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Original Research Article

Fiber content and quality of pomegranate (*Punica granatum* L.) Cultivated in a Coastal Oasis

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A B S T R A C T

An organoleptic characterization of the edible portion of the seeds was investigated. Pomegranate juice could be considered a good source of DF with an acceptable SDF/IDF ratio. The total soluble solids vary from 12.2% to 17.7%. The mean titrable acidity is 0.375%. The mean values of the minerals content are about 10.44mg/100 ml for phosphorus, 218.2mg/100 g for potassium and 6.73mg/100g for sodium. Total phenolics content and antioxidant ranged from 7.13 to 12.90 (mg GAE /ml) and from 14.12 to 25.31 (µl/ml) respectively. Statistical analysis reveals four groups.

Introduction

The pomegranate is one of the oldest edible fruits (Evreinoff, 1949). It is considered native of Persia and surrounding areas. During the last decade, the cultivation of pomegranate has known a great extension. World production increased from about 1.5 million t/year in 2005 (FAOSTAT, 2005) to 2,450,000t/year in 2010. By producing around 1.200.000 tons annually, India is the largest producer of this edible fruit in the world (Anonymous 1).

In Tunisia, the pomegranate is a widespread species. Indeed, it is one of the most important pomegranate producers and exporters in the world. The area reserved for this species increased from 5.650 ha in 1980 to 13.000 ha in 2008 (Anonymous 2). The Governorate of Gabes occupies the first place in terms of area and production with 2.600 ha and 24.000 tons per year respectively (GIAF and DGPA, 2008). The national production of pomegranate could record a slight progression from 62.000 tons in 2002 to 75.000 tons in 2009. The variety Gabsi, one of the well-known pomegranate cultivars in Tunisia with very appreciable sensory quality, and therefore with high value, representing approximately 35% of this tonnage (Emna,

2010). In 2010, the national production has reached to 80.000 tons surrounding the production of Spain (80.000 t / year) and Turkey (85.000t / year) (GIF 2010). However, until this time, exports remain relatively low and rarely exceed 1% of the total production.

The edible part contains juice, pulp and seeds and represents about 65 to 75% of the total weight of the fruit (Tehranifar *et al*., 2010). This portion is sweet, sweet–sour or sour, its color is white, pink or red and it is rich in sugars, minerals and phenolic compounds (Viuda-Martos *et al*., 2010). The pomegranate is also a good source of dietary fiber. The acceptability of pomegranate to the consumer and processor depends on a combination of several quality attributes that are related to its chemical composition. Fruit quality depends largely on sugar and acid content of the juice.

Thus, the flavor of the pomegranate, sweet and slightly acidulated, is the result of a harmonious balance of these two constituents. In the world, the production and the consumption of pomegranate has been increased because it is used in various fields. Indeed, besides its use fresh, it is used for making refreshing drinks, aromas, jam and other preparations (cakes, wines, etc) (Aviram and Dornfeld, 2001). Recently, pomegranate juice was demonstrated to be high in antioxidant activity (Gil *et al*., 2000).

So, to meet the requirements of the sector, it has become increasingly important to characterize the different clones to obtain the most appropriate one for commercial use. Therefore, the aim of this work is to evaluate the quality of 21 pomegranate accessions cv. Gabsi cultivated in a coastal oasis in the South-East of Tunisia.

Materials and Methods

Chemicals

4-Morpholinoethanesulfonic acid (MES), tris (hydroxymethyl) aminomethane, Amylase thermostable Thermamyl 120 L, protease from Bacillus licheniformis, amyloglucosidase solution from Aspergillus niger, Folin−Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH• free radical) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material

21 pomegranate accessions cv. Gabsi were collected from mature trees in October 2010 in the region of Gabes in the South-East of Tunisia, which is characterized by an arid bioclimate of Mediterranean type with a mild winter. Fruits were transferred to a +4°C store room on the same day as they were harvested. Table 1 presents more information about geographical origin of the accessions.

