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THE COMPARISON OF THE PHYSICOCHEMICAL PARAMETERS AND ANTIOXIDANT ACTIVITY OF HOMEMADE AND COMMERCIAL POMEGRANATE JUICES*

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ABSTRACT

Background. The pomegranate (Punica granatum L.) has gained a reputation as a fruit with many healthpromoting properties. It is considered to help prevent or treat various disease risk factors including high blood pressure, high cholesterol, oxidative stress, hyperglycemia and inflammatory activities. It has been demonstrated that certain components of pomegranates, such as polyphenols, have potential antioxidant, anti-inflammatory and anticarcinogenic effects.

Materials and methods. Five commercially available (CA) and three homemade (HM) pomegranate (Punica granatum L.) juices were evaluated for their physicochemical properties including titratable acidity (TA), pH and total soluble solids (TSS), as well as antioxidant properties such as anthocyanin content, ascorbic acid content (AA), antioxidant activity (DPPH), total phenolic compounds (TPC) and ferric reducing antioxidant power (FRAP). Moreover, the concentrations of 18 different elements (Ca, K, Mg, P, S, Na, Cr, Cu, Fe, Mn, Mo, Sr, Zn, Al, Cd, Ni, Pb, Hg) were determined using the ICP-OES method, with prior wet mineralization **Results.** TA was significantly lower (P < 0.05) in homemade than in commercial juices (0.380 and 1.318%) citric acid respectively). The TPC and FRAP parameters were about 50% higher in commercial than in homemade juices. Polyphenols were strongly correlated with antioxidant activity measured by FRAP and DPPH tests (r = 0.958 and 0.886 respectively), and a significant correlation for anthocyanins and vitamin C (r = 0.849) was observed. The most common mineral in each tested juice was potassium (132.69–3151.87 mg/dm³) and a high level of magnesium (23.42–123.63 mg/dm³) was found.

Conclusions. Obtaining juices from whole fruits (peel and arils), as in commercial production, enhanced the content of polyphenol compounds and the antioxidant activity of the juice. However, a better mineral composition was observed in homemade juices.

Keywords: pomegranate juice, homemade, antioxidant activity, polyphenols, minerals, ICP-OES

INTRODUCTION

The pomegranate (Punica granatum L.), which oldest edible fruits and has been cultivated widely belongs to the *Punicaceae* family, is one of the in many tropical and subtropical regions. Recently,

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pomegranate cultivation has significantly increased (Zarei et al., 2011). The edible part of the fruit constitutes about 52% (w/w) of the total fruit weight, comprising 70–78% juice and 22–30% seeds (Kulkarni and Aradhya, 2005). The composition of the fruit is dependent on the cultivation techniques and climatic conditions (Ozgen et al., 2008; Tzulker et al., 2007). In addition, the physical and chemical characteristics of pomegranate are highly dependent on the stage of fruit development and maturity (Zarei et al., 2011).

Pomegranate fruits contain considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals (Akpinar-Bayizit, 2010; Ekşi and Özhamamcı, 2009). Moreover, pomegranate juice is an important source of anthocyanins, especially 3-glucosides, as well as 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin (Alighourchi et al., 2008). Additionally, many of the phenolic compounds and tannins such as punicalin, pedunculagin, punicalagin and ellagic acid are present in the pomegranate (Kulkarni and Aradhya, 2005). The popularity of the pomegranate is mainly due to its beneficial biological actions, most of which are attributed to the phenol content (Gil et al., 2000; Lansky and Newman, 2007; Tezcan et al., 2009). Studies have shown that pomegranates demonstrate many health benefits, such as the prevention of cardiovascular disease, cancers and neurological damage in humans (Lansky and Newman, 2007). These beneficial effects have been attributed to a high level of antioxidant activity (Gil et al., 2000). Recently, the high antioxidant activity of the extracts from different parts of the pomegranate fruit such as the peel, juice and seeds have been reported (Shiban et al., 2012). Phenolic acids, anthocyanins and ascorbic acid, either alone or in combination, are responsible for the antioxidant activity of pomegranate (Eddebbagh et al., 2016). It has been shown that pomegranate juice contains the highest concentration of total polyphenols compared to other studied fruit juices (Vrhovsek et al., 2004).

The aim of the study was to investigate the differences in bioactive components and physicochemical parameters between homemade and commercially available pomegranate juices.

