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ORIGINAL ARTICLE

Evaluation of unexplored pomegranate cultivars for physicochemical characteristics and antioxidant activity

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Abstract The aim of present study was to evaluate the different pomegranate cultivars for physicochemical properties and to identify the best cultivars suitable for food processing and future breeding. Various attributes of fruit (including length, weight and diameter), rind (thickness and weight), calyx (length), aril (weight, percentage, length and width), seed (length and width) and juice (titratable acidity and soluble solids or TSS) were evaluated. Total and reducing sugar, pH, ascorbic acid, total phenolic contents, antioxidant activity and maturity index were also evaluated. A significant difference in all tested parameters was noted amongst the tested cultivars except calyx length, rind thickness, arils and seed dimensions. The antioxidant activity and total phenolic contents of pomegranate cultivars were ranged from 15.77 to 42% and 1158.9 to 1540.7 mg GAE L^{-1}. The cultivars Tor-390 and Sorkhak-859 were sweet in taste, while the rest of the cultivars have sweet sour taste. The study concluded that the majority of cultivars were rich source of total phenolics, ascorbic acid, total soluble solid and total sugars, which are beneficial to health. Some of the elite cultivars (NKP-561, SRK-296, SZR-385, SRK-878) showed high quality attributes and were suitable for future breeding programs.

Keywords Pomegranate cultivars · Physico-chemical properties · Antioxidant activity and total phenolics

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Introduction

Pomegranate (*Punica granatum* L.) is regarded as a fruit with high commercial value and one of the tasty and delicious fruit, which is consumed throughout the world. It is widely distributed to different parts of the world, i.e. the Mediterranean, Middle East, North Africa and Asia (Sarkhosh et al. 2006). The fruits were first cultivated in Afghanistan, Iran, eastern India and China some 1000 years ago. It was then distributed around the world through western part of Persia and Mediterranean region (Celik and Ercisli 2009). The fruits of pomegranate are usually consumed as fresh (Al-Said et al. 2009), but with the advancement in science, it can be processed into products, like syrup and juices, sauces and jams. The consumable part of the pomegranate fruit accounts for approximately 55–60% of the total weight depending on the cultivar, out of which 75–85% is juice and 15–25% seeds (Al-Maiman and Ahmad 2002).

In recent times, researches have been focused on finding the best sources of antioxidant to tackle life threatening diseases. An extraordinary antioxidant activity has been discovered in many fruits, fruit parts and products (Doshi et al. 2015; Kumar et al. 2016; Mishra et al. 2015; Shanmugam et al. 2016), including the pomegranate seeds, juice and peel, juice (Gil et al. 2000; Tehranifar et al. 2010). Certainly, pomegranate juice stands among the fruit juices

and beverages with health benefits and high antioxidant activity (Seeram et al. 2007). The presence of high levels of antioxidants can be due to the abundance of phenolics in the pomegranate fruits. Pedunculagin, ellagic acid, anthocyanins, punicalin, punicalagin and flavanols are the main phenolics present in the pomegranate (Gil et al. 2000). But the variation in the composition of these compounds largely depends on climate, cultural practices, cultivar, maturity and growing environment (Ozkan 2005). Likewise, a variation in other quality attributes (i.e. vitamins, sugars and organic acids) of pomegranate have also been reported previously (Kulkarni and Aradhya 2005; Tezcan et al. 2009). Furthermore, the maturity of the fruit can be evaluated on the basis of rind, aril and juice color, as well as the acidity of the juice. Similarly, the acceptability of the pomegranate fruit by the consumers depends on the acidity, rind color, fruit size, sugar content and flavor. Despite of huge production of pomegranate in Afghanistan, most of the cultivars are still unexplored for their physiochemical properties. A step is, therefore, taken to examine the physico-chemical characteristics along with the antioxidant activity of 10 available pomegranate cultivars.

