

Comparison of fruit quality characteristics and polyphenolic compounds in seven Iranian pomegranate cultivars

Abstract

Present study was carried out in order to compare and evaluate some quality biochemical characteristics and different polyphenolic compounds in juice of seven commercial Iranian pomegranate cultivars. Malas Mommtaz Saveh, Shishe Kab, Zagh Aghda, Naderi Badroud, Malas Daneh Ghermez Yazd, Shirin Pust Daneh Ghermez Yazd and Zard Anar Arsenjan cultivars were studied. Fruits were harvested randomly from a collection orchard in Arsenjan regin, Fars province. Some physical characteristic of fruits and biochemical parameters of juice were determined. Also polyphenolic composition of juices were measured using HPLC analysis. Significant differences were found among studied pomegranate cultivars for various physical fruit characteristics, quality parameters and polyphenolic composition of juice in present study. Malas Daneh Ghermez Yazd had the highest fruit physical characteristics such as fruit weight, length, diameter, and aril weight and juice percentage. Also this cultivar had the maximum of TSS and total polyphenolic compounds and gallic acid concentration of juice. The highest TSS, antioxidant activity and caffeic acid, chloregenic acid, vanilin and ellagic acid concentration were detected in juice of Shishe Kab. Thus these two pomegranate cultivars evaluated as cultivars with better health and nutritional value in comparison to other cultivars.

Keywords: anthocyanins, ellagic acid, Iran, *Punica granatum*

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Introduction

Pomegranate (*Punica granatum* L.) is a nutrient dense fruit with a high health value¹ and one of the most important Iranian fruit crop. Iran is the world's leading producer of this fruit with a production of over 700,000 tons/year.² According to historical evidences, Iran is the origin of pomegranate. This fruit has been spread from this region to other areas.³ A vast range of pomegranate varieties can be found in Iran; 760 original, decorative and wild ones.⁴ Pomegranate is considered as one of the most tolerant fruit crops capable of growing under arid and semi-arid climatic conditions.⁵

Researches have proved that pomegranate fruits or their processed products prevent disease and have a beneficial and profound impact on human health.⁶ Pomegranate juice contains a high amount of total soluble solid (TSS), anthocyanins, polyphenolic compounds, vitamin C, sugars and proteins, in addition to 85% moisture content.⁷ Antioxidant, anticancer and anti-atherosclerotic effects of pomegranate chemical compounds have been confirmed in numerous works.⁸⁻¹⁰

Cultivar, growing region, climate, maturity and cultural practice are the main factors determining chemical composition of pomegranate fruits.¹¹⁻¹⁵ Significant differences in various fruit quality parameters such as organic acids, phenolic compounds, sugars and water-soluble vitamins have been reported in previous studies.¹⁶⁻¹⁸

Although Iran and its neighboring regions are considered as center of origin of this species and in spite of numerous pomegranate cultivars native to Iran studies on the properties of the cultivars are limited. Various authors reported a vast range of different phenolic compounds in all parts of pomegranate tree such as bark, leaves,

peel, seeds and fruits,¹⁹⁻²³ however similar investigation on Iranian cultivars are rare. Cultivar selection for commercial production for meeting market requirements can be carried out based on such data. The aim of present study was to compare and evaluate some quality biochemical characteristics and different polyphenolic compounds in juice of seven commercial Iranian pomegranate cultivars.

Materials and methods

Samples

Seven Iranian commercial pomegranate cultivars were studied including; Malas Mommtaz Saveh (MMS), Shishe Kab (SK), Zagh Aghda (ZA), Malas Daneh Ghermez Yazd (MDGY), Naderi Badroud (NB), Shirin Pust Daneh Ghermez Yazd (SDGY) and Zard Anar Arsenjan (ZAA). Fruits were harvested randomly from uniform trees with the same age (4-year-old trees) in a collection orchard in Arsenjan regin, Fars province in october 20th (according to long term data in the area, pomegranate fruit quality parameters reach their optimum content at this date of year). The average temperature, rainfall and relative humidity in growing season of 2016 were 27 °C, 170 mm and 30%, respectively. The trees were planted in randomized block design and spaced 5 and 3 m between and along the rows, respectively. The trees were grown under drip irrigation and routine cultural practices suitable for commercial fruit production. Orchard management programs (application of fertilizers, pests, diseases and weeds control) were conducted uniformly according to optimized available recommendations (based on soil and water samples analysis) for the orchard site. 7 kg of pomegranate fruits was harvested for each cultivar approximately. They were kept at 4°C till analysis. Three replicates were maintained for each analysis and each replicate indicating four pomegranate fruits.

