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Physico-chemical characteristics of main pomegranate (*Punica granatum* L.) cultivars grown in Dalmatia region of Croatia

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Summary

Consumers are increasingly expecting the fruits to be tasty and attractive while being safe and healthful. We determined the external and internal fruit quality properties of main pomegranate cultivars widely grown in Croatia. Of the three cultivars, 'Pastun' produced the biggest fruits (460 g) followed by 'Konjski zub' as 309 g and 'Ciparski' as 341 g. Cultivars exhibited a range of flavor (from sour to sweet) and acidity (0.9 to 4.3% in juice). Aril colour of 'Ciparski' and 'Pastun' were red while 'Konjski zub' had light pink aril colour. 'Pastun' had the highest soluble solids and acidity in both fruit juice and peel. 'Ciparski' was in high for total anthocyanins content (12.8 mg of cyanidin-3,5-diglucoside equivalents per 100 g of fresh mass) while 'Pastun' is high for total phenolics content (144.7 mg gallic acid equivalent per 100 g fresh mass) in juice. Our results indicated that there were important quality differences among pomegranate cultivars grown in Croatia.

Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and more recently its fruits are gained more interest for its antioxidant and nutritional values that are important for human health (Tezcan et al., 2009; Tehranifar et al., 2010). The edible part of the fruit contains considerable amounts of sugars, vitamins, polysaccharides, polyphenols and minerals (Ercisli et al., 2007; Ozgen et al., 2008). Moreover recent clinical studies revealed its effective antimicrobial (McCarrell et al., 2008), antiviral, anticarcinogenic and anti-inflammatory activities (Viuda-Martos et al., 2010).

The cultivation of the pomegranate is mainly confined to semi-arid mild-temperate to subtropical climates in the main growing areas including most of Mediterranean countries (Stover and Mercure, 2007) where pomegranate trees are particularly adapted to saline and poor soils (Martinez et al., 2006).

In Croatia, pomegranates grow mainly in Neretva valley located in East coast of Adriatic and it has traditionally been consumed as fresh fruit and also processed as juice. In the country pomegranates are spread mainly as a minor fruit and traditional plantations use a few local cultivars. However more recently, there is high demand and public awareness to its cultivation and it is getting more commercialisation in the country. The fruits are usually sold in nearby local markets and supermarkets for fresh consumption.

The quality of pomegranate fruits is strongly dependent on the cultivars, growing regions, climate, maturity and cultural practices (Poyrazoglu et al., 2002; Tehranifar et al., 2010).

Fruit maturity time of pomegranate is commonly determined based on external (peel colour, size) and internal (aril colour, sugar content and acidity) factors (Ercisli et al., 2007; Al Said et al., 2009). Appearance, especially red colour and size, effects the consumers behaviour and they are accepted as the most important external quality parameters for pomegranate (Alighourchi and Barzegar, 2009; Celik and Ercisli, 2009). Fruit maturity occurs in general in September and October according to growing areas in Croatia. There are no standard maturity indices or postharvest quality attributes to assist in the harvesting and management of postharvest fruit quality in pomegranates grown in Croatia. Previously, only a few published results on the limited physical properties of pomegranate cultivars have been reported in Croatia (Marinović and Vego, 2009; Ugarković et al., 2009). No published data were found on their chemical and textural properties related to eating quality and processing in particular comparing pulp and juice. Obtaining these data can help breeders and consumers to select genotypes with high level of desirable compounds along with better physical properties.

Scientific assessment on physical and chemical content of commercially grown pomegranate cultivars in different countries is also needed for proper evaluation on this unique fruit. Therefore, one of the main objectives of this study was to characterize important external and internal quality attributes of main pomegranate cultivars grown in south Dalmatia, Neretva valley in Croatia. In addition another important aim of the study was to compare the evaluated fruit characteristics of cultivars in order to determine which cultivars have the best quality features.

Materials and methods

Plant Materials

The fruits at commercially ripe stage from three main pomegranate cultivars such as 'Ciparski', 'Konjski zub' and 'Pastun' were harvested from seven-year-old trees in the Neretva valley in south Dalmatia of Croatia in October 2009. The trees were spaced 5 and 3 m between and within rows. All cultivars were grown under the same geographical conditions and took the same agronomic and cultural practices. After harvest, fruits were quickly transported in cold chain to the research laboratory at the Institute for Adriatic Crops and Karst Reclamation in Split. Twelve pomegranate fruits sampled from each cultivar and four replicates were maintained for each analysis and each replicate includes three fruits.