Materials and methods

Pomegranate cultivars

Ten best performing pomegranate cultivars [Sorkhak-878 (SRK-878), Maykosh Shinki-5025 (MKS-5025), Manayi Sor-384 (MS-384), Sor Zod Ras-385 (SZR-385), Tor-390 (T-390), Tashkurghani-860 (TSK-860), Sorkhak-296 (SRK-296), Sorkhak-859 (SRK-859), Nazek Post-561 (NKP-561) and Tashkurghani-6063 (TSK-6063)] were selected for the present study. Fruits that were commercially ripened were picked randomly from mature trees in October 2014. After harvesting the were transported to the laboratory through a well-ventilated vehicle on the same day. The fruit samples on arrival were stored at 4 °C till the analysis. The experiments were designed in replication and each replicate further represented three pomegranate fruits. An analytical grade chemicals, solvents and standards were used during the analysis of this research.

Physical properties

A total of nine fruits from cultivar was analysed for various physical attributes. The fruits were thoroughly cleaned and weighed by using analytical balance with 3 decimal places. The length FL (mm), diameter FD (mm) and calyx length CL (mm) of the fruits were measured with the help of a digital Vernier Caliper. The length of the fruit was calculated from the polar axis, i.e. from the apex of the fruit to the bottom of the stem. The width was measured in the direction perpendicular to the polar axis. The arils were first separated manually and then 100 arils AW (g) was weighed and recorded. Also, aril length AL (mm), arils width AW (mm), seed length SL (mm), seed width (mm), rind weight RW (g) and rind thickness RT (mm) per fruit were computed. The extracted fruit juice was then utilized in the experiments for the determination of chemical composition of fruits.

Titratable acidity

Total titratable acidity (TA) was determined by the method defined by AOAC (2005).

Total soluble solid (°Brix)

The total soluble solid (TSS) content of the pomegranate juice was measured by Digital Refractometer (RX 5000, ATAGO, Japan).

Sugars

Fruit sugars were described as the sum of reducing and non-reducing sugars. Reducing and total sugars were estimated by the method as described by AOAC (2005).

Vitamin C

Ascorbic acid (mg/100 g) were assessed by the method described by Ruck (1963).

pH

The pH measurements were done using a digital pH meter (Metrohm 601).

Total phenolics

The total phenolics (TPC) were analysed by Folin–Ciocalteu (FC) reagent as explained by Ainsworth and Gillespie (2007). To 1 ml of the extract 9 ml of the distilled water was added in a 25 ml of volumetric flask. After that, 1 ml of FC-reagent was added to the mixture upon shaking. The mixture was allowed to stand for 5 min and was then supplemented with 7% of Na_2CO_3 solution. The final volume of the solution was adjusted to 25 ml with distilled water, which was then incubated at room temperature for 2 h. Sample (200 µL) was transferred to a clear 96-well plate and the absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as Gallic acid equivalent (GAE) juice.

Antioxidant activity

Antioxidant activity of pomegranate juice was assessed by the method of Brand-Williams et al. (1995). Briefly, mixed 0.1 ml of pomegranate juice 0.6 ml of absolute ethanol and 0.06 mL of DPPH radical solution (0.5 mM in ethanol). The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a Cecil 2010 UV–visible spectrophotometer. The reaction mixture without DPPH was used as a blank. The antioxidant activity was calculated using the following equation:

$$\mathit{Antioxidant\ activity}(\%) = [1 - (\mathit{sample}\ 517\,\mathrm{nm}/\mathit{control}\ 517\,\mathrm{nm})] \times 100.$$

Data analysis

All the experiments were performed in triplicate and the recorded data were analyzed by using the statistical program Statistix 8.1 using ANOVA.

