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An Evaluation of the Phytochemical Properties of Some Pomegranate Cultivars during Fruit Development and Ripening

Hossein Meighani[1*], Mahmood Ghasemnezhad[2], Davood Bakhshi[2]

1. Assistant Professor, Department of Horticultural Science, Faculty of Agriculture, University of Jiroft, Jirot, Iran.

2. Associate Professor, Department of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran.

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Abstract

The pomegranate fruit is a good source of bioactive compounds. The present study has investigated the biochemical and sensory characteristics of the arils of four Iranian commercial pomegranate cultivars namely Malase Shirine Saveh (MSS), Malase Torshe Saveh (MTS), Alak Shirine Saveh (ASS) and Agha Mohammad Ali (AMA) for several developmental and ripening stages during 45–180 days after fruit set (DAFS). The results showed that the total soluble solids (TSS), individual and total anthocyanin concentrations, and color parameters including chroma and a* values significantly increased, in contrast, the total phenolic concentration and color parameters (L* and hue angle) gradually decreased during developmental stages. Six anthocyanin pigments were found responsible for the red color of pomegranate juice. The quantity and the quality of the anthocyanin pigments were different among the cultivars and the various developmental stages. The predominant anthocyanin pigment at all developmental stages in all cultivars was cyanidin 3,5-diglucosides. The maximum total phenolic concentration was recorded at 45 DAFS for all cultivars. The highest antioxidant activity was recorded at 45 DAFS, and gradually decreased until 135 DAFS. Similar decrease was observed in total phenolic and flavonoid concentrations. Notably, increasing of antioxidant activity at the late-developmental stage was due to induction of the flavonoids and anthocyanins accumulation.

Keywords: Anthocyanins, Antioxidant activity, Color parameters, Phenolic compounds, Pomegranate.

Abbreviations: AMA, Agha Mohammad Ali; **ASS**, Alak Shirine Saveh; **AA**, Antioxidant activity; **C**, Chroma; **DAFS**, Days after fruit set; **GAE**, Gallic acid equivalents; **h°**, Hue angle; **MSS**, Malase Shirine Saveh; **MTS**, Malase Torshe Saveh; **TA**, Titratable acidity; **TAC**, Total anthocyanin concentration; **TFC**, Total flavonoid concentration; **TPC**, Total phenolic concentration; **TSS**, Total soluble solids.

Introduction

Pomegranate (*Punica granatum* L.) is a tropical and subtropical deciduous or evergreen shrub belonging to the Punicaceae family. However, It is natively grows in Iran, it has been domesticated to the Himalayas region in northern India (Holland et al., 2009), and cultivated in countries such as Iran, India, Egypt, China, Israel, Tunisia,

*Corresponding Author, Email: hmeighani@ujiroft.ac.ir

Syria, Lebanon, Turkey, Greece, Cyprus, Italy, France, Spain, Chile, Portugal, USA, Oman, and South Africa (Fawole and Opara, 2013). Iran is one of the main pomegranate-producing countries in the world. Although Iran's total pomegranate production fluctuates annually, annual production of pomegranate has been recorded as 10,866,300 tons (Anonymous, 2015).

In recent years, the popularity of pomegranate fruit has increased due to its medicinal properties and nutritional benefit for human health. The edible part of the fruit (aril) contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (Al-Maiman and Ahmad, 2002). The beneficial effects of pomegranate juice are attributed to the high level of antioxidant activity (Gil et al., 2000) that could be due to the high level of phenolic compounds and sugar-containing polyphenolic tannins and anthocyanins (Çam et al., 2009; Gil et al., 2000). Phenolic compounds are among the most important groups of secondary metabolites in fruits, as they are responsible for color, astringency, bitterness, flavor, and nutritional qualities in fruits and vegetables (Kalaycıoglu and Erim, 2017).

The red color of the pomegranate fruit is considered as one of the most appreciated marketable attributes by consumers. The red color of pomegranate juice depends on the concentration and the type of anthocyanins available in the fruit. Six anthocyanin pigments such as delphinidin, cyanidin, and pelargonidin 3-glucosides and 3, 5-diglucosides are responsible for the red color of pomegranate juice and peel (Gil et al., 2000; Miguel et al., 2004; Alighourchi et al., 2008).

Various biochemical changes occur during the maturation and ripening of the pomegranate fruit. Therefore, to obtain the highest quality and nutritional value, it is very important to harvest the pomegranate fruits at maturity stage. Early harvesting of the fruit causes the depletion of traits such as color, taste, and aroma, and the late harvested fruit is associated with reduced shelf life and grater susceptibility to physiological disorders and pathogens during storage (Schwartz et al., 2009). Pomegranate is a non-climacteric fruit with a low respiration rate, and therefore the fruits should be harvested at the fully ripening stage (Kulkarni and Aradhya, 2005). The biochemical changes during fruit development and ripening have been studied by many researchers (Kulkarni and Aradhya, 2005; Schwartz et al., 2009; Fawole; and Opara, 2013); however, few studies on this regard have been focused on Iranian cultivars. Therefore, due to differences among cultivars, growing region, climates, cultural practices, and degree of fruit maturity on fruit biochemical composition, further studies are required.

The main objective of this study was to characterize the important internal quality attributes of four pomegranate cultivars during different stages of development and maturation. In addition, the evaluated fruit characteristics were compared in order to determine which cultivars have the best quality features as well as to determine the best harvest time.

Materials and methods

Plant material and pomegranate juice preparation

Four commercial pomegranate cultivars Malase Torshe Saveh (MTS), Malase Shirine Saveh (MSS), Alake Shirine Saveh (ASS) and Agha Mohammad Ali (AMA) grown on the Agricultural Research Center of Saveh Markazi province, Iran (34˚99′ N, 50˚15′ E and 962m above sea level) were used for this study during the 2014 growing season. The trees used for this study were eight years old with planting distances of 4 m and 2.5 m between and along the rows, respectively. Nine trees of each cultivar were selected for this study. The trees received routine cultural practices suitable for commercial fruit production including winter and summer pruning, fertilization, and irrigation. Two

fruits per tree were randomly harvested at different stages of development and maturation, 45, 75, 105, 135, 150, 165, and 180 days after fruit set, and they were immediately transferred to the horticultural laboratory, University of Guilan, Iran. The arils were manually separated and used for color parameter measurement or squeezed by using a garlic press. The aril juice was centrifuged (1252×g for 15 min at 4˚C) and used for biochemical composition and antioxidant activity or stored in −80˚C for anthocyanin analysis.

