis
Cluster analysis

ABSTRACT

The antioxidant capacity of pomegranate juices (PJs) obtained from eight cultivars were determined by the scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and β-carotene-linoleate model system. Total phenolic content (TPC) and total anthocyanin content (TAC) were also determined and found to vary between the range of 208.3–343.6 mg catechin equivalents and 8.1–36.9 mg cyanidine-3-glucoside equivalents per 100 ml of PJ, respectively. Cultivar Izmir 8 showed the highest scores for trolox equivalent antioxidant capacity (TEAC) as 418.3 ± 5.2 mg/100 ml of PJ, anti-lipid peroxidative activity (ALPA) as 93.5 ± 1.8% and efficient concentration (EC_{50}) as 29.8 ± 2.9 ml of PJ/g of DPPH. The hierarchy of PJs for antioxidant capacity with respect to their TEAC and ALPA values was I8 > I1499 > I10 > I1264 > I1479 > I26 > I23 > Zivzik. Interrelationships among the analyzed parameters and PJs obtained from eight cultivars were investigated by principal component analysis (PCA). Dimension of data set was reduced to two components by PCA accounting for the 93% of the total variance. Eight PJs were classified into three groups by cluster analysis (CA).

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits widely grown in many tropical and subtropical countries (Fadavi, Barzegar, Azizi, & Bayat, 2005). Over 1000 cultivars of *Punica granatum* exist, originating from the Middle East, extending throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New World (Lansky & Newman, 2007). There is growing interest in this fruit because it is considered to be a functional product of great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction (Martinez, Melgarejo, Hernandez, Salazar, & Martinez, 2006).

In general, comparable juice or extracts from other common fruits show antioxidant activity *in vitro* inferior to that of the pomegranate (Lansky & Newman, 2007). Pomegranate juice (PJ) has been proposed as chemopreventive, chemotherapeutic, antiatherosclerotic and anti-inflammatory agent. Accordingly, its consumption has grown tremendously (Faria, Monteiro, Mateus, Azevedo, & Calhau, 2007). The antioxidant capacity of commercial PJ is three times higher than those of red wine and green tea (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000). PJ was found to inhibit low-density lipoprotein oxidation, macrophage foam cell formation and atherosclerosis development in atherosclerotic mice. These beneficial effects of the PJ were attributed to the antioxidative properties of pomegranate polyphenols and sugar-containing polyphenolic tannins and anthocyanins (Gil et al., 2000). The soluble polyphenol content in PJ varies within the limits of 0.2–1.0%, depending on variety, and includes mainly anthocyanins (such as cyanidin-3-glucoside, cyanidin-3,5-diglucoside, and delphindin-3-glucoside), catechins, ellagic tannins, and gallic and ellagic acids (Aviram et al., 2000).

Significant differences have been reported in the concentration of some individual phenolic compounds of different pomegranate cultivars affecting the antioxidant capacity of PJ (Hernandez, Melgarejo, Tomas-Barberan, & Artes, 1999; Poyrazoğlu, Gökmen, & Artık, 2002). Antioxidant capacity of PJ, like of other fruits, depends on cultivar, growing region, climate, maturity and cultural practice. Also technology used to obtain PJ may affect the antioxidant capacity. The potent antioxidant and antiatherosclerotic properties of commercial PJs have been attributed to their high content of polyphenols including ellagitannins as punicalagin anomers (Seeram, Lee, & Heber, 2004).

Antioxidants may act in various ways such as scavenging the radicals, decomposing the peroxides and chelating the metal ions. Therefore, the antioxidant capacity must be evaluated with different tests for different chemical mechanisms (Kulkarni, Aradhya, & Divakar, 2004). According to the chemical reaction used, methods to measure antioxidant capacity can be mainly grouped into two

* Corresponding author. Tel.: +90 232 388 4000x3010; fax: +90 232 342 7592.
E-mail address: mustafa.cam@ege.edu.tr (M. Çam).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.009

classes: hydrogen atom transfer (HAT) and electron transfer (ET) based methods. HAT methods measure the ability of an antioxidant to quench free radicals by hydrogen donation. ET methods measure the ability of a potential antioxidant to transfer one electron to reduce radicals, metals or carbonyls (Huang, Ou, & Prior, 2005). ET and HAT reactions may occur in parallel, and the mechanism dominating in a given system will be determined by antioxidant structure and properties, solubility and partition coefficient, and system solvent (Prior, Wu, & Schaich, 2005). Different results obtained from different antioxidant tests could be regarded as a dilemma in first sight. But it is a known fact that antioxidant mechanism in biological matrixes is quite complex and several different factors play role in these mechanisms. Due to this complexity only a single antioxidant test system is not enough to reach a conclusion, instead, different antioxidant tests are applied and different characteristics are determined (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

