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EFFECTS OF CLARIFICATION AND STORAGE ON ANTHOCYANINS AND COLOR OF POMEGRANATE JUICE CONCENTRATES

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ABSTRACT

Whole pomegranates with rinds were processed into juice and then concentrate. Effects of cold clarification and storage temperatures (–23C, 5C, 12C and 20C) on anthocyanins (ACNs), ACN composition and color were determined. Major ACNs in pomegranate juice concentrate (PJC) were identified as cyanidin-3,5-diglucoside (47.9%), delphinidin-3,5-diglucoside (23.2%) and cyanidin-3-glucoside (18.5%). Diglucosides were more stable than monoglucosides during storage. ACN degradation and polymeric color formation were fitted to first-order reaction models. Higher storage temperatures increased the rate of ACN degradation and polymeric color formation in PJCs. Good correlation ($r = –0.988$) was found between ACN degradation and polymeric color formation during storage. Rate of ACN degradation and polymeric color formation were slower in the PJC obtained from unclarified juice than PJC obtained from clarified juice during storage.

PRACTICAL APPLICATIONS

There has been great interest in pomegranates and their products for their potential health benefits due to their impressive antioxidative properties, which are highly correlated with their polyphenol content, including tannins and ACNs. During pressing of pomegranates, polyphenols especially high molecular weight tannins present in the rinds pass to the juice. And excessive polyphenols cause haze and sediment formation, astringent, taste and color loss. This is serious industrial problem in the production of clear pomegranate juice. Attractive red-violet color of pomegranate products is due to their ACN contents. However, ACNs are unstable and susceptible to degradation leading to a brownish color during juice processing and storage. Results of this study showed that clarification with gelatin as well as high storage temperatures adversely affected the ACNs of pomegranate juice concentrates (PJCs). Therefore, PJC should be produced from unclarified juice and frozen-stored. The reconstituted juice should then be cold-clarified right before marketing.

INTRODUCTION

Pomegranates have been grown in a large geographical area. India, Iran, China, Turkey, U.S.A., Azerbaijan and Spain are the major producers of pomegranates. There are many pomegranate varieties grown in the world, with great variations in the composition, color and acidity. For example, only in Turkey, over 100 local and 30 registered (MEYED 2011) pomegranate varieties are grown. On the basis of acidity and sweetness, Turkish pomegranate varieties can be classified into three groups: sour, sour-sweet and sweet. The color of Turkish pomegranate varieties also varies greatly: from pink

to violet. Hicaznar and Silifke aşısı are the most preferred varieties by Turkish juice industry (MEYED 2011) due to their violet-red color and sweet-sour taste.

There has been great interest in pomegranates and their products for their potential health benefits due to their impressive antioxidative properties, which are highly correlated with their polyphenol content, including tannins (especially hydrolyzable tannins) and anthocyanins (ACNs) (Gil *et al.* 2000). The beneficial effects of polyphenols are mainly due to their scavenging action on free radicals. The diets rich in polyphenols are highly correlated with reduced coronary heart disease and cancer mortality. With all these health benefits, the demand for pomegranate juice and concentrates in the world has been increased tremendously for the last 10 years. This demand resulted in the steady increase in pomegranate plantation in Turkey. For example, the production of pomegranates in metric tons was 80,000 in 2005, 91,000 in 2006 and 102,000 in 2007 in Turkey (Ege İnternet Yayıncılık Merkezi 2009).

Other than health benefits, the polyphenols in pomegranate juice are also responsible for color, astringency and bitterness, as well as for haze and sediment formation during concentration and storage. During the pressing of the pomegranates, polyphenols, especially high molecular weight tannins present in the rinds, pass to the juice. Therefore, the pomegranate juices and concentrates contain more polyphenols than the eaten parts of fruits, called as arils. While high polyphenol content increases the antioxidant activity of pomegranate juice, excessive polyphenols cause haze and sediment formation, astringent taste along with color loss. This is a serious industrial problem in the production of clear pomegranate juice. The color and taste of pomegranate juice have great importance for consumer acceptance.