MATERIALS AND METHODS

Material

The research materials consisted of 5 pomegranate juices (Photo 1) commercially available (CA) on the



Photo 1. Tested commercial pomegranate juices



Photo 2. Tested pomegranate fruits

Polish market and 3 handmade (HM) pomegranate juices obtained from fresh fruit (Photo 2). Handmade juices were made in the laboratory from three (differing visually) kinds of fruit present on the local market by squeezing the pomegranate arils with a hand press manual juicer (as for citrus fruits). The obtained juices were filtered through paper. Details of the fruits and juices used (label information) are presented in Table 1. The approximate cost of home-produced juice was calculated based on the price of the pomegranate fruit and juice yield per kilogram of fruit.

Physicochemical properties

Titratable acidity (TA) was determined according to AOAC (2005) by titration with 0.1 M NaOH until pH 8.1 in agreement with the pH-meter. Titratable acidity was expressed as % w/v of citric acid. The pH measurements were performed using a digital pH-meter (CP-401,

Method of extraction and samples	Country (producer)	Label information/appearance	
CA			
1	Turkey	100% juice, pasteurized	11.42
2	Israel	100% juice, pasteurized	
3	Azerbaijan	100% juice, directly pressed, pasteurized	
4	Russia	pomegranate juice	
5	Azerbaijan	juice with sugar syrup and citric acid addition	3.33
HM			
1	India	shiny, smooth, vivid red skin; large hard fruits	~6.90
2	India	shiny, slightly rough, wrinkled skin with small brown spots, a medium-sized fruit	~7.40
3	India	rough, dull skin with numerous brown spots; a medium-sized fruit	~6.67

Table 1. Characteristics of used pomegranate fruits

Elmetron, Poland). The total soluble solids (TSS) were determined with Abbe's refractometer (calibrated with distilled water) at 20°C according to the Determination... (2005) method. Results were expressed as °Brix [°Bx]. All analyses were performed in duplicate. The maturity index (TSS/TA) was calculated based on the classification made by Martinez et al. (2006).

Anthocyanins

Total anthocyanins were determined spectrophotometrically, as described by Rapisarda et al. (2000) with minor modifications. An aliquot of each juice sample (100 μ L) was diluted up to 2400 μ L with a 0.25 mM potassium chloride buffer pH 1 and 0.4 M sodium acetate buffer pH 4.5. The absorbance of both solutions was measured at 510 nm (Abs_{pH1} and Abs_{pH4.5}). Anthocyanin concentrations were expressed as cyanidin-3-glucoside equivalent [mg/dm³] calculated by the equation:

$$C_{\rm mg/L} = (Abs_{\rm pH1} - Abs_{\rm pH4.5}) \times 484.82 \times 1000 / / 24.825 \times DF$$

where:

484.82 - molecular mass of cyanidin-3-glucoside,

24 825 – molar absorptivity of cyanidin-3-glucoside at 510 nm in the pH 1 solution,

DF – dilution factor.

Vitamin C

The ascorbic acid content was estimated using 2,6-dichloroindophenol (DCPiP) dye as an indicator (Robinson and Stotz, 1945). The principle of the method involves the oxidation of L-ascorbic acid to dehydroascorbic acid with an excess of DCPiP. An excess of dye was extracted with xylene and determined spectrophotometrically at 500 nm. Results were expressed as mg per 100 cm³ of juice.

Ferric reducing/antioxidant power assay

The antioxidant properties of the aqueous pomegranate solution (20% v/v) were determined using a Ferric reducing/antioxidant power assay (FRAP method) according to a modified version of the procedure described by Benzie and Strain (1999). A calibration curve was prepared using a Trolox standard solution at concentrations 0–80 µmol/sample. The results were expressed as the FRAP value (mmol Trolox/dm³ of juice).

DPPH radical scavenging activity

Radical scavenging activity (RSA) was investigated using the 2.2-diphenyl-picrylhydrazyl radical (DPPH) according to the procedure performed by Arendse et al. (2014). 10-fold diluted aqueous solutions of pomegranate juices were used. The decrease in DPPH absorbance (*A*) was measured at 517 nm according to the blank (A_0). The DPPH radical scavenging activity expressed as % was calculated as: RSA, % = ($[A_0 - A]$ / A_0) × 100%.

Total phenolic content

The total phenolic content (TPC) of the juices was determined using the method of Singleton and Rossi (1965) with minor modifications. A 20-fold diluted aqueous solution of each pomegranate juice was used. A calibration curve was made using gallic acid at a concentration of 0–30 mg/cm³. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per dm³ of juice [mg/dm³].