There are many parameters used in the literature to describe the antioxidant capacities of samples (e.g. antioxidant activity, antioxidant efficiency, antioxidant potential). But these terms are more independent of specific reactions and have similar chemical meanings (Huang et al., 2005). We used the term "antioxidant capacity" throughout the text to refer to the results obtained from four different methods which we used.

Purposes of the present study were to determine the antioxidant capacity of eight PJs with four commonly used spectrophotometric methods, obtain relationships between the analyzed parameters and eventual classification of PJs with multivariate statistical techniques.

2. Materials and methods

2.1. Materials

Eight pomegranate cultivars widely grown in Turkey were studied: Izmir 8 (I8), Izmir 10 (I10), Izmir 23 (I23), Izmir 26 (I26), Izmir 1264 (I1264), Izmir 1479 (I1479), Izmir 1499 (I1499) and Zivzik. The selected cultivars are popular for fresh consumption, and preferred by industry in production of fruit juice and concentrate. Pomegranate cultivars were classified as sweet (I8, I10, I23, I26 and I1479), sour-sweet (Zivzik) and sour (I1264 and I1499) according to the maturity index values (Martinez et al., 2006). The cultivar Zivzik, one of the most popular cultivar in Turkey, was supplied from Directorate of the Ministry of Agriculture (Siirt, Turkey). The other cultivars were harvested in October 2006 from the orchard of Aegean Agricultural Research Institute when all greenness had disappeared from fruit rind surface and red or yellow colour appeared.

Approximately 7 kg of pomegranate fruit was sampled for each cultivar. After discarding injured and sunburnt fruits, pomegranate fruits were peeled and the skins covering the seeds were removed manually. The juice of the seeds was extracted with a pilot plant packaged-type press (Bucher, Switzerland). The juices were kept at −40 °C until analyzed for no longer than three months. Before the experiments, PJs were defrosted and then centrifuged at 4000 g for 15 min at +4 °C in order to remove water insoluble particles.

2.2. Chemicals

Catechin hydrate, potassium chloride, sodium acetate, sodium carbonate, Folin & Ciocalteau's phenol reagent, potassium peroxodisulfate, tris–HCl buffer solution, β-carotene, tween-20, linoleic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (1,1-diphenyl-2- picrylhydrazyl) were purchased from Sigma/Aldrich (St. Louis, MO), chloroform and methanol from Merck (Darmstadt, Germany).

2.3. Methods

2.3.1. Total anthocyanin content (TAC)

TAC of PJs was determined by pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) (Lako et al., 2007). Briefly, 0.4 ml of PJ sample was mixed with 3.6 ml of corresponding buffers and read against water as a blank at 510 and 700 nm. Absorbance (A) was calculated as

$$A = (A_{510} - A_{700})\mathrm{pH}_{1.0} - (A_{510} - A_{700})\mathrm{pH}_{4.5}$$

Total anthocyanin content of samples (mg cyanidin-3-glucoside/100 ml of PJ) was calculated by following equation:

$$\mathrm{TAC} = \left(\frac{A \times \mathrm{MW} \times \mathrm{DF} \times 100}{\mathrm{MA}}\right)$$

where A: absorbance; MW: molecular weight (449.2); DF: dilution factor (10); MA: molar absorptivity of cyanidin-3-glucoside (26,900).

