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PHYSICAL AND BIOCHEMICAL CHANGES IN POMEGRANATE (*PUNİCA GRANATUM* L. cv. 'HİCAZNAR') FRUITS HARVESTED AT THREE MATURITY STAGES

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Abstract

This study was carried out to determine the physical and biochemical changes in fruits harvested at three different maturity stages in pomegranate cultivar 'Hicaznar'. Fruits were harvested at the periods of 1) the time of the beginning of the color change in arils (August 15), 2) the time of the pink color of arils (September 6) and 3) the time of the red color of arils (October 3). In the study, fruit weight, fruit juice, colour (L, a*, b*), titratable acidity, pH, total soluble solids, total phenolics, phenolic composition (gallic, chlorogenic, ellagic and syringic acids) and organic acids (malic and citric acids) were investigated. Significant increases in fruit weight, total soluble solids, malic acid and colour value of a* were detected with progress of maturity. On the contrary, titratable acidity, total phenolics, gallic, ellagic and sitric acids were significantly decreased with progress of maturity. The changes in chlorogenic and syringic acids were not statistically significant.*

Key words: *pomegranate, maturity, phenols, organic acids.*

INTRODUCTION

Pomegranate belongs to the *Punica* genus of Punicaceae family, the most important species being *Punica granatum* L. Pomegranate is one of the oldest known fruit species and its cultural history dates back to around 3000 BC. It can grow up to 1000 m above sea level in tropical and subtropical climates. Pomegranate is cultivated widely in the Mediterranean Basin, South West Asia and America in the world. Pomegranate plants have many advantages such as being easily adaptable to various climatic and soil conditions, being easy to replicate and having high productivity. Pomegranates are also grown as ornamental plants or hedge plants in Turkey. Pomegranate production, which is 59 000 tonnes in 2000 in Turkey, reached 445 750 tonnes in 2015 with a big increase. Pomegranate is a very rich fruit species in terms of phenolic substances, flavonoids, tannins, fatty acids, aromatic compounds, amino acids, tocopherols, sterols and terpenoids (Ozgen et al., 2008; Wang et al., 2010).

In addition to many factors such as increasing environmental pollution, ultraviolet rays and smoking, stressful living conditions, environmental and psychological factors negatively affect human health and cause free radicals formation. It has became important to consume natural nutrients instead of using drugs to remove the negative effects of free radicals and to prevent disease formation (Hochstein and Atallah, 1988; Benzie, 2003). Fruits that are rich in terms of phenolic substances prevent these diseases by preventing these free radicals and strengthen the immune system. In addition to that, they have positive effects on health due to their antimicrobial and antioxidative effects. The most important factor affecting the nutritional content of fruits evaluated as functional food is genotype. However, it is a known fact that environmental conditions also influence the nutritional contents of fruits. Researchers have reported that the antioxidant capacities of fruits vary significantly with respect to fruit maturity levels as well as ecological (temperature, soil characteristics and night-time temperature difference) and cultural (irrigation, fertilization) conditions (Gao et al., 2012). There are many studies interested in the increase of the nutrient contents of fruits by different applications (Rossi et al., 2003, Ancos et al., 2000). It has been reported that the nutritional content of fruit species changes significantly with maturity level. For example,

in a study of two different species of *Zizipus*, it was determined that the amounts of phenolic compounds in green fruits were higher than those in ripe ones in *Z. mauritiana* and *Z. nummularia* species (Choi et al., 2012; Wu et al., 2012).

The objective of this study was to evaluate the pomegranate fruit harvested at different maturity levels in terms of their physical and biochemical contents.

MATERIALS AND METHODS

Materials

In the study, the fruits harvested in three maturity levels of pomegranate cultivar Hicaznar were used. The fruits were obtained from a farmer's orchard in Serik region of Antalya, Turkey.

Methods

In the study, fruits were harvested at 3 different maturity levels. In this regard, pomegranate fruits were harvested at 1) the time of the beginning of the color change in arils (August 15), 2) the time of the pink color of arils (September 6) and 3) the time of the red color of arils (commercial harvest time, October 3).

***Determination of physical properties*.** Fruit width, fruit height, fruit weight, peel weight and aril weight were determined in the harvested fruit. Peel color of fruits were measured using a colorimeter (Chroma Meter CR-400, Minolta) and expressed as L*, a* and b* values.

