

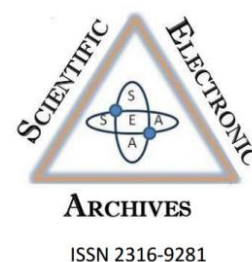
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Antioxidant activity and chemical composition from different parts of pomegranate (*Punica granatum* L.) cultivars

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Abstract. Pomegranate fruit is rich in phenolic compounds and antioxidant properties and can show difference in the composition according to cultivars, part of fruit, environmental conditions and analysis method. Therefore, the aim of the present study was to analyse and to compare the chemical composition and antioxidant properties from the different parts of fruit Valenciana and Wonderful pomegranate cultivars. The pomegranate fruits were separated into aril, peel, membrane and seeds manually and analysed by phenolic compounds, flavonoids, antioxidant activity (DPPH method), soluble solids, titratable acidity, pH and vitamin C content. The phenolic content and flavonoids were significantly affected by pomegranate cultivars and part of fruit. In the part of fruit showed differences by the DPPH, however the pomegranate cultivars not showed. Significant differences were revealed between the pomegranate cultivars and part of fruit for total soluble solids, titratable acidity and pH. The different parts of pomegranate fruit (aril, membrane peeling and seed) has influences on the phenolic content, flavonoids, antioxidants, total soluble solids, pH and acidity. The cultivar does not show differences on the antioxidants by DPPH method, pH and vitamin C. This study suggests the importance of the selection of the different parts of fruit and cultivar that will be used as raw material in the preparation of pomegranate products with higher antioxidant activities.

Keywords: Phenol. DPPH method. Flavonoids.

Introduction

Pomegranate (*Punica granatum* Linn.), main species of Punicaceae family, is a widely grown culture crop in many tropical and subtropical countries. The pomegranate family has a single genus *Punica* with two species, *P. granatum* and *P.*

protopunica (VERMA; MOHANTY; LAL, 2010; CHANDRA *et al.*, 2010). In the species *Punica granatum*, there are botanical variations within the same (varieties): the variety Wonderful that is characterized by high sweetness, attractive purple color and medium acidity and the variety Valenciana

which is characterized by sweetness, juiciness and low acidity. To consumption human, the desirable characteristics are high sweetness, moderate to low levels acidity, red wine and fruity odor, low bitterness and astringency (MAYUONI-KIRSHINBAUM; PORAT, 2014).

Pomegranate fruits are characterized by being globose or flattened; with a smooth and leathery skin, it may present a brownish-yellow to red when ripe; the mesocarp is spongy, with chamber divisions by a horizontal diaphragm and vertical septal membranes made of papery tissue, each chamber is filled with many seeds agglomerated in thick, spongy placentas, with the arils not attaching to the septal membranes (SILVA et al., 2013). The seeds are surrounded by the juicy arils, which comprised the edible portion of the fruit (SILVA et al., 2013). The edible portion (aril) of fruit is about 55-60% of the total fruit weight and consists of about 75-85% juice and 15-25% seeds (AL-MAIMAN; AHMAD, 2002).

One hundred g pomegranate fruit arils provide 72 kcal of energy, 1.0 g protein, 16.6 g carbohydrate, 1.0 mg sodium, 379.0 mg potassium, 13.0 mg calcium, 12.0 mg magnesium, 0.7 mg iron, 0.17 mg copper, 0.3 mg niacin and 7 mg vitamin C (GROVE; GROVE, 2008). The fruit is also too rich in phenolic compounds and antioxidant properties and showed different in the composition according to cultivars, parts of fruit, environmental conditions and analysis method.

Tehranifar et al. (2010) to investigate the physicochemical characteristics and antioxidant activity of twenty pomegranate cultivars cultivated in Iran, found values for the total soluble solids content ranging from 11.37 to 15.07°Brix, while the titratable acidity content ranged from 0.33 to 2.44 g 100 g⁻¹, total sugar content from 13.23 to 21.72 g 100 g⁻¹, ascorbic acid ranged from 9.91 to 20.92 mg 100 g⁻¹, anthocyanins between 5.56 and 30.11 mg 100 g⁻¹, phenolics from 295.79 to 985.37 mg 100 g⁻¹ and finally the antioxidant activity by the DPPH method between 15.59 and 40.72 %. Fischer, Carle and Kammerer (2011) observed that the peel, mesocarp, aril and differently produced pomegranate juices markedly affected the profiles and contents of phenolics, underlining the necessity to optimize these parameters for obtaining products with well-defined functional properties. Nuncio-Jáuregui et al. (2014) showed that the position within the pomegranate tree had no significant effects on total soluble solids, the titratable acidity, maturity index, pH, organic acids, sugars profiles, proline, antioxidant activity and total phenolic compounds.