Mineral composition

Before the determination of elements, wet mineralization was carried out. The samples of pomegranate juices were weighed (about 1 g) into Teflon vessels and added to 8 cm³ of concentrated HNO₃ (65% POCH). The mineralization of the samples was performed using a microwave mineralizer Milestone Ethos Ultrawave-One (Milestone SRL, Italy). A microwave-assisted heating program was implemented using 15 min for ramp time and 15 min for hold time at applied adjustable power (up to 1800 W). Temperatures were set at 150 and 200°C. Maximum pressure was set at 120 bar. After cooling, the samples were transferred quantitatively to flasks with a capacity of 50 cm³ and supplemented with redistilled water to the mark. The concentrations of 17 metals (Ca, K, Mg, P, S, Na, Cr, Cu, Fe, Mn, Mo, Sr, Zn, Al, Cd, Ni, Pb, Hg) were determined by optical emission spectrometry with inductively-induced plasma (ICP-OES) using a Thermo iCAP 6500 spectrophotometer (Thermo Fisher Scientific Inc., USA). The detection threshold obtained for each element was not lower than 0.01 mg/kg (with an assumed detection capacity of the measuring apparatus at a level exceeding 1 ppb). A curve fit factor for the studied elements was above 0.99. All the analyses were done in three independent

repetitions for each sample. The targeted repeatability expressed as the relative standard deviation (RSD) and targeted recovery were 20% and 97% to 102% respectively. The method was validated using certified reference material (NIST – 1515). In order to identify the relevant measurement lines and avoid possible interferences, the method of adding an internal standard was applied. ⁸⁹Y, ¹⁷³Yb ions (at concentrations of 2 mg/dm³ and 5 mg/dm³ respectively) as internal standards were used. Results were expressed as mg/dm³.

Statistical analysis

Results were expressed as mean value \pm SD and the variation coefficient was calculated (%VC). Correlation analysis was made based on Pearson's correlation coefficient. All data were analyzed by Statistica software version 12.0 using analysis of variance (ANOVA) and differences between the two groups of juices were determined for significance at P < 0.05 using Tukey's test.

RESULTS AND DISCUSSION

Physicochemical parameters

The titratable acidity (TA), pH and total soluble solids (TSS) of the tested juices are shown in Table 2. The titratable acidity of the HM pomegranate juices was stable and ranged from 0.358-0.397% of citric acid, while in CA pomegranate juices this parameter varied from 0.896-1.882%. The pH value of the tested juices was higher in HM juices and ranged from 3.5 to 3.9, while the pH of commercial juices varied from 2.2–2.7, but the differences were not significant (P > 0.05). Such a low pH indicates the possible longterm microbiological stability of the tested juices. The titratable acidity was inversely correlated with pH value (r = -0.754). Juices which had a low acid content had a correspondingly higher pH. The results obtained by Vazquez-Araujo et al. (2010) are in agreement with the present study; TA of fresh pomegranate juices was $1.02 \pm 0.02\%$ of citric acid and the pH ranged from 3.1 to 3.3. It was proved by Zarei et al. (2011) that TA content decreased significantly and pH value increased significantly during fruit ripening. Therefore, TA reduction can be used as a standard criterion to determine the maturity phase of the pomegranate fruit. Moreover, Ismail et al. (2014), who tested pomegranate juices made from arils by electric juice centrifuge and from whole fruits by mechanical pressing, observed that the method of juice extraction has a slight influence on the pH and TA of pomegranate juices.

In general, the TSS content of HM juices was relatively stable (13.7–14.5°Bx), while in the commercially

Table 2. Effect of extraction methods on physicochemical parameters of pomegranate juices

Method of extraction and samples	Titratable acidity % of citric acid	pH	Total soluble solids °Bx	
HM				
1	$0.358 \pm \! 0.023$	3.85 ± 0.13	$14.40\pm\!\!0.23$	
2	$0.397 \pm \! 0.053$	3.84 ± 0.06	$13.70\pm\!\!0.10$	
3	$0.384 \pm \! 0.035$	$3.50 \pm \! 0.04$	$14.50 \pm \! 0.07$	
Mean \pm SD	$0.380^{a} \pm 0.020$	$3.73^a{\pm}0.20$	$14.20\pm\!\!0.436$	
CA				
1	1.728 ± 0.163	$2.60 \pm \! 0.09$	16.23 ± 0.15	
2	$0.896 \pm \! 0.187$	$2.70 \pm \! 0.07$	$14.20\pm\!\!0.10$	
3	$1.882 \pm \! 0.099$	$2.40 \pm \! 0.03$	$13.70\pm\!\!0.10$	
4	1.152 ± 0.123	$2.18 \pm \! 0.08$	$13.70\pm\!\!0.10$	
5	$0.935 \pm \! 0.018$	$2.50\pm\!\!0.02$	11.83 ± 0.06	
Mean ±SD	$1.318^{b}\pm 0.458$	$2.48^b \pm 0.20$	13.93 ± 1.572	

^{a,b}Significant differences (P < 0.05) between methods of juice extraction.

available juices, it varied from $11.8-16.2^{\circ}Bx$. This is in agreement with other authors who found TSS in fresh pomegranate juices to be $16.5 \pm 0.1^{\circ}Bx$ (Vazquez-Araujo et al., 2010) and $14.94-14.04^{\circ}Bx$ (Ismail et al., 2014) depending on the method of juice production.

