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The Physico-Chemical Properties of Pomegranate Juice (*Punica granatum* L.) Extracted From Two Egyptian Varieties

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Abstract: The present study was carried out to analyze and compares the physico-chemical properties of fresh juice obtained from two pomegranate cultivars (Wardey and Manfalouty). It also aimed to study the effects of extraction methods on the physico-chemical changes of its juices. The obtained results demonstrated that the two cultivars are different in all measured parameters for physical properties of the pomegranate cultivars. With respect to the effect of extraction methods of tested juices, blender had a higher juice yield (72.60 % and 73.21 %) compared to mechanical press (53.08 % and 45.02 %) in two pomegranate cultivars (Manfalouty & Wardey varieties, respectively). The total soluble solids were 15.41 and 14.46 in blending, while it was 14.08 and 14.00 °Brix in mechanical press (Manfalouty & Wardey varieties, respectively). Little differences were detected in pH and total acidity values of juice obtained from whole fruit compared to juice obtained from seeds. The highest amount of total sugars was observed for Manfalouty (pressing extraction), while Wardey variety had the highest content of ascorbic acid in pressing extraction. The antioxidant activity, tannins, polyphenols and pectin contents were higher in juices extracted from whole pomegranates than in experimental juices obtained from the arils alone.

Key words: Extraction methods · Pomegranate juice · Physico-chemical properties

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important commercial fruit crop that is extensively cultivated in parts of Asia, North Africa, the Mediterranean and the Middle East [1-3]. Pomegranate is one of the oldest known edible fruit and more recently its fruits has been gained much interest in different countries as in Portogal [4], in Egypt, Youssef *et al*. [5] and Hassan *et al*. [6], in Turkey, Kazankaya *et al*. [7], Ercisli *et al*. [8], Ozgen *et al*. [9] and Gundogdu and Yilmaz [10], in Sultanate of Oman Opara *et al*. [11], in Iran, Mousavinejad *et al*. [12] and Muradoglu *et al*. [13], in Italy, Ferrara *et al*. [14], in Spain, Mena *et al*. [15], in Croatia, Cadze *et al*. [16], in Morocco, Martinez *et al*. [17], in USA, Rajasekar *et al*. [18] and in Tunisia, Hasnaoui *et al*. [19] and Zaouay *et al*. [20]. Pomegranate has been used for folk medicine for many centuries with health-beneficial effects of derived products rich in polyphenols and organic acids, high in antioxidant activity and contain considerable amounts, of sugars, polysaccharides and minerals [11,16, 21-24, 25-27]. Pomegranate fruits have been widely consumed fresh or processed into juice, jams, syrup and sauce [15, 16, 28, 29]. 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 [16, 30]. However, Youssef *et al.* [5] reported that the edible portion of pomegranate comprised 40.25-45.98% juice, 16.41-17.41% seed's waste and 43.34% -36.61 of Wardey and Manfalouty, respectively. Cultivar type, environmental and postharvest factors and processing methods have been found among the factors affecting the composition of pomegranate juice [15, 17, 18, 31, 32]. Recently, Faria and Calhau [33] noticed some differences in phenolic composition between commercial juices and experimental ones of pomegranate as the use of the arils alone or the whole fruit to make juice has an enormous impact on polyphenols content and consequently on antioxidant capacity of the juice. In the

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eastern Mediterranean region of Turkey, Alagoz and Safder [34] reported that total phenolics, anthocyanins and antioxidant capacity profiles among pomegranate grown accessions varied depending on aril color and maturity index group. In southern Tunisia, Zaouay *et al.* [20] observed great differences in physico-chemical characteristics, chemical composition and antioxidant activity of the pomegranate cultivars and found significant correlations between total phenolics content and antioxidant activity and between the red color intensity and antioxidant capacity, or total phenolic content and some anthocyanins pigments. The objectives of the present study were to analysis and compare investigating the effect of processing extraction methods on the physico-chemical properties change of pomegranate fresh juices of two Egyptian pomegranate varieties namely: Wardey and Manfalouty.