To date no work reported on the chemical constituents and antioxidant activity from each part of fruit and different pomegranate cultivars. Zhang, Fu and Zhang (2011) compared the in vitro antioxidant properties of different parts of pomegranate flowers. Therefore, the aim of the present study was to analyze and to compare the chemical composition and antioxidant properties

from aril, peel, membrane and seeds of Valenciana and Wonderful pomegranate cultivars.

Material and methods

Plant material and sample processing

Two different cultivars were evaluated: Wonderful cultivar that show high sweetness, attractive purple color and medium acidity and the Valenciana cultivar which is characterized by sweetness, juiciness and low acidity. The Wonderful fruits were purchased from a municipal market São Paulo city, São Paulo state, Brazil and the Valenciana fruits were harvested from a commercial orchard located in the Assaí city, Paraná state, Brazil. The pomegranate fruits were separated into aril, peel, membrane and seeds manually and after stored to -4°C until analysis.

Analytical Procedures

All the reagents were of analytical grade from different sources.

Phenolic compounds

Phenolic compounds were determined according by Stratil, Klejdus and Kuban (2006) with modifications. Each piece of fruit (aril, peel, membrane and seeds) (0.1 g) was extract with 10.0 mL of 80 % ethanol (v/v), followed by stirring for 20 min at 200 rpm shaker table (Tecnal, TE-145, Brazil) and at ambient temperature (25°C). The material was centrifuged at 2500 g (Fanem, Excelsa 3 Model 280, Brazil) and the supernatant collected. The quantification of total phenolic compounds was analyzed spectrophotometrically using Folin-Ciocalteu colorimetric method. For colorimetric reaction, we used a 1.0 mL aliquot of extract appropriate dilution and added with 1.0 mL of aqueous 0.9 N Folin-Ciocalteu reagent and 1.0 mL of sodium carbonate solution 10 % (w/v). The mixture was incubated for 30 min in the dark at room temperature. Absorbance was measured at 760 nm in a spectrophotometer Micronal, AJX-1600. Gallic acid was used as standard and the total phenolic results, mean of three replicates, were expressed as mg Gallic Acid Equivalents per 100 g sample (mg GAE 100 g⁻¹).

Flavonoids

Flavonoids compounds were analyzed by Woisky and Salatino (1998) method. The extraction of flavonoids was performed according to the phenolic compounds describe above. Then, in the tubes were added 2.0 mL of extract, 2.0 mL of aluminum chloride 5% (w/v) and 2.0 mL of methanol. For the reaction, the samples were allowed to stand for 30 min in the dark and the reading was carried out on a spectrophotometer (Micronal, AJX- 1600) at 425 nm. Quercetin was used for construction of the calibration curve. From the equation of the line obtained it was calculated the content of flavonoids and the results, average of

three replicates, were expressed in mg of Quercetin per 100 mg of sample.

Antioxidant activity

Antioxidant activity was determined according to the methodology described by Aruoma (2003). For extraction, 1.0 g of the sample was placed in Falcon-type centrifuge tubes with a volume of 50.0 mL, adding 20.0 mL of methanol 50%. The samples were left to rest for 30 min at room temperature at 25 °C. Then, they were centrifuged (Centrifuge, TDZ5) at 25000 rpm for 5 min, and after this period, the supernatant was transferred to new tubes. In the first extraction supernatant, 20.0 mL of acetone 70% were added, homogenized and allowed to stand for 30 min at room temperature. Centrifugation was performed under the same conditions as described above, and the supernatant was used for analysis. Aliquots of 0.1 mL of each diluted extracts were placed in a test tube with 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH), leaving to allowed to stand for 30 min. After this period, the reading was performed using a Micronal spectrophotometer, AJX-1600 at 515 nm. For the construction of the calibration curve, the DPPH solution was used. From the calibration curve, the DPPH activity was calculated and the results, mean of three replicates, were expressed in % DPPH.

Total soluble solids, titratable acidity, pH and vitamin C

Total soluble solids (SST) were measured with a digital refractometer (model N-20; Atago, Bellevue, Wash., USA) at 25 °C, with values expressed in °Brix. For pH and titratable acidity (TA) were analyzed using an acid-base potentiometer (877 Titrimo plus, Metrohm ion analysis CH9101,

Herisau, Switzerland), using 0.1 N NaOH solution up to pH 8.1, being the AT expressed in % of citric acid. Finally, ascorbic acid content was determined by employing the Tillmans method (INSTITUTO ADOLFO LUTZ, 2008). Results were expressed as mg ascorbic acid per 100.0 mL of sample. All these analyses also were made in triplicate.

