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Physical and Chemical Properties of Pomegranate (*Punica granatum* L.) Fruit in Maturation Stage

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Abstract: Twelve pomegranate (*Punica granatum* L.) cultivars obtained from different growing regions of Iran were analyzed for their physical and chemical properties. These properties included fruit fresh weight, volume and density, peel thickness, soluble solids (TSS), titratable acidity (TA), EC, pH, vitamin C, ellagic acid content of juice and peel, total antioxidant activity of peel and juice and etc. Fruit weight ranged from 103.38 to 505.00 g and fruit volume from 99.41 to 547.88 cm^3. Similarly, average fruit density ranged from 0.91 $g.cm^{-3}$ to 1.04 $g.cm^{-3}$ and peel thickness of the fruit was recorded from 1.60 to 6.01 mm. Reducing sugars ranged between 13.89 to 29.83 g/100 ml, total soluble solids ranged from 15.17 to 22.03 (°Brix) and titratable acidity ranged between 0.35% and 3.36% in pomegranate juices. pH and vitamin C content also ranged between 2.75-4.14 and 9.68-17.45 mg/100 ml, respectively. According to the results of HPLC, ellagic acid content of juice and peel ranged between 1-2.38 mg/100 ml and 10-50.00 mg/100 g, respectively. Total antioxidant activity measured by FRAP assay with a range of 225.17-705.50 (mmol/100 g) and 157.33-419.33 (mmol/100 ml) in peel and juice, respectively.

Key words: Pomegranate · Physical properties · Antioxidant activity · Juice · Peel

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Punicacea family [1]. It is one of the important and commercial horticultural fruits which is generally very well adapted to the Mediterranean climate [2]. It has been cultivated extensively in Iran, India and some parts in the U.S.A (California), China, Japan and Russia. Pomegranate fruits are consumed fresh or processed as juice, jellies and syrup for industrial production [3-5]. Different parts of its tree (leaves, fruits and bark skin) have been used traditionally for their medicinal properties and for other purposes such as in tanning [6]. It is proved to have high antioxidant activity [7] and good potency for cancer prevention [8]. The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals [9,10]. Studies to determine weather great variability exists in the antioxidant activity and other physical and chemical properties among different pomegranate cultivars allow breeders to select and breed genotypes with higher levels of compounds. It also provides a way of increasing the dietary intake of antioxidant compounds. Consumers can be addressed toward healthier pomegranate products based on possible relationships between fruit properties, such as fruit fresh weight, skin thickness etc, with juice chemical characteristics, such as the antioxidant activity, vitamin C content, sugars and etc.

Physical and chemical properties of pomegranate have been evaluated in Turkey [11], Italy [12] etc. Historical evidence reveals that its primary origin is Iran where is grown in every area, both coastal and mountainous areas and that from there it has been spread to other areas [13]. The total pomegranate production in Iran was 665,000 tons in 2003 [14]. Thus according to its culture in Iran, fruit characteristics determination is of significant importance to evaluate.

The present study describes the variation among pomegranate fruits of twelve cultivars selected from different areas of Iran. The objectives of this study were to determine the variability in the juice and peel antioxidant activity and other physical and chemical properties of selected pomegranate cultivars. Due to the influence of environment and cultivar differences on

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physico-chemical values of the fruit, more work is required.

MATERIALS AND METHODS

Plant Material: Pomegranate fruits of 'Rabbab', 'Malas-e-Yazd', 'Malas-e-Saveh', 'Shishe-Kap', 'Khazar-e-Bardeskan', 'Naderi', 'Alak', 'Abdandan', 'Tabrizi', 'Poost Syah-e-Saveh', 'Poost Syah-e-Badrood' and 'Lamsari-e-Behshahr' cultivars were harvested from pomegranate orchards located in the Neiriz, Yazd, Saveh, Ferdows, Bardeskan, Badrood and Behshahr cities in Iran. They were brought to the laboratory of Gorgan University of agricultural sciences and natural resources in 2009. The ripe fresh fruits were from different mature trees randomly (completely randomized design of four trees per variety in a sample of ten fruits per replications) selected to represent the population of the plantation.

Physical Properties: Harvested fruits were sorted for size and uniformity of shape and weight. All fruits were first flushed by tap water before the peel, pulp and seed fractions were carefully separated. The peel and pulp were separated manually after fruit fresh weight, volume measurement and fruit density determination.