Results

Physical characteristic

The results of various physical characteristics of pomegranate cultivars are summarized in Fig. 1. All observed parameters showed a significant difference ($p < 0.05$) except calyx length, rind thickness, arils length, seed length and seed width. The average fruit weight was in the range of 608 g for MKS-5025 to 206 g for T-390. Similarly, MKS-5025 had significantly higher value for the fruit length (102 mm), fruit diameter (110 mm) and rind weight (332 g). However, T-390 had lower values for the fruit length (70 mm), fruit diameter (72 mm), rind thickness (3.6 mm), rind weight (112 g), aril weight (30 g) and aril length (9.5 mm). The cultivar SRK-878 had longer calyx and thicker rinds, but had fewer arils compared to all other tested cultivars. A higher number of arils were recorded in the MS-384 cultivars with smaller seeds, whereas NKP-561 cultivar was found to have longer, thicker and heavier arils. A lower aril width (6.6 mm) and seed width (3.2 mm) was observed in fruits from TSK-6063, while the cultivars SZR-385, SRK-296 and SRK-859 were found in between the other cultivars for various physical properties (Fig. 1a–d).

The results of correlation analysis showed that fruit weight was positively correlated with the fruit length, fruit diameter and rind width (Table 1). The values of the correlation coefficients with fruit width were in the order of fruit diameter > fruit length > rind width with 0.9878, 0.8950, 0.8739, respectively. Furthermore, a very strong positive correlation was also observed between fruit length and fruit diameter, fruit length and rind width. A negative correlation between fruit length and seed weight was also witnessed. The data regarding arils of the pomegranate demonstrated an existence of positive correlation between AW–AL, AW–AWD and AL–AWD. Additionally, the rest of the parameters were found independent of each other.

Chemical composition and maturity index

The data regarding various chemical properties and maturity index of the different pomegranate cultivars has been presented in Fig. 2a–c. The results of present study showed a significant difference ($p < 0.05$) among the pomegranate cultivars for all the tested parameters. The cultivars, SZR-385 and TSK-860 had the highest levels of total soluble solids (17 °Brix) among the tested cultivars of the pomegranate from Afghanistan. The cultivar T-390 had lower total soluble solids (13.90 °Brix), reducing sugar (3.87%), pH (3.08) and maturity index (5.3), yet the maximum total acidity 2.65) (Fig. 2a, b). The concentration of total sugars was between 8.7% (SRK-878) and 6% (NKP-561), however SRK-878 had lower reducing sugars (<50%) of the total sugars (Fig. 2b). The ascorbic acid contents were highly varied among the tested pomegranate cultivars, i.e. 191 mg/100 g was recorded in SRK-296 and 45 mg/100 g was observed in MKS-5025 (Fig. 2c). Similarly, the total phenolics revealed significant variation among the evaluated cultivars and the values were in the range of 1159–1541 mg GAE L^{-1}. The highest value for total phenolics was found in NKP-561 that exhibited highest antioxidant activity, while MKS-5025 was low in total phenolics and had low antioxidant activity (Fig. 2a, c). The maturity index values were also varied among the cultivars, which were in the range of 18.4 for TSK-6063 to 5.3 for T-390 (Fig. 2a). A significant positive correlation was existed between the TPC-AO (0.9307) and RS-AO (0.6511), whereas the correlation between MI–TA was highly negative (Table 2).

Discussion

Physical appearance (including fruit weight, length, diameter, calyx length, rind thickness and width, aril numbers, weight, width and length and seed dimensions) of a pomegranate fruit is an important quality parameter that can attract the consumer. This quality parameter may strongly be dependent on the cultivar as well as ecological conditions. The present results, have demonstrated higher values, regarding FW, FL, FD and CL of the fruits as