Fruit peel and aril colour

Fruit peel and aril colour of pomegranates was measured on each fruit computed as means of three measurements taken from opposite sides at the equatorial region of the fruit by using chromometer (ColorTec-PCM, USA) and results were represented as L*, a* and b* value. Chroma and hue angle were calculated by using L*, a* and b* values (Perkins-Veazie, 1992).

Physical properties

Fruits were weighed individually on balance of accuracy of 0.001 g. Length and diameter of the fruit and calyx were measured with a digital Vernier calliper. The measurement of fruit length was made on the polar axis of the fruit, i.e. between the apex and the end of the stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. Arils were manually separated from the fruits, and total aril content was weighed. Replicate measurements of the peel thickness on the op-

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posite sides were made using a digital Vernier calliper (TEHRANIFAR et al., 2010). Fruit juice yield was determined by extracting the contents of replicate samples of 100 g of arils per fruit using a juice extractor. Then the pulp and juice was analyzed for their chemical properties.

Dry matter, soluble solid, acidity, pH and ascorbic acid

Total dry matter (DM) was obtained by drying homogenised pomegranate arils at 105 °C until constant mass (AOAC, 1995). Soluble Solid Content (SSC, %) was measured using an Abbe refractometer (A. Krüss Optronic, Germany) calibrated against sucrose. Acidity was measured according to the AOAC method (1995) and expressed in g/L citric acid. pH value was measured with a pH meter (Mettler Toledo, Switzerland). Ascorbic acid (AA) was determined by the 2.6-dichloroindophenol titrimetric method according to the standard method (AOAC, 1995).

Sugars

Sucrose, D-glucose and D-fructose contents were determined by enzymatic test kits (R-Biopharm, France), measuring the formation of NADPH at 340 nm, according to the described protocol of the kits (ROSALES et al., 2007).

Total phenolics and total flavonoids

Total phenolics and total flavonoids were determined using the Folin-Ciocalteu colorimetric method (OUGH and AMERINE, 1988). Total phenolics were extracted from 10 g of fresh samples using 40 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted (in water bath at 80 °C), kept for 20 min in inert atmosphere, and filtered through a Whatman filter paper using a Büchner funnel. Extraction of the residue was repeated under the same conditions. The filtrates were combined and diluted to 100 mL in a volumetric flask with 80% aqueous ethanol, and the obtained extract was used for determination of total phenolics and total flavonoids. The formaldehyde precipitation was used to determine total flavonoids in fruit samples (KRAMLING and SINGLETON, 1969). Total phenolics and total flavonoids were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh mass of pulp and juices.

Total anthocyanins

The total anthocyanins content in the extract from fruits was determined by PELLEGRINI et al. (1965). Fruit anthocyanins were extracted from 2 g of fresh samples using 2 mL of 0.1 % HCl (by volume) in 96 % ethanol and 40 mL 2 % aqueous HCl (by volume). The mixture was centrifuged at 5500 rpm for 10 min. The obtained supernatant was used for the determination of total anthocyanins. The absorbance was measured at 520 nm. The molar absorbance value for cyanidin-3.5-diglucoside was used as a standard value. Results were expressed as mg of cyanidin-3.5-diglucoside equivalents per 100 g fresh mass of pulp and juices.

Statistical Analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by LSD test at $P<0.05$ significant level (SAS, 1990).

Results and discussion

Physical properties

Physical properties of three pomegranate cultivars are shown in Tab. 1. Statistically significant differences were recorded among cultivars ($P < 0.05$).