Fruit fresh weight was determined by weighting the fruits in the air on a precision digital balance (Mettler AJ50) with an accuracy of 0.0001 g. Fruit volume was determined by water displacement method and the weight density of the fruit was obtained by the ratio of weight to volume. Then peel thickness was measured by a digital caliper with 0.01 mm accuracy and oven-dried to constant weight to calculate its moisture content. Aril, juice and seed weight was measured as above. Fruit juice content measured using an electric extractor (Toshiba 5020). Then the arils were analyzed for major chemical compositions and antioxidant activity.

Chemical Analysis: Total soluble solids (°Brix) in the juice were determined with a digital refractometer (ATAGO RX-5000) at 20°C, calibrated using distilled water. Titrable acidity was estimated by juice titration with 0.1 N NaOH to the titration end point of pH 8.3, monitored with a pH meter (Labtron) and expressed as citric acid content ($mg.100ml^{-1}$). Reducing sugars was determined according to the method of Lane and Eyon as described by Ranganna [15] and expressed as mg sugar per ml.

For pH and electrical conductivity (EC, dSm^{-1}) determinations, the samples were homogenized and measured with a pH meter (Labtron, Iran) and conductivimeter (ABB-100), respectively. Vitamin C was determined by employing the Jacob's method [32] and expressed as mg per 100 ml.

Ellagic acid tannin was determined by a HPLC method described by Yoshiaki *et al.* [16] as follows:

Peel and juice of fruits (10 g, 10 ml) were homogenized in methanol (30 ml) and then the homogenate was refluxed for 1 h. After refluxing, the reflux sample was filtered in vacuo. The filtrate with 10 ml of water added was evaporated to ca. 10 ml and then 0.1 *M* HCl solution (100µl) was added. The extract was directly loaded on to the Sep-Pak Plus tC_{18} cartridge, previously conditioned with 10 ml of methanol, followed by 10 ml distilled water and washed with 10 ml of distilled water. Ellagic acid on the cartridge was eluted with 10 ml of methanol. The eluted was collected in a flask and then evaporated to dryness under reduced pressure below 40°C using a rotary evaporator. The residue was dissolved in 5 ml of methanol and the sample solution was filtered through a 0.5 µm filter before injection to HPLC [16].

Antioxidant activity of pomegranate juice was determined by the FRAP method described by Benzie and Strain [17]. A portion of 5 ml of juice was measured followed by addition of distilled water (1:9 w/v). The homogenized were centrifuged at 6000 g for 10 minutes. The supernatant was recovered and used directly for FRAP assay without storage. For the peels a portion of 5 g of dry powdered peel was weighted and diluted with methanol followed by homogenization and used for FRAP assay.

All solvent/chemicals used were of analytical grade and obtained from Sigma (MO, USA) and Merck (Germany).

Statistical Analysis: Data were analyzed statistically (ANOVA) using analysis of variance and differences among the means were determined for significance at $P<0.05$ using Least Significant Difference test and the system programme SAS.

RESULTS AND DISCUSSION

A considerable variation was observed in some of the physico-chemical and antioxidant properties of studied pomegranate cultivars. Some of the physical characteristics of the pomegranate fruits are presented in Table 2.

Table 1: Analysis of variance of physical characteristics of the studied pomegranate cultivars

SOV	df	FrFw	FrV	FrD	FrPTh	FrAw	FrJ	SeFrW
Cultivars	11	67803.93**	80787.64**	0.004**	5.99**	28220.31**	459.76**	72.98**
Error	36	1564.84	2126.82	0.001	0.50	951.55	47.44	23.55
CV		13.53	14.85	3.43	26.26	17.38	17.30	33.86