The MI of tested fruits from which HM juices were made ranged between 34.5–40.2, which indicates that the varieties of all tested fruits were sweet (Martinez et al., 2006). According to Vazquez-Araujo et al. (2010), the maturity index appeared to be a good indicator of fruit maturity as it increases significantly during fruit ripening. Authors calculated the MI in fresh pomegranate juices as 16.2 ± 0.3 .

Antioxidant activity

Parameters expressing the antioxidant activity of tested pomegranate juices are summarized in Table 3. Higher levels of ascorbic acid (AA) were found in HM juices than CA ones. Ascorbic acid, commonly known as vitamin C, plays an important role in the human body. It is necessary for the synthesis of collagen, a protein that has many connective functions in the body. As an antioxidant, it reacts with histamine and peroxide for reducing inflammatory symptoms (Barrita and Sánchez, 2013). Ascorbic acid was found in tested juices at the levels of 15.7–26.8 mg% (HM) and

9.1–16.1 mg% (CA). This is in agreement with Ranu and Uma (2012) as well as Arendse et al. (2014) who found AA levels of 19.8 mg% and 16.82 mg% respectively in fresh pomegranate juices.

The content of anthocyanins in tested HM juices ranged from 103.51-256.81 mg/dm3 while in CA juices it was significantly lower (P < 0.05) and ranged from 6.84-67.38 mg/dm³. Anthocyanins are some of the phenolic compounds that contribute to the red, blue or purple colors of many fruits, including pomegranate juice, and they are well known for their antioxidant activity (Alighourchi et al., 2008). Results reported by Hasnaoui et al. (2011), who tested 30 samples of Tunisian pomegranate fruits and found that the total anthocyanin content ranged from 9-115 mg/dm³ of juice, were similar to own results obtained for HM juices, but significantly higher than those obtained for CA juices. The obtained values of total anthocyanins were slightly lower than those of 493.306 mg/dm³ and similar to those of 252 mg/dm³ reported for Turkish (Orak, 2008) and Iranian (Alighourchi et al., 2008) pomegranates respectively.

Antioxidant activity was measured in terms of its radical scavenging potential by DPPH test. The antioxidant activity of CA juices ranged from 51.16 to 76.29% (66.13% on average), excluding sample no. 4

Method of extraction and samples	Vitamin C mg %	Anthocyanins mg/L	DPPH % inhibition	FRAP mmol TE/L	TPC mg GAE/L
HM					
1	26.76 ± 1.77	$256.81 \pm\! 10.30$	69.99 ± 0.07	24.89 ± 0.16	$6\ 753.54\ {\pm}76.34$
2	16.91 ± 0.99	103.51 ± 3.20	38.94 ± 1.05	$22.09 \pm \! 0.88$	$6\;135.59\pm\!\!88.15$
3	15.66 ± 0.85	$214.82 \pm \!$	44.46 ± 1.46	25.68 ± 1.01	$7\ 436.93\ {\pm}76.60$
Mean \pm SD	$19.78^{\text{a}}\pm\!6.08$	191.72ª ±79.22	51.13 ± 16.56	$24.22\pm\!\!1.89$	$6\ 775.35\ {\pm}650.95$
СА					
1	$13.89 \pm \! 1.07$	16.60 ± 0.70	$76.29 \pm \! 1.08$	57.17 ± 0.39	$13\;318.43\pm\!92.53$
2	16.16 ± 1.43	67.38 ± 3.10	51.16 ± 0.94	30.86 ± 0.85	$7844.06\pm\!\!61.69$
3	10.10 ± 0.00	13.67 ± 0.40	72.60 ± 0.42	70.33 ± 0.78	$13\ 023.99\ {\pm}77.11$
4	$9.09 \pm \! 1.43$	6.84 ± 0.10	$3.35 \pm \! 0.80$	$8.23 \pm \! 1.03$	$1\ 442.75\ {\pm}15.42$
5	9.60 ± 0.71	26.36 ± 0.90	64.56 ± 0.31	47.96 ± 1.21	$10\ 003.27 \pm \! 15.42$
Mean ±SD	$11.77^{b}\pm 3.10$	$26.17^{b}\pm\!24.08$	53.59 ± 29.69	42.91 ± 24.13	9 126.50 ±4 853.25