MATERIALS AND METHODS

Materials: Pomegranate Fruit Samples: Pomegranate (*Punica granatum* L.) fruits namely: Manfalouty and Wardey varieties were obtained from Horticulture Department Farm, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt during seasons of 2009 and 2010.

Chemicals: All reagents, solvents and standards were analytical reagent grade and were purchased from El-Naser Pharm. & Chem. Ind. Co. Cairo, Egypt.

Methods

Physical Properties of Fresh Pomegranate Fruits: Twenty fresh fruits of each cultivar were individually analyzed for physical characteristics. Fresh fruits were weighed on a balance of accuracy of 0.001g. Fruits were weighted. After measurement the whole fruit size, the arils were manually separated from the fruits then total arils and peel per fruit were measured as above.

Extraction of Juice: The fruits were washed with water and wiped completely dry. Fruits from each cultivar were then divided into equal portions for juice extraction. Two methods of extraction were applied for extracting pomegranate juice. The first method consisted of manually peeling the fruits, separating the seeds and extracting the juice by an Electric juice centrifuge (blending of seeds). In the second method, fruits were cut in two halves and the juice was immediately extracted using a hand operated juice extractor/mechanical press. The obtained raw juice from each extraction was filtered through muslin cloth. The juices were immediately stored at 4°C in the dark until analysis.

Chemical Analysis: Expressed juice was used to determine the following chemical properties.

Turbidity: The juice was subjected to turbidity measurement using UV-VIS spectrophotometer with absorbance set at 600 nm [35].

Dry Matter: Dry matter was determined by oven drying at 70°C until a constant weight was obtained [36].

Titratable Acidity, pH, Total Soluble Solids (TSS %) Content and Maturity Index: Titratable acidity (TA) was determined by titration to pH 8.1 with 0.1M NaOH solution and expressed as g of citric acid per 100g of juice [36]. The pH and soluble solids content of the juice were measured immediately after extraction using pH meter and digital refractometer, respectively. The refractometer was calibrated using distilled water and measurement was done with the temperature compensated mode. The soluble solids content was expressed as °Brix. All measurements were made in triplicate and average results reported. Maturity index (TSS/TA): was calculated based on the classification made by Martinez *et al.* [37].

Sweet varieties: MI = 31-98
Sour –sweet varieties: MI =17-24
Sour varieties = MI = 5-7

Total Sugars: The total sugars (g/100g juice) were estimated according to the method described by Ranganna [38].

Ascorbic Acid: Ascorbic acid contents (g/100g juice) of samples were determined according to the titration method using 2, 6-dichlorophenol indophenol as was reported by AOAC [36].

Total Anthocyanins: The total anthocyanins content in the extract from fruits was estimated by Giusti and Wrolstad [39]. The samples were diluted by a potassium chloride buffer until the absorbance of the sample at 510nm wavelength was within the linear range of the spectrophotometer (Cecil 2010 UV-visible). This dilution factor was later used to dilute the sample with the sodium acetate buffer. The wavelength reading was performed after 15 min of incubation, four times per sample, diluted in the two different buffers and at two wavelengths of 510 nm and 700 nm:

The total anthocyanins = {(A x MW x DF x 100)/ MA}

where A = $\{A_{510} - A_{700}\}$ pH 1.0 – $\{A_{510} - A_{700}\}$ pH 4.5; MW: molecular weight (499.2); DF: dilution factor; molar absorptive coefficient of cyaniding -3-glucosid (26.900). Results were expressed as mg cyaniding -3-glucoside 100 g^{-1} of juice.

Total Phenols: The concentration of phenolic compounds was determined as described by Jayaprakasha *et al.* [40] and results were expressed as a tannic acids equivalent. The juices were dissolved in a mixture of methanol and water (6:4 v/v). Sample (0.2 ml) was mixed with 0.1 ml of ten-fold diluted Folin-Ciocalteu reagents and 0.8 ml of 7.5 % sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using spectrophotometer.