2.3.2. Total phenolic content (TPC)

TPC of PJs was determined spectrophotometrically using the Folin & Ciocalteu assay described by Vinson, Dabbagh, Mamdouh, and Jang (1995). One millilitre of PJ sample was mixed with 1 ml of 6 M HCl and 5 ml of 75% methanol/water solution in a screw-capped tube. The tube was vortexed and placed in a 90 °C waterbath and shaken for 2 h. Then, the tube was allowed to cool to room temperature and diluted to a 10 ml volume with distilled water. One millilitre of this solution was mixed with 5 ml of previously tenfold diluted Folin & Ciocalteau reagent. Fifteen millilitres of Na_2CO_3 (7 g/100 ml) was added to this mixture to produce basic conditions. The mixture was diluted to 100 ml with distilled water. The absorbance versus prepared blank was read at 760 nm until it reached steady state. The same procedure was applied for six standard solutions of catechin (50–300 mg/100 ml). Final results were expressed as mg catechin equivalent per 100 ml of PJ.

2.3.3. Antioxidant capacity determinations

Two different antioxidant capacity methods were followed using DPPH radical and the results expressed as efficient concentration (EC_{50}) and antioxidant activity (AA).

EC_{50} was determined according to the method of Brand-Williams, Cuvelier, and Berset (1995) with slight modifications. 0.1 ml of appropriately diluted PJ samples was mixed with 3.9 ml of a 25 mg/l methanolic solution of DPPH·. The control sample was prepared with the same volume of methanol. Absorbance at 515 nm was measured at different time intervals on a Carry 50 Scan UV–vis. spectrophotometer until the reaction reached steady state. The DPPH· concentration in the reaction medium was calculated with equation: $A_{515\,\mathrm{nm}} = 35.762[\mathrm{DPPH}\cdot] - 0.0516$, ($r^2 = 0.999$), as determined by linear regression containing different concentrations of DPPH·. The % of remaining DPPH· ($\%\mathrm{DPPH}^{\cdot}_{\mathrm{Rem}}$) at steady state was calculated as follows:

$$\%\mathrm{DPPH}^{\cdot}_{\mathrm{Rem}} = \frac{[\mathrm{DPPH}\cdot]_t}{[\mathrm{DPPH}\cdot]_{t=0}}$$

where $[\mathrm{DPPH}\cdot]_{t=0}$ and $[\mathrm{DPPH}\cdot]_t$ are the initial concentration of DPPH· and the DPPH· concentration at steady state, respectively. The % of remaining DPPH· at steady state was plotted against the sample concentration to obtain EC_{50} value which is defined as the amount of sample necessary to decrease the initial DPPH· concentration by 50%. EC_{50} was expressed as ml sample to g DPPH·.

AA was determined according to the method of Kulkarni and Aradhya (2005). 0.1 ml of PJ was mixed with 0.9 ml of 100 mM Tris–HCl buffer (pH 7.4) to which 1 ml of DPPH (0.500 μM in ethanol) was added. The control sample was prepared similar way by adding 0.1 ml of water instead of PJ. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a Carry 50 Scan UV–vis. spectrophotometer. The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation:

$$AA(\%) = \left[1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right] \times 100$$

Trolox equivalent antioxidant capacity (TEAC) was determined by ABTS radical according to the method of Re et al. (1999) with slight modifications. ABTS· stock solution was prepared by dissolving 30 mg ABTS in 7.8 ml of 2.46 mM potassium peroxodisulfate. After 16 h, this stock solution was diluted with 100 mM phosphate buffer (pH 7.6) to give 0.700 ± 0.005 absorbance at 734 nm. Samples were also diluted with the same buffer by 1:20 (v:v). 50 μl of diluted samples were mixed with 1950 μl of ABTS· solution and absorbance was measured after 6 min of incubation. Results were expressed as mg TEAC per 100 ml of PJ.

Millers' (1971) method of β-carotene/linoleate model system was used with slight modifications. Two milligrams of crystalline β-carotene was dissolved in 10 ml chloroform. One millilitre of this solution was added to 40 μl linoleic acid and 400 μl tween-20 in a round-bottom flask. After removing chloroform under nitrogen flush for 5 min, 100 ml demineralised water was added with vigorous stirring to form an emulsion. Five millilitres of this emulsion was added to each tube containing 0.5 ml of diluted (with tris–HCl buffer by 1:10) samples. The control samples were prepared by adding 0.5 ml of tris–HCl buffer (diluted with water by 1:10) instead of samples. Absorbances were measured against blank, which was prepared as β-carotene emulsion but without adding β-carotene, at 470 nm. Mixtures were left in a water bath at 50 °C until the control sample was bleached almost completely (~90 min). Tubes were cooled to room temperature and absorbances were remeasured. ALPA (anti-lipid peroxidative activity) was calculated using the following equation:

$$\text{ALPA} = \left(\frac{A_{S:90} - A_{C:90}}{A_{S:0} - A_{C:90}}\right) \times 100$$

where $A_{S:90}$ is the absorbance of sample at 90th min, $A_{C:90}$ is the absorbance of control at 90th min and $A_{C:0}$ is the absorbance of control at 0th min.