Average fruit mass of pomegranate cultivars ranged from 310 g ('Ciparski') to 460g ('Pastun'). The fruit length and diameter values were between 73.3 mm ('Konjski zub') and 83.1 mm ('Pastun'), and 79.1 mm ('Konjski zub') and 95.3 mm ('Pastun'). With regard to fruit shape (L/D ratio), the 'Konjski zub' is more rounded. Calyx length and diameter values were 16.1 mm ('Konjski zub') and 20.8 mm ('Pastun') and 18.0 mm ('Konjski zub') and 22.7 mm ('Pastun'), respectively. Among cultivars, cv. 'Pastun' had significantly different physical properties than 'Ciparski' and 'Konjski zub' that has similar physical properties. There was significant difference observed in aril ratio among the cultivars and 'Ciparski' had the highest aril ratio (58.8%) while 'Pastun' had the lowest one (53.6%). There were no significant differences in fruit peel thickness among the cultivars that varied from 3.9 mm ('Pastun') to 4.2 mm ('Konjski zub') (Tab. 1).

Tab. 1: Physical properties of three pomegranate cultivars grown in Croatia

	Cultivars		
	Ciparski	Konjski zub	Pastun
Fruit mass (g)	341b	309b	460a
Fruit length (mm)	75.8b	73.3b	83.1a
Fruit diameter (mm)	84.6b	79.1b	95.3a
Fruit length/diameter	0.9NS	0.9	0.9
Calyx length (mm)	18.0b	16.1b	20.8a
Calyx diameter (mm)	20.9NS	18.0	22.7
Calyx length/diameter	0.9NS	0.9	1.1
Peel thickness (mm)	4.0NS	4.2	3.9
Peel weight (g)	141b	129b	202a
Aril ratio (%)	58.8a	55.1ab	53.6b
Aril colour	Red	Light pink	Red
Taste	Sweet	Sour-sweet	Sour

Data are expressed as average value of four replicates. Different letters in same row indicate significant differences at the 5 % level by LSD test.

In several previous studies, a wide variation was found on fruit mass of pomegranate cultivars that varied between 150 and 568 g (ERCAN et al., 1992; YILMAZ et al., 1992; AL-MAIMAN and AHMAD, 2002; KAZANKAYA et al., 2003; OZKAN, 2005; TEHRANIFAR et al., 2010). Our fruit mass results are within these limits. Some studies conducted on fruit length and width of pomegranate cultivars ranged from 61 to 91 and 36 to 104 mm (YILMAZ et al., 1992; AL-MAIMAN and AHMAD, 2002; KAZANKAYA et al., 2003) which supports our findings. Calyx length and calyx diameter of pomegranate genotypes were found between 13.5 29.9 mm and 12.5 25.0 mm (SARKHOSH et al., 2009; TEHRANIFAR et al., 2010) which in agreement with our findings.

These dimensions can be used to discriminate the cultivars among each other and also designing machine components and parameters for pomegranate processing.

Fruit peel and aril colour

Fruit peel and aril colour of pomegranate cultivars are shown in Tab. 2. Cultivar 'Pastun' had significantly higher red coloration (*a* value) on its peel while 'Ciparski' had the least red coloration among the cultivars. Cv. 'Ciparski' had significantly more light peel colour (*L* value) while 'Pastun' had the least lightness. The chroma value (*C*), which represents colour intensity, was similar among the cultivars. Fruit aril colour varied in all measured parameters.

Tab. 2: Fruit peel and aril colour of three pomegranate cultivars grown in Croatia

	Peel colour				
Cultivars	*L**	*a**	*b**	*Chroma*	*Hue*
Ciparski	69.7a	10.5b	24.3a	27.3^{NS}	63.6a
Konjski zub	59.6b	11.4b	25.7a	28.7	64.6a
Pastun	46.6c	17.0a	24.3a	30.3	54.8b
			Aril colour		
Ciparski	67.7a	12.1a	30.8a	33.6a	68.7a
Konjski zub	43.2b	-4.6b	16.7b	17.5b	105.5b
Pastun	33.3c	-6.2b	17.9b	20.2c	117.1b

Data are expressed as average value of four replicates. Different letters in the same column indicate significant differences at the 5 % level by LSD test. NS: Non significant.

Red colour intensity (*a* value) of arils was significantly higher in 'Ciparski'. Cultivars 'Konjski zub' and 'Pastun' have significantly less yellowness (*b* value) and less lightness (*L* value) compared to cv. 'Ciparski'. However, hue value (*h*) was significantly higher in cultivars 'Konjski zub' and 'Pastun', but colour intensity (*C*) was lowest in cv. 'Konjski zub'. The visible aril colour of this cultivar was as light pink while 'Pastun' and 'Ciparski' have red arils. Attractive red aril colour is one of the most important physical characteristics of pomegranate.