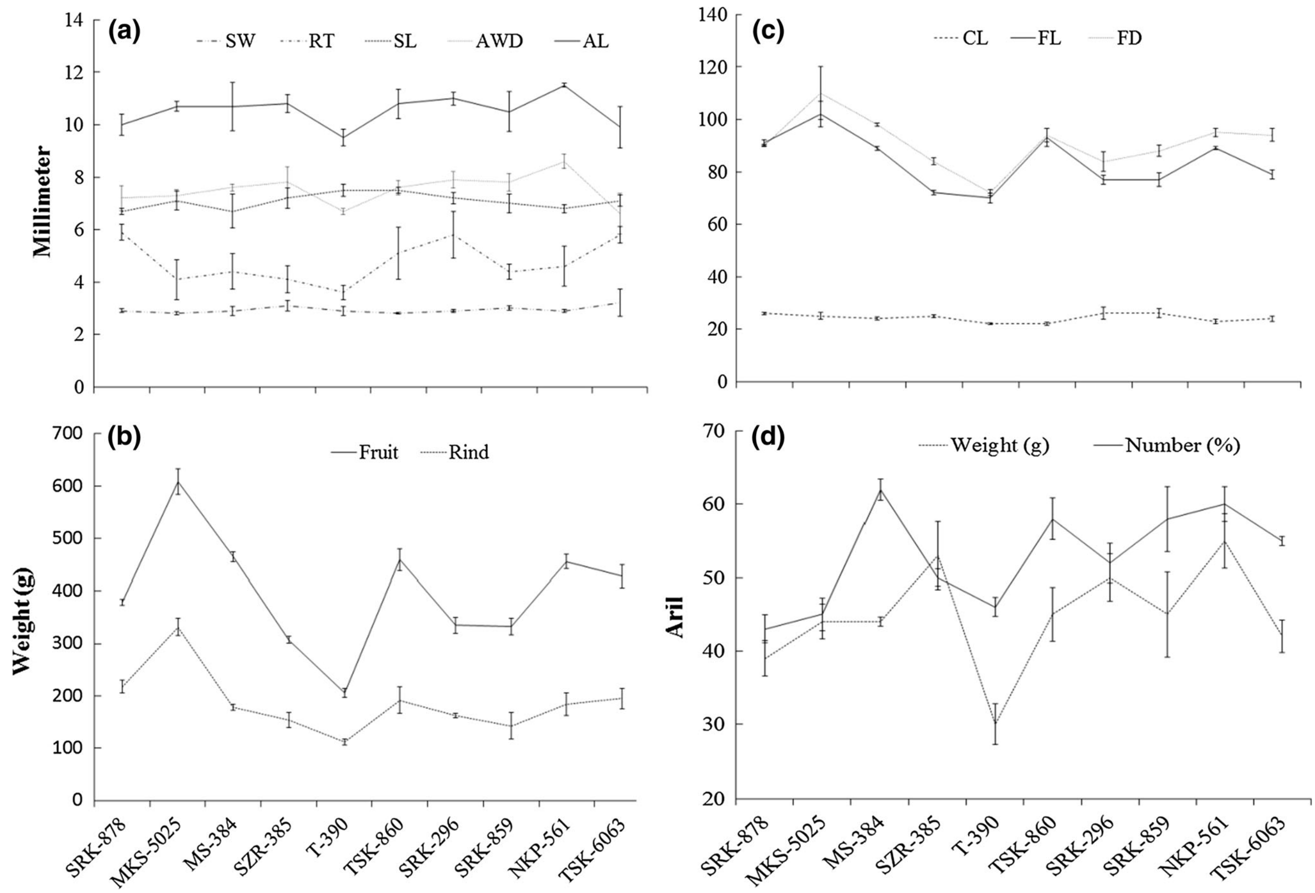

Fig. 1 Physical properties of ten pomegranate cultivars. **a** *SW* means seed weight; *RT* means rind thickness; *SL* means seed length; *AWD* means aril diameter and *AL* means aril length expressed in millimeter. **b** Fruit and rind weight in grams. **c** *CL* stands for calyx length; *FL* stands for fruit length and *FD* stands for Fruit diameter in millimeter. **d** Aril weight (g) and aril number (%). The *error bars* represent ± SE at $p = 0.05$ of the replicated data