Table 2: Physical characteristics of the studied pomegranate cultivars

Cultivars	FrFw (g)	FrV (cm^3)	FrD (g/cm^3)	FrPTh (mm)	FrAw (%)	FrJ (%)	SeFrW (%)
Rabbab	441.25^{b}	467.82^{b}	0.95b^{cd}	6.01^{a}	46.30^{d}	38.63^{cd}	7.67^{e}
Malas-e-Yazd	369.25^{dc}	380.20^{c}	0.97^{b}	4.06^{b}	57.82^{c}	44.88^{bc}	10.10^{de}
Malas-e-Saveh	203.75^{f}	212.60^{e}	0.96^{bc}	1.92^{cd}	62.24^{b}	36.88^{cd}	18.67^{ab}
Shishe-Kap	348.75^{d}	364.66^{cd}	0.96^{bcd}	2.53^{cd}	65.04^{b}	50.13^{ab}	12.89^{bcde}
Khazar-e-Bardeskan	505.00^{a}	547.88^{a}	0.93^{cd}	2.10^{cd}	71.26^{a}	59.833^{a}	11.71^{cde}
Naderi	281.25^{e}	301.46^{d}	0.94^{bcd}	2.91^{c}	59.05^{c}	37.11^{cd}	11.62^{cde}
Alak	168.50^{fg}	172.55^{e}	0.98^{b}	2.62^{cd}	57.85^{c}	39.85^{cd}	13.01^{bcde}
Abdandan	328.75^{de}	350.08^{cd}	0.94^{bcd}	2.13^{cd}	72.06^{a}	52.358^{ab}	15.05^{abcd}
Tabrizi	422.50^{bc}	465.25^{b}	0.91^{d}	2.57^{cd}	54.80^{c}	35.845^{cd}	12.12^{bcde}
Syah-e-Saveh	140.00^{gh}	152.61^{ef}	0.92^{cd}	1.60^{d}	60.22^{c}	20.18^{e}	17.318^{abc}
Syah-e-Badrood	195.00^{fg}	212.62^{e}	0.92^{cd}	2.33^{cd}	57.65^{c}	30.50^{d}	21.57^{a}
Lamsari-e-Behshahr	103.38^{h}	99.41^{f}	1.04^{a}	1.727^{d}	68.94^{b}	31.515^{d}	20.24^{a}
Least Significant Difference	56.73	66.136	0.0468	1.02	3.12	9.88	6.96

The means followed by similar letters in each column are not significantly different at 5% level.

Table 3. Analysis of variance of Chemical characteristics of the studied pomegranate cultivars

SOV	df	RS	TSS	TA	FI	EC	pH	VitC	TanJ	TanP	AP	AE
Cultivars	11	113.22**	11.53**	2.44**	596.20**	0.87**	0.63**	22.19*	0.76**	657.60**	99598.77**	21113.44*
Error	36	7.43	1.54	0.084	31.90	0.17	0.11	8.64	0.10	22.10	16205.30	10171.98
CV		14.31	6.83	24.41	26.29	9.80	9.41	22.20	21.07	16.10	33.61	38.03

Table 4: Chemical characteristics of the studied pomegranate cultivars

Cultivars	RS (g/100cc)	TSS (°B)	TA (%)	FI	EC	pH	VitC (mg/100ml)	TanJ (mg/100ml)	TanP (mg/100g)	AP (mmol/100g)	AE (mmol/100ml)
Rabbab	16.45^{ef}	19.88^{b}	1.49^{bc}	13.41^{dc}	4.52^{b}	3.09^{de}	11.00^{cd}	1.00^{d}	10.00^{h}	229.67^{d}	284.33^{abcd}
Malas-e-Yazd	15.93^{ef}	18.20^{bcd}	0.68^{e}	29.08^{b}	3.96^{bcde}	3.33^{cd}	15.62^{ab}	2.00^{ab}	30.00^{ef}	234.33^{d}	180.33^{cd}
Malas-e-Saveh	16.25^{ef}	18.30^{bcd}	1.53^{b}	12.01^{dc}	4.09^{bcd}	3.42^{cd}	12.83^{bcd}	1.50^{c}	20.00^{g}	440.50^{c}	248.33^{bcd}
Shishe-Kap	15.33^{ef}	18.25^{bcd}	1.21^{bc}	16.38^{c}	4.45^{b}	3.42^{cd}	9.68^{d}	1.50^{c}	10.00^{h}	303.83^{cd}	157.33^{d}
Khazar-e-Bardeskan	27.09^{ab}	16.75^{de}	0.35^{e}	50.24^{a}	4.10^{bcd}	3.49^{bcd}	12.76^{bcd}	2.00^{ab}	25.00^{gf}	640.17^{ab}	271.04^{bcd}
Naderi	29.83^{a}	17.70^{cd}	1.24^{bc}	14.95^{c}	3.67^{cde}	3.50^{bcd}	10.12^{cd}	1.25^{dc}	30.00^{ef}	225.17^{d}	323.08^{abc}
Alak	18.98^{ed}	18.93^{bc}	1.29^{bc}	15.35^{c}	4.24^{bc}	3.70^{abc}	12.11^{bcd}	1.00^{d}	20.00^{g}	463.50^{bc}	248.67^{bcd}
Abdandan	13.89^{f}	15.17^{e}	1.10^{cd}	14.13^{c}	4.33^{b}	4.14^{a}	14.02^{abc}	1.62^{bc}	37.75^{dc}	284.93^{cd}	196.40^{cd}
Tabrizi	15.38^{ef}	17.65^{cd}	0.74^{de}	25.22^{b}	4.40^{b}	3.96^{ab}	17.45^{a}	1.02^{d}	32.50^{de}	374.38^{cd}	242.03^{bcd}
Syah-e-Saveh	24.06^{bc}	22.03^{a}	0.73^{de}	31.37^{b}	3.41^{e}	3.79^{abc}	14.30^{abc}	1.43^{dc}	50.00^{a}	314.89^{cd}	269.54^{bcd}
Syah-e-Badrood	21.11^{cd}	16.83^{de}	0.58^{e}	30.12^{b}	3.57^{de}	4.00^{a}	13.09^{bcd}	2.38^{a}	40.00^{bc}	327.63^{cd}	341.81^{ab}
Lamsari-e-Behshahr	14.27^{f}	18.23^{bcd}	3.36^{a}	5.57^{d}	5.11^{a}	2.75^{e}	15.84^{ab}	1.62^{bc}	45.00^{ab}	705.50^{a}	419.33^{a}
Least Significant Difference	3.91	1.78	0.42	8.10	0.58	0.48	4.21	0.46	6.74	182.56	144.64

