The arils characterization of five different pomegranate (*Punica granatum*) genotypes stored after minimal processing technology

Mahmood Ghasemnezhad · Somayeh Zareh ·
Mohammad Ali Shiri · Zahra Javdani

Revised: 25 September 2013 / Accepted: 13 November 2013 / Published online: 3 December 2013
© Association of Food Scientists & Technologists (India) 2013

Abstract In this study, the quality of minimally processed different Iranian pomegranate genotypes was investigated during storage at 4 °C for 14 days. The results showed that at the end of storage time, the lowest microbial count was found on the arils of “Torsh Syabe Lorestan” genotype. There was a significant difference in titratable acidity, total soluble solids, total anthocyanin, catechin, and quercetin content in most genotypes after 14-day cold storage; while no difference was found between studied genotypes in antioxidant capacity, total phenolic, and flavonoid content. In general, total phenolic content and antioxidant capacity of pomegranate arils gradually decreased over storage time. Diphenolase activity of polyphenol oxidase (PPO) enzyme with dopamine hydrochloride substrate and peroxidase (POD) decreased over storage time, whereas diphenolase activity of PPO with pyrocatechol substrate significantly increased. The lowest diphenolase activity with dopamine hydrochloride and pyrocatechol substrates, as well as POD activity was found in *Torsh Syabe Lorestan*. The results suggested that the genotype of *Torsh Syabe Lorestan*, which showed the lowest microbial count on the arils and enzymes, could be more appropriate for minimal processing technology.

Keywords Pomegranate · Antioxidant · Anthocyanin · Minimal processing · Polyphenols · Polyphenol oxidase

Introduction

Pomegranate (*Punica granatum* L.) is one of the most important fruits native to Iran and some parts of the Mediterranean

area. It is also extensively cultivated in Spain, Egypt, Russia, India, France, China, Japan, and USA in recent years (Varasteh et al. 2012). It has been cultivated since ancient times throughout the Mediterranean basin to India, and is highly adaptable to adverse climatic conditions and different soil types (Muharrem and Nazan 2009). Aril is the edible part of pomegranate fruit that represents 50–70 % total weight of the fruit (Safa and Khazaei 2003). It is composed of 10 % sugar (mainly fructose and glucose), 1.5 % organic acids (principally ascorbic acid, citric, and malic acid) and bioactive compounds such as anthocyanins and other phenolic compounds (Safa and Khazaei 2003). The skin and aril color of pomegranate genotypes, depend on the cultivar, growing region, ecological conditions during fruit maturation, and ripening (Holland et al. 2009). Therefore, the arils of the pomegranate have differences in skin color and thickness, amount of water, taste, and hardness; these are the most important features of the different pomegranate genotypes. The color is most effective on consumer purchase decision. In addition, the others important parameters are texture, flavor, and nutritional value (Holland et al. 2009).

Pomegranate consumption is limited due to difficulties in peeling to obtain the arils. On the other hand, pomegranate is very sensitive to sunburn, cracking, cuts, or bruises in the husk, as well as to chilling injury (Artes et al. 2000). Despite their excellent internal quality, these diverse external defects make the injured fruit unsuitable for fresh marketing and consumption, and usually, they are destined for industrial use or animal consumption (Artes et al. 2000). The processing of the externally damaged pomegranates that are unacceptable for fresh marketing and consumption could be an excellent way to obtain a commercial profit from discarded pomegranate fruits (Lopez et al. 2005).

Just as society has evolved over time, food system has evolved over centuries into a global system of immense size

M. Ghasemnezhad (✉) · S. Zareh · M. A. Shiri · Z. Javdani
Department of Horticultural Science, Faculty of Agriculture,
University of Guilan, Rasht, Iran
e-mail: Ghasemnezhad@guilan.ac.ir

and complexity. Production of pomegranate arils in “ready-to-eat” form would be a convenient and desirable alternative to the consumption of fresh fruits and may increase pomegranate demand by consumers (Ghasemnezhad et al. 2013). Minimally processed pomegranate arils would be more appealing to customers than whole fruits and increase fresh consumption of pomegranate fruits (Muharrem and Nazan 2009).

Raw material entering the food industry represents a potential source of microbial contamination. The potential growth of pathogens and microorganisms will be affected by the initial level of contamination and the processing steps in eliminating bacteria in the food. Generally, different microbial populations are present in contaminated food, and some groups become more predominant after a certain storage period. This selection might depend on several physico-chemical factors and processing and storage conditions. In the last years, different procedures have been reported for the establishment of shelf-life, mainly based on the detection of microbial alteration, as well as physico-chemical and sensorial changes (Valero et al. 2012).

However, maintaining the nutritional and microbial quality of pomegranate arils is a major challenge as minimally processed arils easily deteriorate in texture, color, overall quality, and consequently, shelf-life is reduced (Caleb et al. 2012). This is due to the active metabolic processes related to endogenous enzymatic activity, enhanced respiration rate with increased production of ethylene (Muharrem and Nazan 2009), and increased microbial load, that some of which may be potentially harmful to human health (Caleb et al. 2012). If not controlled, these changes can lead to rapid senescence and deterioration of the product (Muharrem and Nazan 2009).

Compared to whole fruits, minimally processed pomegranate arils have a greatly reduced postharvest life (Gil et al. 1996). Therefore, finding pomegranate genotypes appropriate for minimal processing technology could be very important. Research carried out in Spain on minimally processed pomegranates had shown that browning is the result of oxidation of phenolic compounds and the internal composition of pomegranate changes during storage time, indicating that the stabilization of anthocyanin pigments is essential for achieving high quality, since its attractive color is one of the most important sensory characteristics of fruits and pomegranate arils (Labbe et al. 2010).