Physical parameters

Fruits were weight using a digital scale with accuracy of 0.001 g and reported as gram. The length and diameter of the fruit and calyx were measured with a digital caliper and reported as centimeter. The measurement of fruit length was made on the polar axis, i.e. between the apex and the end of stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. The arils were manually separated from the fruits, 10 arils were weighed using a digital scale with accuracy of 0.0001 g and average weight of one aril was expressed as milligram. Fruits for each cultivar were manually peeled and, by using a manual device with a pedal for pressing the arils, the juice passed through a perforated plate and the seeds and pulp remained on the plate, juice weight ratio to the whole fruit was calculated and expressed as juice percentage. This juice was used for biochemical analysis and determination of some quality parameters.

Biochemical analysis

TSS (Total soluble solids) expressed in Brix° was measured using a refractometer.

The titrable acidity (TA) was determined by titration to pH 8.1 with 0.1M NaOH solution and expressed as percentage.²⁴ Total anthocyanins were measured spectrophotometrically using pH differential method with two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Briefly, 0.4 ml of juice was mixed with 3.6 ml of corresponding buffers and read against water as blank at 510 and 700 nm. Absorbance (A) was calculated as

$$A = (A_{515} - A_{700}) \text{ pH 1.0} - (A_{510} - A_{700}) \text{ pH 4.5}$$

Then total anthocyanins content was calculated using the equation:

$$\text{Anthocyanin (mg} \cdot 100^{-1} \text{ml juice}) = (A \times \text{MW} \times \text{DF} \times 100) / (\epsilon \times 1)$$

Where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ϵ in this formula correspond to the predominant anthocyanin in the sample. The pigment content was calculated as cyanidin-3-glucosid, where MW=449.2 and $\epsilon=26.900$.²⁵ The total polyphenols concentration of fruits was measured by the Folin-Ciocalteau reagent using gallic acid as standard. The juice (1ml) was mixed with 5 ml Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and 4ml sodium bicarbonate (7.5%w/v), and the mixture was diluted to 100 ml with distilled water. The solution was kept in the dark at room temperature for 2 hours; the absorbance was then measured at 765 nm with spectrophotometer. Total polyphenolics content was expressed as gallic acid equivalents (the concentration of gallic acid was established from a calibration curve) in mg/100 ml juice (mg GAE.100 ml⁻¹ juice).²⁵ Ascorbic acid concentration in fruits was measured spectrophotometrically. To 100 μ l of fruit juice 10 ml of metaphosphoric acid (1%) was added, then to 1 ml of this mixture 9 ml of indophenol (50 μ M) was added and vortexed and read with spectrometer at 515 nm. A calibration curve was prepared with known ascorbic acid concentrations. The results were expressed as mg.100⁻¹ ml juice.²⁵ Antioxidant activity was assessed according to the method

of Brand-Williams et al.²⁶ Briefly, 100 μ l of pomegranate juice diluted in the ratio of 1:100 with methanol:water (6:4 v/v) was mixed with 2 ml of 0.1 mM DPPH in methanol. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm spectrophotometrically. The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation: antioxidant activity (%) = [1 - (sample 517 nm/control 517 nm)] × 100.

HPLC analysis

Before injection, each juice was centrifuged in an eppendorf tube (8min at 5000×g) and the centrifuged supernatant was allowed to pass through a 0.2 μ m PTFE filter (Sigma-Aldrich, Germany). The chromatographic analysis was carried out on Agilent Technologies 1200 series HPLC system. Injection volume was 20 μ l, Zorbax eclipse C18, 5 μ m (ID), 4.6×150 mm (FT) column was used for the separation of sample components. Oven was adjusted at 30°C. Mobile phase gradient consisted of methanol: formic acid 1%: (10:90), hold time: 0 min, methanol: formic acid 1%: (25:75), hold time: 10 min, methanol: formic acid 1%: (60:40), hold time: 20 min, methanol: formic acid 1%: (70:30), hold time: 30 min. Run time was 40 min and flow rate was 1.0 ml·min⁻¹. Chromatograms were recorded at 280 and 320 nm. Each compound was quantified by comparing its peak area against the standard curves. To obtain the standard curves, five different concentrations of each compound including caffeic acid, carvacrol, chatechin, chloregenic acid, coumarin, p-coumaric acid, eugenol, ellagic acid, gallic acid, hesperetin, hesperidin, quercetin, rutin, sinapic acid, trans-ferulic acid, vanilin (all HPLC grade, Merck Chemical Company, Germany) were injected.