Chemical properties of pomegranate juice and pulp

The results on the qualitative fruit traits obtained from juice and pulp in arils of pomegranate cultivars are given in Tab. 3 and 4. As indicated on both tables, significant statistical differences ($P < 0.05$) among cultivars on all searched parameters are observed (Tab. 3, 4).

The soluble solid content values in juice and pulp were the highest with 15.2 and 15.6 in cv. 'Pastun' and followed by 'Ciparski' as 14.8 and 15.1% and 'Konjski zub' (13.1 and 15.0%). Acidity of fruit juice was significantly higher in 'Pastun' (4.3 %) compared to cv. 'Ciparski' (0.9 %) and 'Konjski zub' (1.4 %). These cultivars were found in same order in pulp acidity. The pH value ranged from 2.6 ('Pastun') to 3.4 ('Ciparski') in juice and 2.9 ('Pastun') to 4.0 ('Ciparski') in pulp.

Tab. 3: Chemical properties of fruit juice of three pomegranate cultivars grown in Croatia

	Cultivars		
	Ciparski	Konjski zub	Pastun
Dry matter (%)	14.5ab	14.2b	14.8a
Soluble Solid Content (%)	14.8a	13.1b	15.2a
Acidity (%)	0.9c	1.4b	4.3a
pH	3.4a	3.3a	2.6b
Vitamin C (mg/100 ml)	18.8c	20.1b	26.0a
Total anthocyanins (mg/100 ml)	12.8a	2.5c	10.3b
Total phenolics (mg GAE/100 ml)	105.1b	77.7c	144.7a
Total flavonoids (mg GAE/100 ml)	55.9b	50.2c	111.8a
Sucrose (%)	0.3b	0.9a	0.2c
Glucose (%)	7.4a	6.4b	7.3a
Fructose (%)	8.3a	7.5c	8.1b

Data are expressed as average value of four replicates. Different letters in the same row indicate significant differences at the 5 % level by LSD test.

Tab. 4: Chemical properties of fruit pulp of three pomegranate cultivars grown in Croatia

	Cultivars		
	Ciparski	Konjski zub	Pastun
Dry matter (%)	20.6a	15.7c	17.3b
Soluble solid content (%)	15.1b	15.0b	15.6a
Acidity (%)	0.4c	0.6b	1.8a
pH	4.0a	3.7a	2.9b
Vitamin C (mg/100 ml)	21.6a	17.3c	20.2b
Total anthocyanins (mg/100 ml)	5.5b	1.5c	6.9a
Total phenolics (mg GAE/100 ml)	124.7b	104.6c	179.1a
Total flavonoids (mg CE/100 ml)	62.8c	70.3b	87.8a
Sucrose (%)	0.3b	0.2c	1.4a
Glucose (%)	7.6b	9.8a	5.7c
Fructose (%)	8.6b	11.1a	6.5c

Data are expressed as average value of four replicates. Different letters along the row indicate significant differences at the 5 % level by LSD test.

In pulp, sugar / acid ratio is an accepted main flavor quality in most fruit species and it seems that there were modest variations on SSC and substantial variations on acidity of these cultivars. Similar finding was reported by (Ugarković et al., 2009) on pomegranates.

According to acidity values, pomegranate cultivars are classified as sweet (<1 %), sour-sweet (1-2 %) and sour (>2 %) (Onur and Kaska, 1985). Therefore 'Ciparski' is sweet, 'Konjski zub'is sour-sweet and 'Pastun' is sour cultivar. Sweet, sour-sweet and sour taste are commonly reported among pomegranate genotypes (Sarkhosh et al., 2009; Cam et al., 2009; Tehranifar et al., 2010).

High dark red aril colour, high SSC and relatively high acidity of pomegranate arils are considered to be a good choice for both fresh fruit and juice markets (Ozgen et al., 2008).