Titrable acidity, pH and total soluble solids

The titrable acidity (A) was determined by titration to pH 8.1 with 0.1M NaOH solution and expressed as g of citric acid per 100g of juice (AOAC, 1984). The pH measurements were performed using a digital pH meter InoLab (WTW, Weilheim, Germany) at 21°C. The total soluble solids (TSS) were determined with a digital refractrometer (Model 10430; Cambridge Instruments Inc., Buffalo, NY, USA).

Mineral analysis

For the determination of mineral content (sodium, potassium, and phosphorus),

sample was totally dried at 100 ± 5°C, than 1g of each dry sample was incinerated during 4h at 550°C. Ashes were mixed with 4ml of distilled water and 1ml of concentrated HCl. The solution was heated until boiling, then filtered and adjusted to 100ml with distilled water. This solution will be used for mineral analysis such as potassium, sodium and phosphorus.

The sodium and potassium contents were determined with a Sherwood 410 flame photometer regulated on the filter of sodium or potassium. The contents of sodium (%Na^+) or of potassium (%K+) in the dry matter plant were calculated as: % Na+ or % K^+ = (C * DF) / (100 * m), where C is the concentration of sodium or potassium (mg/l), DF is the factor of dilution and m is the mass of the extract (g).

The phosphorus concentration was determined using a Secomam spectrophotometer. Extracts were diluted if needed. The absorbances were measured at 430nm. P concentrations were calculated with the formula: %P = (C x DF)/ (100 x m), where C: P content (mg/l), DF Dilution factor, m extract mass (g).

Total phenolics

The total phenolics (TP) were determined by using Folin–Ciocalteu method (Jayaprakasha *et al*., 2001). 300µl of diluted pomegranate juice was mixed with 0.5 ml of 10-fold-diluted Folin–Ciocalteu reagent and after 3min, 4ml of 7.5% sodium carbonate was added. The mixture was allowed to stand for 90 min at room temperature before the absorbance was measured at 765nm using spectrophotometer. The results were expressed as mg gallic acid equivalent in ml of fruit juice (mg GAE/ml of juice).

DPPH radical scavenging activity

Antioxidant activity was assessed according to the method of Okonogi *et al*. (2007). Briefly, pomegranate juice (PJ) was diluted in methanol to prepare a methanolic test solution of different concentrations prepared from a stock solution of each PJ. DPPH (100µM) was dissolved in methanol and mixed with a 100µl of the methanolic test solution. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The amount of DPPH was determined spectrophotometrically at 517 nm. All measurement was performed in triplicate. The radical-scavenging activity was calculated as % inhibition from the following equation:

$$DPPH_{radical\text{-}scavenging}\ (\%) = (DO\ control - DO\ sample/DO\ control) * 1000$$

Analysis of Dietary Fiber

Samples preparation

Fresh pomegranate fruits were peeled manually. The edible portion was weighed and homogenized thoroughly in a mixer then it was quantitatively transferred into 500 ml round bottom flasks (glass) and lyophilized. Freeze-dried were then powdered using mechanical grinder to get a fine powder.

Determination of TDF, IDF and SDF

The amount of DF was determined using the protocol described by Lee *et al*. (1992) with slight modifications, using a TDF assay kit (Bioquant TDF-100 kit, Merck, Germany). As samples contained a high level of sugar, they were previously

extracted with 80% ethanol to remove most of the sugars. Triplicate dry and desugared samples (1 g each) were suspended in 40mL of MES-Tris buffer and treated with 50µL of Thermamyl (heat-stable α-amylase) at 100°C for 30min and then digested with 100µL of a 50mg/mL protease solution (60°C, 60 min), followed by incubation with 100µL of amyloglucosidase (60°C, 1h) to remove protein and starch. The enzyme digestates were filtered through tared fritted glass crucibles (no. 2) using the Fibertec E system consisting of the 1023 filtration module. The crucibles containing insoluble dietary fiber (IDF) were rinsed with dilute alcohol and acetone, and dried overnight in a 105°C oven. The filtrates and washings were mixed with 4 volumes of 95% ethanol to precipitate materials that were soluble in the digestates.