The oxidation of phenolic substrates by polyphenol oxidase (PPO) is thought to be the major cause of the brown coloration of many fruits and vegetables during storage and processing (Vidhan et al. 2010). Previous study of Ghasemnezhad et al. (2013) had confirmed that PPOs play an important role in oxidation and degradation of anthocyanins in pomegranate arils. The difference in the browning reactions of different genotypes is also related to the different amounts of sugar and amino acids in the fruits. The response

of genotypes depends on the amount of phenolic compounds and concentration of inhibitors of PPO activity (Labbe et al. 2010). Therefore, one of the most important goals regarding marketing is the maintenance of color transparency of pomegranate arils; it is necessary to identify genotypes that show less color change during storage (Gil et al. 1996). The use of appropriate genotypes is the most consistent way of maintaining quality and controlling decay of pomegranate arils (Caleb et al. 2012). This study seeks to compare the potency of some Iranian pomegranate genotypes to minimal processing technology.

Material and methods

Plant materials and treatments The fruits of five commercial pomegranate genotypes, including “Malas Syabe Lorestan,” “Malas Yazdi,” *Torsh Syabe Lorestan*, “Malas Torsh Saveh,” and “Naderi Kashan” were harvested at the commercial mature stage from a commercial orchard in Saveh Pomegranate Research Station, Iran. Immediately, the fruits were transported to the horticulture laboratory at University of Guilan, Rasht, Iran. Fruits with defects (sunburn, cracks, bruises, and cuts in the husk) were discarded and only fruit with healthy outer skins and uniform in size and appearance were used. Husks were carefully cut at the equatorial zone with sharpened knives, and arils manually extracted as described by Gil et al. (1996) and washed in distilled water at 5 °C for 2 min. After rinsing, the excess water removed from the arils surface by clean paper towels. The arils were directly placed in rigid polyethylene boxes (10×6×5 cm³) and the caps were not completely air-tight. The arils were randomly distributed into five groups and three boxes were used for each genotype (as replication). All packages were stored in a cold room at 4 °C and 95 % relative humidity for 14 days.

Chemicals Pyrocatechol, pyrogallol, dopamine hydrochloride, 3-methyl-2-benzothiazolinone hydrazine (MBTH), dimethylformamide, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Potato dextrose agar (PDA), nutrient agar (NA), and Folin were obtained from Merck (Darmstadt, Germany). Cyaniding-3-glucoside, quercetin 3-galactoside (Hyperoside) and (+)- Catechin were obtained from Extrasynthese (France).

Microbial analysis Microbial analyses of pomegranate arils were done after 14-day storage at 4 °C, as described in AOAC (2000) with some modifications. The samples were serially diluted and the plates were incubated at 35 °C for 48–72 h. Total fungal counts (molds and yeasts) were carried out on PDA medium. Total bacterial counts were carried out on

plates of NA. Microbial data were converted into logarithms of the number colony forming units ($\log \text{CFU mL}^{-1}$).

Measurements of total soluble solids (TSS) and titratable acidity (TA) TSS ($^{\circ}\text{Brix}$) was determined by a digital refractometer (CETI-Belgium). TA was determined by titrating aliquots (5 mL) of juice samples to an endpoint of pH 8.2 with 0.1-N NaOH and expressed as percentage of citric acid.

Total phenolic content Total phenolics content in arils were analyzed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method as described by Ghasemnezhad et al. (2011) with some modifications. For this purpose, 0.5-g arils was ground in the presence of liquid nitrogen by mortar and pestle; then 10-mL pure methanol was added to extract phenolic compounds. The extract was centrifuged at $705\times g$ for 5 min. Three hundred microliters of methanolic extract was brought to a volume of 500 μL with distilled water in test tubes, followed by addition of 2.5 mL of 10 % Folin–Ciocalteu reagent and allowed to stand for 6 min. Thereafter, 2 mL of 7.5 % sodium carbonate solution was added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm using a UV/Vis spectrophotometer model PG Instrument $^{+}80$ (Leicester, UK). Results are expressed as milligram gallic acid 100 g^{-1} fresh weight.

Total flavonoids content Total flavonoids content, were analyzed spectrophotometrically as described by Blasa et al. (2005) with some modifications. One hundred fifty microliters of methanolic extract were mixed with 0.3-mL NaNO_2 (5 %), and 0.3-mL AlCl_3 (10 %) were added after 5 min. The pomegranate peel extract samples were mixed and after 6 min, neutralized with 2-mL NaOH solution (1 M). The absorbance was measured at 510 nm and the quantification was carried out using a standard curve prepared from authentic catechin. Finally, the results are expressed as milligram catechin equivalents (RE) per 100 g of sample.