Statistical Analysis: Statistical analysis of data was done according to SAS [41] using statistical software. Data were analyzed by Statistical Analysis of Variance (ANOVA) and differences among means were determined for significant at $P<0.05$ using LSD test.

RESULTS AND DISCUSSION

Physical Characteristics of Two Egyptian Pomegranate Cultivars: Physical characteristics of the two pomegranate cultivars (Manfalouty and Wardey) are shown in Table 1. The results for the physical properties of the pomegranate cultivars demonstrated that the two cultivars are significantly different in all measured parameters. Data showed some differences between the pomegranate cultivars for fruit weight, peels (outer peels and central lamella) and internal seeds in flesh seeds, juice as % of pits and juice in whole fruits. Fruit weight value of pomegranate Manfalouty cultivar (280.0±10.12 g) was significantly higher than that of Wardey (277.75±8.03g). However, internal seeds in flesh seeds and peels (outer peels and central lamella) of the total fruit weight in Manfalouty (24.55±0.08 and 30.12±2.25%) were significantly lower than those in Wardey (28.67±0.16 and 51.46±4.23%), respectively. Juice from whole fruits (by Mechanical pressing) of Manfalouty variety (53.01 ±4.10) was significantly higher compared to that of Wardey (45.02±3.28). Meanwhile, Juice as % of pits (Blending seeds) is significantly lower for Manfalouty (72.60±0.92%) than that for Wardey (73.21±0.58%). Data indicated that there was a significant difference ($p\leq 0.05$) between the blender and mechanical press methods of extraction. These data indicated that extraction by blender cultivar Wardey gave higher juice yield, while using mechanical press cultivar Wardey showed lower yield. The obtained results are in the same line with those of Zarei *et al*. [42], who reported that yield of obtained juice using an electric extractor for cultivars grown in Iran varied between 48.02% and 63.52% FW. In this concern, Martinez *et al*. [37] reported juice percentage between 17.63% and 50.01% FW. These results are also consistent with those previously reported by Al-Said *et al.* [43] on pomegranate cultivars grown in Oman. In this respect, Zaouay *et al.* [20] declared that variation of fruit weight depended on the cultivar and ecological condition and one of the most important factors from an industrial point of view is the juice content of the aril. Blender had a higher juice yield compared to mechanical press [18]. Both of the cultivars would be useful especially in developing cultivars with the high agronomic potential. The Manfalouty cultivar with higher percentage of aril and juice, being a highly desirable property in the food processing and beverage industry, could be more promising than Wardey variety.

Effect of Extraction Methods of Pomegranate Juice Parameters: Results in Table 2 present the effect of extraction methods on the physico-chemical parameters of pomegranate juice. There was significant differences were detected in total soluble solids (TSS), pH, titratable acidity (TA) and maturity index values. TSS levels ranged from 14 to 15.41°Brix. The content of TSS in pomegranate juice for cultivar Manfalouty by blending was significantly higher than TSS in those for Manfalouty by pressing and for cultivar Wardey by both mechanical press and blending. The obtained results are similar to those reported by Martinez *et al.* [37] for five Spinach cultivars and Tehranifar *et al*. [44] for cultivars grown in Iran. As shown in Table 2, significantly higher contents of titrable acidity (1.3343 and 1.5237 mg/100 g) were observed in Manfalouty than those in Wardey (0.6422 and 0.7425 mg/100g) by pressing and blending methods, respectively. Cultivar Manfalouty had low pH value of the cultivar in blender (3.15) and mechanical press (3.14) extractions. The highest pH value reached a maximum of 4.28 for cultivar Wardey, in blender extracted juice, followed by that for Wardey by pressing (4.25). The highest significant maturity index value was found for Wardey juice by pressing, following it that for Wardey by blending and then those of Manfalouty by either blending or pressing that were insignificantly different.