After 1h, the precipitates were filtered through tared fritted glass crucibles. One of each set of duplicate insoluble fiber residue samples and soluble fiber residues was washed in a muffle furnace at 525°C for 5 h, and then weighed. The second set of residues was used for protein determination (as Kjeldahl nitrogen 6.25). The dietary fiber values in the sample and blank fractions were calculated as the soluble dietary fiber (SDF) or IDF residues (% original sample weight) minus the % ash and the % crude protein measured in the residues. The TDF was calculated as the sum of SDF and IDF.

Statistical analysis

Three measurements were taken on each analysis, and the results were expressed as the mean of those values ± standard error. Analysis of variance procedure (ANOVA) was performed. Results were significant when $p < 0.05$. A hierarchical classification between cultivars was performed by SPSS 18.0.

Results and Discussion

Titrable acidity, pH, total soluble solids, volume of juice and maturity index

The results for pH, volume of juice (VJ), total soluble solids (TSS), Acidity (A) and ripeness index (RI) of the pomegranate from the different accessions are given in Table 2. Variation of the physico-chemical characters were significant ($P < 0.001$). As shown in table 2, GK2 gives the less juicy fruits (64.67%), whereas GC2 gives the juiciest one (85.50%). The mean titrable acidity and the pH were respectively 3.65 ± 0.5% and 0.37 ± 0.21. The lowest pH and the highest acidity were obtained for GG3 (3.03 and 1.14% respectively). GM1 have the highest pH and the lowest acidity, which are respectively 4.60 and 0.16%. The TSS varies between 12.2% for GO2 to 17.7% for GC5.

Total phenolics and antioxidant activity

The total phenolics and antioxidant activity analysis results for the pomegranate accessions investigated are presented in Table 2. A significant variation in total phenolics concentration was found among the twenty one accessions of pomegranate studied ($P < 0.001$) and the values ranged from 7.13 to 12.90 (mg GAE /ml). The highest level of total phenolics was observed in GC3 and the lowest one in GO2 (Table 2).

The differences in antioxidant activity among the pomegranate cultivars were statistically significant ($P < 0.001$) and the values ranged from 14.12 to 25.31 µl/ml. The highest and the lowest antioxidant activity were detected in GO2 and GC4, respectively (Table 2).

Mineral characterization

The mineral analysis of the different studied accessions revealed significant variations in the studied nutrients between the accessions ($p<0.001$). The most abundant nutrient in all juice samples was the potassium, its content ranged from 149.33 mg/100ml for GO3 to 291.77 mg/100ml for GC4. The phosphorus and the sodium contents varied respectively from 6.23mg/100ml for GM3 to 14.73mg/100ml for GO2 and from 5.22 mg/100g for GME3 to 10.18 mg/100ml for CE3 (Figure 1).

Dietary fiber content

Table 3 gives the TDF, IDF, SDF, SDF as % of TDF and SDF/IDF ratio. The results of analysis of variance showed a highly significant effect recorded for all variables studied ($P < 0.001$). The TDF ranged from 0.57 ± 0.02 g/100g (GME1) to 1.15 ± 0.10 g/100g in GC1, with an average of 0.82 ± 0.15 g/100g. The insoluble DF was the major fraction in pomegranate DF concentrates with an average of 0.64±0.16 g/100g. GC1 has the highest value (1.00 g/100g), the lowest value was registered in GME1 (0.44 g/100g). The SDF was low in GC3 (0.12 g/100g) and high in GC5 (0.34 g/100g). The SDF as % TDF ranged between 11.47% in GC3and 35.69 % in GG3, but most of the pomegranate accessions had more than 20% of their TDF as SDF.

Variability according to chemical characterization, total phenolics, antioxidant activity and dietary fiber contents.

Grouping of accessions based on the combination of chemical characters, total phenolic and dietary fiber contents and antioxidant activity divided them into four main clusters (Figure 2). The first cluster was consisted of six individuals which are characterized by the lowest volume of juice and mineral content. The second group holds five accessions with the highest TSS and mineral content. GO2, GG3, GC2 and GC5 constitute the third group. These accessions have the highest volume of juice, the highest acidity and SDF/IDF ratio. The fourth group comprises six accessions. This group was characterized by the highest phenolic content and antioxidant activity.