Table 3. Effect of extraction methods on the antioxidant activity of pomegranate juices

^{a,b}Significant differences (P < 0.05) between methods of juice extraction.

which was completely different. The antioxidant activity of HM juices was slightly lower (51.3% on average; P > 0.05). The higher antioxidant activity of commercial pomegranate juices compared to homemade juices suggests that the industrial process of extracting the juices either increased the content of pomegranate antioxidants or enhanced their activity. This could be due to the fact that whole fruits (skin and arils) are used for industrial production of juices. This is in agreement with Gil et al. (2000) who observed the same trend. According to Eghdami and Asli (2010), the antioxidant activity of pomegranate aril juices made by mechanical pressing, measured with DPPH, ranges from 23.8–38.01%.

The antioxidant capacity of the commercial juices, measured by FRAP method, was not stable (VC = 56%) and ranged from 30.86-70.33 mmol/dm³, excluding sample no. 4. 2-fold higher values in CA than in HM samples (24.22 ±1.89 mmol/dm3) were observed, supporting the statement that the method of juice extraction has an important role in determining their antioxidant activity. The antioxidant capacity of pomegranate seed juices extracted using a food processor, tested by Ozgen et al. (2008), showed a variable level of this parameter, between 4.63 ± 0.44 and 10.9 ± 0.72 mmol TE/dm^3 . It has been demonstrated by Gil et al. (2000) that the higher antioxidant activity of commercial juices is due to the punicalagins and ellagic acid derivatives, which are mostly located in the skin, more than the other hydrolyzable tannins from the arils. Therefore it is recommended that the juice should be extracted from whole fruits for a higher antioxidant capacity.

In the tested juices the total phenolic content (TPC) ranged from 1442.75 to 13 209.38 mg GAE/dm³.

The total phenolic content in HM juices was stable (VC = 9.6%) while in commercial juices this parameter was variable (VC = 53%). It is supported by the research of Tezcan et al. (2009), in which the TPC of pomegranate juices was found to range from 144 to 10 086 mg GAE/dm³. The phenolic content in juices is strongly dependent on the production process. As reported by Gil et al. (2000) and later by Tzulker et al. (2007), juices with higher contents of peel residues have from a 2-fold (fruits squeezed in juice extractors) to 20-fold (homogenates of the whole fruit) higher phenolic content compared to juices prepared only from the arils. Moreover, it was stated that TPC in pomegranate juices depends on the botanical and geographical origins of fruits (Mousavinejad et al., 2009; Tzulker et al., 2007). Analysis of the phenolic content of pomegranates (whole fruits and juices) from different countries showed the following levels of these components: 2380-9300 mg/dm³ in Turkish cultivars (Mousavinejad et al., 2009); 990-2260 mg pyrogallol equivalents/dm³ in Iranian cultivars (Borochov-Neori et al., 2009), 2566 mg/dm³ in commercial juices from the United States (Gil et al., 2000), and from 2602 to 10 086 mg GAE/dm³ in commercial juices from Turkey (Tezcan et al., 2009). According to Vrhovsek et al. (2004), the recommended daily intake (RDI) of polyphenols is 1 g, which means that the consumption of approximately 300 cm³ of pomegranate juice will meet the RDI for this nutrient.

The obtained results show a strong correlation coefficient between TPC and DPPH (r = 0.886), TPC and FRAP (r = 0.958) as well as DPPH and FRAP (r = 0.797; Table 4) which is in accordance with the previously published reports (Eddebbagh et al., 2016; Ozgen et al., 2008).

	pН	TA	TSS	DPPH	FRAP	TPC	Vitamin C
ТА	-0.800	1					
TSS	0.158	0.209	1				
DPPH	0.111	0.286	0.206	1			
FRAP	-0.380	0.730	0.074	0.797	1		
TPC	-0.192	0.588	0.232	0.886	0.958	1	
Vitamin C	0.823	-0.621	0.350	0.280	-0.315	-0.134	1
Anthocyanins	0.863	-0.786	0.172	0.109	-0.415	-0.247	0.849