Statistical analysis

Data was analyzed by SAS software and means were compared using Duncan's multiple range test at 5% probability level.

Results and discussion

Table 1 indicates some fruit physical parameters in studied pomegranate cultivars. The highest average of fruit weight was obtained from MDGY (345.50 g) which was significantly higher in comparison to ZA (233.83 g) cultivar. Other cultivars were not statistically different compared to MDGY. The maximum of fruit length was observed in MDGY (8.17 cm) which was significantly higher than SK (6.67 cm), other studied pomegranate cultivars did not show significant difference in comparison to MDGY. Fruit diameter of MDGY (7.83 cm) was significantly higher than ZA and SK cultivars. The maximum of calyx length (2.60 cm) was observed in ZA which was significantly higher than all other pomegranate cultivars. ZA had the lowest calyx diameter (1 cm), however ZAA and SK were not statistically different compared to ZA. The highest average weight of arils was observed in MDGY (470 mg) which was significantly higher than all other cultivars. MMS, SK and ZA were not statistically different. MDGY and SK had significantly higher juice percentage in comparison to other pomegranate cultivars.

Table 1 Some physical characteristics in fruits of seven commercial Iranian pomegranate cultivars

Cultivars	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Calyx length (cm)	Calyx diameter (cm)	Aril weight (mg)	Juice percentage (%)
MMS	296.00 ab	7.33 ab	7.33 abc	1.33 c	2.80 ab	256.67 cd	44.17 b
SK	271.50 ab	6.67 b	6.83 bc	1.60 bc	1.50 c	264.67 cd	49.43 a
ZA	233.83 b	7.00 ab	6.50 c	2.60 a	1.00 c	246.33 d	44.60 b

Table Continued....

Cultivars	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Calyx length (cm)	Calyx diameter (cm)	Aril weight (mg)	Juice percentage (%)
NB	304.17 ab	7.50 ab	7.50 ab	1.50 bc	2.33 b	294.83 bc	45.30 b
MDGY	345.50 a	8.17 a	7.83 a	1.50 bc	3.10 a	470.00 a	51.00 a
SDGY	272.33 ab	7.33 ab	7 abc	1.50 bc	2.50 ab	287.13bc	43.63 b
ZAA	300.00 ab	7.33 ab	7.67 ab	1.73 b	1.27 c	316.57 b	44.40 b

Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

Tehranifar et al.¹³ compared some physico-chemical properties in 20 Iranian commercial pomegranate cultivars. Their findings indicated that parameters such as fruit length and diameter and also calyx length and diameter have close correlation with cultivars. They observed the highest (315 g) and lowest fruit weight (196 g) in Shirin Pust Sefeed and Shirin Pust Ghermez cultivars respectively. Nikdel et al.¹⁵ compared five Iranian pomegranate cultivars and observed the highest and lowest fruit mass in Shishe-Kap (109.27 g) and in Ghand, Shalghami and Shahvar (78.07, 78.42, 80.94 g) respectively. Variation of fruit weight is due to the cultivar and ecological conditions.²⁷ One of the most important parameters from an industrial point of view is the juice content of the aril. In our study (whole fruit) juice percentage varied from 43.63% (SDGY) to 51% (MDGY). Our findings were in accordance to Fadavi et al.²⁸ Tehranifar et al.¹³ obtained more varied levels for this parameter as they showed that the juice percentage varied from 26.9% (Torsh Shahvar Kashmar) to 46.5% (Shirin Pust Ghermez), this can be contributed to higher number of studied

cultivars in their experiment. Zarei et al.¹⁴ reported similar results as they evaluate juice percentage (whole fruit) in 6 Iranian pomegranate cultivars (from 48.02% in Rabbab Fars to 60.49% in Shahvar).

Table 2 shows some biochemical quality parameters in juice of seven commercial Iranian pomegranate cultivars. TSS in SK and MDSY was significantly higher than MMS, ZA, NB, SDGY and ZAA. Significant difference was observed between cultivars for total anthocyanin content, ZA and SK had significantly higher total anthocyanin content in comparison to ZAA and NB. Total polyphenolic compounds were significantly 11.5% higher in MDGY compared to NB. Other cultivars were not statistically different. Ascorbic acid concentration was not statistically different among all studied cultivars. Highest antioxidant activity (52.63%) was observed in SK. This parameter was significantly higher in MDGY compared to ZA and MMS. Total acids in MMS (3.43%) was significantly higher than all other cultivars. SK, ZA, NB, MDGY and ZAA were not statistically different.

