Harran Tarım ve Gıda Bilimleri Dergisi 18 (1), 1-15, 2014
ISSN 0099246, **Research Article**

Physicochemical Properties of Zivzik and Gorumlu Pomegranates

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Abstract
One of the largest pomegranate plantation areas in the World is Anatolia where is also considered as to be the native lands of pomegranate cultivation. Even though presence of many pomegranate accessions in the region only *Hicaz* variety has world-wide reputation and is generally used for production of pomegranate juice concentrate. The objective of this study is illuminating the physicochemical properties of special pomegranate types grown in the Eastern and Southeastern regions of Anatolia. Four different pomegranate varieties namely *Zivzik*, *Ali Ağay*, *Hacı Hesin*, *Radişu* were assayed for their phenolic, anthocyanin, ascorbic acid contents and antioxidant activities. The anthocyanin compositions of pomegranates were identified by using HPLC system equipped with photodiode array detector. Results indicated that these pomegranate varieties have comparable natural compound and ascorbic acid contents, and they have higher antioxidant activity than the other pomegrante varieties and fruit types. The HPLC-PDA analysis revealed that there are at least three individual anthocyanins such as Cyanidin 3,5 –diglucoside, Pelargonidin 3,5 –diglucoside, Cyanidin 3 –glucoside in assayed pomegranates.

Keywords: Pomegranate, *Zivzik*, Anatolia, Phenolic compounds, Anthocyanins

Zivzik ve Görümlü Narlarının Fizikokimyasal Özellikleri

Özet
Dünyanın en geniş nar plantasyon alanları arasında Anadolu bulunmaktadır ve aynı zamanda bu topraklar nar bitkisinin ilk kültüre alındığı bölgeler arasında sayılmaktadır. Bölgede çok sayıda nar tipleri bulunmasına rağmen özellikle Hicaz narları dünya çapında tanınmaktadır ve genellikle bu çeşit narlar meyve suyu konsantresi yapımında kullanılmaktadır. Bu çalışmanın amacı Doğu ve Güneydoğu Anadolu yörelerinde yetiştirilen bazı nar tiplerinin fizikokimyasal özelliklerinin özelliklerinin tespit edilmesidir. *Zivzik*, *Ali Ağay*, *Hacı Hesin*, *Radişu* olmak üzere dört farklı nar çeşidi fenolik, antosiyanin, askorbik asit ve antioksidan içerikleri bakımından incelenmiştir. Narların antosiyanin kompozisyonları fotodiot detektör kullanılarak HPLC (HPLC-PDA) vasıtasıyla tespit edilmiştir. Elde edilen sonuçlara göre üzerinde çalışılan narlar önemli ölçüde doğal bileşikler ve askorbik asit içermekte ve ayrıca yüksek antioksidan aktiviteye sahiptirler. HPLC-PDA analizine göre narlarda Siyanidin 3,5-diglukozit, Pelargonidin 3,5-diglukozit, Siyanidin 3-glukozit antosiyanin bileşikleri bulunmaktadır.

Anahtar sözcükler: Nar, Zivzik, Anadolu, Fenolik bileşikler, Antosiyaninler

Introduction

Demand to pomegranate products seemed to reach new heights reflecting the depth of its ‘health promoting’ perception among people. Pomegranate fruit is one of the most important sources of phenolic compounds (polyphenolic tannins, anthocyanins), which are known to act as natural antioxidants and antimicrobial agent. Thus, these natural antioxidants can protect humans against the oxidative stress and reduce consequently the risk of chronic diseases (Gil et al., 2000; Faria, 2007; Lansky and Newman, 2007). Spreading of reports indicating its medicinal properties (Curtis CL et al., 2004) serves better appreciation of the nutritional value of pomegranates.

Pomegranate (*Punica granatum* L.) is generally considered to have originated in the vicinity of the Southeastern Anatolia, mid-mesapotomia and Persia. The total pomegranate production of Turkey is around 250.000 tons per annum as of 2012. The most prevalent pomegranate variety cultivated in Anatolia is *Hicaz*. *Hicaz* pomegranate is specifically suitable to industrial processing because of its high acidity, high fruit juice yield, and bright red color. The origin of *Hicaz* pomegranate variety is Alanya where is located at the South West region of Anatolia and this variety was subjected to many scientific investigations. In additition to this, *Hicaz* pomegranates are the major raw material of fruit juice concentrate imported to European and North American countries from Turkey. Even though *Hicaz* variety is the most common variety cultivated all over the Anatolia there are many other pomegranate types locally produced and consumed. These varieties await for studies shedding light on their physicochemical properties and the introduction to the people on the World caring better quality diet and nutrition. Especially Sirnak (Gorumlu) and Siirt (*Zivzik*) regions known for the diverse and rich flora of plants including fruit-bearing pomegranate trees. Although many pomegranate types/varieties have been cultivated in Eastern Anatolia only a few studies was carried out for determining physicochemical properties of these pomegranate types (Poyrazoglu et al., 2002; Gündoğdu et al., 2010; Gozlekci et al., 2011).