***Determination of biochemical properties*.** In order to determine the biochemical properties, the arils separated from their peels were squeezed and the obtained fruit juice was filtered through filter paper. The pH of the fruit juice was determined by a pH meter (Hanna). Total soluble solids were measured with a hand refractometer and expressed as %. Titratable acid content was determined according to the method described by Karaçalı (1990) and calculated as % citric acid.

The total phenolic content of fruit juices was determined using the Folin-Ciocalteu method. For this purpose, the aril juice was diluted 1:5 with etanol. 100µl of fruit juice was added and 3ml of purified water was added. Then, 200 µL of Folin-Ciocalteu (0.2N) and 100 µL of sodium carbonate (20%) were added and incubated in the dark for 2 hours. The absorbance values were then read on a spectrophotometer adjusted to a wavelength of 765 nm. The total amount of phenolic substances in pomegranate juice was calculated from the standard calibration curve. For the determination of standard calibration curve, 50, 100, 150, 200, 250, 300, 350 and 400 mg/L gallic acid solutions were prepared and their absorbances were read at 765 nm in a spectrophotometer by the same method.

Phenolic contents were analyzed according to the modified procedure of Caponio et al. (1999). The 5 ml of fruit juice was mixed with 10 ml of methanol (80%). The sample was incubated in an ultrasonic bash for 10 min and centrifuged at 4000 rpm for 10 min. The upper phase was filtered through a 0.45 µm membrane filter (Millipore) and 20µl of the sample was injected into an HPLC (Shimadzu Inc) equipped with a diode array detector (lmax = 278), Agilent Eclipse XDB-C18 column (250x4,6 mm, 5µm) operated at 30^{o}C, a SIL-10AD vp autosampler, a LC-10AD vp pump, a CTO-10Avp column oven, and a DGU-14A degasses. The mobile phase consisted of 3% acetic acid (A) and methanol (B). The flow rate was 0.8 mL/min. The gradient program was given in Table 1. The peaks were identified by comparison with the peak of standard of gallic acid, catechine, chlorogenic acid, vanillic acid, syringic acid, ellagic acid, quercetin and kaempherol (Sigma Chemical Co) (Figure 1). The phenolics were expressed as µg per g fruit juice.

Table 1. The linear solvent gradient system used in HPLC analysis of phenolics.

Time (min)	**0.1**	**20**	**28**	**35**	**50**	**60**	**62**	**70**	**73**	**75**	**80**	**81**
A *(%)	93	72	75	70	70	67	58	50	30	20	0	93
B (%)	7	28	25	30	30	33	42	50	70	80	100	7

*Solvent A: 3% Acetic acid, Solvent B: Methanol

Table 2. Some physical properties of fruits of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest date	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Shape index	Peel weight (g)	Aril weight (g)	Aril yield (%)
August 15	260.8 c*	70.7 c	79.5 c	1.1	131.6 c	129.1 c	0.49
September 6	326.9 b	76.7 b	86.7 b	1.1	172.6 b	154.2 b	0.47
October 3	438.5 a	87.9 a	97.2 a	1.1	235.3 a	203.2 a	0.46