The means followed by similar letters in each column have no significant difference at 5% level.

Fruit weight ranged from 103.38 to 505.00 g and fruit volume from 99.41 to 547.88 cm^3. It was observed that, on average, the weight and volume of 'Khazar-e-Bardeskan' were 505.00 g and 547.88 cm^3, respectively. 'Lamsari-e-Behshahr' had the lowest fruit weight (103.38 g) and fruit volume (99.41 cm^3).

Similarly, average fruit density ranged from 0.91 $g.cm^{-3}$ to 1.04 $g.cm^{-3}$ and peel thickness of the fruit was recorded from 1.60 to 6.01 mm. However, differences were obtained in fruit aril percent, fruit juice percent and seed percent (Table 2). Peel thickness of the fruit was found to be the maximum for 'Rabbab' and the minimum

for 'Syah-e-Saveh'. In this study, 'Khazar-e-Bardeskan' had the highest fruit juice (59.83) and 'Syah-e-Saveh' had the lowest fruit juice (20.18). Different proportion of pomegranate juice to fruit has been reported for Spanish varieties, which ranged from 50.26% to 64.17% [18]. In other varieties, such as Ganesh, the juice to fruit ratio was 60% and in two selected varieties of Muscat, 84% [19]. In some Indian varieties, this ratio has been reported ranging from 44.96% to 68.55% [20].

'Syah-e-Badrood' and 'Rabbab' fruits had the maximum (21.57%) and minimum (7.67%) seed content, respectively and showed a significant difference to other studied cultivars.

Chemical properties of studied pomegranate fruits showed significant statistical differences in all parameters (Table 3).

Reducing sugar ranged from 13.89 to 29.83 g/100 ml and total soluble solids from 15.17 to 22.03 (°Brix). Reducing sugar content was the highest (29.83 g/100 ml) in 'Naderi' and the lowest (13.89 g/100 ml) in 'Abdandan' (Table 4). This finding is quite more than the findings of Al-Kahtani [21] and Saxena *et al.* [22].

'Syah-e-Saveh' fruits had significantly more total soluble solids (22.03) than other cultivars (Table 4). Mustafa Ozgen *et al.*, [23] reported 16.7% TSS in pomegranate fruits, which is lower than our result. Total soluble solids in some Spanish cultivars was reported as ranging from 12.36% to 16.32% [18]. In Turkish cultivars, total soluble solids was the minimum of 16% and the maximum of 19% [24]. This value was 16.9% in Taief cultivar of Saudi Arabia [25] and 14.5% in Asaria cultivar of Portugal [26].