The objective of this study was to assesss physicochemical properties of special pomegranate varieties grown in Gorumlu and Siirt namely *Hacı Hesin*, *Ali Ağay*, *Radişu* and *Zivzik* pomegranates, respectively. In this study physical and chemical properties of pomegranate varieties grown in the Eastern and Southeastern regions of Anatolia were investigated. In addition, the fruits were divided three classes as small, medium, large and the effects of fruit size on the physico-chemical characteristics of the fruits were investigated.The individual anthocyanin compounds present in pomegranate cultivars were examined by using HPLC.

Materials and Methods

Plant material

Zivzik pomegranate fruits used in this study were obtained from the plantation areas located in Şirvan province of Siirt. The other three pomegranate types (Hacı Hesin, Ali Ağay, Radişu) were collected from trees grown in pomegranate plantation areas located in Görümlü province of Şırnak. Pomegranate fruits were immediately transferred to the laboratory and stored at 4°C until used in experiments.

Determination chemical -physical properties of pomegranates

pH analyses of samples were carried out using a pH meter (Fisher sci. model 10 (Denver, U.S.)), color analyses were completed with Hunter Lab colorimeter (Color Quest XE, USA), Brix° values of the samples were determined using a ABBE refractometer (Atago Brand). Formol value of the samples were measured as described by Yenice (1974) and titrable acidity of the samples

Determination of total phenolic content (TPC)

TPC of arils were determined by the Folin-Ciocalteu method with gallic acid standard. Briefly, 30 µl of sour cherry extract were pipetted into test tubes containing 2.370 ml deionized water and 150 µl Folin-Ciocalteu's reagent were added onto the mixture (Karaaslan et al., 2011). The mixture was then vortexed and kept at dark for 8 minute. Then 450 µl saturated Na_2CO_3 was added and allowed to stand for 30 minutes in the dark medium. The absorbance of each sample was read at 750 nm in a spectrophotometer (Libra, Biochrom, UK) against the blank. Various concentrations of gallic acid solution (from 50 to 500 µg/ml) were used to plot a calibration curve. Results were expressed as mg gallic acid equivalent per gram pomegranate (mg GAE/kg).

Determination of total anthocyanins (TA)

TA contents of the sour cherries were determined by using pH-differential method (**Wrolstad, 1976**). The results were expressed as cyanidin-3- glucoside (mg/kg) according to the following equation.

$$TA = \frac{AxMWxDFx1000}{\varepsilon xl}$$

where A = $(A_{\lambda 520} - A_{\lambda 700})_{pH\ 1.0} - (A_{\lambda 520} - A_{\lambda 700})_{pH\ 4.5}$; MW (molecular weight)=445.2 g/mol; DF=dilution factor; l=path length in cm; Ɛ (molar extinction coefficient)=29,600 in L/mol/cm for cyanidin-3- glucoside.

Radical DPPH scavenging capacity

The free radical scavenging capacities of sour cherries were measured according to DPPH method reported by Blois (1958) with minor modifications. A 0.1 ml of each extract was pipetted onto 2.9 ml of DPPH solution (0.1 mM in ethanol). After 30 minutes of incubation at room temperature the absorbance at 517 nm was measured. Radical scavenging capacity was expressed as percent scavenging effect according to the following formula.

DPPH Scavenging Effect (%) = [(Ac - As)/Ac] x 100

Purification of phenolics

Anthocyanin and non-anthocyanin phenolics were purified on a C-18 Cartridge (Finisterre, Teknokroma) using a vacuum manifold. Previously, cartridge was activated with 5 ml ethyl acetate (EtaC), 5 ml methanol (0.01% HCl, v/v) and 2 ml acidified water (0.01% HCl) respectively. A 1 ml sample extract was loaded and then the cartridge was firstly washed with 2 ml acidified water (0.01% HCl, v/v). After the cartridge was well dried by means of nitrogen, the cartridge was washed with 5ml EtaC to elute non-anthocyanin phenolics; and finally washed with 2 ml MetOH (0.01% HCl, v/v) to elute anthocyanins within separate test tubes. The EtaC and MetOH extracts were put in a water bath (40 ºC) and solvents were removed under a stream of nitrogen. Phenolics were dissolved in acidified water (0.01% HCl), filtered through 0.45 µm PVDF filter and transferred to vials.