2.4. Data processing

All the analysis were performed in triplicate. Results were expressed as means ± standard deviation. Descriptive statistical analysis, Pearson correlation coefficients, one-way analysis of variance (ANOVA) and Tukey's HSD test were performed using the SPSS 10.0.1 statistical package for Windows (SPSS Inc., Chicago, USA). Pattern recognition tools were applied to the data sets including principal component analysis (PCA) as a visualisation method, and cluster analysis (CA) as an unsupervised learning method.

PCA was applied to the standardised (z-scores) data set to observe interrelationships of samples and analyzed parameters using the Unscrambler software package (Version 9.6; CAMO AS, Oslo, Norway). CA was applied to the standardised data to establish cluster the PJs using XLSTAT (Version 2007.08.01; Addinsoft). To obtain the hierarchical associations, Euclidean distance and the Ward's method were used as dissimilarity measure and amalgamation rule, respectively.

3. Results and discussion

3.1. Total anthocyanin content (TAC) and total phenolic content (TPC)

Table 1 shows the TAC, TPC, EC_{50}, AA, TEAC and ALPA results of eight PJs. TAC of sour (I1264 and I1499) and sour-sweet (Zivzik) cultivars were more than sweet cultivars (I8, I10, I23, I26, I1479). TAC of I1264 was about 4.6-fold higher than that of I23. The highest TPC was found in I1499 (343.6 mg/100 ml) and the lowest in the I23 (208.3 mg/100 ml) cultivar. Gil et al. (2000) have reported the TAC and TPC of "Wonderful" pomegranate cultivar as 38.7 and 248.7 mg/100 ml, respectively. In another study, TAC and TPC of "Suruç" cultivar were reported as 8.9 and 156.4 mg/100 ml, respectively (Vardin & Fenercioğlu, 2003). These variations are due to differences among cultivars, growing seasons, agricultural practices and variations in applied total phenolic determination assays.

Red fruit juices have received great attention due to their phenolic content and antioxidant activity. To assess the relative phenolic content of PJs, we have compared their TPC values with those corresponding to other red fruit juices determined by the same methodology. TPC of PJs were more than that of the other juices such as turnip juice (77.2 mg/100 ml) and sour-cherry juice (79.7 mg/100 ml) (Gündüç & El, 2003), red grape juices (172.8 mg/100 ml) and red wine (186.9 mg/100 ml) (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999) while TPC of the juice of *Viburnum opulus* (351.3 mg/100 ml) (Çam & Hışıl, 2007) was higher than PJs.

3.2. Antioxidant capacity

Four methods were used to test the antioxidant capacities of PJs including one based on HAT (β-carotene bleaching method) and the others based on ET reaction mechanisms.

ABTS radical scavenging capacities of PJs were expressed as TEAC, an ET based method (Huang et al., 2005; Prior et al., 2005). While the highest result observed for I8 cultivar with 418.3 ± 5.2 TEAC, the lowest result was of Zivzik with 221.2 ± 2.4 TEAC (Table 1). The hierarchy for antioxidant capacity with respect to their