Determination of aril color

The color of the pomegranate arils was determined by using a colorimeter (Chroma Meter CR-400, Minolta, Japan) and expressed as L*, a*, b*, chroma (C), and hue angle (H˚) color values. L* indicates lightness, taking values within the range 0–100 (black–white, respectively), and a* and b* are the chromatic coordinates, green–red and blue–yellow coordinates, respectively. a* expresses positives values for reddish colors and negative values for the greenish of the samples, whereas b* express positive values for yellowish colors and negative values for bluish of the samples. Chroma (C) and hue angle (h°) were calculated according to the following equations:
$C= (a^{*2} + b^{*2})^{1/2}$ and $h°= \tan^{-1} (b^*/a^*)$.

Total soluble solids (TSS), titratable acidity (TA), and pH

The measurements were made on fresh aril juice. TSS was measured by using a digital refractometer (Euromex RD6535, Holland), and the results were expressed as ˚Brix at room temperature. The pH of the juice was directly determined at room temperature by using a pH meter (WTW 526, Germany). TA was determined with 0.1 N NaOH to a pH 8.2 endpoint, using 5 mL of diluted juice in 35 mL distilled water, and the results were calculated as a percentage of citric acid concentration.

Total phenolic and flavonoid concentrations

The total phenolic concentration was determined by using of the Folin–Ciocalteu method (Brand-Williams et al., 1995) with a slight modification in a UV-Vis spectrophotometer ($T80^+$, PG instruments Ltd). Briefly, 300 µl of diluted pomegranate juice in the ratio of 1:10 and with methanol:water (6:4) was mixed with 1.5 ml of diluted Folin–Ciocalteu reagent (1:10 in distilled water) and a short vortex of 1.2 ml of 7.5% sodium carbonate was done and samples were incubated for 90 min at room temperature. The absorption at 760 nm was measured, and the results were expressed as gallic acid equivalents (GAE) per 100 ml juice (mg GAE/100 mL of juice).

The total flavonoid concentrations were determined spectrophotometrically according to Park et al. (2008). Catechin was used as a standard. The flavonoid concentrations were expressed as mg catechin equivalents (RE) per 100 mL of juice.

Antioxidant activity (AA)

Antioxidant activity was measured by using the 2,2 diphenyl-1-pic-rylhydrazyl (DPPH) radical-scavenging method described by Brand-Williams et al. (1995) with slight modification. 50 µL of juice diluted in the ratio of 1:20 with methanol:water (6:4) was mixed with 0.1 mol L^{-1} DPPH in methanol. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517 nm by using a UV-Vis spectrophotometer. For each sample, three separate determinations were recorded. Antioxidant activity was measured as the percentage decline in absorbance relative to the control, corresponding to the percentage of DPPH scavenged (% DPPHsc), which was calculated as:

$$\%DPPHsc = [(Acontrol - Asample)/Acontrol] \times 100$$

HPLC analysis of anthocyanins

The HPLC analysis and the individual anthocyanin identification and quantification were performed as reported

previously (Gil et al., 1995). The juice pomegranate was centrifuged at 7,829 g and filtered through a 0.45 µm filter. Next, 10 µL of clarified juice was injected into a Unicam Crystal 200 HPLC system equipped with a UV-Visible detector (Waters model 2487). A column bondapak C18 from Waters was used for the separation. The elution was carried out at room temperature by using 5% formic acid aqueous solution (A) and methanol (B) in a linear gradient from 15% to 35% B at 15 min, followed by an isocratic run until 20 min. The flow rate was 1 mL min^{-1} with the UV-Vis detector at 510 nm (Miguel et al., 2004). Anthocyanins were identified by comparing their retention times with those of the pure standards.

Statistical analysis

Experiment was designed as a split plot in time with a randomized complete block design (factor A: cultivar and factor B: ripening stage). Three replicates were considered for each analysis and each replicate indicating six pomegranate fruits (two fruits per tree). Data for analytical determinations were subjected to analysis of variance (ANOVA). A mean comparison using the Duncan's Multiple Range Test (DMRT) at the 5% level was performed using SAS software Version 9.1.

Results

Color parameters

Fruit peel and aril pigmentation play important role in defining pomegranates fruit quality. There were significant differences ($p<0.05$) in color parameters L*, a*, chroma, and hue angle between the cultivars and maturation stages. The L* value that indicates lightness progressively reduced in the four cultivars during development and ripening. No differences were observed among MSS, MTS, and ASS from 75 DAFS up to the harvest time. The greatest L* value at harvest time was related to the AMA cultivar with the mean of 36.9. The most notable changes were detected in a* value, which increased persistently in the four cultivars by ripening. This is in consistent with the replacement of the white color with the red color, which increased during development. The greatest changes occurred between 45–75 DAFS for MSS and ASS cultivars, whereas it was 75–105 DAFS for AMA and MTS cultivars. The level of a* in the AMA arils was clearly less than in the other cultivars. At the maturity stage, the highest and lowest values for a* were recorded in the ASS and AMA cultivars with means of 25.4 and 2.7, respectively.

The results of the analysis of variance showed that there were no differences in a* value between MSS and MTS pomegranate cultivars during 75 to 180 DAFS. The C* values, which indicated purity or intensity, gradually increased from 105 DAFS until the full maturity stage in MSS and MTS cultivars. In the ASS cultivar, the C* value constantly increased up to the full maturation stage, but without differences up to 130 DAFS. In contrast to others, the C* value in the AMA cultivar reduced until the maturity stage (165 DAFS). The highest level of C* was recorded in ASS with a mean of 27.73 at the last sampling date, and the lowest value was found in MSS with a mean of 14.58 at 105 DAFS. The hue angle (h°) value gradually decreased by development of maturity in all cultivars, as the aril color changed from white to red. The maximum and minimum decreases rate between the initial and full maturity stages was related to AMA (73.4%) and ASS (12.5%), respectively (Table 1).

TSS, TA, and pH

The results of TSS, TA, and pH of the four different pomegranate cultivars and the stages of maturation and ripening are shown in Table 1. The TSS of the juice increased up to the maturity stage in the four cultivars (Table 1). At full maturity stage, the highest TSS was 17.82 (°Brix) for MTS, followed by 17.6, 17.4, and 16.7 (°Brix) for the MSS, ASS, and AMA

cultivars, respectively. No significant difference was observed among the cultivars from 105 DAFS till the full maturity stage (Table 2).

The pattern of changes in the juice TA for the four cultivars studied was similar. The TA rapidly increased from 45 up to 75 DAFS, and thereafter gradually reduced till the maturity stage. At 75 DAFS, the highest and lowest increments of 104% and 9% were related to the MTS and AMA cultivars, respectively. TA Level was remarkably higher in MTS cultivar in comparison with other cultivars throughout the development process of the fruits, (Table 2).