HPLC analysis of phenolic compounds

Identification and quantifaction of phenolic compounds were performed on Waters 2795 HPLC (Waters, USA) equipped with a Waters 2996 photodiode array detector. Autosampler (Set at 7°C) was controlled with EMPOWER™ 2 software. Samples (mixtures of anthocyanin and non-anthocyanin, 1:1, v/v) were injected into Supelcosil (Supelco) LC-18 (25 cm X 4.6 mm X 3µm) column. The mobile phase was a mixture of 0.1% (v/v) trifluoroacetic acid in water (A) and 0.1% (v/v) trifluoroacetic acid in acetonitrile (B). Column temperature was 35 °C and flow rate was 1 ml min-1. The gradient conditions were 5%, 35%, 75%, 75%, 5% and 5% of solvent B at 0, 45, 47, 52, 54 and 65 minutes, respectively. Anthocyanins were detected at 520 nm; non-anthocyanins were detected at both 280 and 320 nm wavelengths. Identification of phenolic compounds was carried out by comparing retention times and spectral data of separated peaks with those of authentic standards. Working standard solutions were prepared in the concentration range from 0.01 to 0.5 mg/ml diluting with 0.1% (v/v) trifluoroacetic acid in water solution. Quantitative determination were carried out using calibration curves of standards (neochlorogenic acid: y= (3E + 0.7)x, R^2 = 0.9933; catechin: y = (5E + 0.6)x, R^2 = 0.9965; epicatechin: y = (5E + 0.6)x, R^2 = 0.9989; caffeic acid: y = (5E + 0.7)x, R^2 = 0.9991; cyanidin-3- glucoside: y = (5E + 0.7)x, R^2 = 0.9971; cyanidin-3-rutinoside: y = (5E + 0.7)x, R^2 = 0.9980). For anthocyanins lacking reference standards (cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside), identification was done according to their retention times, polarity, characteristic spectra and previous reports (Garofulić et al., 2013; Kirakosyan et al., 2009; Simunic et al., 2005; Tomàs-Barberàn, 2001) and quantification was done according to the cyanidin-3-glucoside and cyanidin-3-rutinoside calibration curves.

Table 1. Physical properties of four pomegranate cultivars.

Cultivars	FW (g)	FL (cm)	FD (cm)	AL (cm)	AD (cm)	HGW (g)	JY (%)	AP (%)
Zivzik	261±8^{a}	7.1±0.1^{a}	7.7±0.1^{a}	1.04±0.01^{c}	0.80±0.01^{c}	41.65±0.5^{d}	38±0.6^{b}	55±0.6^{b}
Hacı Hesin	317±31^{a}	7.5±0.2^{a}	8.2±0.3^{a}	1.16±0.02^{b}	0.92±0.1^{a}	51.81±1.9^{b}	44±0.7^{a}	65±0.9^{a}
Ali Ağay	281±18^{a}	7.4±0.2^{a}	7.9±0.2^{a}	1.21±0.02^{a}	0.88±0.1^{ab}	59.37±1.5^{a}	46±1.6^{a}	66±1.7^{a}
Radişu	275±17^{a}	7.3±0.2^{a}	7.8±0.2^{a}	1.1 ±0.2^{b}	0.86±0.1^{b}	48.17±0.8^{c}	35±0.9^{b}	52±1.2^{b}

^{abcd} Different letters in the same column indicate statistically significant difference ($p<0.05$).