HPLC analysis of flavonoids Flavonoids were determined using high-performance liquid chromatography (HPLC) as described by Bakhshi and Arakawa (2006). For polyphenol extraction, after 14-day storage, 1 mL of solvent (methanol/acetic acid, 85:15, v/v) was added to 1 g of arils powder and then kept in refrigerator overnight. Aqueous part of the samples was centrifuged for 10 min at $7,826\times g$. The supernatant of centrifuged samples were filtered through disposable 0.45- μm syringe filter. Fifty microliter of the filtered sample was injected into HPLC (Waters, 1525, Milford, CT, USA) equipped with a UV-visible detector (Waters Dual k Absorbance 2,487), a column (Waters Symmetry C18; 5- μm pore size; $4.6\times 150 \text{ mm}$; Waters, Dublin, Ireland). Samples and the column were held at 25°C , the eluent flow was at

1.0 mL min^{-1} , and the run time was 40 min. Flavonoids were identified by comparing their retention time with that of a corresponding standard and by spiking samples with the standard. Flavonoids concentration was calculated from the absorbance at 280 and 350 nm and was expressed in microgram per milliliter.

Total anthocyanin The juice samples were diluted with distilled water to give an absorbance reading between 0.6 and 0.8 units. The pH values of the diluted pomegranate juice were 3.32 and 3.50 (Mehmet 2002). Total anthocyanin content was determined according to pH differential method as described by Lako et al. (2007). Absorbance was measured in a UV/Vis spectrophotometer at 510 and 700 nm in buffers at pH 1.0 and 4.5 using:

$$A = [(A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}]$$
 with molar extinction coefficients of cyanidin-3-glucoside (26,900) for pomegranate fruit juice. Results were expressed as milligrams of cyanidin-3-glucoside per 100 mL of pomegranate juice.

Antioxidant activity The antioxidant activity was evaluated by DPPH radical scavenging method according to the procedure of Shiri et al. (2011) with some modifications. Briefly, 50 μL of pomegranate arils methanolic extracts was added to 950 mL of DPPH radical and mixed by vortex and allowed to stand at room temperature in darkness. The absorbance of the samples was measured at 515 nm after 15 min by UV/Vis spectrophotometer. The antioxidant activity was expressed as the percentage of decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (%DPPHsc), was calculated using:

$$\% \text{DPPHsc} = [(A_{\text{cont}} - A_{\text{samp}}) \times 100] / A_{\text{cont}}$$

Where A_{cont} is the absorbance of the control and A_{samp} is the absorbance of the sample.

Peroxidase (POD) enzyme assay The pomegranate arils (0.5 g) were frozen in liquid nitrogen followed by grinding with 1-mL extraction buffer (50-mM phosphate buffer, pH=7) containing 0.5-mM EDTA and 2 % polyvinylpolypyrrolidone (PVPP) (w/v). Homogenate was centrifuged for 15 min at 15,339g and the supernatant used to determine enzyme activity. Peroxidase activity was assessed according to the method of In et al. (2007) with some modification. Peroxidase activity was determined spectrophotometrically by adding 250 μL of enzyme extract to 375- μL guaiacol 10 mM and 375- μL H_2O_2 10 mM. Absorbance was read at 470 nm. The activity is expressed as units per milligram fresh weight.

PPO enzymes assay The pomegranate arils (0.5 g) were frozen in liquid nitrogen followed by grinding with 1-mL

extraction buffer (50-mM phosphate buffer, pH=6.8) containing 0.5-mM EDTA and 6 % PVPP (*w/v*). Homogenate was centrifuged for 15 min at 17,530g and the supernatant used to determine enzyme activity. Monophenolase and diphenolase activity of PPO in arils was assessed with different substrates according to the method as described by Ghasemnezhad et al. (2013).

Monophenolase activity of PPO was determined spectrophotometrically by adding 200 μ L of enzyme extract to 200- μ L pyrogallol 0.02 M and 2,100- μ L phosphate buffer solution (0.1 M, pH=7.2). Absorbance was read at 420 nm every 5 s up to 3 min according to Narpinder et al. (1999). The activity is expressed as unit per 100 g fresh weight per minute.

Diphenolase activity of PPO was determined using two different substrates, pyrocatechol, and dopamine hydrochloride. Briefly, for the first substrate, 200 μ L of enzyme extract was added to 300 μ L pyrocatechol 0.5 M and 2,500- μ L phosphate buffer solution (0.1 M, pH=7.2). Absorbance was read at 420 nm according to Vidhan et al. (2010) and the activity is expressed as units per milligram fresh weight. With the second substrate, 10 μ L of enzyme extract was added to 500- μ L dopamine hydrochloride solution 60 mM with MBTH 5 Mm, 2 % (*v/v*), phosphoric acid 5 % and 490- μ L phosphate buffer solution (0.1 M, pH=7.2). Absorbance for dopamine hydrochloride substrate was read at 475 nm every 5 s up to 3 min according to Espin et al. (1995). The activity is expressed as unit per 100 g fresh weight per minute.

Statistical analysis Data were analyzed as a two-factor linear model by PROC MIXED procedure by SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC), where genotype and storage times were the factors. Before analysis of variance, data were tested for normality and homoscedasticity using the Kolmogorov–Smirnov and Cochran tests, respectively. To determine statistical differences between treatments, variance analysis and Tukey tests were performed ($p \leq 0.01$). All determinations were performed in triplicate.

Results and discussion

Microbial count There was a significant difference in bacterial and fungal population on the arils of different pomegranate genotypes after 7- and 14-day storage at 4 °C (Fig. 1a and b). The lowest bacterial and fungal growth was found by *Torsh Syabe Lorestan* arils at the end of cold storage time. As the results showed, in most studied genotypes, microbial growth increased over storage time, which was consistent with the results reported by Raybaudi-Massilia et al. (2007) in fresh-cut “Fuji” apples. Moreover, Soliva-Fortuny and Martín-Belloso (2003) reported that fruit physico-chemical properties such as pH and TA have an important effect on microbial shelf-life of fresh-cut fruit.