The pH values of unripe pomegranate juice decreased till 75 DAFS for all the studied cultivars. The greatest reduction at this stage was observed for MTS (39%). An increase in pH was observed among MSS, MTS, and ASS cultivars during maturity stage. This increase in the AMA cultivar was not different from the previous sampling date. In addition, no differences were observed from 105 DAFS till the maturity stage among the cultivars, except for MTS. The minimum pH value at the maturity stage among all the studied cultivars was belong to the MTS cultivar (Table 2).

Table 1. Changes in the arils color parameters among four pomegranate cultivars at different developmental stages.

Traits	Cultivar	Sampling date (days after fruit set)						
		45	75	105	135	150	165	180
L*	Malase Shirine Saveh	38.9 ^{Ca}	38.7 ^{Ca}	32.8 ^{Bb}	29.8 ^{Bbc}	26.4 ^{Bcd}	24.0 ^{BCde}	21.5 ^{Be}
	Malase Torshe Saveh	46.2 ^{ABa}	44.7 ^{ABa}	37.6 ^{Bb}	29.8 ^{Bc}	27.5 ^{Bc}	26.3 ^{Bcd}	22.0 ^{Bd}
	Alake Shirine Saveh	40.7 ^{BCa}	39.1 ^{BCa}	34.2 ^{Bb}	30.6 ^{Bbc}	27.5 ^{Bc}	22.2 ^{Cd}	22.0 ^{Bd}
	Agha Mohammad Ali	51.6 ^{Aa}	47.1 ^{Aab}	43.8 ^{Abc}	39.1 ^{Acd}	37.2 ^{Ad}	36.9 ^{Ad}	36.9 ^{Ad}
a*	Malase Shirine Saveh	-0.3 ^{Bf}	3.2 ^{Be}	4.5 ^{Be}	8.8 ^{Bd}	11.0 ^{Bc}	15.4 ^{Bb}	21.8 ^{Ba}
	Malase Torshe Saveh	0.1 ^{Af}	1.3 ^{Cf}	3.4 ^{Be}	9.0 ^{Bd}	13.2 ^{Bc}	17.2 ^{Bb}	23.1 ^{ABa}
	Alake Shirine Saveh	0.5 ^{Ae}	6.5 ^{Ad}	9.5 ^{Ac}	11.5 ^{Ac}	17.2 ^{Ab}	25.2 ^{Aa}	25.4 ^{Aa}
	Agha Mohammad Ali	-0.5 ^{Cd}	-0.1 ^{Dd}	1.1 ^{Cc}	1.2 ^{Cc}	2.0 ^{Cb}	2.6 ^{Ca}	2.7 ^{Ca}
Chroma	Malase Shirine Saveh	15.1 ^{Bde}	14.9 ^{Be}	14.6 ^{Ce}	16.2 ^{Acd}	16.9 ^{BCc}	19.3 ^{Bb}	24.7 ^{Ba}
	Malase Torshe Saveh	15.6 ^{Bd}	15.4 ^{Bd}	15.2 ^{BCd}	16.4 ^{Ad}	18.7 ^{Bc}	21.2 ^{Bb}	26.0 ^{ABa}
	Alake Shirine Saveh	14.8 ^{Bc}	15.7 ^{Ac}	16.6 ^{ABc}	17.6 ^{Ac}	21.2 ^{Ab}	27.7 ^{Aa}	27.7 ^{Aa}
	Agha Mohammad Ali	17.9 ^{Aa}	17.9 ^{Aa}	17.9 ^{Aa}	16.4 ^{Ab}	15.8 ^{Cb}	15.4 ^{Cb}	15.4 ^{Cb}
Hue	Malase Shirine Saveh	91.6 ^{Aa}	78.2 ^{Cb}	72.0 ^{Cc}	56.8 ^{Bd}	49.4 ^{Be}	37.1 ^{Bf}	27.8 ^{Bg}
	Malase Torshe Saveh	88.1 ^{Ba}	85.3 ^{Ba}	77.0 ^{Bb}	56.7 ^{Bc}	44.9 ^{Cd}	36.1 ^{Be}	27.4 ^{Af}
	Alake Shirine Saveh	88.3 ^{Ba}	65.5 ^{Db}	55.0 ^{Dc}	49.0 ^{Cd}	35.8 ^{De}	23.7 ^{Cf}	23.5 ^{Cf}
	Agha Mohammad Ali	91.6 ^{Aa}	90.4 ^{Aa}	86.5 ^{Ab}	85.7 ^{Ab}	82.9 ^{Ac}	80.5 ^{Ad}	80.2 ^{Ad}

For each sampling date, values with different capital letters show significant difference ($p<0.05$) among cultivars. For each cultivar, values with different small letters show significant difference ($p<0.05$) during different development stages (n=3).

Table 2. Changes in total soluble solids (TSS), titratable acidity (TA) and pH among four pomegranate cultivars at different maturation and ripening stages.

Traits	Cultivar	Sampling date (days after fruit set)						
		45	75	105	135	150	165	180
TSS (%)	MSS	5.7 ^{ABf}	11.8 ^{Ae}	15.2 ^{Ad}	16.1 ^{Ac}	16.6 ^{Abc}	17.2 ^{Aab}	17.6 ^{Aa}
	MTS	5.7^{ABf}	9.6 ^{Ce}	13.1 ^{BCd}	15.6 ^{Ac}	16.4 ^{Abc}	17.4 ^{Aab}	17.8 ^{Aa}
	ASS	5.3 ^{Be}	11.5 ^{Ad}	13.2 ^{Bc}	15.7 ^{Ab}	16.6 ^{Aab}	17.3 ^{Aa}	17.4 ^{Aa}
	AMA	6.4 ^{Ae}	10.6 ^{Bd}	12.2 ^{Cc}	14.0 ^{Bb}	15.8 ^{Aa}	16.6 ^{Aa}	16.7 ^{Aa}
TA (%)	MSS	0.81 ^{Bb}	1.47 ^{Ba}	0.75 ^{BCc}	0.71 ^{BCc}	0.64 ^{Cd}	0.50 ^{Ce}	0.48 Ce
	MTS	2.39 ^{Ac}	4.90 ^{Aa}	3.30 ^{Ab}	2.14 ^{Abc}	1.96 ^{Ac}	1.61 ^{Ad}	1.44 ^{Ad}
	ASS	0.87 ^{Bc}	1.69 ^{Ba}	0.87 ^{Bb}	0.78 ^{Bbc}	0.75 ^{Bc}	0.62 ^{Bb}	0.63 ^{Bd}
	AMA	0.75 ^{Ccd}	0.82 ^{Ca}	0.66 ^{Cb}	0.59 ^{Cc}	0.52 ^{Ccd}	0.46 ^{Cd}	0.46 ^{Cd}
pH	MSS	4.36 ^{Ba}	3.53 ^{Bc}	3.67 ^{Abc}	3.71 ^{Ab}	3.75 ^{Ab}	3.75 ^{Ab}	3.77 ^{Ab}
	MTS	3.78 ^{Ca}	2.70 ^{Cd}	2.75 ^{Bd}	3.20 ^{Bc}	3.20 ^{Bc}	3.33 ^{Cbc}	3.43 ^{Bb}
	ASS	4.72 ^{Aa}	3.47 ^{Bc}	3.72 ^{Ab}	3.80 ^{Ab}	3.80 ^{Ab}	3.82 ^{ABb}	3.82 ^{Ab}
	AMA	4.44 ^{Ba}	3.70 ^{Ac}	3.79 ^{Abc}	3.86 ^{Abc}	3.87 ^{Ab}	3.90 ^{Ab}	3.90 ^{Ab}