Table 4. Person's correlation coefficients of tested parameters

Mineral composition

Analysis of the mineral content of pomegranate juices by ICP-OES (Table 5) has shown that the predominant mineral is potassium, followed by phosphorus, sulfur and magnesium. However, potassium was found in a very wide range, especially in CA juices, which could be explained variety differences and technology juice production. Sodium content ranged from 7.02– 26.87 mg/dm³ for HM and from 1.99–62.09 mg/dm³ for CA. In commercial juices, sodium content was significantly higher (P < 0.05) compared to the homemade juices. Like most fruit varieties, fresh pomegranates naturally contain traces of sodium; however, the high sodium content of commercial pomegranate juice might be related to the clarification process using sodium bentonite, as mentioned by Akpinar-Bayizit (2010). Similarly, calcium content was almost twice as high in commercial juices. According to the Association of the Industry of Juices and Nectars (AIJN, 2008), the calcium content of pomegranate juice is generally between 50 and 100 mg/dm³. However, it is stated that the amount of Ca could decrease due to possible oxalate sedimentation during the clarification process. All tested samples were free from Cd, Pb and Hg (<1 ppm). Most of the findings of the present study with regard to the mineral components of pomegranate

Table 5. The effect of extraction methods on the mineral composition of pomegranate juices

M:1		HM, mg/L		CA, mg/L			
Mineral -	minimum	maximum	mean ±SD	minimum	maximum	mean ±SD	
Ca	10.85 ^{HM1}	45.75 ^{HM2}	29.62 ± 17.60	12.90 ^{CA2}	120.45 ^{CA1}	48.60 ±43.41	
K	2 016.07 ^{HM1}	3 040.49 ^{HM2}	$2\ 691.80 \pm 585.30$	132.69 ^{CA4}	3 151.07 ^{CA3}	$1\ 782.14 \pm 1\ 282.52$	
Mg	25.24 ^{HM1}	83.81^{HM2}	49.15 ± 30.73	23.42 ^{CA4}	123.63 ^{CA1}	70.23 ± 43.67	
Р	118.39 ^{HM1}	166.36 ^{HM2}	$143.19\ {\pm}24.03$	5.25 ^{CA4}	201.66 ^{CA1}	98.89 ± 81.24	
S	102.12 ^{HM1}	162.05 ^{HM2}	132.19 ± 29.96^{a}	24.69 ^{CA5}	78.32 ^{CA1}	$53.32 \pm \! 19.51^{\rm b}$	
Na	7.02 ^{HM1}	18.87 ^{HM2}	$13.79\pm\!\!6.10^{\rm a}$	19.98 ^{CA2}	62.09 ^{CA1}	$38.60 \pm 19.00^{\text{b}}$	
Cr	n.d. ^{HM1}	0.055 ^{HM2}	$0.030\pm\!\!0.028$	0.010 ^{CA4}	0.191 ^{CA1}	0.094 ± 0.073	
Cu	0.337 ^{HM3}	0.566 ^{HM1}	0.453 ±0.115	0.031 ^{CA5}	0.548 ^{CA2}	$0.296\pm\!\!0.230$	
Fe	n.d. ^{HM1}	3.799 ^{HM3}	2.440 ± 2.118	0.318 ^{CA5}	4.112 ^{CA1}	2.635 ± 1.548	
Mn	0.499 ^{HM1}	0.837 ^{HM3}	0.620 ± 0.188	0.367 ^{CA4}	2.659 ^{CA1}	0.994 ± 0.960	
Мо	n.d. ^{HM1}	0.019 ^{HM3}	0.012 ± 0.011	0.001 ^{CA3}	0.014 ^{CA2}	0.005 ± 0.005	
Sr	n.d. ^{HM1,3}	0.020^{HM2}	0.007 ± 0.011	0.015 ^{CA2}	1.179 ^{CA1}	0.567 ± 0.487	
Zn	0.076^{HM1}	1.256 ^{HM2}	0.718 ± 0.597	n.d. ^{CA4,5}	8.699 ^{CA2}	2.814 ± 3.872	
Cd		n.d.			n.d.		
Pb		n.d.			n.d.		
Al	0.930 ^{HM1}	1.547 ^{HM3}	1.223 ±0.310	0.381 ^{CA4}	2.563 ^{CA3}	1.635 ± 1.095	
Hg		n.d.			n.d.		
Ni	0.016 ^{HM1}	0.158 ^{HM2}	0.097 ± 0.073	n.d. ^{CA4,5}	0.378 ^{CA2}	0.140 ± 0.183	

n.d. – below the level of detection (<1 ppm).

^{a,b}Significant differences (P < 0.05) between elements according to methods of juice extraction.

juice are consistent with the results of other studies (Akpinar-Bayizit 2010; Ekşi and Özhamamcı, 2009; Fischer-Zorn and Ara, 2007).