Table 1: Physical characteristics of two pomegranate cultivars grown in Egypt (on fresh basis)

Parameters	Cultivars Wardey	 Manfalouty	LSD 0.05
Fruit average weight (g)	277 [b] ±8.03	280 [a] ±10.12	0.04
Peels (outer peels and central lamella) (%)	51.46[a] ±4.23	30.12 [b]±2.25	0.01
Internal seeds in flesh seeds (%)	28.67[a] ±0.16	24.55[b]±0.08	0.03
Juice as % of pits (Blending seeds)	73.21[a] ±0.58	72.60[b]±0.92	0.04
Juice in whole fruits % (Mechanical pressing)	45.02[b] ±3.28	53.01[a]±4.10	0.02

Means of 20 fruits in each row followed by different letters are significantly different ($p<0.05$) ±standard Deviation.

Table 2: Effect of extraction methods on some pomegranate juice parameters of two pomegranate cultivars grown in Egypt

Extraction Methods	TSS(°Brix)	pH	TA(% citric acid)	Maturity index(MI)
Pressing (Whole fruits)				
Wardey	14.00 [b]	5.25[b]	0.6422[d]	21.55[a]
Manfalouty	14.08 [b]	3.14[c]	1.3343[b]	10.70[c]
Blending (Seeds)				
Wardey	14.46 [b]	4.28[a]	0.7425[c]	19.66[b]
Manfalouty	15.41[a]	3.15[c]	1.5237[a]	10.11[c]
LSD 0.05	0.05	0.04	0.03	0.05

Means of 20 fruits in each column followed by different letters are significantly different ($p<0.05$) ±standard Deviation.

Table 3: Effect of extraction methods on reducing, non-reducing and total sugars percentage in juice of two pomegranate varieties (mg/100 g)

Extraction Methods	Total sugars %	Reducing sugars %	Non-reducing sugars %
Pressing (Whole fruits)			
Wardey	13.00[c]	12.65[c]	0.35[c]
Manfalouty	14.02[b]	14.01[b]	0.51[a]
Blending (Seeds)			
Wardey	13.06[c]	12.71[c]	0.33[c]
Manfalouty	14.65[a]	15.13[a]	0.49[b]
LSD 0.05	0. 04	0.03	0.03

Means of 20 fruits in each column followed by different letters are significantly different ($p<0.05$) ±standard Deviation.

Table 4: Effect of extraction methods on some compounds of pomegranate of pomegranate juice

Extraction methods	Tannins (%)	Pectin (%) (as calcium pectate)	Total phenol (mg/g)	Total anthocyanins (mg/100 ml)	Ascorbic acid (g/100 g)
Pressing (Whole fruits)					
Wardey	0.206[b]	0.30[b]	5.543[b]	33.34[a]	5.50[b]
Manfalouty	0.216[a]	0.40[a]	5.761[a]	26.06[c]	4.28[c]
Blending (Seeds)					
Wardey	0.120 [d]	0.25[c]	4.892[d]	33.01[b]	6.80[a]
Manfalouty	0.132[c]	0.30[b]	5.138[c]	25.85[d]	5.26[b]
LSD 0.05	0.01	0.03	0.02	0.02	0.04

Means of 20 fruits in each column followed by different letters are significantly different ($p<0.05$) ±standard Deviation

The differences were detected in pH and total acidity values might be due to the phenolic pattern of the juice obtained from rind compared to those present in the juice obtained from grains [28].

Effect of Extraction Methods of Pomegranate Juice on Reducing, Non Reducing and Total Sugars: Data in Table 3 demonstrates the effect of extraction methods on reducing, non-reducing and total sugars % of pomegranate juice. Total sugars contents of the two studied cultivars ranged from 13.00-14.65 %. The highest significant contents of total and reducing sugars was observed for Manfalouty in blending extraction juice, while the lowest amount of total sugars was for Wardey (pressing extraction). Data also shows that Manfalouty had the highest values of reducing and non-reducing sugars. On the other hand, non-reducing sugars in the juice extracted by the two used methods of extraction ranged between 0.33-0.51percent. The lowest significant non-reducing sugar percentages were found in Wardey variety juices that extracted by pressing and blending that were similar. However, the highest significant non-reducing sugar percentage in Manfalouty was noticed for juice extracted by pressing, followed by that of the same cultivar juice by blending. The total sugars contents were slightly lower than observed values by Poyrazoglu *et al.* [22], being 13.9-16.06 g/100g. In this respect, Aviram *et al*. [45] found that the fresh pomegranate Wonderful cultivar juice contained 10% total sugars.