Table 1
Values for the analyzed parameters in pomegranate juices

Cultivars	TAC (mg/100 ml)	TPC (mg/100 ml)	EC_{50} (ml PJ/g DPPH)	AA (%)	TEAC (mg/100 ml)	ALPA (%)
I8	8.2 ± 0.2^{a}	309.0 ± 2.0^{d}	29.8 ± 2.9^{a}	74.6 ± 0.3^{c}	418.3 ± 5.2^{f}	93.5 ± 1.8^{c}
I10	14.1 ± 0.1^{c}	286.1 ± 9.5^{c}	41.3 ± 4.1^{bc}	76.4 ± 0.3^{e}	372.2 ± 7.4^{d}	90.6 ± 1.6^{c}
I23	8.1 ± 0.1^{a}	208.3 ± 1.9^{a}	72.3 ± 0.1^{f}	73.8 ± 0.1^{b}	229.8 ± 4.6^{a}	76.4 ± 0.8^{b}
I26	12.3 ± 0.3^{b}	221.2 ± 1.2^{b}	64.5 ± 1.6^{e}	73.0 ± 0.2^{a}	255.8 ± 5.0^{b}	81.6 ± 2.7^{b}
I1264	36.9 ± 0.1^{e}	283.1 ± 0.6^{c}	38.9 ± 1.2^{b}	91.8 ± 0.3^{g}	363.4 ± 2.3^{d}	89.5 ± 2.5^{c}
I1479	11.4 ± 0.5^{b}	280.0 ± 1.0^{c}	51.8 ± 1.3^{d}	73.0 ± 0.2^{a}	327.8 ± 4.6^{c}	87.8 ± 0.1^{c}
I1499	21.7 ± 1.1^{d}	343.6 ± 6.4^{e}	47.2 ± 1.8^{cd}	89.7 ± 0.2^{f}	392.4 ± 4.6^{e}	91.6 ± 2.5^{c}
Zivzik	21.5 ± 0.3^{d}	231.6 ± 1.6^{b}	70.5 ± 1.1^{f}	75.6 ± 0.1^{d}	221.2 ± 2.4^{a}	66.1 ± 2.4^{a}

Values that are followed by different letters within each column are significantly different ($p < 0.05$).

TEAC was I8 > I1499 > I10 > I1264 > I1479 > I26 > I23 > Zivzik. PJ production methods may affect the type and the concentration of phenolics which contribute to the antioxidant capacity of PJ. Commercial PJs were produced usually by pressing the whole pomegranate fruits into juice. The phenolic pattern of the commercial PJ includes additional phenolics to those present in the arils juice and that the industrial process to produce pomegranate juices also extracts some phenolic compounds from the fruit rind (Gil et al., 2000). TEAC of eight PJs in the present study was approximately two and three times lower than commercial PJs obtained from Wonderful variety reported by Gil et al. (2000) and Seeram et al. (2008), respectively.

In β-carotene bleaching method, PJs capability to prevent β-carotene from bleached during accelerated oxidation conditions were measured. Ranking of the cultivars with respect to their ALPA was the same as that observed TEAC assay. This method is based HAT reaction mechanism and most relevant to human biology because peroxyl radicals formed *in vitro* may more closely reflect *in vivo* conditions (Prior et al., 2005).

The reaction between DPPH and antioxidant(s) was believed to involve HAT reaction but recently suggested that the reaction in fact behaves like an ET reaction (Huang et al., 2005). In the first DPPH test, reaction between antioxidant(s) of PJs and DPPH free radical reached the steady state in 1 h. Final results were expressed using the term EC_{50}. The lower the EC_{50} the higher the antioxidant capacity (Brand-Williams et al., 1995). All cultivars showed scavenging effects against DPPH radical ranging from 29.8–72.3 ml PJ/g DPPH. The hierarchy for antioxidant capacity with respect to their EC_{50} values was I8 > I1264 > I10 > I1499 > I1479 > I26 > Zivzik > I23.

Eight PJs showed higher AA values (73.0–91.8%) than PJ from Ganesh variety (69%) reported by Kulkarni and Aradhya (2005). When expressing the antioxidant capacity with AA parameter, sour PJs (I1499 and I1264) showed striking results in comparison to the other three parameters especially with EC_{50}. The hierarchy for antioxidant capacity with respect to their AA values was I1264 > I1499 > I10 > Zivzik > I8 > I23 > I26 ≈ I1479. Although reaction type (based on ET) and the DPPH radical were the same for EC_{50} and AA parameters, the hierarchy of PJs was different for those two parameters. Brand-Williams et al. (1995) classified the antioxidants according to their kinetic behaviour as rapid, intermediate and slow. As pointed out in the methods section, we determined the EC_{50} parameter after the reaction reached steady state conditions approximately at 60th min. On the other hand, AA parameter was determined relying on a fixed time of 30 min. In the case of slower kinetic behaviour, an AA value determined at 30 min would be erroneous because the reaction would still be progressing as pointed out by Brand-Williams et al. (1995). The solvents used for preparing the radical solutions were methanol for EC_{50} and ethanol for AA. Type of solvent and polarity may affect the ET and HAT, which are key aspects in the measurement of antioxidant capacity (Péréz-Jiménez & Saura-Calixto, 2006). Sour cultivars (I1264 and I1499) contain approximately 10 times higher amount of citric acid than the other six cultivars (unpublished results). High AA results of sour cultivars might be due to the synergistic effects of citric acid by promoting activity of antioxidants. These results clearly show that when determining antioxidant capacity of PJs or any kind of food materials with DPPH radical it is necessary to constitute steady state conditions which changeable for each food material even the same food material from different varieties. The results of the four antioxidant capacity assays reinforce the opinion of some authors (Frankel & Meyer, 2000; Prior & Cao, 1999) that antioxidants are multi-functional and their activity and mechanism largely depend on the composition of materials and conditions of the antioxidant capacity test system, and therefore more than one type of antioxidant capacity measurement are necessary to take into account the various mechanisms of antioxidant action.