It was found that the quality of pomegranate juices available on the market is price-dependent and cheaper juices (4 and 5) were characterized by lower antioxidant activity. The antioxidant properties of more expensive commercial juices (1 and 2) were even better than homemade juices, excluding vitamin C and anthocyanins. However, both components are thermally unstable, so their level is reduced by the pasteurization process, which is commonly applied in commercial juice production. Moreover, tested homemade juices were prepared manually without using a juicer machine. This method allowed the majority of bioactive compounds in the fruit juice to be preserved. In our earlier study, we found that using an electric juicer to extract pomegranate juice caused a loss of vitamin C (up to 60%) and anthocyanins (up to 40%) compared to manually pressed juice (Dżugan et al., 2016). Moreover, the presence of active enzymes in raw juice can result in the loss of bioactive components in juices even during short-term storage. Overall, this research has confirmed that it is possible to replace manually prepared juices with commercially available juices. However, this is associated with higher purchase costs (calculated according to prices in Table 1), but this estimation is imprecise due to the season-dependent price of pomegranate fruits.

CONCLUSION

Due to its high antioxidant activity, pomegranate juice is highly valued for its strong health-promoting benefits. The obtained results have shown that the method of production has a strong influence on the physicochemical properties and some antioxidant properties of pomegranate juice. A good and stable composition of HM juices was observed. However, higher antioxidant parameters were found for CA juices, probably as a result of the presence of polyphenols from whole fruits (peel and arils). Additionally, studies have shown that only the highest quality and most expensive commercial pomegranate juice can be as beneficial as homemade pomegranate juice.

REFERENCES

- AIJN (2008). Association of the Industry of Juices and Nectars (EU). Provisional reference guideline for pomegranate juice. Brussels: European Fruit Juice Association.
- Akpinar-Bayizit, A. (2010). Analysis of mineral content in pomegranate juice by ICP-OES. Asian. J. Chem., 22, 8, 6542–6546.
- Alighourchi, H., Barzegar, M., Abbasi, S. (2008). Anthocyanins characterisation of 15 Iranian Pomegranate (*Punica* granatum L.) varieties and their variation after storage and pasteurization. Eur. Food Res. Technol., 227, 881– 887. http://dx.doi.org/10.1007/s00217-007-0799-1
- AOAC (2005). Official method of analysis. 17 Edition. Washington D.C., USA: Association of Official Agricultural Chemists.
- Arendse, E., Fawole, O. A., Opara, U. L. (2014). Effects of postharvest storage conditions on phytochemical and radical-scavenging activity of pomegranate fruit (cv. Wonderful). Sci. Hortic., 169, 125–129. http://dx.doi. org/10.1016/j.scienta.2014.02.012
- Barrita, J., Sánchez, M. (2013). Antioxidant role of ascorbic acid and his protective effects on chronic diseases. In: J. A. Morales-Gonzalez (Ed.), Oxidative stress and chronic degenerative diseases – A role for antioxidants. InTech. http://dx.doi.org/10.5772/52181 Available from: http:// www.intechopen.com/books/oxidative-stress-andchronic-degenerative-diseases-a-role-for-antioxidants/ antioxidant-role-of-ascorbic-acid-and-his-protectiveeffects-on-chronic-diseases.
- Benzie, I. F., Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method Enzymol., 299, 15–27. PMID: 9916193.
- Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I., Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. J. Food Compos. Anal., 22, 89–95. http://dx.doi.org/10.1016/j. jfca.2008.10.011
- Determination of soluble solids (indirect method by refractometry). Analysis no. 8 (2005). Paris: International Federation of Fruit Juice Producers.
- Dżugan, M., Kisała, J., Wesołowska, M. (2016). Zawartość składników bioaktywnych w soku z owoców granatu (*Punica granatum*) w zależności od metody wytwarzania [The content of bioactive components in juice from pomegranate fruit (*Punica granatum*) in relation to the production method]. In: A. Duda-Chodak, D. Najgebauer-Lejko, I. Drożdż, T. Tarko (Eds.), Rola procesów technologicznych w kształtowaniu jakości żywności

(pp. 143–153). Kraków: Oddz. Małopol. Pol. Tow. Techn. Żywn. [in Polish].