Titrable acidity was found to be the maximum for 'Lamsari-e-Behshahr' (3.36%) and the minimum for 'Khazar-e-Bardeskan' (0.35%). The Brix/acid ratio was the highest in 'Khazar-e-Bardeskan' (50.24) and the lowest in 'Lamsari-e-Behshahr' (5.57). The pH ranged from 2.75 to 4.14, which is borne out by the acidic taste of the fruit [25,27]. pH characterized the acidic taste of juice [28] and the findings of this research are more than average (3.34) reported by Mustafa Ozgen *et al.*, [23].

Pomegranate studied contains fairly high amount of vitamin C, ranging from 9.68 to 17.45 mg/100 ml. As the results showed, 'Tabrizi' and 'Shishe-Kap' had the highest (17.45 mg/100 cc) and the lowest (9.68 mg/100 cc) amounts of vitamin C, respectively. The average value of vitamin C of fruits studied was more than that found by Al- Khatani [21].

In this study, 'Syah-e-Badrood' fruit had the maximum (2.38 mg/100 ml) ellagic acid content of juice and followed by 'Malas-e-Yazd', 'Khazar-e-Bardeskan', 'Abdandan', 'Lamsari-e-Behshahr', 'Malas-e-Saveh', 'Shishe-Kap', 'Syah-e-Saveh', 'Naderi' and 'Tabrizi' (Table 4). Both 'Rabbab' and 'Alak' had the lowest amount of ellagic acid content of juice (1 mg/100 ml).

The amount of ellagic acid in fruit peel fluctuates considerably among the studied cultivars with a maximum of 50.00 mg/100 g ('Syah-e-Saveh') and a minimum of 10.00 mg/100 g ('Rabbab' and 'Shishe-Kap') (Table 4).

The amount of total antioxidant activity of fruit peel varied in different cultivars and ranged from 225.17 to 705.50 mmol/100 g. The highest antioxidant activity level was detected in 'Lamsari-e-Behshahr' and the lowest in 'Naderi' (Table 4).

Fruit juices exhibited a range of antioxidant activity (from 157.33 to 419.33 mmol/100 ml). 'Lamsari-e-Behshahr' fruit juice had the highest antioxidant activity while 'Shishe-Kap' had the lowest (Table 4).

The variation was much higher than those reported by Guo *et al.* [29] who studied antioxidant activities of peel, pulp and seed fractions of common fruits in China.

In this study, antioxidant activity of the peel was higher than juice, which was similar to the results reported by Guo *et al.* [29], Maria *et al.* [30] and Yunfeng *et al.* [31]. According to mentioned researchers' results, this difference in antioxidant activity seems to be due to the presence of pomegranate peel tannins. The main antioxidant compounds in pomegranate juice are hydrolysable tannins, but anthocyanins and ellagic acid derivatives also contribute to the total antioxidant capacity of the juice. Gil *et al.* [9] reported that the amount of total phenolics in peel was markedly higher than arils of pomegranate fruit.

CONCLUSION

This study showed considerable variation in some of the chemical and antioxidant properties of pomegranate cultivars widely grown in Iran. The studied cultivars probably represent only a portion of the native germplasm. It is important to evaluate and conserve local genetic materials, not only for general consumption, but also for their health advantages. The variation could originate from the pomegranate cultivar and agro-climatic as well as environmental conditions.

This study provides important data for compositional information of the fruits (e.g. reducing sugars, vitamin C, titrable acidity, antioxidant activity and etc), emphasizing that pomegranate fruit can be a good source of nutrients. More studies of physical and chemical relationship among different cultivars need to be undertaken.

In conclusion, a comparison of the results obtained by us with those found in other studies reveals that pomegranate fruit contains important amounts of antioxidant and high amount of nutrients both in arils and peel that play a valuable role in people's daily diet.

Nomenclature

FrFw	Fruit fresh weight
FrV	Fruit volume
FrD	Fruit density
FrPTh	Fruit peel
FrAw	thickness
FrJ	Fruit aril percent
SeFrW	Fruit juice
RS	Percent in fruit
TSS	Fruit seed percent
TA	Reducing sugars
FI	Total soluble
EC	Solids
pH	Titrable acidity
VitC	Harvest index
TanJ	Electric
Tanp	Conductivity
AP	pH
AE	Vitamin C
	Ellagic acid content of juice
	Ellagic acid content of peel
	Total antioxidant activity of peel
	Total antioxidant activity of juice

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