Results and Discussion

Physical properties

The physical characteristics of *Zivzik*, *Hacı Hesin*, *Ali Ağay* and *Radişu* pomegranate cultivars are given in Table 1. Average fruit weights of pomegranate cultivars are ranged from 26 g (*Zivzik*) to 317 g (*Hacı Hesin*). Similarly, a study was carried out to determine the physical properties of pomegranates grown in Pervari town of Siirt reported that pomegranate fruit weight ranges between 197 and 310 g (Kazankaya et al., 2003). These values are close to the results presented in this study. Juice yield was measured in order to determine the appropriateness of pomegranates for industrial processing. The highest juice yield was found in *Ali Ağay* pomegranates (46 %) and the lowest juice yield value was determined in *Radişu* pomegranates (35 %). Gozlekci et al. (2011) reported that juice yield of some Anatolian pomegranate cultivars are between 37 % and 48 % which is comparable to our findings presented in this study. Hundred grain weight refers to approximate size of grain and juice yield and also influences consumer perception of better quality pomegranate fruit. The higher hundred seed weight indicates the higher fruit yield and this type pomegranates are more suitable for industrial use. In this study it was found that *Ali Ağay* has the highest hundred grain weight (HGW) (59.37 g) and *Zivzik* has the lowest HGW (41.65 g). Gozlekci et. al (2011) reported that HGW ranges between 35.56 and 45.29 g for the varieties they examined. Especially, it was determined in this study that *Hacı Hesin* and *Ali Ağay* varieties grown in Görümlü region have higher grain size and more suitable for fresh consumption and thus these pomegranates have higher HGW compared to the HGW data presented in the previous studies. Physical properties of three different size (small, medium and large) pomegranates are displayed in Table 2. Statistically significant differences were observed among three different size (small, medium and large) pomegranates investigated in this study within the measured parameters such as weight, length ($p<0.05$). This indicates that the difference in size of the fruit have an effect on physical properties.

Physical and chemical properties of pomegranate juice

The Brix°, pH, titrable acidity and color intensity values of the pomegranates belonging to different cultivars are shown in Table 3. Significant differences (p<0.05) were found among the pomegranate cultivars. The highest total soluble solids content was in *Zivzik* (18.4 Brix°) and the lowest was in *Ali Ağay* (16.5 Brix°). Our results were higher than values observed by Tehranifar et al. (2010) (11.37-15.07° Brix), while our data were in agreement with values (between 14.7 and 17.9) reported by Çam et al. (2008). The pH values ranged between 3.39 (*Ali Ağay*) and 3.63 (*Hacı Hesin*). The titrable acidity content varied from 0.96 % (*Zivzik*) to 1.33 % (*Ali Ağay*). Similar results were reported by Gündoğdu et al. (2010) (between 0.47% and 1.08%) for the pomegranates grown in Siirt.

It was determined that color intensity value was almost 1.5 fold higher in red colored *Zivzik* (4.4) pomegranates compared to *Hacı Hesin* (3.0) and *Ali Ağay* (2.6) pomegranates (p<0.05) (Table 3). The formol values found in *Zivzik*, *Ali Ağay* and *Radişu* pomegranate juice were 16.2, 14.5, 15.1, 13.5 (mL 0.1 mol NaOH/100 mL), respectively (Table 3).

Our results were higher than values observed (9.16 mL 0.1mol NaOH/100 mL) by Ünal et al. (1995), were in agreement with values (4-20 mL 0.1 mol NaOH/100 mL) reported by Türkmen and Ekşi (2010). These data demonstrated that the cultivar type was the main parameter influencing the physical and chemical properties of pomegranates. However, the composition of the fruit is dependent on the climatic conditions of the region, pre and postharvest agricultural practices, the degree of maturation stage, altitude and the storage and transport conditions (Poyrazoğlu et al., 2002; Özgen et al., 2008; Tezcan et al., 2009). These factors affect biochemical reactions, gene expression, enzyme synthesis, and regulation of biochemical pathways involving in secondary metabolite accumulation during cell and plant growt.

The physical and chemical characteristics of different size (small, medium and large) of Zivzik, Hacı Hesin, Ali Ağay and Radişu pomegranate cultivars are described in Table 4. Statistically significant differences were not observed between three different size (small, medium and large) pomegranates for Brix°, pH, titrable acidity and color intensity of fruit (p<0.05). These findings indicatethat the difference in size of the fruit does not have an effect on some chemical properties. There was no statistically significant difference in Zivzik, Hacı Hesin, and Radişu pomegranates in terms of their Brix° values on the contrary to Ali Ağay pomegranates. On the other hand, titrable acidity of Haci Hesin, Ali Ağay, and Radişu was different from each other for the fruits at different sizes while there was no variation among the Zivzik pomegranates at various sizes. The highest color intensity value of different sizes in Radişu pomegranates was found in small sizes (Table 4). pH value of small and medium size Ali Ağay pomegranates were similiar while for large size pomegranates were different (p<0.05).