Statistical analysis

A completely randomized experimental design was used in factorial arrangement: two cultivars and four pieces of fruit. Data were analyzed by software SISVAR version 5.4 (FERREIRA, 2011) using analysis of variance (ANOVA) and differences among means were determined for significance at $p < 0.05$ using Tukey's test.

Results and discussion

The results of phenolic compounds, flavonoids and antioxidant activity in aril, membrane, seeds and peels from Valenciana and Wonderful pomegranate cultivars are shown in Table 1. The phenolic content was significantly ($p < 0.05$) affected by pomegranate cultivars and each part of the fruit analyzed. The highest phenolic contents were founded in peel (1196.76 mg GAE 100 g⁻¹) and membrane (1129.14 mg GAE 100 g⁻¹) to the Valenciana cultivar. The Wonderful cultivar had the highest content of phenolics in membrane (1090.10 mg GAE 100 g⁻¹). The Valenciana cultivar showed the highest phenolic content in the peels when compared with Wonderful cultivar and the Wonderful showed the highest level in the aril. The phenolic compounds total values (2919.43 mg GAE 100 g⁻¹ Valenciana and 2568.96 mg GAE 100 g⁻¹ Wonderful) agree with those reported by Nuncio-Jáuregui *et al.* (2014) in different pomegranate cultivars (4210 to 2647 mg GAE 100 g⁻¹).

Table 1. Phenolic compounds (mg GAE 100 g⁻¹), Flavonoids (mg Quercetin 100 mg⁻¹) and Antioxidant activity (%) in aril, membrane, seeds and peels from Valenciana (VAL) and Wonderful (WON) pomegranate cultivars.

	Phenolic (mg GAE 100 g ⁻¹)		Flavonoids (mg Quercetin 100 mg ⁻¹)		Antioxidant activity (%)	
	VAL	WON	VAL	WON	VAL	WON
Aril	326.29 bB	718.67 abA	0.01 cA		0.47 cA	1.35 b
Membrane	1129.14 aA	1090.10 aA	30.06 bA		19.66 bA	84.49 a
Seeds	267.24 bA	381.52 bA	39.99 abB		59.38 aA	0.77 b
Peels	1196.76 aA	378.67 bB	55.22 aA		33.69 bB	88,76 a
CV (%)	27,61		25,12		9,81	

Note: Means followed by the different letter (capital in the line and small in the column) differ by Tukey at 5% probability. Source: Research data.

According to Legua *et al.* (2016) comparing our values of phenolic compounds (90-145 mg GAE 100 mL⁻¹) of nine pomegranate cultivars with those reported in other fruit juices, such as grape, apple and Spanish blueberry juice, with 70.5-117 mg 100 mL⁻¹, 25.4 mg 100 mL⁻¹ and 128 mg mL⁻¹ (DÁVALOS; BARTOLOME; GOMEZ-CORDOVES, 2005; CALÍN-SÁNCHEZ *et al.*, 2013; SHARMA; KORI; PARMAR, 2015), respectively, showed that the content of total phenols in pomegranate juice

has high values because the pomegranate fruit is a rich source of natural phenolic compounds.

Fischer, Carle and Kammerer (2011) identified and quantified of phenolic compounds from pomegranate peel, mesocarp, aril and juices by Folin-Ciocalteu method and found highest values for the mesocarp, followed by pomegranate peels. The authors reported and the importance of selection of raw materials, i.e., co-extraction of arils and peel, markedly affected the profiles and

contents of phenolics in the pomegranate juices, underlining the necessity to optimize these parameters for obtaining products with well-defined functional properties. This study agrees with the results showed by Fisher, Carle and Kammerer (2011).

Regarding to flavonoids content, the Valenciana cultivar showed the highest content (55.22 mg Quercetin 100 mg⁻¹) in the peel followed by in the seed (39.99 mg Quercetin 100 mg⁻¹) and the less content in the aril (0.01 mg Quercetin 100 mg⁻¹). The Wonderful pomegranate cultivar showed the highest content in the seed (59.38 mg Quercetin 100 mg⁻¹) and the less content in the aril with 0.47 mg Quercetin 100 mg⁻¹. When compared the pomegranate cultivars, it was observed the highest content in the seed to the Wonderful and in the peel to the Valenciana.

The activity antioxidant was significantly ($p < 0.05$) affected by part of fruit (Table 1). The pomegranate cultivars not showed difference each other and it was not observed effect of the interaction of cultivars with the parts of fruit. The highest values were observed in the membrane (87.03 % to Valenciana and 81.95 % to Wonderful) and peel (87.55 % to Valenciana and 89.97 % to Wonderful). Not was detected activity antioxidant in the aril and seeds to Valenciana pomegranate cultivar by the DPPH method.