Table 2
Correlation coefficients (with 6 df) among analyzed parameters

Trait	EC_{50}	AA	TEAC	ALPA	TPC
AA	−0.388				
TEAC	−0.963[a]	0.466			
ALPA	−0.881[a]	0.374	0.924[a]		
TPC	−0.813[b]	0.634	0.919[a]	0.773[b]	
TAC	−0.182	0.852[a]	0.154	0.030	0.282

a,b = significant at $p < 0.01$ or $p < 0.05$, respectively.

Pearson's correlation coefficients between the means of each variable were computed with 6 degree of freedom (df). Statistically significant ($p < 0.01$) correlation coefficient were found between TEAC and EC_{50} ($r^2 = -0.963$), TEAC and ALPA ($r^2 = 0.924$), TEAC and TPC ($r^2 = 0.919$). Correlation coefficients were high among three antioxidant capacity values (TEAC, ALPA and EC_{50}) but AA value was not significantly correlated with the other antioxidant activity values (Table 2). Due to the low correlation coefficients of TAC with TEAC, ALPA and EC_{50} values we could say that anthocyanins did not play major role in antioxidant mechanisms with these tests. In a study, it was found insignificant correlation between TAC and antioxidant activity (by DPPH radical) but significant correlation between TAC and ferric reducing antioxidant potential in 29 PJs (Tzulker et al., 2007). On the other hand, correlation coefficient between AA and TAC was significant ($r^2 = 0.852$) at $p < 0.01$. TPC values indicated high correlation coefficients with TEAC, ALPA and EC_{50} values (Table 2).

3.3. Principal component analysis (PCA)

PCA is a technique that, by the reduction of data dimensionality, allows their visualisation retaining as much as possible the information present in the original data. So, PCA transforms the original measured variables into new uncorrelated variables called principal component (Berrueta, Alonso-Salces, & Héberger, 2007).

PCA was applied to the standardised values of analyzed parameters of 8 PJs. The loadings, eigenvalues and percent of cumulative variance for the first two principal components are shown in Table 3. After PCA, the dimensionality of data was reduced from six partially correlated variables to two uncorrelated PC1 and PC2 with almost 6.7% loss of variation. First two PCs accounted for 93.3% of the total variability. PC1 and PC2 were responsible for 67.4% and 25.9% of the total information, respectively. Evidently, PC1 is generally more correlated with the variables than PC2. This is to be expected because PCs are extracted successively, each one accounting for as much of the remaining variance as possible. The absolute value of the loadings (their actual sign depends on the calculation algorithm used) is an indicator of the participation of the analyzed parameters in the PCs (Helena et al., 2000). While PC1 correlates highly with the original variables in decreasing order as TEAC,

Table 3
Loadings, eigenvalues and percent of cumulative variance for the first two principal components

Variable	PC1	PC2
AA	0.677	0.706
EC_{50}	−0.920	0.260
TEAC	0.965	−0.253
ALPA	0.883	−0.354
TAC	0.389	0.894
TPC	0.937	−0.031
Eigenvalues	4.046	1.155
% Cumulative variance	67.433	93.343