Attractive red-violet color of pomegranate juice and concentrates is due to their ACN contents, which vary greatly from one variety to other variety. For example, Özgen *et al.* (2008) found marked differences in ACN content of six different local pomegranate varieties, ranging from 6.1 to 219 mg ACN/L. Although the major ACNs of pomegranates depend on the variety, the 3-glucosides and 3,5-diglucosides of cyanidin, delphinidin and pelargonidin were reported (Hernandez *et al.* 1999; Gil *et al.* 2000; Marti *et al.* 2002).

ACNs are unstable and susceptible to degradation, leading to a brownish color during processing and storage as a result of the oxidation of ACNs due to their antioxidant properties. The polymerization of ACNs with tannins is associated with both color deterioration as well as original color (Rommel *et al.* 1992). During juice processing, fining agents and heat treatments (e.g., pasteurization and concentration) cause changes in the color of ACN containing products (Rommel *et al.* 1992). Knowledge of the changes that ACN pigments undergo during processing and storage is important with respect to their role in color quality.

The major objective of our investigation was to evaluate the effects of clarification and storage on the stability of ACNs and color of pomegranate juice concentrate (PJC) samples. A secondary objective was to characterize the ACNs of the Hicaznar variety by high-performance liquid chromatography (HPLC) techniques and monitor changes during clarification and storage.

MATERIALS AND METHODS

Chemical and Reagents

Standards of cyanidin 3-*O*-glucoside and cyanidin 3,5-*O*-diglucoside were purchased from Sigma (St Louis, MO), pelargonidin 3-*O*-glucoside and pelargonidin 3,5-*O*-diglucoside from Fluka (Seelze, Germany) and delphinidin-3-*O*-glucoside from Polyphenols Laboratories AS (Sandnes, Norway). All reagents used for liquid chromatography were of HPLC grade and obtained from Merck (Darmstad, Germany). The other reagents used in this study were of analytical grade and obtained from Merck.

Samples

Pomegranates (*Punica granatum* L. var. Hicaznar) were obtained from Alata Horticultural Research Institute (Erdemli, Mersin) in October 2006. Hicaznar variety is native to Turkey and highly cultivated in Mediterranean region. Pomegranates were processed into juice in the fruit juice pilot plant at Ankara University. They were stored only for a very short time after harvest (at 4C for 2 days) before processing into juice. A flow diagram for the processing of PJC is shown in Fig. 1. Before juice extraction, pomegranates were washed in cold tap water and drained. The top and bottom of pomegranate rinds were removed with a sharp stainless steel knife to prevent microbial contamination. The pomegranates with rinds (50 kg) were cut into four pieces and processed into juice. The pomegranate quarters were pressed on a rack-and-cloth press (Bucher-Guyer, Niederweningen, Switzerland).

Since pomegranates contain an insignificant amount (0.02–0.04%) of pectin (Benk 1971) or no pectin (Magerramov *et al.* 2007), pomegranate juice was not depectinized. Similarly, pomegranates contain insignificant amount of protein; therefore, bentonite was not used. Therefore, among the clarification agents, only gelatin was used for clarification. The primary function of gelatin was to remove the high molecular weight polyphenols, such as hydrolyzable tannins, by forming flocks. The tannins in pomegranate juice are responsible for astringent taste and cause turbidity during storage.

The cloudy juices from press were divided into two parts. Halves were clarified using only gelatin at 5C (cold clarification). The remaining juices were not clarified. The gelatin