The studied chemical characteristics showed considerable variations between accessions for all of characters. The results of analysis of variance showed a highly significant effect recorded for all variables. Most accessions had an average TP higher than 11.47 mg/ml. The reported levels of this total phenolics in literature were 12.45 and 207.6mg/ml by Ozgen *et al*. (2008); 20.83mg/ml by Cam *et al*. (2009); 14.4mg/ml and 10.08 mg/ml by Tezcan *et al*. (2009). Their results were in agreement with our results. The total phenolics content of pomegranate juices were greater than other juices such as turnip, sour cherry and red grape juice (Cam *et al*., 2009). Antioxidant activity has been reported for seven commercial pomegranate juices from Turkey 10.37–67.46% (Tezcan *et al*., 2009) and eight pomegranate juices from Iran 18.6–42.8% (Mousavinejad *et al*., 2009). In this study the values are ranged between 14.12 and 25.31 µl/ml. The variation in comparison with the data of the present research may be the result of other factors such as the

Table.1 Accessions of *Punica granatum* l. (cv. Gabsi), their codes and their places of origin

Accessions	Codes	Origin	Latitude (N)	Longitude (E)	Altitude (m)
GME1, GME3, GME5	1, 2, 3	Metouia	33°57’	10°00’	22
GO1, GO2, GO3	4, 5, 6	Ouedhref	33°59’	9°58’	26
GG1, GG4, GG5	7, 8, 9	Gabès ville	33°52’	10°04’	12
GC1, GC2, GC3, GC5, GC6	10, 11, 12, 13, 14	Chenini	33°52’	10°04’	24
GM1, GM2, GM3, GM4	15, 16, 17, 18	Mareth	33°37’	10°17’	48
GK1, GK2, GK4	19, 20, 21	Kettana	33°45’	10°11’	20

Table.2 Mean and standard error of measured chemical traits in studied pomegranate accessions

Accessions	TP (GAE mg/ml juice)	DPPH (IC_{50} ml/ml)	VJ (%)	TSS (%)	PH	A (%)	RI
GME1	9.12±0.20	22.14±0.14	71.00±2.65	14.60±0.53	3.73±0.19	0,20±0.009	71.59 ±1.30
GME3	8.14±0.15	21.52±0.031	76.13±1.06	15.00±0.60	3.51±0.11	0.35±0.005	42.73±1.700
GME5	10.19±0.21	19.54±0.10	77.00±2.00	13.80±1.97	3.45±0.65	0.43±0.045	32.36±6.27
GO1	7.57±0.20	23.13±0.14	77.47±4.39	15.20±2.09	3.46±0.43	0.42±0.013	36.03±5.88
GO2	7.13±0.12	25.31±0.04	80.27±1.10	12.20±0.20	3.34±0.08	0.42±0.006	28.77±0.44
GO3	7.80±0.23	23.44±0.06	76.50±3.12	17.47±1.13	3.45±0.35	0.20±0.007	32.02±1.15
GG1	11.47±0.22	16.36±0.06	74.93±1.11	14.20±0.44	3.23±0.15	0.49±0.009	28.62±0.44
GG4	10.89±0.14	19.37±0.05	82.77±4.13	14.83±0.11	3.23±0.25	0.51±0.026	28.93±1.29
GG5	11.33±0.11	16.82±0.04	80.00±1.73	13.77±1.36	3.03±0.16	1.14±0.060	12.07±0.86
GC1	12.51±0.11	15.21±0.05	82.73±0.64	14.93±0.38	3.27±0.25	0.44±0.062	34.34±4.46
GC2	11.93±0.10	15.19±0.03	85.50±1.80	13.80±0.36	4.34±0.11	0.20±0.007	89.53±3.90
GC3	12.90±0.08	14.33±0.05	83.13±1.03	16.10±0.90	4.44±0.21	0.19±0.004	84.76±5.40
GC5	12.82±0.03	14.12±0.08	85.33±0.58	16.80±0.69	4.22±0.29	0.20±0.002	84.40±2.40
GC6	12.54±0.05	14.55±0.06	77.03±1.00	17.70±0.95	4.49±0.09	0.18±0.002	97.27±5.51