Table 1 Coefficient of correlation (r) between physical properties of ten pomegranate cultivars

	FW	FL	FD	CL	RT	RW	AW	A	AL	AWD	SL	SW
FW	1											
FL	0.89**	1										
FD	0.98**	0.86**	1									
CL	−0.01^{ns}	−0.03^{ns}	0.09^{ns}	1								
RT	0.11^{ns}	0.12^{ns}	0.09^{ns}	0.39^{ns}	1							
RW	0.87**	0.84**	0.86**	0.19^{ns}	0.11^{ns}	1						
AW	0.31^{ns}	0.11^{ns}	0.33^{ns}	0.21^{ns}	0.11^{ns}	0.10^{ns}	1					
A	0.22^{ns}	0.03^{ns}	0.22^{ns}	−0.32^{ns}	0.01^{ns}	−0.27^{ns}	0.46^{ns}	1				
AL	0.44^{ns}	0.33^{ns}	0.42^{ns}	0.01^{ns}	−0.01^{ns}	0.17^{ns}	0.90**	0.54^{ns}	1			
AWD	0.17^{ns}	0.17^{ns}	0.18^{ns}	0.07^{ns}	−0.09^{ns}	−0.05^{ns}	0.82*	0.48^{ns}	0.91**	1		
SL	−0.36^{ns}	−0.38^{ns}	−0.44^{ns}	−0.53^{ns}	−0.21^{ns}	−0.27^{ns}	−0.22^{ns}	−0.16^{ns}	−0.22^{ns}	−0.30^{ns}	1	
SW	−0.35^{ns}	−0.65*	−0.28^{ns}	0.28^{ns}	0.19^{ns}	−0.41^{ns}	0.18^{ns}	0.17^{ns}	−0.18^{ns}	−0.17^{ns}	−0.11^{ns}	1

FW fruit weight, *FL* fruit length, *FD* fruit diameter, *CL* calyx length, *RT* rind thickness, *RW* rind weight, *A* aril, *AW* aril weight, *AL* aril length, *AWD* aril diameter, *SL* seed length, *SW* seed weight