Effect of Extraction Methods of Pomegranate Juice on Some Compounds: The effect of two different pomegranate juices (from Wardey and Manfalouty varieties) obtained by the two extraction methods on some compounds were shown in Table 4. Data showed that, there were significant differences between the percentages of tannins, pectin, total phenol, anthocyanin and ascorbic acid contents in the obtained juices extracted by two extraction methods. The juice obtained from the whole fruit had higher content of tannins (0.206-0.216 %) compared to those obtained from the seeds (0.120-0.132 %) in the Wardey and Manfalouty varieties, respectively. These results are in the same line with those obtained by Gil *et al*. [28], who reported that pomegranate juice content of tannins depending on the extraction pressure and that juice obtained by blending the seeds contained less tannins than that obtained from whole fruits. Results also revealed significant differences in pectin content of the juices obtained by the two tested extraction methods. Pectin content of juice extracted from whole fruits varied from 0.3% to 0.4%, while the extracted juice from seeds fluctuated between 0.25% and 0.30% in the Wardey and Manfalouty varieties, respectively. Such variations might be due to the effect to extraction method from the whole fruits. The pressure used might extract pectin from the rind, which passes onto the juice. In this respect, Rajasekar *et al.* [18] indicated that chemical analysis of juice showed significant differences among cultivars and extraction methods.

The total phenolics content was in the range of 4.892-5.7612 mg/g. In mechanical press extracted juice, higher total content was found in Wardey and Manfalouty cultivars (5.543 & 5.760mg/g), respectively. However, in blending extracted juice, Cultivar Wardey had the lower total phenol content (4.892 mg/g) than Manfalouty (5.1383 mg/g). The highest total phenols were found in Manfalouty cultivar juice extracted by pressing. These results indicated that the composition of pomegranate juice and its content of bioactive compounds, depends on factors such variety and maturity index as mentioned by Miguel *et al.* [4]. On the other hand, results in the same table showed some differences in the total phenols content of the juices by two tested varieties. The total phenols content of juice obtained from the seeds alone was higher than that obtained from whole fruit. In this concern, Faria and Calhau [33] reported that there are some differences in phenolic composition between commercial juices and experimental ones. With respect to anthocyanins, data in Table 4 revealed that anthocyanins contents of Wardey cultivar were significantly higher (33.01–33.34 mg/100 ml) than those of Manfalouty cultivar (25.85 – 26.01 mg/100ml) in both blender and mechanical press extracted juice, respectively. These results indicate that juice obtained from Wardey cultivar had higher anthocyanins compared to those from Manfalouty variety, regardless the method of extraction. Similar results have been reported with Gil *et al*. [28]. From the same table, the data showed that the fresh juice of Wardey had the higher content of ascorbic acid (5.50 and 6.80mg/100g), than variety Manfalouty that recorded the lower values (4.28 and 5.26 mg/100g) by pressing and blending methods, respectively. Ascorbic acid contents were higher in juices extracted by blending than that by pressing for the two cultivars. The concentration of extracted ascorbic acid was found to be more by mixer extraction method in both Manfalouty and/or Wardey varieties as reported by Youssef *et al.* [5].

CONCLUSIONS

The obtained results would be a guide in the selection of potential cultivars used in food industry and fresh market. The composition information of fruits (e.g. vitamin C, acidity, antioxidant etc.), highlighting that the pomegranate fruit could be a good source of nutrient. It also provides important data for extraction method affect physic-chemical properties of the juice.

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