Significant variations in soluble solid contents (11-23 %), acidity (0.1-4.5 %) and pH (3.3-4.3) of pomegranates juices have been reported over the years by various researchers (Ercan et al., 1992; Yilmaz et al., 1992; Al-Maiman and Ahmad, 2002; Kazankaya et al., 2003; Ozkan; 2005).

There was significant difference in ascorbic acid, total anthocyanins, total phenolics and total flavonoids content in both pulp and juice of the cultivars at $P<0.05$ (Tab. 3, 4).

Vitamin C values were between 18.8 ('Ciparski') and 26.0 mg/100 ml ('Pastun') in juice and 17.3 ('Konjski zub') and 21.6 mg/100 ml ('Ciparski') in pulp (Tab. 3, 4). Tehranifar et al. (2010) reported vitamin C in pomegranate genotypes between 9.9-20.9 mg per 100 g.

Total anthocyanins, total phenolics and total flavonoids ranged from 2.5 ('Konjski zub') to 12.8 mg of cyanidin-3.5-diglucoside equivalents per 100 g of fresh mass ('Ciparski'); 77.7 ('Konjski zub') to 144.7 mg GAE per 100 g fresh mass ('Pastun') and 50.2 ('Konjski zub') to 111.8 mg of GAE per 100 g of fresh mass ('Pastun') in juice and 1.5 ('Konjski zub') to 6.8 mg of cyanidin-3.5-diglucoside equivalents per 100 g of fresh mass ('Ciparski'); 104.6 ('Konjski zub') to 179.1 mg of GAE per 100 g fresh mass ('Pastun') and 62.8 ('Ciparski') to 87.8 mg GAE per 100 g of fresh mass ('Pastun') in pulp, suggesting that cv. 'Pastun' had significantly higher amount of bioactive content for human health than 'Ciparski' and 'Konjski zub' in considering both juice and pulp (Tab. 3, 4). Cam et al. (2009), Tehranifar et al. (2010) and Fawole

et al. (2011) reported total anthocyanins in different pomegranate cultivars grown in Iran, Turkey and South Africa were between 9.9-20.9; 8.10-36.9 and 16.5-26.9 mg per 100 g of juice, respectively. Total phenolic contents of pomegranate cultivars reported from different countries between 14.4 and 1008 mg GAE per 100 g fresh mass (Ozgen et al., 2008; Cam et al., 2009; Tezcan et al., 2009; Tehranifar et al., 2010; Fawole et al., 2011). Their results were in agreement with our results.

Among the different compounds that could serve as unequivocal markers in a fruit juice product, anthocyanins and phenolic compounds are potentially the most useful because of their ubiquity, specificity and multiplicity. Phenolic compounds are important for their contribution to sensory attributes, as well as for their potential health benefits in fruits and vegetables. The effects of phenolic compounds on low-density lipoproteins and aggregation of platelets are beneficial because they reduce some of the major risk factors for coronary heart disease (Poyrazoglu et al., 2002).

Glucose and fructose were found to be dominant sugars an all analyzed cultivars in both juice and pulp. The glucose and fructose concentration were found between 6.4 ('Konjski zub') to 7.4 % ('Ciparski') and 7.5 ('Konjski zub') to 8.3 % ('Ciparski') in juice and 5.7 ('Pastun') to 9.8 % ('Konjski zub') and 6.5 ('Pastun') to 11.1 % ('Konjski zub') in pulp. Sucrose was found in trace amounts and it ranged from 0.2 % in cv.'Pastun' to 0.9 % in cv. 'Konjski zub' in juice and 0.2 % in cv.'Konjski zub' to 1.4 % in cv. 'Pastun' in pulp (Tab. 3, 4). Ozgen et al. (2008) reported average 6.4 and 6.8 % fructose and glucose and negligible amount of sucrose in juice of six pomegranate cultivars from Turkey.

Conclusions

The quality of pomegranate fruits is strongly dependent on the cultivars. The physical properties of the pomegranate cultivars in this research demonstrated that the cultivar is important factor to determine fruit quality. Cv. 'Pastun' has bigger fruit size, attractive red skin colour, higher red aril coloration, high SSC and acidity, high bioactive content including ascorbic acid, anthocyanins, total phenolics and total flavonoides. The other cultivars are also promising because they have diverse taste that is important for table consumption.

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