Table 2. Average fruit weight (FW), fruit length (FL), fruit diameter (FD), aril lenght (AL), aril diameter (AD), hundred grain weight (HGW), juice yield (JY) and aril proportion (AP) % of different size pomegranate cultivars

Cultivars	Size	FW(g)	FL(cm)	FD(cm)	AL(cm)	AD(cm)	HGW(g)	JY(%)	AP (%)
Zivzik	S	159±7[c]	5.9±0.05[c]	6.4±0.04[c]	0.9±0.01[b]	0.8±0.01[a]	37.6±0.74[c]	36±1[a]	54±2[a]
	M	254±5[b]	7.2±0.05[b]	7.8±0.07[b]	1.0±0.01[a]	0.7±0.01[a]	41.7±0.55[b]	36±1[a]	56±1[a]
	L	370±9[a]	8.0±0.07[a]	8.7±0.08[a]	1.0±0.01[a]	0.8±0.01[a]	45.5±0.99[a]	35±1[a]	54±1[a]
Hacı Hesin	S	147±6[c]	6.0±0.09[c]	6.6±0.06[c]	1.0±0.01[a]	1.0±0.12[a]	48.6±3.20[a]	44±1[ab]	65±1[a]
	M	326±13[b]	7.8±0.12[b]	8.6±0.16[b]	1.1±0.03[a]	0.8±0.09[a]	51.3±3.78[a]	46±1[a]	66±2[a]
	L	476±48[a]	8.5±0.23[a]	9.4±0.32[a]	1.1±0.04[a]	0.8±0.06[a]	55.4±3.17[a]	41±2[b]	63±2[a]
Ali Ağay	S	158±8[c]	6.1±0.09[c]	6.5±0.14[c]	1.1±0.03[a]	0.8±0.02[a]	53.3±2.63[b]	55±2[a]	67±2[a]
	M	266±13[b]	7.3±0.15[b]	7.9±0.17[b]	1.1±0.02[a]	0.8±0.01[a]	59.2±1.39[ab]	40±2[b]	67±3[a]
	L	418±17[a]	8.5±0.13[a]	9.2±0.15[a]	1.2±0.03[a]	0.9±0.02[a]	65.5±2.91[a]	45±2[b]	63±3[a]
Radişu	S	175±9[c]	6.3±0.12[c]	6.8±0.15[c]	1.0±0.01[b]	0.8±0.01[a]	43.5±1.19[b]	39±1[a]	57±2[a]
	M	251±8[b]	7.3±0.10[b]	7.79±0.17[b]	1.2±0.02[a]	0.8±0.01[a]	49.5±1.47[a]	35±1[ab]	48±2[b]
	L	397±26[a]	8.3±0.23[a]	8.9±0.19[a]	1.0±0.03[b]	0.8±0.01[a]	51.4±0.82[a]	34±2[b]	49±1[b]

[abcd] Different letters in the same column indicate statistically significant difference ($p<0.05$). (Small; S , Medium; M, Large; L

Table 3. Avarage brix, pH, titrable acidity and color intensity value of pomegranate juice

Pomegranate Juice	Brix°	pH	Titratable Acidity (%)	Color Intensity	**Formol value**(mL 0.1 M NaOH/100 mLfruit juice)
Zivzik	18.4±0.1[a]	3.55 [a]	0.96±0.03[b]	4.4±0.2[a]	16.2±0.3[a]
Hacı Hesin	17.9±0.2[b]	3.63 [b]	1.03±0.02[b]	3.0±0.4[bc]	15.1±0.7[a]
Ali Ağay	16.5±0.1[c]	3.39 [c]	1.33±0.04[a]	2.6±0.5[c]	14.5±2[a]
Radişu	17.9±0.1[b]	3.52 [a]	1.22±0.05[a]	3.9±0.5[ab]	13.5±0.9[a]

[abcd] Different letters in the same column indicate statistically significant difference ($p<0.05$).