Significant at $p \leq 0.05$ (*) or 0.01 (**); *ns* non-significant

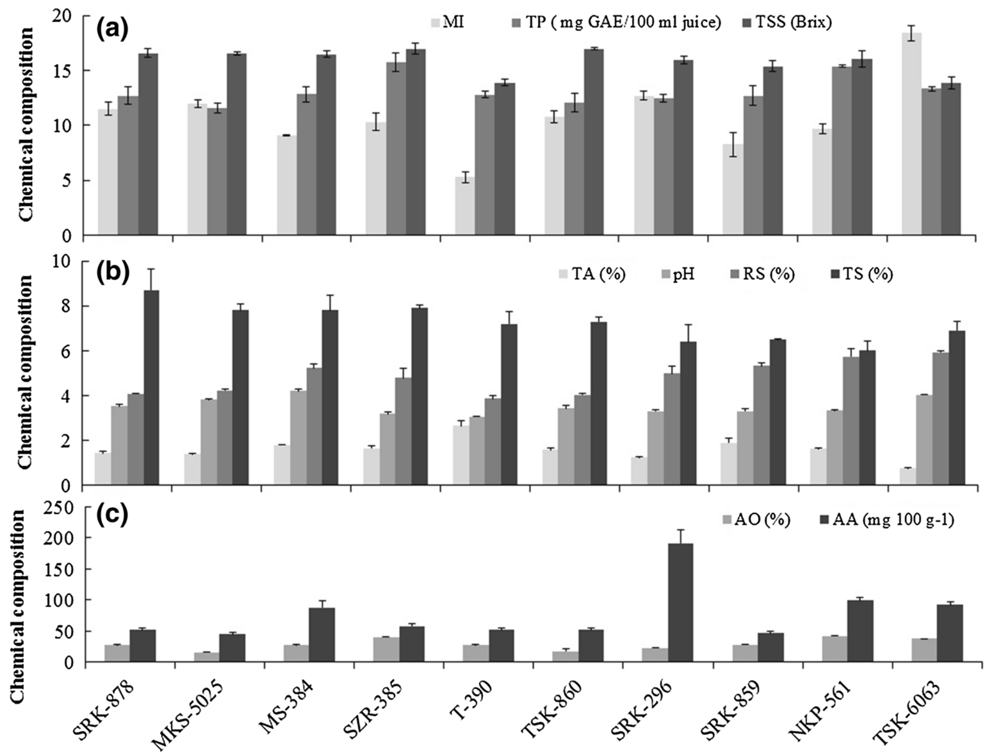

Fig. 2 Chemical composition of ten pomegranate cultivars. **a** *MI* means maturity index; *TP* means total phenolics expressed as mg GAE/100 ml juice and *TSS* means total soluble solids. **b** *TA* means titratable acidity; *RS* means reducing sugar and *TS* means total sugar. **c** *AO* means antioxidant activity and *AA* means Ascorbic acid. The *error bars* represent ± SE at $p = 0.05$ of the replicated data

Table 2 Coefficient of correlation (r) between various chemical properties of the juice from ten pomegranate cultivars

	pH	TSS	TA	TS	AA	AO	MI	RS	TPC
pH	1								
TSS	0.02^{ns}	1							
TA	−0.50^{ns}	−0.13^{ns}	1						
TS	0.26^{ns}	0.39^{ns}	0.01^{ns}	1					
A	0.02^{ns}	−0.06^{ns}	−0.37^{ns}	−0.51^{ns}	1				
AA	−0.12^{ns}	−0.24^{ns}	−0.07^{ns}	−0.25^{ns}	0.08^{ns}	1			
MI	0.52^{ns}	−0.08^{ns}	−0.96**	−0.01^{ns}	0.33^{ns}	0.11^{ns}	1		
RS	0.34^{ns}	−0.24^{ns}	−0.44^{ns}	−0.58^{ns}	0.43^{ns}	0.65*	0.43^{ns}	1	
TPC	−0.28^{ns}	0.03^{ns}	0.03^{ns}	−0.23^{ns}	0.08^{ns}	0.93**	−0.05^{ns}	0.49^{ns}	1

TA titratable acidity, *TSS* total soluble solids, *TS* total sugar, *RS* reducing sugar, *AA* ascorbic acid, *TPC* total phenolic content, *AO* antioxidant activity, *MI* maturity index

Significant at $p \leq 0.05$ (*) or 0.01 (**); *ns* non-significant

compared to Iranian varieties. The values for FW, FL, FD and CL of the best Iran cultivar was 376 g; 64 mm; 68 mm; 16 mm (Sarkhosh et al. 2009). Similarly, the values regarding fruit skin thickness were greater than values reported by Çam et al. (2009), but were comparable to that of Riyahi et al. (2011). Contrary to that the data for various physical parameters of all tested cultivars in this study were lower than the Glavas cultivar from Herzegovina (Gadže et al. 2013). Furthermore, the results regarding the physical properties of the various cultivars were significantly different except the calyx length, rind thickness, arils and seed dimensions. These differences in the physical properties can be attributed to the genetic makeup of the cultivars. Among the evaluated cultivars, MKS-5025 had bigger fruits size that might prove it as a promising cultivar of greater agronomic potential.