One of the main limiting factors for long-term storage of pomegranate fruits is decay, that is caused by various pathogens such as *Botrytis cinerea*, *Aspergillus niger*, *Penicillium* spp., and *Alternaria* spp. (Caleb et al. 2012). The susceptibility to pathogen attacks is also variable in different genotypes of whole pomegranate fruit (Asgrai and Shahedy 1996). The pomegranate genotypes with red skin are more susceptible to microbial contamination during storage than white-skin fruits (Caleb et al. 2012). In the present study, we also found the arils of *Torsh Syabe Lorestan* with white fruit skin showed lower fungal growth than red skin fruits during cold storage (Fig. 1b).

Microbial quality criteria are often used to determine the acceptability limits and the shelf-life of minimally fresh processed produce and this is used as a minimal standard for processed produce having a limited microbial count and free of pathogenic microorganisms (Rojas-Graü et al. 2007). Fresh-cut fruits are a fertile environment for microorganisms to growth due to the high amount of moisture and sugar present on the surface; therefore, the study of the development of microbial growth is required to ensure the microbial safety of those products (Rojas-Graü et al. 2007).

TSS and TA The results showed a significant difference of TA and TSS content immediately after arils extraction and at the end of 14-day storage at 4 °C (Table 1). Generally, in most genotypes, TSS and TA content increased at the end of storage time. These findings are in accordance to Gil et al. (1996), who indicated that TSS and TA increased significantly in arils of different pomegranate genotypes during storage. Banjongsinsiri et al. (2004) concluded that TSS content of “Kent” mango is most likely moderated by changes in the solubility of large molecular weight insoluble pectin and non-pectin components, such as cellulose and hemicellulose, in the cell wall. Furthermore, Ghasemnezhad et al. (2013) also found that TSS and TA content of both chitosan-coated and uncoated pomegranate arils gradually increased during 12-day storage at 4 °C.

Increase in TSS and TA content may be due to water loss (Labbe et al. 2010). In this study, reduction in TA in some genotypes could be explained by increase of respiration rate after peeling and slicing of fruits (Perez-Gago et al. 2006). TA and organic acids might have provided carbon skeletons for the synthesis of secondary metabolites during storage (Mazza and Miniati 1993). The consumption of carbohydrates in the respiration process also reduces TSS; that could explain the decrease in TSS content in *Torsh Syabe Lorestan* arils after storage time (Tanada-Palmu and Grosso 2005).

Total phenolic and flavonoids The results showed a significant difference of total phenolic and flavonoids content in the arils of different pomegranate genotypes immediately after harvest and after 14-day storage at 4 °C (Table 1). In most

Fig. 1 Changes in bacterial (a) and fungal (b) population on aril surface of different pomegranate genotypes after 7- and 14-day storage at 4 °C. The value indicates the mean $\pm$ standard error

Table 1 Changes of TSS, TA, total phenolic and flavonoid content, anthocyanin concentration, and antioxidant activity in the arils of different pomegranate genotypes at harvesting time and after 14-day storage at 4 °C

Genotypes	Storage time (days)	TSS (°Brix)	TA (%)	Total phenolic (mg 100 g ⁻¹ FW)	Total flavonoid (mg 100 g ⁻¹ FW)	Anthocyanin (mg 100 mL ⁻¹)	Antioxidant activity (% DPPHsc)
<i>Malas Torsh Saveh</i>	0	11.7 $\pm$ 0.97 fg	0.19 $\pm$ 0.04 e	568.9 $\pm$ 12.3 ef	62.3 $\pm$ 9.0 f	84.1 $\pm$ 16.7 de	72.5 $\pm$ 4.1 a
	14	12.4 $\pm$ 0.63 d	0.27 $\pm$ 0.02 b	579.5 $\pm$ 12.8 ef	134.0 $\pm$ 13.7 cd	62.3 $\pm$ 17.2 f	65.7 $\pm$ 3.1 de
<i>Malas Yazdi</i>	0	13.5 $\pm$ 0.51 bc	0.27 $\pm$ 0.07 b	699.7 $\pm$ 19.5 ab	127.3 $\pm$ 10.1 cd	113.1 $\pm$ 33.6 b	68.8 $\pm$ 2.9 bc
	14	14.0 $\pm$ 0.56 a	0.23 $\pm$ 0.08 d	652.2 $\pm$ 18.6 c	150.7 $\pm$ 11.3 c	123.9 $\pm$ 23.8 a	69.3 $\pm$ 3.2 bc
<i>Torsh Syabe Lorestan</i>	0	11.8 $\pm$ 0.33 fg	0.39 $\pm$ 0.05 a	713.1 $\pm$ 26.4 a	174.3 $\pm$ 12.4 abc	53.9 $\pm$ 27.6 fg	69.3 $\pm$ 5 bc
	14	8.7 $\pm$ 0.41 h	0.38 $\pm$ 0.12 a	635.5 $\pm$ 24.6 c	200.7 $\pm$ 13.2 a	80.5 $\pm$ 10.5 de	67.9 $\pm$ 4.8 de
<i>Malas Siyabe Lorestan</i>	0	12.2 $\pm$ 0.11 de	0.17 $\pm$ 0.01 ef	701.7 $\pm$ 28.3 ab	37.3 $\pm$ 3.4 h	102.3 $\pm$ 8.3 c	69.1 $\pm$ 5.3 bc
	14	12.5 $\pm$ 0.28 d	0.27 $\pm$ 0.01 b	700.6 $\pm$ 27.1 ab	127.3 $\pm$ 11.5 cd	89.0 $\pm$ 4.7 d	64.8 $\pm$ 4.6 e
<i>Naderi Kashan</i>	0	13.9 $\pm$ 0.41 b	0.19 $\pm$ 0.04 e	640.6 $\pm$ 20.7 c	42.3 $\pm$ 3.8 fg	87.3 $\pm$ 14.4 d	71.9 $\pm$ 5.9 a
	14	13.5 $\pm$ 0.48 bc	0.26 $\pm$ 0.01 bc	609.7 $\pm$ 18.9 cd	99.0 $\pm$ 9.2 e	63.9 $\pm$ 14.5 f	65.3 $\pm$ 5.2 de