For each sampling date, values with different capital letters show significant difference ($p<0.05$) among cultivars. For each cultivar, values with similar small letters are not significantly different ($p<0.05$) during different development stages (n=3).

Table 3. Biochemical composition and antioxidant activity among four cultivars of pomegranate juice at different maturity stages.

Traits	Cultivar	Sampling date (days after fruit set)						
		45	75	105	135	150	165	180
Total phenolic concentration (mg 100 mL^{-1})	MSS	392.6 ^{Aa}	237.4 ^{Ab}	183.7 ^{Ac}	177.8 ^{Acd}	164.8 ^{Acde}	156.7 ^{Ade}	144.8 ^{Ae}
	MTS	344.4 ^{Ba}	210.7 ^{Bb}	178.5 ^{Ac}	152.6 ^{Bd}	146.7 ^{Ad}	141.5 ^{Bd}	135.2 ^{ABd}
	ASS	223.0 ^{Ca}	181.9 ^{Cb}	167.4 ^{Abc}	159.6 ^{ABbc}	155.6 ^{Ac}	154.0 ^{ABc}	150.4 ^{Ac}
	AMA	251.1 ^{Ca}	159.3 ^{Cb}	135.2 ^{Bc}	123.0 ^{Ccd}	119.6 ^{Bd}	119.1 ^{Cd}	118.5 ^{Bd}
Total flavonoid concentration (mg 100 mL^{-1})	MSS	167.3 ^{Aa}	120.7 ^{Ab}	101.4 ^{Ac}	88.1 ^{Acd}	80.3 ^{Ad}	82.4 ^{ABd}	85.4 ^{Ad}
	MTS	134.8 ^{Ba}	111.2 ^{ABb}	97.4 ^{Ac}	81.8 ^{Ad}	75.0 ^{Ad}	79.9 ^{Bd}	82.5 ^{Ad}
	ASS	117.0 ^{Ca}	94.9 ^{BCb}	88.2 ^{Abc}	85.7 ^{Abc}	83.9 ^{Ac}	90.2 ^{Abc}	90.9 ^{Abc}
	AMA	129.1 ^{BCa}	85.3 ^{Cb}	69.4 ^{Cb}	54.1 ^{Bd}	49.5 ^{Bd}	46.3 ^{Cd}	46.3 ^{Bd}
Total anthocyanin concentration (mg L^{-1})	MSS	0.0 ^{Bf}	9.4 ^{Bef}	24.5 ^{Be}	51.4 ^{Bd}	94.4 ^{Bc}	178.2 ^{Cb}	248.6 ^{Ba}
	MTS	0.0 ^{Be}	5.2 ^{Cf}	14.4 ^{Ce}	34.8 ^{Cd}	108.7 ^{Bc}	201.1 ^{Bd}	266.0 ^{ABa}
	ASS	6.5 ^{Af}	23.7 ^{Ae}	59.4 ^{Ad}	118.8 ^{Ac}	184.9 ^{Ab}	268.8 ^{Aa}	279.6 ^{Aa}
	AMA	0.0 ^{Bd}	0.0 ^{Bd}	1.9 ^{Dcd}	5.3 ^{Dc}	25.0 ^{Cb}	34.4 ^{Da}	35.9 ^{Ca}
Antioxidant activity (%DPPHsc)	MSS	79.5 ^{Aa}	52.0 ^{ABd}	43.2 ^{Bd}	38.4 ^{Bcd}	43.6 ^{Bcd}	48.0 ^{ABbc}	50.6 ^{Ab}
	MTS	75.4 ^{Aa}	46.3 ^{BCbc}	40.0 ^{BCe}	36.2 ^{Bde}	43.7 ^{Bcd}	46.3 ^{Bbc}	49.7 ^{Ab}
	ASS	72.4 ^{ABa}	56.4 ^{Ab}	50.7 ^{Abc}	45.8 ^{Ac}	49.9 ^{Abc}	51.9 ^{Abc}	53.4 ^{Ab}
	AMA	64.0 ^{Ba}	41.6 ^{Cb}	36.1 ^{Cc}	29.9 ^{Cd}	31.5 ^{Ccd}	33.2 ^{Ccd}	34.6 ^{Bcd}

For each sampling date, values with different capital letters show significant different ($p<0.05$) among cultivars. For each cultivar, values different capital letters show significant different ($p<0.05$) during different development stages (n=3).

Total phenolic and flavonoid concentration

The results revealed that the total phenolic concentration (TPC) and the total flavonoid concentration (TFC) changed during fruit development and ripening (Table 3). Pomegranate juice showed a rapid and significant decrease in TPC during the initial stages of fruit development from 45 to 75 days. TPC gradually reduced till maturity stage after 75 days. Overall, there were approximately 63.1%, 59.6%, 32.6%, and 52.4% decreases in TPC for the MSS, MTS, ASS, and AMA cultivars from 45 to 180 DAFS, respectively. The highest and the lowest values of TPC at the maturity stages were recorded in the ASS and AMA cultivars with means of 150.37 and 118.52 mg GAE 100 mL^{-1} juice, respectively.

The change of pattern in TPC and TFC decline during the early stages of fruit development were recorded from 45 to 75 DAFS for the ASS cultivar and until 105 DAFS for the others. This decrease continued till the full maturity stage for AMA and to 150 DAFS for the other cultivars, and slightly increased till the full maturity stage without differences. The highest value of TFC at the full maturity stage was 90.9for ASS, followed by 85.4, 82.5, and 46.3mg RE 100 mL^{-1} juice for MSS, MTS, and AMA, respectively (Table 3).