Table 4. Average brix, pH, titrable acidity and color intensity value of different size pomegranates

Variety	Size	Brix° (%)	pH	Titratable acidity (%)	Color Intensity
Zivzik	S	18.5±0.1[a]	3.51[a]	1.03±0.04[a]	5.1±0.3[a]
	M	18.4±0.1[a]	3.59[b]	0.92±0.05[a]	4.7±0.4[a]
	L	18.4±0.1[a]	3.56[ab]	0.93±0.04[a]	3.6±0.7[a]
Hacı Hesin	S	17.8±0.04[a]	3.57[a]	1.06±0.04[a]	3.3±0.1[a]
	M	17.9±0.4[a]	3.62[b]	1.12±0.01[a]	1.7±0.1[b]
	L	18.1±0.4[a]	3.72[c]	0.91±0.03[b]	0.9±0.1[c]
Ali Ağay	S	16.5±0.2[ab]	3.35[b]	1.34±0.05[ab]	1.3±0.1[a]
	M	17.0±0.2[a]	3.36[b]	1.47±0.07[a]	0.6±0.1[c]
	L	16±0.3[b]	3.46[a]	1.18±0.04[b]	0.8±0.01[b]
Radişu	S	18.3±0.1[a]	3.47[a]	1.3±0.04[a]	5.9±0.01[a]
	M	17.6±0.3[a]	3.50[ab]	1.21±0.04[a]	5.6±0.01[b]
	L	17.9±0.2[a]	3.59[b]	1.17±0.15[a]	5.6±0.02[b]

[abcd] Different letters in the same column indicate statistically significant difference ($p<0.05$) (S; Small, M; Medium, L; Large)

Total phenolics, total anthociyanins and antioxidant activities of pomegranates

The total phenolic compounds, total anthocyanins, antioxidant and ascorbic acid content of the pomegranates from different cultivars are displayed in Table 5. The total phenolic content is one of the most important parameters for appraising pomegranate characterization. Phenolic compounds prevents oxidation of fatty acids, vitamins and enzymes in cells (Harborne and Williams, 2000; Martens and Mithöfer, 2005; Zaouay et al., 2012). The highest total phenolic compounds were found in *Zivzik* (973 mg GAE/L) pomegranates, and followed by *Radişu* (927 mg GAE/L), *Hacı Hesin* (786 mg GAE/L) and *Ali Ağay* pomegranates (735 mg GAE/L) respectively. The differences in the phenolic contents of pomegranates were statistically significant ($p<0.05$). Özgen et al. (2008) reported that total phenolic content ranges from 1245 to 2076 mg GAE/L for the pomegranates grown in Mediterranean region of Turkey. Total phenolic compound of eight pomegranate cultivars grown in Turkey were measured at the value of between 208.3 mg GAE/100 mL and 343.6 mg GAE/100 mL as reported by Çam et al. (2008). This can be related to the varietal factors of the studied cultivars, harvesting time and performing time of the experiments, maturity status, genetic diversity of the metabolic pathways responsible for varied amount of phenolics biosynthesis (Tehranifar, 2010). Gunduc et al. (2003) reported that among widely consumed fruit juices in Turkey; total phenolic compound of turnip juice is 772 mg/L, and cherry juice is around 797 mg/L. The data presented in this study for total phenolics content were among 927 and 973 mg GAE/L. The phenolics content of the assayed pomegranates juice in this study were higher than those of fruit juices reported previously (Gonduc et al. 2003).

Anthocyanins contribute to the formation of red, blue or purple colors in many fruits and they are well-known compounds for their antioxidant activity (Tehranifar et al., 2010; Mena et al., 2011; Zaouaya et al., 2012). The highest amount of total anthocyanin was detected in *Zivzik* pomegranates (145 mg cyanidin-3-glucoside/L), the lowest anthocyanins was detected in yellow-pink colored *Ali Ağay* pomegranates (64 mg GAE/L) (Table 5). Moderately significant differences were found between cultivars in terms of their anthocyanins content. Vardin and Fenercioğlu (2003) were reported that total anthocyanin content of Suruç pomegranate cultivars are 8.9 mg/100 mL and for some Tunisian pomegranate cultivars the total anthocyanin content was found between 50.52 and -490.42 mg/L as reported by Zaouay et al. (2012). The difference in anthocyanin content of pomegranate cultivars largely is due to differences in cultivar, maturation stage, production region and climatic conditions (Gil et al. 1995; Borochov-Neori et al., 2009).

In this study, the differences in antioxidant activitiy (DPPH, antioxidant ability to scavenge free radical) among the pomegranate cultivars were found statistically non-significant (*p*<0.05). Antioxidant activity of *Zivzik*, *Hacı Hesin*, *Ali Ağay* and *Radişu* pomegranates was found as 86%, 88%, 87% and 87%, respectively (Table 5). Mousavinejad et al., (2009) has been reported antioxidant activity for İran pomegranates among 18.6-42.8% (according to DPPH), Çam et al. (2009) reported that antioxidant activity of eight pomegranate cultivars grown in Turkey ranges between 73.02% and 91.8% (according to DPPH method). Our result were higher than the data reported by Mousavinejad et al. (2009), and was similiar to those reported by Çam et al. (2009). The difference in antioxidant activity might be variety specific and stems from the differences in their total phenolic and anthocyanin content.