Values followed by column with same letters are not significantly different at $P < 0.01$ according to the Tukey test. The value indicates the mean $\pm$ standard error

pomegranate genotypes, total phenolic content slightly decreased at the end of cold storage, except for *Malas Torsh Saveh*. The highest total phenolic content was observed by *Malas Syabe Lorestan* (700.62 mg 100 g⁻¹ FW) at the end of storage.

Genetics plays an important role in controlling fruit phenolic compound synthesis; and distribution of phenolic compounds between the different genotypes is significantly different. Previous study of Ghasemnezhad et al. (2013) showed that concentration of total phenolics of both chitosan-coated and uncoated pomegranate arils decreased significantly during cold storage. The decrease in phenolic compounds might be due to cell structural breakdown as part of senescence during storage and depends on the type of genotype (Lata et al. 2009). In addition, the reduction of phenols might be due to oxidation of phenolic compounds accelerates with PPO and POD activities (Gil et al. 1996).

In contrast, total flavonoids content of the arils increased significantly over storage time, independently of genotypes (Table 1). These results are consistent with Harbaum et al. (2008), who indicated a significant increase of flavonoid concentration in the pakchoi variety Xue Li Hong, during cold storage. However, the amount of flavonoids depends on many factors such as genotype, environmental conditions, production methods, transportation, and handling system of fruit (Vieira et al. 2009). Similar results were obtained by Tudela et al. (2002), who found that in potato, fresh-cutting induced the biosynthesis of flavonoids. In general, phenylpropanoid pathway for the conversion of the amino acid L-phenylalanine to trans-cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL) and the subsequent reactions producing new compounds such as flavonoids are well known (Tomás-Barberán et al. 1997a,b). PAL activity can be stimulated by different stimulants such as wounding and low temperatures. Therefore, it seems that increasing in total flavonoids content may be due to the increasing in PAL activity during cold storage of arils.

Anthocyanin content Total anthocyanin content of pomegranate arils was determined immediately extraction and after 14-day cold storage (Table 1). The results indicated that anthocyanin content of arils decreased significantly at the end of storage, except for *Torsh Syabe Lorestan* and *Malas Yazdi*. In these genotypes, anthocyanin content increased after 14-day storage. At the end of storage time, the highest anthocyanin content was observed in *Malas Yazdi* (123.97 mg 100 mL⁻¹). As mentioned previously by Ghasemnezhad et al. (2013), along extension of storage time, total anthocyanin content decreased in pomegranate arils. Anthocyanin degradation may be due to damage to pomegranate arils during the fruit peeling process. Juice leakage probably occurs through wounding of arils and is followed by decreased levels of anthocyanins (Gil et al. 1996). Moreover, it seems that

anthocyanin degradation during senescence may be due to oxidative processes. Increases in POD activity can lead to a reduction of color (Yuan et al. 2010). In contrast, Kalt et al. (1999) reported that during storage of fresh small fruits such as strawberries and raspberries, anthocyanin content increased. Increase in anthocyanin content during storage may be related to increase in water loss or continued pigment biosynthesis after harvest (Amarowicz et al. 2009). However, another factor that caused the difference in anthocyanins content in pomegranate genotypes maybe the variable activity of PAL during senescence; this is the main enzyme to synthesis of phenolic compounds such as anthocyanins (Sakihama et al. 2002).

Antioxidant activity The results showed a significant reduction of antioxidant activity in the most genotypes, except for *Malas Yazdi* at the end of storage (Table 1). This result is in agreement with Ghasemnezhad et al. (2013), who reported that the antioxidant activity of both chitosan-coated and uncoated pomegranate arils decreased significantly over cold storage time. But Lopez et al. (2005) found that the antioxidant activity of pomegranate arils did not significantly change during cold storage. Previous studies have shown that there is a positive correlation between antioxidant activity and total phenolic content (Rapisarda et al. 1999). Therefore, as this study showed, reduction of antioxidant capacity could be attributed to reduction of total phenolic content. Labbe et al. (2010) showed a significant difference in antioxidant capacity of pomegranate genotypes, with decline during storage time. Genetic characteristics and environmental conditions usually affect antioxidant capacity (Vieira et al. 2009).

Phenolic compositions The flavonoids content such as quercetin, catechin, and cyanidin-3-glucoside after 14-day storage were depended on the pomegranate genotypes (Table 2). The *Naderi Kashan* and *Malas Torsh Saveh* genotypes showed the highest quercetin content, but the highest catechin content was observed in *Malas Yazdi* (94.76 µg mL⁻¹). For cyanidin-3-glucoside, *Malas Torsh Saveh* and *Torsh Syabe Lorestan* showed the lowest content but there was no significant difference between *Malas Yazdi*, *Malas Syabe Lorestan*, and *Naderi Kashan* genotypes for it.