Antioxidant activity (AA)

The AA of pomegranate juice at different stages of fruit maturation and ripening is shown in Table 3. Unripe fruits at 45 DAFS showed relatively high values of AA in all the cultivars. These values reduced rapidly till 75 DAFS. After 75 days, reduction occurred with lower ratio so that the lowest values were recorded at 135 DAFS. Later, AA increased up to the full maturity stage. At this stage, the highest value was 49.4% ($DPPH_{SC}$) for ASS and the lowest value of 31.6% ($DPPH_{SC}$) was obtained for the AMA cultivar. Overall, the decreases in AA from 45 DAFS to the maturity stage were 50.6%, 41.4%, 39.4%, and 31.8% for the AMA, MTS, MSS, and ASS cultivars, respectively (Table 3).

Anthocyanins

The biosynthesis of individual anthocyanins in pomegranate fruit was measured during different maturity stages. Six different anthocyanins were detected in the juices of all the cultivars, including 3-

glucosides and 3,5-diglucosides of cyanidin, delphinidin, and pelargonidin. However, the values of these anthocyanins were different among the cultivars and the stages of maturation and ripening. Anthocyanin compounds were not detected in MSS and MTS cultivars till 45 DAFS, and till 75 DAFS for the AMA cultivar. At 45 DAFS, cyanidin 3,5-diglucosides, delphinidin 3,5-diglucosides, and delphinidin 3-glucosides were identified in the ASS cultivar and at 75 DAFS cyanidin 3-glucosides was also identified. During fruit development, mono- and di-glucosylated derivatives of pelargonidin were identified gradually. All the six anthocyanin compounds were identified at 135 DAFS for all the cultivars. The abundance of individual anthocyanins at the full maturity stage for all the studied cultivars was in the order of cyanidin 3,5-diglucoside > cyanidin 3-glucoside > delphinidin 3,5-diglucoside > delphinidin 3-glucoside > pelargonidin 3,5-diglucoside > pelargonidin 3-glucoside (Fig. 1). However, the mean values of these anthocyanins were different among the studied pomegranate cultivars.

The total anthocyanin concentration (TAC) was calculated as the sum of the mean amounts of individual anthocyanins detected in each chromatogram (Table 3). As shown in Table 3, the TAC in all studied cultivars gradually increased up to the full maturity stage. At this stage, the highest value of TAC was 279.4 mg L^{-1} for ASS, followed by 265.9, 248.6 and 35.94 mg L^{-1} for the MTS, MSS, and AMA cultivars, respectively. TAC in AMA was markedly lower than the other cultivars (Table 3).

The quantity of 3,5-diglucosides derivatives was greater than that of 3-monoglucosides during seasonal growth, but as the fruits progressed to maturity, the level of monoglucoside derivatives increased (Fig. 3). For example, the ratio of monoglucoside derivatives to TAC in the MTS cultivar increased from 18% at 75 DAFS to 24% at the full maturity stage.

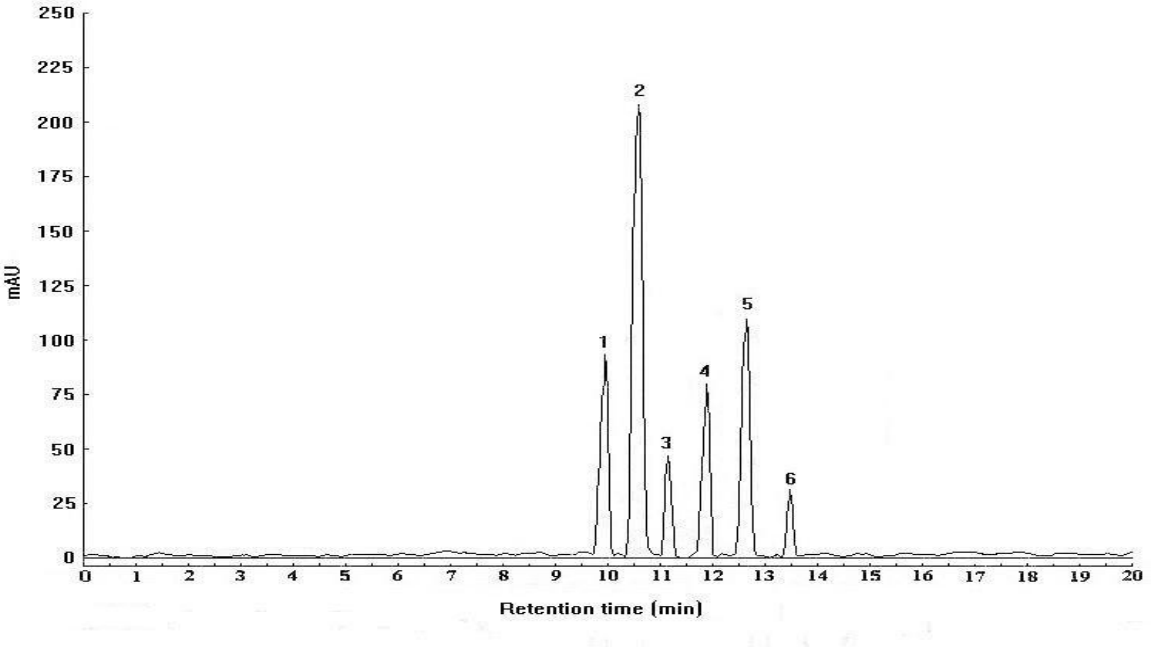

Fig. 1. A representative chromatogram of the separated anthocyanins in the aril juice of 'Malase Torshe Saveh' pomegranate at full maturity stage. (Peaks: 1, delphinidin 3,5-diglucoside; 2, cyanidin 3,5-diglucoside; 3, pelargonidin 3,5-diglucoside; 4, delphinidin 3-glucoside; 5, cyanidin 3-glucoside; 6, pelargonidin 3-glucoside).