- Eddebbagh, M., Messaoudi, M., Abourriche, A., Berrada, M., Attaleb, M., Benbacer, L., Bennamara, A. (2016). Correlation of the cytotoxic and antioxidant activities of moroccan pomegranate (*Punica granatum*) with phenolic and flavonoid contents. J. Pharm. Pharmacol., 4, 511– 519. http://dx.doi.org/10.17265/2328-2150/2016.09.006
- Eghdami, A., Asli, D. E. (2010). Determination of antioxidant capacity of pomegranate juice by using 2,2-diphenyl-1-picrylhydrazyl. Organic. Chem. J., 1, 30–33.
- Ekşi, A., Özhamamcı, I. (2009). Chemical composition and guide values of pomegranate juice. GIDA, 34, 5, 265–270.
- Fischer-Zorn, M., Ara, V. (2007). Pomegranate juice chemical composition and potential adulteration. Fruit Proces., 17, 4, 204–213.
- 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. J. Agr. Food Chem., 48, 4581–4589. http://dx.doi.org/10.1021/jf000404a
- Hasnaoui, N., Jbir, R., Mars, M., Trifi, M., Kamal-Eldin, A., Melgarejo, P., Hernandez, F. (2011). Organic acids, sugars, and anthocyanins contents in juices of Tunisian pomegranate fruits. Int. J. Food Prop., 14, 741–757. http://dx.doi.org/10.1080/10942910903383438
- Ismail, F. A., Abdelatif, S. H., Abd, El-Mohsen, N. R., Zaki, S. Z. (2014). The physico-chemical properties of pomegranate juice (*Punica granatum* L.) extracted from two egyptian varieties. World J. Dairy Food Sci., 9, 29–35.
- Kulkarni, A. P., Aradhya, S. M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chem., 93, 319–324. https://doi. org/10.1016/j.foodchem.2004.09.029
- Lansky, E. P., Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for the prevention and treatment of cancer and inflammation. J. Ethnopharmacol., 109, 177–206. https://doi.org/10.1016/j. jep.2006.09.006
- Martinez, J. J., Melgarejo, P., Hernandez, F., Salazzar, D. M., Marinez, R. (2006). Seed characterization of five new pomegranate (*Punica granatum* L.) varieties. Sci. Hortic., 110, 241–246. https://doi.org/10.1016/j. scienta.2006.07.018
- Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K., Hossein, M., Khodaparast, H. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem., 115, 1274–8. https://doi.org/10.1016/j.foodchem.2009.01.044
- Orak, H. H. (2008). Evaluation of antioxidant acitivity, colour and some nutritional characteristics of

pomegranate (*Punica granatum* L.) juice and its sour concentrate processed by conventional evaporation. Int. J. Food Sci. Nutr., 60, 1–11. http://dx.doi. org/10.1080/09637480701523306

- Ozgen, M., Durgaç, C., Serçe, S., Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chem., 111, 3, 703–706. https://doi.org/10.1016/j.food chem.2008.04.043
- Ranu, P., Uma, G. (2012). Effect of thermal treatment on ascorbic acid content of pomegranate juice. Indian J. Biotechnol., 11, 309–313.
- Rapisarda, P., Fanella, F., Maccarone, E. (2000). Reliability of analytical methods for determining anthocyanins in blood orange juices. J. Agr. Food Chem., 48, 6, 2249. http://dx.doi.org/10.1021/jf991157h
- Robinson, W. B., Stotz, E. (1945). The indophenol-xylene extraction method for ascorbic acid and modifications for interfering substances. J. Biol. Chem., 160, 217–225.
- Shiban, M. S., Otaibi, M. M., Al-Zoreky, N. S. (2012). Antioxidant activity of pomegranate (*Punica granatum* L.) fruit peels. Food Nutr. Sci., 3, 991–996. http://dx.doi. org/10.4236/fns.2012.37131
- Singleton V. L., Rossi J. A. (1965). Restricted access colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. Am. J. Enol. Vitic., 16, 144–158.
- Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chem., 115, 873–877. https:// doi.org/10.1016/j.foodchem.2008.12.103
- 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. J. Agr. Food Chem., 55, 9559–9570. http:// dx.doi.org/10.1021/jf071413n
- Vazquez-Araujo, L., Chambers, IV E., Adhikari, K., Carbonell-Barrachina, A. A. (2010). Sensory and physicochemical characterization of juices made with pomegranate and blueberries, blackberries, or raspberries. J. Food Sci., 75, 7, 398–404. http://dx.doi. org/10.1111/j.1750-3841.2010.01779.x
- Vrhovsek, U., Rigo, A., Tonon, D., Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. J. Agr. Food Chem., 52, 6532–6538. http://dx.doi.org/10.1021/ jf049317z
- Zarei, M., Azizi, M., Bashir-Sadr, Z. (2011). Evaluation of physicochemical characteristics of pomegranate (*Punica* granatum L.) fruit during ripening. Fruits, 66, 121–129. http://dx.doi.org/10.1051/fruits/2011021