Previous results demonstrated that climate and environmental factors such as light and temperature, geographical conditions, and different genetic factors are important in the synthesis of quercetin compounds. Levels of quercetin and cyanidin-3-glucoside are highly correlated. Genetically, any factor that causes expression of genes leading to anthocyanin synthesis, also results in the expression of genes necessary for quercetin synthesis (Bakhshi and Arakawa 2006). Moreover, Lata et al. (2009) indicated that there was a significant difference between different apple genotypes for catechin. Our results showed that addition of more cyanidin-3-glucoside to

Table 2 Changes of quercetin, catechin, and cyanidin-3-glucoside content in the pomegranate arils in different genotypes after 14-day storage at 4 °C

Genotypes	Quercetin ($\mu\text{g mL}^{-1}$)	Catechin ($\mu\text{g mL}^{-1}$)	Cyanidin-3-glucoside ($\mu\text{g mL}^{-1}$)
<i>Malas Torsh Saveh</i>	22.37 $\pm$ 3.05 ab	45.52 $\pm$ 7.06 c	118.4 $\pm$ 25.0 b
<i>Malas Yazdi</i>	20.20 $\pm$ 2.6 b	94.76 $\pm$ 12.7 a	382.3 $\pm$ 37.2 ab
<i>Torsh Syabe Lorestan</i>	6.47 $\pm$ 0.28 c	79.65 $\pm$ 10.5 b	197.7 $\pm$ 29.6 b
<i>Malas Syabe Lorestan</i>	9.83 $\pm$ 0.53 c	77.12 $\pm$ 7.8 b	590.8 $\pm$ 48.3 a
<i>Naderi Kashan</i>	27.66 $\pm$ 3.7 a	77.07 $\pm$ 6.5 b	410.5 $\pm$ 39.3 ab

Values followed by column with same letter are not significantly different at $P < 0.01$ according to the Tukey test. The value indicates the mean $\pm$ standard error

Malas Syabe Lorestan and *Malas Yazdi* leads to higher levels of total anthocyanin too. Therefore, these genotypes could be more appropriate in the minimal processing of pomegranate, due to high anthocyanin content and the role of this antioxidant in red aril color.

PPO activity Pyrogallol, as a monophenolic substrate and pyrocatechol and dopamine hydrochlorite, as diphenolic substrates, were assayed and their activity were compared in different pomegranate genotypes arils.

Monophenolase activity by pyrogallol substrate There was a significant difference in monophenolase activity among pomegranate genotypes immediately after arils extraction and over 14-day cold storage (Fig. 2a). The change of monophenolase activity during storage time was dependent on pomegranate genotypes. Monophenolase activity increased significantly during first week of storage, except for *Malas Torsh Saveh*; and thereafter declined. The lowest monophenolase activity was found in *Malas Syabe Lorestan*. In contrast, *Malas Yazdi* showed the highest PPO activity at the end of storage, when pyrogallol was used as a substrate (Fig. 2a).

The monophenolase activity of PPO is generally considered as the first step in enzymatic browning, as it catalyzes the hydroxylation of monophenols to *o*-diphenols (Sánchez-Ferrer et al. 1995). Previous finding of Ghasemnezhad et al. (2013) demonstrated that pomegranate arils treated with chitosan showed the lower monophenolase activity, with the higher anthocyanin content after 12-day cold storage. Lower monophenolase activity can be interpreted with inhibition of anthocyanins degradation and enzymatic browning (Ghasemnezhad et al. 2013). In this study, we found that the correlation between higher monophenolase activities with anthocyanin degradation completely depends on genotypes. *Malas Yazdi* with the highest PPO activity with pyrogallol as a substrate showed the highest anthocyanin concentration at the end of storage (Table 1).

Diphenolase activity by pyrocatechol and dopamine hydrochloride The diphenolase activity of PPO enzyme was

assayed by two substrates, pyrocatechol and dopamine hydrochloride (Fig. 2b and c). In general, diphenolase activity of PPO with pyrocatechol substrate was higher than when dopamine hydrochloride was used as substrate. Jang and Moon (2011) used different substrates to assay the polyphenol oxidase and peroxidase activities in different apple genotypes and demonstrated that diphenolase activity of PPO was higher than monophenolase activity.

There was a significant difference for diphenolase activity of PPO with pyrocatechol substrate in different genotypes (Fig. 2b). PPO activity increased gradually during storage time independently of genotypes, although in *Malas Yazdi*, its activity decreased at the first week and then increased at the end of storage time. Immediately after arils extraction, the highest PPO activity with pyrocatechol substrate was found in *Malas Yazdi*, but at the end of storage, *Torsh Syabe Lorestan* showed the highest activity and *Naderi Kashan* showed the lowest one (Fig. 2b). In contrast, PPO activity with dopamine hydrochloride as a substrate gradually decreased during storage in all genotypes, except for *Malas Torsh Saveh*. In this genotype, diphenolase activity increased at the first week of storage and thereafter declined to the end of storage. Moreover, *Torsh Syabe Lorestan* showed the lowest enzyme activity over storage periods (Fig. 2c).