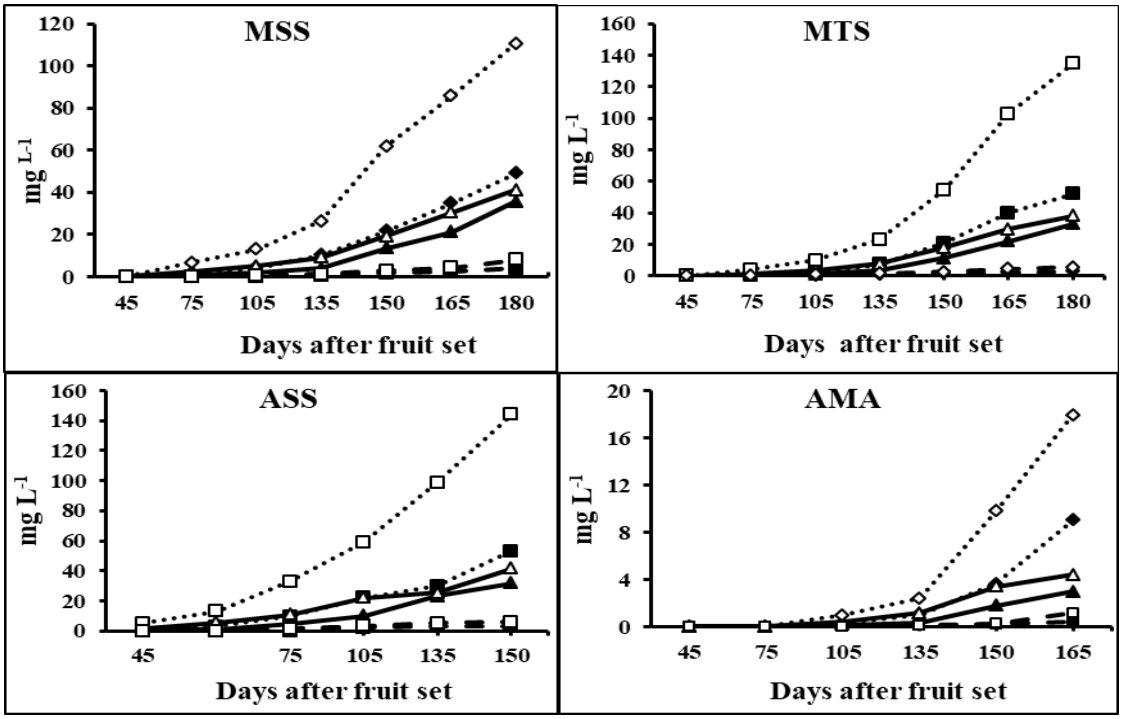

Fig. 2. Individual anthocyanin composition of juice among four pomegranate cultivars at different maturity stages. (MSS, Malase Shirine Saveh; MTS, Malase Torshe Saveh; ASS, Alak Shirine Saveh; AMA, Agha Mohammad Ali. (◊), cyanidin 3,5-diglucoside; (♦), cyanidin 3-glucoside; (Δ) delphinidin 3,5-diglucoside; (▲) delphinidin 3-glucoside; (□) pelargonidin 3,5-diglucoside; (■) pelargonidin 3-glucoside).

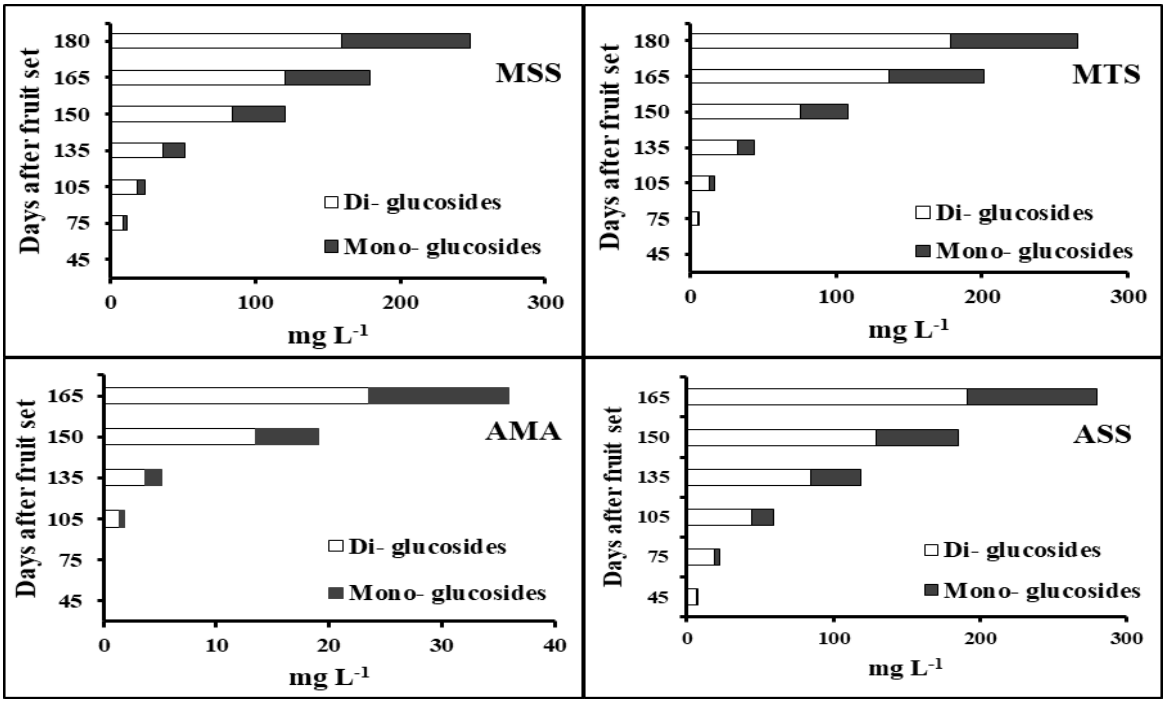

Fig. 3. Mono and di-glucoside anthocyanin compositions of juice in four pomegranate cultivars at different maturity stages. (MSS, Malase Shirine Saveh; MTS, Malase Torshe Saveh; ASS, Alak Shirine Saveh; AMA, Agha Mohammad Ali)

Discussion

In the present study, the L* value progressively reduced in the four cultivars during development and ripening. These results are in agreement with those reported by Fawole and Opara (2013) who reported gradual decreases in L* of arils in the "Bhagwa" and "Ruby" cultivars from 54 days after full bloom until the maturity stage. This trend indicates that the color of arils becomes darker as the fruit matures. However, Shwartz et al. (2009) did not find significant changes during maturation for 121-2 and 101-2 pomegranate accessions. Conversely, the L* value increased during fruit growth in persimmon (Candir et al., 2009).