Table 2 Some biochemical characteristics in juice of seven commercial Iranian pomegranate cultivars

Cultivars	TSS (°Brix))	Anthocyanins mg 100 ml ⁻¹	Polyphenols	Ascorbic acid	Antioxidant activity (%)	Total acids (%)
MMS	15.00 b	18.13 bc	475.67 ab	7.80 a	48.90 c	3.43 a
SK	16.57 a	22.07 ab	503.10 ab	8.47 a	52.63 a	1.97 c
ZA	15.33 b	24.80 ab	493.27 ab	8.60 a	48.93 c	1.63 c
NB	15.17 b	11.57 d	459.07 b	7.63 a	49.83 bc	1.60 c
MDGY	16.57 a	19.13 bc	512.17 a	8.73 a	50.87 ab	1.57 c
SDGY	14.57 b	20.13 bc	488.57 ab	7.33 a	49.63 bc	2.83 b
ZAA	15.23 b	17.07 c	487.67 ab	7.40 a	49.20 bc	1.80 c

Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

Comparison of TSS in different pomegranate cultivars has been carried out previously. Nikdel et al.¹⁵ reported a range between 20.00 °Brix ('Rabbab') to 14.05 °Brix ('Shahvar'), Akbarpour et al.²⁹ reported similar range (15.17–22.03 °Brix). However, Tehranifar et al.¹³ observed a lower range (11.37–15.07 °Brix) in 20 Iranian pomegranate cultivars. Also our findings were in agreement with Poyrazoglu et al.³⁰ results for this characteristic.

Anthocyanins are a member of phenolic compounds that contributes to the red, blue, or purple colour of many fruits, including pomegranate juice, and they are well-known for their antioxidant activity.³¹ Positive correlations between total anthocyanin and both color values and total phenols has been reported previously.³² Among different species or even cultivars of the same species, the anthocyanin content varies considerably, affected by genetic make-up, light, temperature, and agronomic factors.³³ Tehranifar et al.¹³ and Cam et

al.¹² found similar varied levels for this characteristic among studied Iranian and Turkish pomegranate cultivars. Total phenolic compounds concentration was significantly different among studied cultivars and the values ranged from 459.07 to 512.17 (mg 100ml⁻¹). Our findings were in accordance to previous works.^{34,35,4,12,13} The total polyphenolic compounds content in juice of different pomegranate cultivars is higher in comparison to juice of other crops such as strawberry and sour cherry.¹² This parameter is associated with health value of this fruit and its free radical scavenging capacity.²

In contrary to this study, various authors found significant difference between pomegranate cultivars for ascorbic acid content in juice.^{13,14} The loss of ascorbic acid has been reported during the development of fruits.⁸ A rapid depletion in the ascorbic acid content of arils during fruit development has been reported.²

A significant difference was observed for antioxidant activity in studied pomegranate cultivars which was in agreement with previous investigations.^{36,37} Total antioxidants in a variety of plants have been evaluated by Halverson et al.³⁷ systematically. They reported that pomegranate fruits contained very high concentration of antioxidants. For assessing the ability of antioxidant to scavenge free radicals, the DPPH radical scavenging assay is used. Level of discoloration demonstrates the scavenging potentials of the antioxidant extract.⁸ The antioxidant capacity of pomegranate juice (and other fruit juices) depends on cultivar, growing region, fruit maturation, and agricultural factors. The technology used for juice processing may affect antioxidant capacity of pomegranate juice. Alone or in combination, poly phenolic compounds, anthocyanins and vitamin C are responsible for antioxidant activity of pomegranate fruits.²