Ghasemnezhad et al. (2013) demonstrated that PPOs play an important role in oxidation and degradation of anthocyanins in pomegranate arils. Therefore, inactivation of PPO by processing may prevent PPO-catalyzed anthocyanin oxidation in pomegranate arils (Vidhan et al. 2010). The difference in the browning reactions of different genotypes could be related to the different sugar and amino acids content in fruits (Ashoor and Zent 1984). Moreover, the response of genotypes to browning reactions depends on the amount of phenolic compounds, concentration of inhibitors of PPO activity (Labbe et al. 2010). Therefore, one of the most important aims regarding marketing is maintenance of color transparency of pomegranate arils, it is necessary to identify genotypes that show less color change during storage (Gil et al. 1996). This study showed that over storage time, anthocyanin accumulated in arils of *Torsh Syabe Lorestan* genotype had the lowest PPO activity when dopamine hydrochloride used as substrate.

Fig. 2 Changes in PPO activity assayed by pyrogallol (a), pyrocatechol (b), and dopamine hydrochloride (c) in the arils of different pomegranate genotypes during 14-day storage at 4 °C. The value indicates the mean $\pm$ standard error

In contrast, *Malas Yazdi* with the highest diphenolase activity showed the maximum anthocyanin degradation (26 %) as compared to other genotypes.

POD activity assay POD activity of arils in five pomegranate genotypes are presented in Fig. 3. The results showed a

significant difference of POD activity in the arils of different genotypes immediately after extraction and at the end of storage. POD activity decreased at the end of storage time almost at the all pomegranate genotypes. The lowest POD activity at the both harvesting time and over storage time was found in *Torsh Syabe Lorestan* (Fig. 3). The levels of

Fig. 3 Changes in POD activity in the arils of different pomegranate genotypes during 14-day storage at 4 °C. The value indicates the mean $\pm$ standard error

hydrogen peroxide, genetic characteristics, and environmental conditions, harvesting time, and extraction conditions are important factors which affect activity of POD enzyme. Significant increasing in POD activity is associated with the degradation of anthocyanins (Zhang et al. 2005). In this study, we found that *Torsh Syabe Lorestan* pomegranate genotype showed the lowest POD activity followed by the highest anthocyanin content at the end of cold storage.

Conclusions

The present study showed that there was a significant difference for microbial count on the extracted arils of different Iranian pomegranate genotypes. *Torsh Syabe Lorestan* had shown the lowest growth in fungi and bacteria population over 14-day cold storage. Because of less need for chemicals, this genotype can be more appropriate for minimal processing technology. The changes in physiological and biochemical properties of the pomegranate arils over cold storage are highly dependent on genotypes. Overall, we suggest that the use of resistant genotypes is the most consistent way for maintaining quality and controlling decay of pomegranate extracted arils.

Acknowledgments The authors would like to thank the University of Guilan, Rasht, Iran, for funding this research.

References

Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, Maiani G, Piskula MK (2009) Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods: a review. *Mol Nutr Food Res* 53:151–183
 AOAC (2000) *Microbiological Methods*, 17th ed., vol. I. Official Methods of Analysis of AOAC International, USA

Artes F, Tudela JA, Villaescusa R (2000) Thermal postharvest treatments for improving pomegranate quality and shelf life. *Postharvest Biol Technol* 18:245–251
 Asgari SM, Shahedy M (1996) Effect of physical and chemical factors on the weight losing and pomegranate antioxidants. *Iranian food Sci Technol Abstract* 118
 Ashoor SH, Zent JB (1984) Maillard browning in common amino acids and sugars. *J Food Sci* 49:1206–1207
 Bakhshi D, Arakawa O (2006) Induction of phenolic compound biosynthesis with light irradiation in the flesh of red and yellow apples. *J Appl Hort* 8(2):101–104
 Banjongsinsiri P, Kenney S, Wicker L (2004) Texture and distribution of pectic substances of mango as affected by infusion of pectinmethylesterase and calcium. *J Sci Food Agric* 84:1493–1499
 Blasa M, Candiracci M, Accorsi A, Piacentini PM, Albertini MC, Piatti E (2005) Raw millefiori honey is packed full of antioxidants. *Food Chem* 97:217–222
 Caleb OJ, Opara UL, Witthuhn CR (2012) Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food Bioprocess Technol* 5(1):15–30
 Espin JC, Morales M, Varon R, Tudela J, Garcia-Canovas F (1995) A continuous spectrophotometric method for determining the monophenolase and diphenolase activities of apple polyphenol oxidase. *Anal Biochem* 231(1):237–246
 Ghasemnezhad M, Sherafati M, Peyvast A (2011) Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *J Functional Foods* 3(1):44–49
 Ghasemnezhad M, Zareh S, Rassa M, Sajedi RH (2013) Effect of chitosan coating on maintenance of aril quality, microbial population and PPO activity of pomegranate (*Punica granatum* L. cv. Tarom) at cold storage temperature. *J Sci Food Agric* 93(2):368–374
 Gil M, Martínez A, Artes F (1996) Minimally processed pomegranate seeds. *LWT-Food Sci Technol* 29:708–713
 Harbaum B, Hubbermann EM, Zhu Z, Schwarz K (2008) Free and bound phenolic compounds in leaves of pakchoi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) and Chinese leaf mustard (*Brassica juncea* Coss). *Food Chem* 110:838–846
 Holland D, Hatiband K, Bar-Yaakov I et al (2009) Pomegranate: botany, horticulture, breeding, vol 35, Horticultural Reviews. John Wiley & Son, New Jersey
 In BC, Motomura S, Inamoto K, Doi M, Mori G (2007) Multivariate analysis of relation between preharvest environmental factors, post-harvest morphological and physiological factors and vase life of cut Asomi Red Roses. *Jap Soc Hortic Sci* 76:66–72
 Jang JH, Moon K (2011) Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. *Food Chem* 124:444–449
 Kalt W, Fomey CF, Martin A, Prior RL (1999) Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J Agric Food Chem* 47:4638–4644
 Labbe M, Alvaro P, Carmen S (2010) Antioxidant capacity and phenolic composition of juices from pomegranates stored in refrigeration. *International Conference on Food Innovation- Food innova. Valencia (Spain)*, pp 430
 Lako J, Trenerry VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R (2007) Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem* 101:1727–1741
 Lata B, Trampczynska A, Paczesna J (2009) Cultivar variation in apple peel and whole fruit phenolic composition. *Sci Hortic* 121:171–186
 Lopez V, Conesa A, Artes F (2005) Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biol Technol* 37:174–185