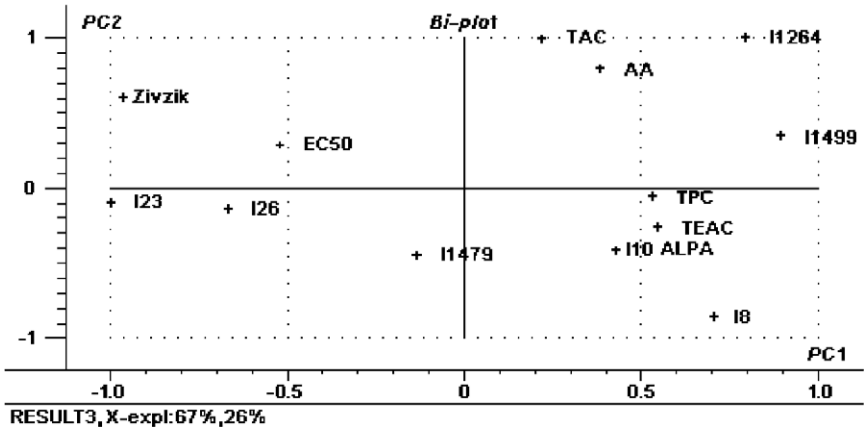

Fig. 1. PCA bi-plot over the scores (cultivars) and loadings (analyzed parameters).

TPC, EC_{50} and ALPA, PC2 correlates highly with TAC and AA. Loadings of all analyzed parameters and individual PJ scores were combined into one bi-plot of PCA1 and PCA2 (Fig. 1). It is possible to detect the relationships between the analyzed parameters and also similarities or differences between PJs by investigating the PCA bi-plot. TAC and AA were the features with positive loadings on PC1 and PC2. TAC and AA were significantly correlated parameters that could be seen from PCA bi-plot (Fig. 1) and Pearson correlation coefficients (Table 2). TEAC, ALPA and TPC were the features with high positive loadings on PC1 and negative loadings on PC2. These parameters were significantly correlated with each other. EC_{50} was the parameter that significantly and negatively correlated with TEAC, ALPA and TPC.

Cultivars at the upper right part of the coordinates were I1264 and I1499, both of which positively correlated with PC1 and PC2. These cultivars can be best described with high content of TAC and AA. Close relationships were observed between I8 and I10 and among I23, I26 and I1479. Zivzik which located at the upper left part of the bi-plot was best characterised by high EC_{50} value.

3.4. Cluster analysis (CA)

In CA, samples are grouped on the basis of similarities without taking into account the information about the class membership. CA calculates the distances (or correlation) between all samples using a defined metric such as Euclidian distance, Manhattan distance, etc. (Berrueta et al., 2007). In the present study, CA was performed to the standardised data. The results obtained from CA are shown as a dendogram in Fig. 2. Three clusters were formed after the application of CA. The first cluster was composed of cultivars I23, I26 and Zivzik. The second cluster grouped the cultivars I8, I10 and I1479. The third cluster was composed of I1264 and I1499.

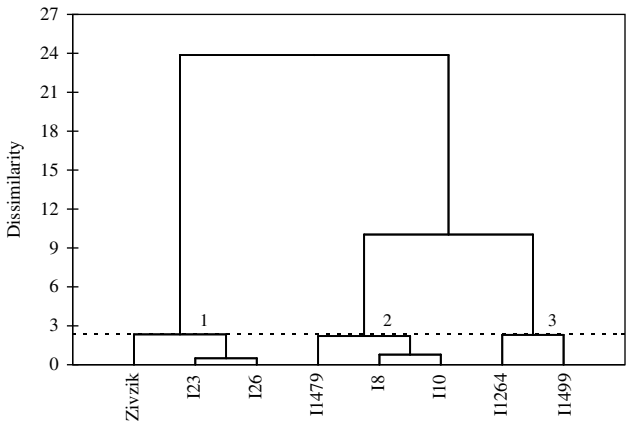

Fig. 2. Dendogram of cluster analysis of pomegranate juices.

4. Conclusions

Statistically significant differences were observed among some pomegranate cultivars in terms of antioxidant capacity, TPC and TAC. The antioxidant capacity of pomegranate juices was influenced by the type of cultivar to a large extent. PJs were ranked as I8 > I1499 > I10 > I1264 > I1479 > I26 > I23 > Zivzik according to TEAC and ALPA values. PJs were ranked with EC_{50} values similarly with TEAC and ALPA values to a great extent but differently with AA values. Antioxidant capacity values including TEAC, ALPA and EC_{50} may be estimated indirectly by using TPC since it showed high correlation with those three antioxidant capacity values. PJs were classified into three groups by CA according to the TAC, TPC and antioxidant capacity values.