Since anthocyanins contribute to fruit color (Shwartz et al., 2009); therefore the variations in L* and a* are related to the accumulation of anthocyanins in the arils during maturation stages. Hence, the variations of L* value were minimal for the AMA cultivar, which had a lower anthocyanin concentration. Borochov-Neori et al. (2009) reported that a* values could provide a proper estimation for anthocyanin concentration. The values recorded for a* in the arils of the "Bhagwa" and "Ruby" cultivars were 0.5–28.5 during the maturity stages (Fawole and Opara, 2013), while the corresponding values for aril juice of 121-2 and 101-2 pomegranate accessions were 3.5–6.5 (Shwartz et al., 2009). The decrease in the hue angle in the investigated cultivars during maturation was probably due to an accumulation of anthocyanins (Fawole and Opara, 2013). Therefore, the variations of hue angle value in the AMA cultivars that had the lowest anthocyanin pigment were minimal. An increase in the C* value for the "Ruby" and "Bhagwa" pomegranate cultivars during maturation was reported by Fawole and Opara (2013).

Several authors have reported significant increases in TSS amount during the maturity and ripening of the pomegranate fruit (Kulkarni and Aradhya, 2004; Shwartz et al., 2009; Fawole and Opara, 2013). An increase in TSS generally leads to the fruit sweetness, especially if accompanied by a decrease in juice acidity (Shwartz et al., 2009). The increase in TSS may be attributed to the hydrolysis of the starch component in the unripe pomegranate, which is desirable for the fruit's taste (Kulkarni and Aradhya, 2004).

In addition to TSS, TA was also used to identify fruit and juice qualities (Shwartz et al., 2009). The reduction of TA during fruit development was reported in pomegranate (Al-Maiman and Ahmad, 2002; Fawole and Opara, 2013), persimmon (Candir et al., 2009), and peach (Moing et al., 1998). Additionally, the TA content in pomegranate accession 101-2 with sour–sweet taste was much higher than the sweet-tasting accession of 121-2 (Shwartz et al., 2009). In guava fruits, TA increased up to the climacteric peak and declined thereafter (Bashir and Abu-Goukh, 2003). However, in strawberry juice, TA increased from 10 days after full bloom stage to the turning stage (onset of maturation) and thereafter decreased till maturity stage (Moing et al., 2001). In our study, decreases in TA coincided with increases in TSS, which led to the promotion of quality fruits at the maturity stage.

The variations of pH inversely coincided with TA during the maturation and ripening stages. In this study, the highest value for pH was recorded at 45 DAFS in all the cultivars, while the maximum pH for the "Taifi" cultivar (3.57) was found at the full-ripe stage (Al-Maiman and Ahmad, 2002), and for the "Ruby" cultivar 3.3 at 54 days after full bloom (Fawole and Opara, 2013). The pH values reported for 20 Iranian pomegranate cultivars at the maturity stage ranged between 3.16 and 4.09 (Tehranifar et al., 2010). According to the results, the cultivar type plays an important role in terms of the TSS, pH, and TA of the pomegranate juice.

A decrease in TPC reduces the astringency of the fruits, which is a desirable sensory attribute in pomegranate (Kulkarni

and Aradhya, 2005). The decrease in TPC is attributed to the oxidation of phenolic compounds by polyphenol oxidase during fruit ripening (Shwartz et al., 2009). In addition, this reduction could be due to the inhibition of the new polyphenols biosynthesis during fruit development and maturation (Fawole and Opara, 2013) or the contribution to the biosynthesis of the flavylium ring in anthocyanins (Kulkarni and Aradhya, 2005). The reduction of TPC during ripening and maturation has been previously reported for pomegranate cultivars (Kulkarni and Aradhya, 2005, Shwartz et al., 2009; Fawole and Opara, 2013), apple (Zheng et al., 2012), and guava fruits (Bashir and Abu-Goukh, 2003).

Our results are in agreement with those reported by Fawole and Opara (2013), who showed a decrease in TFC from 752.18 to 397.27 mg GAE 100 mL^{-1} for the pomegranate "Ruby" cultivar. Similarly, Zheng et al. (2012) reported a TFC reduction in apple from 25 to 185 days after full bloom. The decrease in TFC including tannins is desirable in pomegranate because it reduces the astringent during the fruits developing from the unripe stage to the fully ripened stage (Fawole and Opara, 2013). The values obtained in this study at the full maturity stage were lower than the values reported for the "Ruby" cultivar. The variations of TFC and TPC must be related to the differences among cultivars (genotypes), growing seasons, farming practices, and analysis methods (Nuncio-Jáuregui et al., 2014).

A similar trend was reported for the Ganesh and Ruby pomegranate cultivars (Kulkarni and Aradhya, 2005; Fawole and Opara, 2013). Shwartz et al. (2009) reported a decrease in the AA level from 10.3 to 7.8 mM during the 10 weeks of maturation in pomegranate accession 121-2. Moreover, the "Fuji" apple also showed a decrease in AA from 11.8 to 0.77 mmol TE g^{-1} during fruit growth from 20 to 185 days after full bloom (Zhang et al., 2012). In this study, the reduction of AA was in accordance with the reduction in the TPC level. The decrease of AA during the maturation and ripening of pomegranate fruit could be associated with the decrease of polyphenols in the fruit juice (Fawole and Opara 2013). Some studies have suggested a positive correlation between AA and polyphenol concentration (Shwartz et al., 2009; Zhang et al., 2012; Kalaycıog˘lu and Erim, 2017). The last increase in AA might be attributed to an increased concentration of anthocyanin pigments (Kulkarni and Aradhya, 2005). Anthocyanin, ascorbic acid, and polyphenols are responsible for AA in pomegranate juices (Kulkarni and Aradhya, 2005).

Six different anthocyanins were detected in the juices of all the cultivars, including 3-glucosides and 3,5-diglucosides of cyanidin, delphinidin, and pelargonidin, which confirmed previous reports on other pomegranate varieties (Hernandez et al., 1999; Miguel et al., 2004; Alighourchi et al., 2008; Fawole and Opara, 2013). As shown in Fig. 2, cyanidin 3,5-diglucoside was the predominant pigment in the four pomegranate cultivars. In agreement with our results, cyanidin 3,5-diglucoside was reported as the main pigment in some Iranian pomegranate cultivars (Alighourchi et al., 2008) and the "Ruby" cultivar (Fawole and Opara, 2013). Cyanidin 3-glucoside was the major anthocyanin pigment in the "Wonderful" (Gil et al., 2000) and "Mollar" cultivars (D'Aquino et al., 2010), whereas delphinidin 3-glucoside was the main pigment in the juice of "Assaria" (Miguel et al., 2004). Delphinidin 3,5-diglucoside was the predominant pigment in some Iranian cultivars (Mousavinejad et al., 2009) as well as in the juice of five Spanish pomegranate cultivars (Hernandez et al., 1999).