- Mazza G, Miniati E (1993) Anthocyanins in fruits, vegetable and grains. In: G. Mazza and E. Miniati (eds.). CRC Press, Boca Raton, Fla, pp 1–362
- Mehmet O (2002) Degradation of anthocyanins in sour cherry and pomegranate juices by hydrogen peroxide in the presence of added ascorbic acid. *Food Chem* 78:499–504
- Muharrem E, Nazan E (2009) Maintaining quality of minimally processed pomegranate arils by honey treatments. *Brit Food J* 111:396–406
- Narpinder S, Randhir S, Kulwinder K, Harmit S (1999) Studies of the physico-chemical properties and polyphenoloxidase activity in seeds from hybrid sunflower (*Helianthus annuus*) varieties grown in India. *Food Chem* 66:241–247
- Perez-Gago MB, Serra M, del Rio MA (2006) Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biol Technol* 39:84–92
- Rapisarda P, Tomaino A, Lo Cascio R, Bonina F, De Pasquale A, Saija A (1999) Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J Agric Food Chem* 47:4718–4723
- Raybaudi-Massilia RM, Mosqueda-Melgar J, Sobrino-López A, Soliva-Fortuny R, Martín-Belloso O (2007) Shelf-life extension of fresh-cut “Fuji” apples at different ripeness stages using natural substances. *Postharvest Biol Technol* 45:265–275
- Rojas-Graü MA, Raybaudi-Massilia RM, Soliva-Fortuny RC, Avena-Bustillos RJ, McHugh TH, Martín-Belloso O (2007) Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biol Technol* 45:254–264
- Safa M, Khazaei J (2003) Determining and modeling some physical properties of pomegranate fruits of Saveh area, Iran, related to peeling and packaging. In: International Congress on Information Technology in Agriculture, Food and Environment, Izmir, Turkey, 7–10 October, pp 331–337
- Sakihama Y, Michael F, Cohen S, Grace C, Hideo Y (2002) Plant phenolic antioxidant and prooxidant activities: phenolics- induced oxidative damage mediated by metals in plants. *Toxic* 177:67–80
- Sánchez-Ferrer Á, Rodríguez-López JN, García-Cánovas F, García-Carmona F (1995) Tyrosinase: a comprehensive review of its mechanism. *Biochim Biophys Acta* 1247(1):1–11
- Shiri MA, Ghasemnezhad M, Bakhshi D, Dadi M (2011) Changes in phenolic compounds and antioxidant capacity of fresh-cut table grape (*Vitis vinifera*) cultivar ‘Shahaneh’ as influenced by fruit preparation methods and packaging. *Aust J Crop Sci* 5(12):1515–1520
- Soliva-Fortuny RC, Martín-Belloso O (2003) New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends Food Sci Technol* 14:341–353
- Tanada-Palmu PS, Grosso CR (2005) Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. *Postharvest Biol Technol* 36:199–208
- Tomás-Barberán FA, Gil MI, Castañer M, Artés F, Saltveit ME (1997a) Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. *J Agric Food Chem* 45:583–589
- Tomás-Barberán FA, Loaiza-Velarde J, Bonfanti A, Saltveit ME (1997b) Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *J Am Soc Hortic Sci* 122:399–404
- Tudela JA, Cantos E, Espin JC, Tomas-Barberan FA, Gil MI (2002) Induction of antioxidant flavonol biosynthesis in fresh-cut, potatoes. Effect of domestic cooking. *J Agric Food Chem* 50(21):5925–5931
- Valero A, Carrasco E, García-Gimeno RM (2012) Principles and methodologies for the determination of shelf-life in foods. In: EissaAA (ed.), Trends in Vital Food and Control Engineering. In Tech, Rijeka, Croatia, pp 290
- Varasteh F, Arzani K, Barzegar M, Zamani Z (2012) Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punicagranatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage. *Food Chem* 130:267–272
- Vidhan J, Ara D, John RP (2010) Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punicagranatum* L.). *Food Chem* 118:11–16
- Vieira FG, Borges GS, Copetti C, Gonzaga LV, Nunes EC, Fett R (2009) Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Arch Latinoam Nutr* 59(1):101–106
- Yuan GB, Sun J, Wang Q (2010) Effect of 1-methylcyclopropene on shelf life, visual quality, antioxidant enzymes and health-promoting compounds in broccoli florets. *Food Chem* 118:774–781
- Zhang Z, Pang X, Xuewu D, Ji Z, Jiang Y (2005) Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chem* 90:47–52