Table 3 indicates concentration of some phenolic compounds in juice of studied cultivars. Highest gallic acid concentration (543.72mg⁻¹) was obtained from MDGY which was significantly higher than other cultivars. Gallic acid was not statistically different in MMS and ZA. Catechin was not detected in MMS. NB had the highest concentration of this polyphenolic compound (480.75mg⁻¹) among studied cultivars. Also SDGY (418.76mg⁻¹) had an elevated concentration of Catechin in comparison to other cultivars. The maximum of caffeic acid was detected in SK (204mg⁻¹). This polyphenolic compound was not detected in MMS, NB and ZAA. Also the highest concentration of chloregenic acid was observed in SK (101mg⁻¹) which was approximately two folds higher than MMS and SDGY. p-Coumaric

acid was not statistically different in ZAA and SDGY and also in MMS, SK and MDGY. Vanilin was not detected in MMS, ZA, NB and SDGY. This polyphenolic compound was significantly higher in SK compared to MDGY and ZAA. The highest trans-ferulic acid concentration (29.79mg⁻¹) was observed in SDGY. MMS, NB and MDGY had not this phenolic compound. Hesperedin concentration was not statistically different in MMS, SK, ZA and MDGY. Hesperedin was significantly higher in SDGY in comparison to other cultivars. The highest concentration of ellagic acid was found in SK, however MDGY was not statistically different. Polyphenols, phenolic ring compounds, with multiple hydroxyl groups are the major class of phytochemicals reported in pomegranate and extractable from almost all parts of pomegranate tree, however are most abundant in fruits.⁶ Various authors reported a vast range of different phenolic compounds such as cyanidin, cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, pelargonidin 3,5-di-*O*-glucoside, Ellagic acid, *o*-coumaric acid, *p*-coumaric acid, quercetin, rutin and many more in pomegranate juice.¹⁹⁻²³ In contrary to previous works, in our study presence of rutin, quercetin and eugenol was not detected in any of cultivars which was in agreement with an investigation carried out by Mousavinejad et al.⁴ who did not find quercetin in juice of eight Iranian pomegranate cultivars. In present study ellagic acid showed significant difference among studied cultivars (17.53-68.17 mg⁻¹). Akhavan et al.¹⁸ reported a higher range for this characteristic (17.4-155.9 mg⁻¹) in juice of ten Iranian pomegranate cultivars, Ardestani Torshe Semnan showed the highest concentration of ellagic acid.

Table 3 Concentration of some phenolic compounds in juice of seven commercial Iranian pomegranate cultivars

Cultivars	Gallic acid mg l ⁻¹	Catechin	Caffeic acid	Chloregenic acid	p-Coumaric acid	Vanilin	Trans-ferulic acid	Hesperedin	Ellagic acid
MMS	68.92 cd	nd	nd	53.1 d	17.65 b	nd	nd	14.90 c	37.68 c
SK	53.25 e	156.56cd	204 a	101 a	13.49bc	13.75a	23.75 b	13.81c	68.17 a
ZA	56.65 de	168.34 c	33.9 d	27.9 f	9.82 cd	nd	19.76 b	15.15 c	26.63 d
NB	112.94 b	480.75 a	nd	77.9 c	6.375 d	nd	nd	23.54 b	27.63 d
MDGY	543.72 a	142.14 d	54.7 c	92.1 b	12.74 bc	3.81 c	nd	14.37 c	63.07 a
SDGY	78.35 c	418.76 b	142 b	41.1 e	28.23 a	nd	29.79 a	28.66 a	52.48 b
ZAA	27.35 f	91.014 e	nd	21.8 f	30.98 a	9.55 b	14.85 c	6.86 d	17.53 e

Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

Mousavinejad et al.⁴ measured ellagic acid in juice of 8 Iranian cultivars. They found significant differences for ellagic acid levels in different cultivars. Saveh Black Leather showed the highest level of ellagic acid (160 mg⁻¹) and Sweet Alak contained the least amount of ellagic acid (7 mg⁻¹). One of the most important polyphenolic compound found in pomegranate juice is ellagic acid which contribute greatly to its antioxidant activity and health value. Bell and Hawthorne.³⁸ reviewed some aspects of impact of ellagic acid found in pomegranate fruits on human health.³⁹

Conclusion

In accordance to previous works significant differences were found among studied pomegranate cultivars for different physical fruit characteristics and quality parameters of juice in present study. MDGY had the highest fruit physical characteristics such as fruit weight, length, diameter, and aril weight and juice percentage. Also this cultivar had the maximum of TSS and total polyphenolic compounds and gallic acid concentration in juice. The highest TSS,

antioxidant activity and caffeic acid, chloregenic acid, vanilin and ellagic acid concentration were detected in juice of SK. Thus these two pomegranate cultivars evaluated as cultivars with better health and nutritional value in comparison to other cultivars. Similar studies in different regions of Iran with various cultivars are required for a better assessment of quality differences between Iranian pomegranate cultivars.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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