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

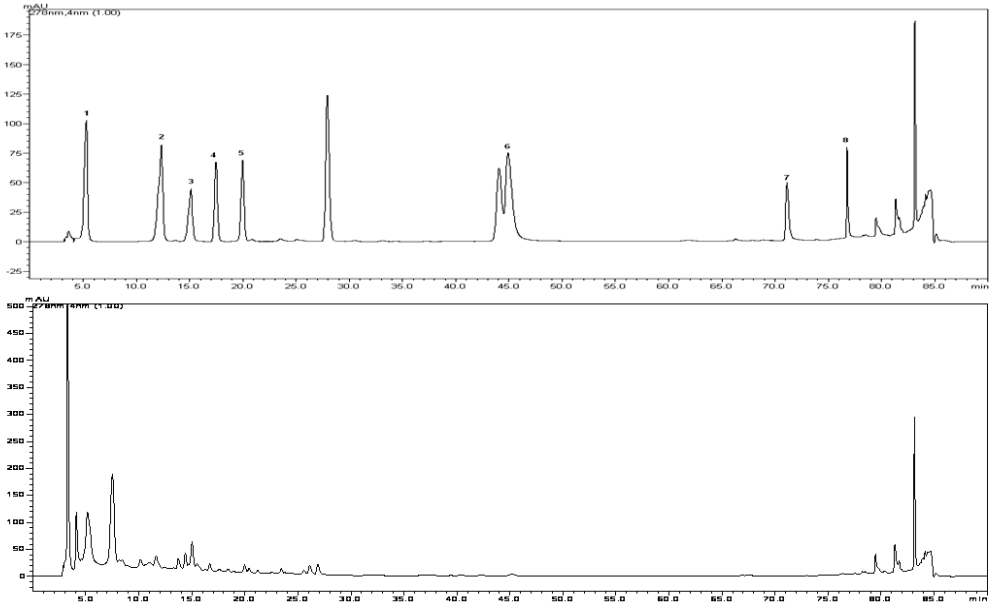

Figure 1: HPLC chromatograms of phenolic standards and extracts of pomegranate fruit juice. 1; Gallic acid 2; Catechin 3; Chlorogenic acid 4; vanilic acid 5; syringic acid, 6; ellagic acid, 7; quercetin 8; Kaempferol

Organic acid contents of pomegranate juice were analized according to the modified procedure of Alhendawi et al. (1997) and Kordis-Krapez et al. (2001). 2 ml of fruit juice was diluted with 2 ml H_3PO_4 (2%) and then 1ml of sample was diluted with 1 ml of extraction solution (0.01M KH_2PO_4, pH: 8.0). 20µl of sample was injected into an HPLC (Shimadzu Inc) equipped with SPD-10Avp UV-VIS detector (210nm), SIL-20AC prominence auto sampler, LC-20AT prominence system controller, LC-20AT prominence Pump, DGU-20A5 degasser and Prodigy ODS-2 (250x4.6mm, 5µm) column operated at 30°C. The mobile phase was distiled water adjusted to pH 2.25 with phosphoric acid. The flow rate was 0.8 mL/min. Peaks were identified by comparison with the peak of standard of tartaric, malic, ascorbic, citric and sucsinic acids (Sigma Chemical Co) (Figure 2). The organic acids were expressed as µg per g fruit juice.

Data analysis: The experiment was planned according to a completely randomized design with three replications. The data were subjected to the analysis of variance using the MINTAB software (MINITAB Inc.) and the means were separated from each other by Tukey's test at the 5 % level of significance.

RESULTS AND DISCUSSION

Fruit weight, fruit height, fruit width, peel weight and aril weight values of pomegranate cultivar 'Hicaznar' fruits harvested in 3 different periods increased significantly with maturity. Fruit shape index and aril yield were not changed significantly by maturity stages in the study (Table 2). The results obtained in the study were found to be similar to those of Özsayın (2012). It has been determined that the

fruit weight increased about 2 times in the last 48 days (from 15 August to 3 October).

Table 3. Changes in values of fruit peel color of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	L *	a*	b*
August 15	55.88 b*	-1.28 c	33.18
September 6	59.47 a	13.31 b	33.09
October 3	58.04 ab	30.49 a	31.39

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

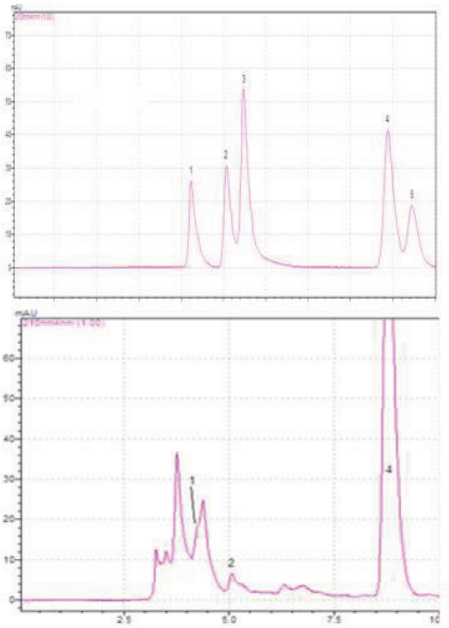

Figure 2: HPLC chromatograms of organic acid standards and extracts of pomegranate fruit juice. 1; tartaric acid 2; malic acid 3; ascorbic acid 4; sitric acid 5; succinic acid