Vitamin C content and anthocyanins content were well correlated. Total anthocyanins content and total phenolic compounds were highest in varieties whichs variety also have the highest vitamin C concentration. The lowest total anthocyanins, vitamin C and phenolics content was found in Ali Ağay (Table 5). Thus it can be concluded that there was a close relationship between the total anthocyanins content, total phenolic compounds and vitamin C content (Gardner et al., 2000; Tehranifar et al., 2010).

Table 5. Average total phenolic content, total anthocyanin content, antioxidant activity % and acorbic acid value of different pomagranate varieties

Variety	**Total phenolic** (mg GAE/L)	**Total anthocyanin** (mg cyanidin-3-glucoside/L)	**Antioxidant activity** (%)	**Ascorbic acid** (mg/100 mL)
Zivzik	973±12[a]	145±6 [a]	86±0.3[a]	16 ±1.1[a]
Hacı Hesin	786±53[b]	93±9 [b]	88 ±0.6[a]	9.7±0.6[b]
Ali Ağay	735±40[b]	64±13 [b]	87 ±0.7[a]	3.2±0.6[c]
Radişu	927±29[a]	144±10 [a]	87±0.5[a]	12±1.0[b]

[abcd] Different letters in the same column indicate statistically significant difference (p<0.05).

Total phenolic compounds, total anthocyanins content, antioxidant activity and ascorbic acid of three different size (small, medium and large) of pomegranate are displayed in Table 6. Highest total phenolic compounds in Zivzik pomegranate was found in small size pomegranates (1043 ± 33 mg GAE/L) (p<0.05). Total anthocyanins content, antioxidant activity (% DPPH) and ascorbic acid content in different size of Zivzik pomegranate were found statistically insignificant (Table 6). It can be concluded that classification of pomegranates depending on their sizes is not useful way of classification for presuming their phytochemical contents.

As classified small, medium and large in Hacı Hesin pomegranate juice was found total phenolic compounds 988, 702 and 582 mg GAE/L respectively; total anthocyanins content 103 , 70 and 34 cyanidin-3- glucoside mg /L; ascorbic acid value 13, 5.9 and 10 mg/100 mL respectively. The effect of fruit size on phytochemicals content was found statistically significant in Hacı Hesin pomegranates for total phenolic compounds, total anthocyanins content and vitamin C ($p<0.05$). It was observed that small (13 mg/100 mL) and large (10 mg/100 mL) Hacı Hesin pomegranates had higher ascorbic acid content than medium size (5.9 mg/100 mL) pomegranates.

For Ali Ağay pomegranates statistically significant properties are total phenolic compounds, total anthociyanin content and ascorbic acid value ($p<0.05$). Small, medium and large size of Ali Ağay pomegranate juice was found average total phenolic compounds 707, 614 ve 577 mg GAE/L respectively; total anthocyanins content 36, 19 ve 25 mg siyanidin-3-glukozit/L respectively; ascorbic acid content 7, 1.4 ve 1.1 mg/100 mL respectively.

It was observed that there are significant difference in phenolics content of Zivzik, Ali Ağay, Hacı Hesin and Radişu pomegranate fruits at different sizes. In all four investigated pomegranate varieties, it was found that the small size pomegranates have the highest phenolics content compared to bigger size pomegranates. This situation indicates that importance of fruit size on assessing their phenolic contents.

Table 6. Averages and error standard of total phenolic content, total anthocyanin content and % antioxidant activity and ascorbic acid value of different size pomegranate fruits

Variety	**Size**	**Total phenolics** (mg GAE/L)	**Total anthocyanins** (mg cyanidin-3-glucoside/L)	**Antioxidant activity** (%)	**Ascorbic acid** (mg/100 mL)
	S	1043±33[a]	174±5[a]	85±0.7[a]	17±1.8[a]
Zivzik	M	951±16[b]	147±11[b]	85±0.9[a]	16±1.4[a]
	L	974±9[b]	128±18[c]	85±0.8[a]	14±0.4[a]
	S	988±15[a]	103±2[a]	90±1.1[a]	13±1.8[a]
Hacı Hesin	M	702±6[b]	70±2[b]	89±1.2[a]	5.9±1.2[b]
	L	582±6[c]	34±1[c]	90±0.5[a]	10±1.2[ab]
	S	707±12[a]	36±1[a]	90±2.3[a]	7±1.7[a]
Ali Agay	M	614±6[b]	19±0.6[c]	89±1.8[a]	1.4±0.04[b]
	L	577±11[c]	25±2[b]	89±1.7[a]	1.1±0.02[b]
	S	1060±12[a]	216±2[a]	86±1.2[a]	12±1.2[a]
Radişu	M	966±18[c]	195±1[b]	86±1.1[a]	10±0.6[a]
	L	1000±10[b]	191±2[b]	87±2.3[a]	13±1.1[a]