GM1	9.42±0.23	21.23±0.03	70.00±1.00	16.57±1.36	4.60±0.14	0.17±0.013	99.38±7.53
GM2	8.96±0.26	22.38±0.02	71.03±0.96	15.27±0.81	3.63±0.19	0.22±0.007	69.20±5.56
GM3	10.63±0.10	19.95±0.04	65.00±2.00	13.73±0.98	3.56±0.14	0.21±0.038	66.11±16.15
GM4	10.42±0.09	20.52±0.03	80.33±0.42	13.60±0.87	3.26±0.10	0.45±0.008	30.47±1.41
GK1	12.09±0.11	15.35±0.05	76.43±1.24	14.03±0.15	3.41±0.27	0.42±0.006	33.25±0.76
GK2	11.33±0.05	17.35±0.08	64.67±1.53	14.53±0.51	3.47±0.16	0.42±0.002	34.42±1.07
GK4	11.51±0.12	16.52±0.09	77.80±2.99	13.97±0.38	3.53±0.10	0.35±0.006	40.51±1.71
Mean ± SE (n = 21)	10.51±1.79	18.78±3.41	76.91±6.06	14.86±1.58	3.65±0.51	0.37±0.21	51.28±26.91

Values are mean ± Standard error.

Table.3 Total, insoluble and soluble dietary fiber contents of 21 pomegranate accessions (Mean ± SE)

Accessions	**TDF**	**IDF**	**SDF**	**SDF as % of TDF**	**SDF/IDF**
GME1	0.57±0.02	0.44±0.02	0.13±0.01	23.39±0.51	0.31±0.01
GME3	0.63±0.04	0.48±0.01	0.15±0.05	23.20±5.41	0.31±0.12
GME5	0.59±0.02	0.45±0.02	0.14±0.04	23.41±6.45	0.31±0.10
GO1	0.65±0.01	0.51±0.02	0.14±0.03	21.92±3.66	0.28±0.06
GO2	0.72±0.03	0.48±0.02	0.24±0.05	33.18±4.57	0.50±0.17
GO3	0.68±0.02	0.53±0.03	0.15±0.01	22.20±1.98	0.28±0.04
GG1	0.83±0.03	0.68±0.03	0.15±0.00	18.16±0.56	0.22±0.01
GG4	0.77±0.02	0.62±0.03	0.14±0.04	18.63±4.93	0.23±0.08
GG5	0.90±0.03	0.58±0.01	0.32±0.02	35.69±0.87	0.55±0.04
GC1	1.15±0.10	1.00±0.09	0.15±0.06	12.73±4.20	0.15±0.06
GC2	0.92±0.03	0.60±0.03	0.32±0.00	34.66±1.32	0.53±0.05
GC3	1.04±0.06	0.92±0.04	0.12±0.02	11.47±1.12	0.13±0.02
GC5	0.85±0.02	0.72±0.03	0.13±0.01	34.93±3.79	0.18±0.02
GC6	0.97±0.03	0.63±0.05	0.34±0.03	14.91±0.99	0.54±0.17
GM1	0.77±0.01	0.59±0.00	0.18±0.01	23.37±1.00	0.31±0.02
GM2	0.87±0.04	0.74±0.02	0.13±0.02	14.50±1.99	0.17±0.03
GM3	0.84±0.01	0.69±0.03	0.16±0.02	18.59±2.56	0.23±0.04
GM4	0.79±0.02	0.65±0.02	0.14±0.02	17.36±2.53	0.21±0.04
GK1	0.89±0.01	0.72±0.03	0.16±0.02	24.43±0.29	0.23±0.03
GK2	0.87±0.03	0.66±0.02	0.21±0.01	16.25±0.44	0.32±0.01
GK4	0.92±0.03	0.77±0.03	0.15±0.00	18.43±1.90	0.19±0.01
Mean ± SE (n = 21)	0.82±0.15	0.64±0.16	0.18±0.12	21.97±12.73	0.29±0.30

Values are mean ± Standard error.

different pomegranate cultivars and sample extraction method used in the experiments. According to the results (Table 2), GC3 and GC4 accessions had the highest and lowest levels of total phenolics and antioxidant activity, respectively. Thus it can be concluded that there was a close relationship between the total phenolics and antioxidant activity.