In the present study, the quantity of 3,5-diglucosides derivatives was higher than 3-monoglucosides level during seasonal growth, but as the fruits progressed to maturity, the ratio of monoglucoside derivatives to TAC was increased. Gil et al. (1995) reported that during the early maturation stages of the "Mollar"

pomegranate cultivar, 3,5-diglucosides were the main pigments, whereas the delphinidin-based derivatives were the predominant compounds at full maturity stage. At the late maturation stages, monoglucoside levels reached to the value (or even higher than) diglucoside levels (Gil et al., 1995).

A rapid increase in TAC during ripening was also reported for the "Ruby" (Fawole and Opara, 2013), "Rabbab-e-Fars" (Zarei et al., 2011), and "Taifi" cultivars (Al-Maiman and Ahmad, 2002). Zarei et al. (2011) reported that TAC of pomegranate increased during fruit ripening, being 3.68, 15.28, and 24.42 mg·100 g^{-1} for 20, 80 and 140 day-old fruits, respectively. Meanwhile, Kulkarni and Aradhya (2005) reported a decrease in TAC in the "Ganesh" pomegranate cultivar from 100 days of fruit set up to the full maturity stage.

Conclusion

In pomegranate, changes in the concentrations of major metabolites were dependent on fruit development. However, the quantity and quality of individual anthocyanins are strongly relied on the type of cultivar. In the current study, cyanidin 3,5-diglucoside was found to be the predominant pigment during all developmental stages for the four studied pomegranate cultivars. Our results revealed that increase in TSS and total anthocyanins, are coupled with decline in TA and total phenolic concentration. This information could help pomegranate juice producers to assess and optimize the juice quality and antioxidant values of these pomegranate cultivars.

References

1. Alighourchi H, Barzegar M, Abbasi S. 2008. Anthocyanins characterization of 15 Iranian pomegranate (*Punica granatum* L.) varieties and their variation after cold storage and pasteurization. European Food Research and Technology 227, 881–887.
2. Al-Maiman S.A, Ahmad D. 2002. Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chemistry 76, 437-441.
3. Anonymous. 2015. Statistical book of agricultural of Iran. Iranian Statistical Centre, Tehran, Iran.
4. Bashir H.A, Abu-Gouck AA. 2003. Compositional changes during guava fruit ripening. Food Chemistry 80, 557-563.
5. Borochov-Neori H, Judeinstein S, Tripler E, Harari M, Greenberg A, Shomer I, Holland D. 2009. Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. Journal of Food Composition and Analysis 22, 189–195.
6. Brand-Williams W, Cuvelier M.E, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28, 25–30.
7. Cam M, Yasar H, Gökhan D. 2009. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food Chemistry 112, 721–726.
8. Candir E.E, Ozdemir A.E, Kaplankiran M, Toplu C. 2009. Physico-chemical changes during growth of persimmon fruits in the East Mediterranean climate region. Scientia Horticulturae 121, 42–48.
9. D'Aquino S, Palma A, Schirra M, Continella A, Tribulato E, La-Malfa S. 2010. Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. Postharvest Biology and Technology 55, 121–128.
10. Fawole O.A, Opara U.L. 2013. Developmental changes in maturity indices of pomegranate fruit: A descriptive review. Scientia Horticulturae 159, 152–161.
11. Gil M.I, Garcia-Viguera C, Artés F, Tomás-Barberán F. 1995. Changes in pomegranate juice pigmentation during ripening. Journal of Agricultural and Food Chemistry 68, 77–81.
12. Gil M.I, Tomas-Barberan F.A, Hess-Pierce B, Holcroft D.M, Kader A.A. 2000. Anioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry 48, 4581–4589.
13. Gould K, Davies K, Winefield C. 2009. Anthocyanins: Biosynthesis, functions and Applications. Springer Science, Business Media, LLC.
14. Hernandez F, Melgarejo P, Tomas-Barberran F.A, Artes F. 1999. Evolution of juice

anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. European Food Research and Technology 210, 39–42.

15. Holland D, Hatib K, Bar-Yaakov I. 2009. Pomegranate: botany, horticulture, breeding. Horticultural Reviews 35, 127–191.
16. Kalaycıoglu Z. Erim F.B. 2017. Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide. Food Chemistry 221, 496–507.
17. Kulkarni A.P, Aradhya S.M. 2005. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chemistry 93, 319-324.
18. Miguel G, Fontes C, Antunes D, Neves A, Martins D. 2004. Anthocyanin concentration of 'Assaria' pomegranate fruits during different cold storage conditions. Journal of Biomedicine and Biotechnology 5, 338–342.
19. Moing A, Svanella L, Rolin D, Gaudillère M, Gaudillère J.P, Monet R. 1998. Compositional changes during the fruit development of two peach cultivars differing in juice acidity. Journal of the American Society for Horticultural Science 123, 770–775.
20. Moing A, Renaud C, Gaudillère M, Raymond P, Roudeillac P, Denoyes-Rothan B. 2001. Biochemical changes during fruit development of four strawberry cultivars. Journal of the American Society for Horticultural Science 126, 394–403.
21. Mousavinejad G, Emam-Djomeh Z, Rezaei K, Haddad-Khodaparast M.H. 2009. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chemistry 115, 1274–1278.
22. Nuncio-Jáuregui N, Calín-Sánchez A, Carbonell-Barrachina A, Hernández F. 2014. Changes in quality parameters, proline, antioxidant activity and color of pomegranate (*Punica granatum* L.) as affected by fruit position within tree, cultivar and ripening stage. Scientia Horticulturae 165, 181–189.
23. Park Y.S, Jung S.T, Kang S.G, Heo B.G, Arancibia-Avila P, Toledo F, Drzewiecki J, Namiesnik J, Gorinstein S. 2008. Antioxidants and proteins in ethylene-treated kiwifruits. Food Chemistry 107, 640-648.
24. Shwartz E, Glazer I, Bar-Yaakov I, Matityahu I, Bar-Ilan I, Holland D, Amir R. 2009. Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate accessions. Food Chemistry 115, 965–973.
25. Singleton V.L, Orthofer R, Lamuela-Raventós R.S. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymology 299, 152-178.
26. Tehranifar A, Zarei M, Nemati Z, Esfandiyari B, Vazifeshenas M.R. 2010. Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. Scientia Horticulturae 126, 180–185.
27. Zarei M, Azizi M, Bashir-Sadr Z. 2011. Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening. Fruits 66, 121-129.
28. Zheng H.Z, Kim Y.I, Chung S.K. 2012. A profile of physicochemical and antioxidant changes during fruit growth for the utilisation of unripe apples. Food Chemistry 131, 106–110.