[abcd] Different letters in the same column indicate statistically significant difference ($p<0.05$). (S; Small, M; Medium, L;Large)

Identification of Anthocyanins Present in Pomegranate Fruits

Anthocyanin compositions of pomegranate arils are given in Table 7. The major anthocyanin in *Zivzik*, *Hacı Hesin* and

Ali Ağay pomegranate cultivars was cyanidin-3,5-di-glucosides (Figure 1). For *Radişu* pomegranate dominant anthocyanin was determined as to be cyanidin-O-glucoside. Highest amount of cyanidin 3,5-diglucoside was found in *Zivzik* pomegranate (42.28 mg/kg) (Table 7) and followed by *Radişu* and *Hacı Hesin*. Mousavinejad et al. (2009) concluded that dominant anthocyanins present in eight Iranian pomegranates were delphinidin 3,5-diglucoside (372–5301 mg/L), cyanidin-3,5-diglucoside (242–2361 mg/L) and pelargonidin 3,5-diglucoside (7–90 mg/L). Difference in pomegranate varieties in terms of their anthocyanin compositions is thought to be the first reason for this observed variation, in addition to this, harvest time, agricultural practices, soil type and structure, storage conditions may be effective on the amount of anthocyanin (Mousavinejad et al., 2009).

Table 7. Anthocyanin composition of Pomegranate arils (mg/kg)

Pomegranate	**Cyanidin 3-5, diglucoside**	**Pelargonidin 3-5, diglucoside**	**Cyanidin -O-diglucoside**	**Total**
Zivzik	42.28±2.1	14.51±0.9	27.70±1.3	84.49
Hacı Hesin	24.64±1.1	7.55±0.4	16.35±1.2	48.55
Radişu	33.68±1.7	25.93±1.2	55.12±2.3	114.73
Ali Ağay	13.88±0.7	5.02±0.2	6.67±0.3	25.57

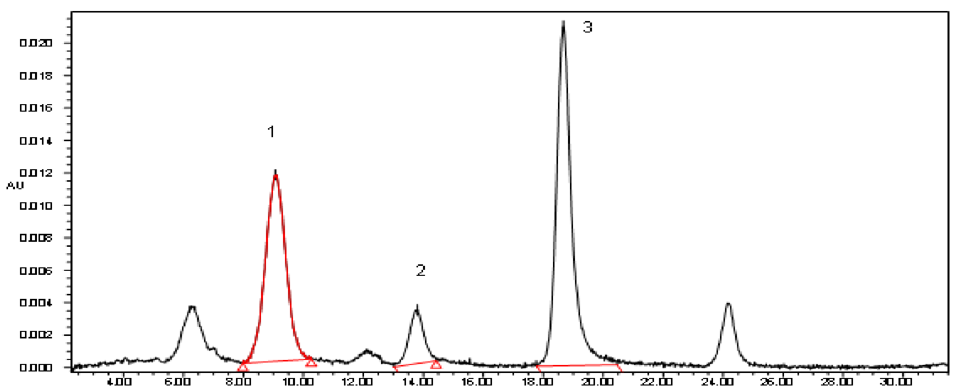

Figure 1. A typical HPLC chromotogram showing the resolved anthocyanin composition of Ali Ağay pomegranate fruit. (1) Cyanidin 3,5 –diglucoside, (2) Pelargonidin 3,5 –diglucoside, (3) Cyanidin 3-glucoside.

Conclusion

In conclusion, it was found that the Zivzik pomegranate cultivar grown in wide areas in Sirvan region is suitable for both fresh consumption and industrial processing. On the other hand, Hacı Hesin, Ali Ağay and Radişu grown in Gorumlu are found to be suitable for fresh consumption. It was moreover suggested that, Radişu and Zivzik pomegranates are especially suitable for processing in the fruit juice industry due to their rich red colour and high anthocyanin, titratable acidity and soluble solids content.

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