The data concerning the chemical properties of the pomegranate fruits bared a considerable difference. Certainly, it is natural for a biological sample to have such differences that might be due to lose in moisture content after postharvest. TSS represents the Brix, which normally increases when the fruit loss water during storage. Another valid explanation for that is the conversion of polysaccharides to smaller sugar molecules by their respective enzymes. Both TSS and total sugars of the tested cultivars were comparable to each other. Similar observation has

also been reported by Valero and Ruiz-Altisent (2000), who reported 10–16.5 °Brix that was lower than the values observed by Zarei et al. (2011). Phenolic compounds are among the health beneficiary components of the food (Rana and Bhushan 2016; Tamuly et al. 2015; Zielinski et al. 2015), which plays important role as an anticancer and antioxidant to protect from CVD. The main reason behind such event might be the low soluble starches in the tested cultivar of pomegranate. The total phenolic contents of NKP-561 were noticed higher in comparison to other cultivars, which mean that the juice from NKP-561 is a best source of the antioxidants. The phenolic compounds have a lots of health benefits thus pomegranate can serve as a best functional food (Al-Muammar and Khan 2012; Chen et al. 2012; Ghasemnezhad et al. 2015; Zanotti et al. 2015; Zarfeshany et al. 2014). The findings of this study are in agreement with Ozgen et al. (2008), who reported that the total phenolics in fruit juice of pomegranate cultivar Kan was 2076 mg GAE L^{-1} and that of Tali was 1245 mg GAE L^{-1}. Similar observations has also been reported by Tezcan et al. (2009) regarding the total phenolic contents (1008.6 mg GAE L^{-1}) of pomegranates. Additionally, all the tested pomegranate cultivars from Afghanistan were rich in ascorbic acid, particularly SRK-296. The SRK-296 has even fourfold higher contents of ascorbic acid than some of the other cultivars. The ascorbic acid is one of the best sources of antioxidants that can be used in various food preparations and food preservations. Previously, most of the researchers also presented higher values of ascorbic acids and antioxidants in the pomegranate juice (Carbonell-Barrachina et al. 2012; Fawole and Opara 2013b; Mena et al. 2011). The pH of the fruits was varied significantly among the tested cultivars. The cultivar MS-384 had high pH value that signifies the active utilization of organic acid into their respective metabolites in this particular cultivar. Besides, titratable acidity was low in all tested cultivars, which is an indicator of maturity, as most of the organic acids (i.e. citric acid, malic acid and acetic acid) decreases during the ripening of fruit (Fawole and Opara 2013a; Kassem et al. 2010; Nuncio-Jáuregui et al. 2014; Workneh et al. 2012). Indeed fruits are living bodies that can continuously respire after harvest, thus consuming the organic acid with increase in pH. Ghafir et al. (2009) also described the dependence of titratable acidity of fruits on their metabolic rate. The taste and flavor of the pomegranate fruit is mainly dependent on the maturity index (TSS/TA), which has been used as a tool for the classification of pomegranate cultivars in recent times (Çam et al. 2009; Martinez et al. 2006). Martinez et al. (2006) has introduced the optimized classification method for Spanish pomegranate cultivars. According to them fruits with maturity index (MI) ranges from 5–7 are considered to be sour, while 17–24 are sweet sour and 31–98 are sweet. Concurring to Martinez et al. (2006), the cultivars T-390 and SRK-859 from the present study, were placed in sour category, while other cultivars can be grouped as sour sweet. The differences among the data of this study in relation to past studies might be due to the differences in the cultivars and/or method of sample extraction during experiments. In general, the results of present study indicated that physicochemical variations in the cultivars can play an important role in the selection of elite genotypes for food processing and future breeding programs.

Conclusion

The data from this study suggested that cultivar difference is the key factor in determining the antioxidant activity and other physico-chemical characteristics in pomegranates. Among the evaluated cultivars, NKP-561, SRK-296, SZR-385, SRK-878 cultivars showed the highest content of total phenolics, antioxidant activity, ascorbic acid, total soluble solid and total sugars, which are beneficial to health. Moreover, these findings provide important information regarding the physico-chemical properties of tested pomegranate cultivars. Such findings can be helpful in selecting the cultivars with quality attributes, i.e. antioxidative, total sugar, taste, etc., which can be valuable for food and nutraceutical industry. In addition to that, the present data will be helpful in selection of elite desirable pomegranate genotypes to be used for commercial production.

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