High juice content is a desirable attribute in pomegranate production and other fruit and it is the most important parameter from an industrial point of view (Cassano *et al*., 2004). In the studied accessions, this varies from the 64.67% to 85.50% which corroborate with those noted by Agrawal and Chandra (1991) who indicates that the percentage of juice in the cultivar Muscat varied between 60 and 84%. This result is better than obtained by other authors who found percentages ranging from 44.96% to 68.55% in Indian and Spanish varieties (Viswanath *et al*., 1999; Martinez *et al*., 2006). The mean titrable acidity is 0.375±0.211 %. If this result is used to compare a variety well known throughout the world (e.g. Wonderful), this cultivar, with an acidity content of around 1.8% (Chace *et al*., 1981), would be considered bitter-sweet or bitter if judged on the same scale. Pomegranate juice is rich in potassium compared with other nutrients. Its mean value was about 218.2 mg/100g which is higher than fresh orange (206.7 mg/100 ml) and grapefruit (166.7 mg/100 ml) (Dominé, 2001).

Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in vegetal products. They have beneficial physiologic effects in humans (Slavin, 2005). The average total dietary fiber content is about 0.82 g/100g. The DF contents of a number of fresh fruits, such as apple, banana, cherry, mango, muskmelon, and peach, were reported by Punna and Paruchuri (2003). The values obtained ranged from 0.8 g/100 g for muskmelon to 2 g/100 g for mango, are similar to those observed for pomegranate. However, the DF contents of other dried fruits reported by USDA (2007) are about 3.7% for raisins, 7.1% for plums and 9.8% for figs. Thus, pomegranate juice could be considered a good source of DF compared with most fresh fruits. The TDF values obtained in the present study were lower than those reported by Ramulu and Rao (2003). This variation could be due to the analytical method used to quantify the fiber content because some authors do not desugar the samples before analysis, so the total DF content would have been overestimated (Al-farsi *et al*., 2007).

The insoluble DF was the major fraction in pomegranate DF concentrates with an average of 0.64g/100g. This fraction is important to intestinal regulation, whereas the soluble fraction is involved in reduction of both blood cholesterol and intestinal glucose absorption (Periago *et al*., 1993). Indeed, fruits fiber can be considered as potential ingredients of food, especially of meat products because of their ability to reduce residual nitrite levels, thus avoiding the possible formation of nitrosamines and nitrosamides (Viuda-Martos *et al*., 2009) and it has been used as fat replace, fat reducing agent during frying, volume enhancer, binder, bulking agent and stabilizer (Aleson-Carbonell *et al*., 2005). Soluble/insoluble fiber ratios are important from both dietary and functional perspectives. It is generally accepted that those fiber sources suitable to be used as a food ingredient should have an SDF/IDF ratio close to 1/2. Dietary fibers from cereals are more frequently used than those from fruits, even though, fruit fibers,

in general, have better nutritional qualities because of their higher levels of associated bioactive compounds and more balanced composition (higher overall fiber content and greater IDF/SDF ratio) (Figuerola *et al*., 2005)

In conclusion, outline of valorization can be advanced. Among the twenty one accessions studied, GME1, GME2, GME3, GO1 and GO2 which showed the highest total soluble solid and mineral contents and the lowest acidity are the most suitable accessions for fresh consumption and health benefits. GO2, GG3, GC2 and GC5 provided the more suitable accessions for food supplementation. They have the highest SDF/IDF ratio and volume of juice with a high content of total phenolics and antioxidant activity. They can made a commercial juice with high level of bioactive substances. In addition, the results provide important information of the physico-chemical properties which can be useful for developing fruit processing industry and selection of superior desirable pomegranate genotypes for bringing to commercial cultivation to meet market demand.

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