Nuncio-Jáuregui *et al.* (2014) also reported that the antioxidant activity was not affected by pomegranate cultivar. The values reported in this

study were higher than the values showed by Tehranifar *et al.* (2010) (15.59% to 40.72%) for activity antioxidant among different pomegranate cultivars. Kulkarni and Aradhya (2005), showed activity antioxidant values ranged from 70 % to 13 % by the DPPH method in pomegranate arils during fruit development. For all analysis (phenolic compounds, flavonoids and antioxidants) the variation in comparison with the data of the literature may be the result of factors such as the different pomegranate cultivars, climatic conditions, growing region, maturity, cultural practice and extraction method used in the experiments.

The results of total soluble solids, titratable acidity, pH and vitamin C in aril, membrane, seeds and peels from Valenciana and Wonderful pomegranate cultivar are shown in Table 2. Significant differences ($p < 0.05$) were revealed between the pomegranate cultivars for total soluble solids and titratable acidity. The interaction cultivars with parts of fruit were significant. However, for pH and vitamin C content not showed differences. The highest content of solid soluble was showed in membrane (22.13 °Brix) and peels (27.87 °Brix) of Wonderful cultivar. It was not detected solid soluble in the seeds. The titratable acidity values ranged from 0.34 to 5.19 % citric acid and was highest in the peels of Wonderful cultivar. The pH showed values ranged from 3.83 to 5.96 and vitamin C content varied from 7.33 to 8.67 mg acid ascorbic 100 mL⁻¹ and not observed differences among the parts of the fruit (aril, membrane, peels and seeds).

Table 2. Total soluble solids (SST, °Brix), titratable acidity (TA) (% citric acid), pH and vitamin C content (mg 100 mL⁻¹) of aril, membrane, seeds and peels from Valenciana (VAL) and Wonderful (WON) pomegranate cultivars.

	SST ¹		Titratable acidity ²		pH ³		Vitamin C ⁴	
	VAL	WON	VAL	WON	VAL	WON	VAL	WON
Aril	13.53bA	15.28cA	0.65bA	1.22cA	3.93bA	3.96bA	7.00aA	8.67aA
Membrane	12.80bB	22.13bA	1.79aB	3.78bA	3.86bA	3.93bA	7.33aA	7.67aA
Seeds	nd	Nd	0.34bA	0.72cA	5.63aA	5.86aA	7.33aA	8.00aA
Peels	17.93aB	27.87aA	2.44aB	5.19aA	4.13bA	3.83bA	7.67aA	7.67aA
CV (%)	8,91		17,87		4,60		16,63	

Note: Means followed by the different letter (capital in the line and small in the column) differ by Tukey at 5% probability. Source: Research data.

Our results were highest than observed by Tehranifar *et al.* (2010), Fadavi *et al.* (2005) and Poyrazoglu, Gokmen and Artik (2002). Nuncio-Jáuregui *et al.* (2014) describe values varied from 16.53 to 14.53 °Brix for the total solids soluble, from 25.10 to 2.29 g L⁻¹ citric acid for the titratable acidity and pH ranged from 5.88 to 3.55 in juices of different pomegranate cultivars.

Statistically significant differences were observed between pomegranate cultivars investigated in parameters measured phenolic compounds, flavonoids, SST and titratable acidity except antioxidants, pH and vitamin C. Also were observed differences among part of fruit (aril, membrane, seeds and peels) in phenolic

compounds, flavonoids, antioxidants by DPPH method, solids soluble, titratable acidity and pH. These results suggesting the influence of cultivar and part of fruit in the determination the chemical characteristics and antioxidant activity in pomegranate.

Our present study confirms the data reported to Tehranifar *et al.* (2010), Fischer, Carle and Kammerer (2011) and Nuncio-Jáuregui *et al.* (2014) that describe the importance to the cultivar was the main parameter which influences the physico-chemical properties and antioxidant activity in pomegranates. Another important result of this present work is about the selection of raw materials that affect the chemical contents and antioxidants in

the pomegranate, indicate the necessity to optimize these parameters for obtaining products with high and well-defined functional properties.

Conclusion

The different part of pomegranate fruit (aryl, membrane peeling and seed) has influences on the phenolic content, flavonoids, antioxidants, total soluble solids, pH and titratable acidity. The cultivar does not show difference on the antioxidants by DPPH method, pH and vitamin C. This study suggests the importance of the selection the different parts of fruit and cultivar that will be used as raw material in the preparation of pomegranate products with higher antioxidant activities.

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