Acknowledgements

This work was financially supported by the Erciyes University Scientific Research Project Unit (Project number, FBD-07-266).

References

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst, 127*, 183–198.

Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M., Coleman, R., et al. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *American Journal of Clinical Nutrition, 71*, 1062–1076.

Berrueta, L. A., Alonso-Salces, R. M., & Héberger, K. (2007). Supervised pattern recognition in food analysis. *Journal of Chromatography A, 1158*, 196–214.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie, 28*, 25–30.

Çam, M., & Hışıl, Y. (2007). Comparison of chemical characteristics of fresh and pasteurised juice of Gilaburu (*Viburnum opulus* L.). *Acta Alimentaria, 36*, 381–385.

Fadavi, A., Barzegar, M., Azizi, M. H., & Bayat, M. (2005). Physicochemical composition of 10 pomegranate cultivars (*Punica granatum* L.) grown in Iran. *Food Science and Technology International, 11*, 113–119.

Faria, A., Monteiro, R., Mateus, N., Azevedo, I., & Calhau, C. (2007). Effect of pomegranate (*Punica granatum*) juice intake on hepatic oxidative stress. *European Journal of Nutrition, 46*, 271–278.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using onedimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic

composition and processing. *Journal of Agricultural and Food Chemistry, 48*, 4581–4589.

Gündüç, N., & El, S. N. (2003). Assessing antioxidant activities of phenolic compounds of common Turkish food and drinks on in vitro low-density lipoprotein oxidation. *Journal of Food Science, 68*, 2591–2595.

Helena, B., Pardo, R., Vega, M., Barrado, E., Fernandez, J. M., & Fernandez, L. (2000). Temporal evolution of groundwater composition in an alluvial aquifer (Pisuerga River, Spain) by principal component analysis. *Water Research, 34*, 807–816.

Hernandez, F., Melgarejo, P., Tomas-Barberan, F. A., & Artes, F. (1999). Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. *European Food Research and Technology, 210*, 39–42.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Kulkarni, A. P., & Aradhya, S. M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry, 93*, 319–324.

Kulkarni, A. P., Aradhya, S. M., & Divakar, S. (2004). Isolation and identification of a radical scavenging antioxidant punicalagin from pith and carpellary membrane of pomegranate fruit. *Food Chemistry, 87*, 551–557.

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry, 101*, 1727–1741.

Lansky, E. P., & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology, 109*, 177–206.

Martinez, J. J., Melgarejo, P., Hernandez, F., Salazar, D. M., & Martinez, R. (2006). Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. *Scientia Horticulturae, 110*, 241–246.

Miller, H. E. (1971). A simplified method for the evaluation of antioxidants. *Journal of American Oil Chemists' Society, 48*, 91–97.

Péréz-Jiménez, J., & Saura-Calixto, F. (2006). Effect of solvent and certain food constituents on different antioxidant capacity assays. *Food Research International, 39*, 791–800.

Poyrazoğlu, E., Gökmen, V., & Artık, N. (2002). Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *Journal of Food Composition and Analysis, 15*, 567–575.

Prior, R. L., & Cao, G. (1999). In vivo total antioxidant capacity comparison of different analytical methods. *Free Radical Biology and Medicine, 27*, 1173–1181.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Sanchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Research International, 32*, 407–412.

Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., et al. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of Agricultural and Food Chemistry, 56*, 1415–1422.

Seeram, N. P., Lee, R., & Heber, D. (2004). Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice.. *Clinica Chimica Acta, 348*, 63–68.

Tzulker, R., Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M., & Amir, R. (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *Journal of Agricultural and Food Chemistry, 55*, 9559–9570.

Vardin, H., & Fenercioğlu, H. (2003). Study on the development of pomegranate juice processing technology: Clarification of pomegranate juice. *Nahrung, 47*, 300–303.

Vinson, J. A., Dabbagh, Y. A., Mamdouh, M. S., & Jang, J. (1995). Plant flavonoids, especially tea flavonols are powerful antioxidants using an *in vitro* oxidation model for heart disease. *Journal of Agricultural and Food Chemistry, 43*, 2800–2802.