With regard to the fruit peel color, the b* value did not change while a significant increase in a* value was determined with maturity. A relative increase was observed with maturity in terms of L* value (Table 3). It has been determined that the highest pH value (2.77) of the fruit juice was in the commercial harvesting period (October 3). Correspondingly, the content of titratable acidity has also decreased regularly with the progress of the maturity. As expected, the total soluble solid content of juices increased steadily with the progress of the maturity and this increase was found to be statistically significant. The content of total soluble solid was found to be 15.7% for the fruits harvested in the commercial harvesting period. Similar to our findings, Kulkarni and Aradhya (2005) reported that the content of total soluble solids increased, while the content of titretable acidity decreased with the progress of the maturity in pomegranate. In another study, it was reported that the total soluble solid content of pomegranate cultivar 'Hicaznar' varied between 14% and 18.2% in the commercial harvest time (Özsayın, 2012), which was similar to our results. The malic acid content of fruits significantly increased with the progress of the maturity. While malic acid content was 317.2 µg/g on August 15, this value increased to 545.2 µg/g at commercial harvest date (October 3). The citric acid content of the fruit was 25323 µg/g on August 15 and decreased by half to 12666 µg/g at the time of commercial harvest (October 3). Tartaric acid was not found in the fruits harvested on August 15 and September 6, but was found to be 466.8 µg/g on October 3 (Table 4). The contents of malic, sitric and tartaric acids of fruits observed in our study were similar to those reported by Karaca (2011). Karaca (2011) reported that sitric acid, malic acid and tartaric acid contents of mature fruit were 17360 µg/g, 500 µg/g and 590 µg/g, respectively. It has also been reported that the content of organic acids in myrtle decreases with maturity (Mulas et al., 2013).

The total phenolic contents of 'Hicaznar' fruit juice were found to be higher in the immature fruit than in the mature fruit. In the study, the total phenolic content in fruit juice was the highest (8308 ± 335 µg / g) at the beginning of coloring (15 August) and decreased significantly with maturity. Similarly, the amounts of gallic acid and ellagic acid in the fruit juice were found to be the highest (97.2 and 13.6 µg/g, respectively) in fruits harvested on August 15 and were reduced to 29.47 and 4.46 µg/g, respectively in fruits harvested on October 3 (commercial harvest date). There were no statistically significant differences in the fruits harvested at the different maturity stages in terms of chlorogenic and syringic acids (Table 5). In support of our results, Al-Maiman and Ahmad (2002) found that the total phenolic amount of pomegranate fruits decreased significantly with the progress of maturity.

Table 4. Changes in pH, total soluble solid, titretable acidity and orhanic acids of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	pH	Total Soluble Solid (%)	Titretable Acidity (%)	Malic acid (µg/g)	Sitric acid (µg/g)	Tartaric acid (µg/g)
August 15	2.70 ab*	10.93 b	3.07 a	317.2 b	25323 a	-
September 6	2.57 b	11.76 b	2.49 ab	354.6 b	16271 ab	-
October 3	2.77 a	15.70 a	1.95 b	545.2 a	12666 b	466.8

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

Table 5. Changes in phenolic compounds of pomegranate cultivar 'Hicaznar' harvested at 3 different periods

Harvest Date	Total phenolics (µg/g)	Gallic Acid (µg/g)	Chlorogenic Acid (µg/g)	Syringic Acid (µg/g)	Ellagic Acid (µg/g)
August 15	8308 a*	97.2 a	83.9	5.7	13.6 a
September 6	5896 b	50.8 ab	84,2	6.3	4.8 b
October 3	5696 b	29.5 b	66.3	5.0	4.5 b

* Means followed by different letters in the same column are significantly different from each other (p≤0.05).

Similarly, Siriamornpun et al. (2015) reported that immature green fruits contained more total phenolic substance than mature fruits in jujube. Moreover, it was reported that total phenolic, gallic acid and ellagic acid contents of myrtle decreased with the progress of maturity which was similar to our results (Fadda and Mulas, 2010; Babou et al., 2016). On the other hand, unlike our findings, it was reported that gallic acid content did not change with the progress of maturity in 'Hicaznar' (Özhan-Tümer, 2006). It has been found that the content of flovonols in *Morus alba* species generally decreases with maturity (Lee and Choi, 2012), but not in *Vitis vinifera* species (Doshi et al., 2006). It has been reported in many studies that the amounts of phenolic substances in fruit species may vary significantly according to genotype, ecological conditions and analysis method (Karaca, 2011; Wang et al., 2010).

CONCLUSIONS

As a result, the biochemical contents of the 'Hicaznar' fruits vary significantly with their maturity level. Malic acid content increases with maturity, while citric acid content decreases. Tartaric acid was not detected in immature fruit. It has been found that the total phenolic substance, gallic acid and ellagic acid contents decrease with maturity. Fruits should still be harvested at commercial harvest time for fresh consumption, even though the amount of some phenolics and total phenolic substance are redused with progress of maturity.

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