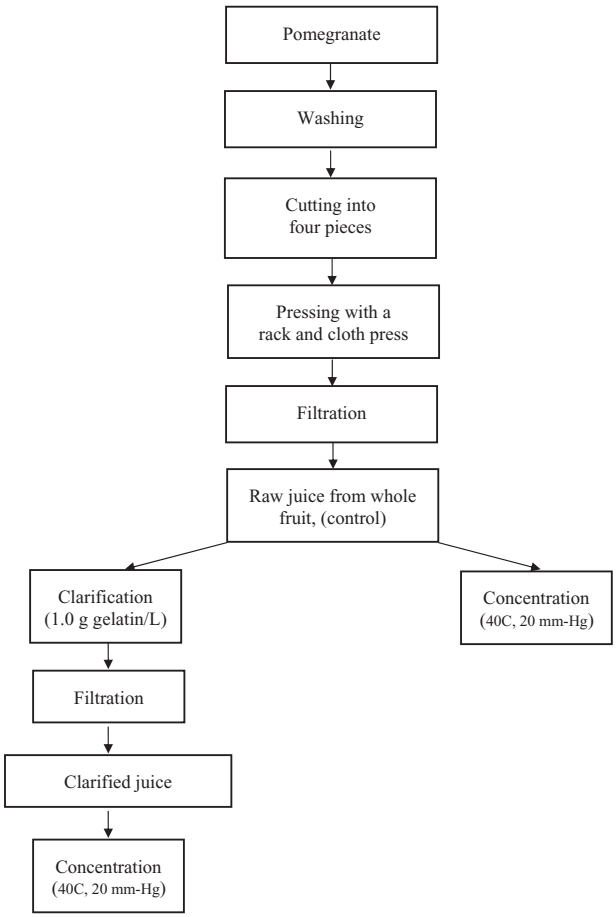

FIG. 1. PROCESSING OF POMEGRANATE JUICE CONCENTRATE

solutions at 1% (w/v) were used for the clarification of juice. The gelatin (A type, 80–100 Bloom strength) was used at a concentration of 2 g/L for juice. Since pomegranate juice contained high amount of polyphenols, kieselsol was not used. After clarification, the turbidity of juice was 1.75 NTU.

The juice samples were concentrated to 68°Bx by a rotary low-pressure evaporator (Heidolph Laborota 4003, Schwabach, Germany) at 40C and 20 mm-Hg pressure.

Compositional Analysis

The total soluble content (°Brix) of PJCs was determined by an automatic digital refractometer (Atago Rx-7000α, Tokyo, Japan). °Brix measurements were carried out at 20C. pH was measured potentiometrically with a pH meter (WTW Inolab Level 1, Weilheim, Germany). Titratable acidity was determined according to the method outlined by IFU (1968) and expressed as "*g anhydrous citric acid/100 g sample*." The turbidity was measured by turbidimeter as NTU (HACH Ratio/XR 43900, Loveland, CO).

ACN Analysis

The total ACN content was determined using the pH-differential method described by Giusti and Wrolstad (2005). The concentrate samples were diluted to their actual °Brix by weighing out 2.5 g concentrate into 25-mL volumetric flask and bringing the final volume with distilled water. The pH of diluted concentrate samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. The dilutions were then allowed to equilibrate for 15 min at room temperature (~22C). Prior to absorbance measurements, the solutions were filtered through a 0.45-μm PVDF (polyvinylidene fluoride) filter (Millipore, Bedford, MA) to remove the haze. The absorbance of equilibrated solutions was measured at 512 nm (λ_{max}) for ACN content and 700 nm for haze correction on a UV-vis double-beam spectrophotometer (ThermoSpectronic Helios-α, Cambridge, U.K.) with 1 cm path length disposable cuvettes (Brand Gmbh, Wertheim, Germany). Absorbance measurements were carried out at room temperature and made against distilled water as a blank. Pigment content was calculated as cyanidin-3-glucoside equivalents with a molecular weight of 449.2 g/mol and extinction coefficient of 26,900 L/cm/mol. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to ACN concentration. ACN measurements were replicated three times.

Polymeric Color Content

Percent polymeric color contents were determined using the bisulfite bleaching method described by Giusti and Wrolstad (2005). The absorbance of bisulfite-treated and nontreated solutions were measured at 420 nm for brown pigments, 512 nm (λ_{max}) for monomeric ACNs and 700 nm for haze correction. Disposable cuvettes of 1 cm path length were used. Absorbance measurements were carried out at room temperature and made against distilled water as a blank. Polymeric color measurements were replicated three times.

Colorimetric Measurements

The color of PJC samples was measured with a light reflectance spectrophotometer (Minolta CM-3600d, Osaka, Japan). Measurements were recorded in L^* (lightness), $+a^*$ (redness) and $+b^*$ (yellowness) CIE (Commission Internationale de l'Eclairage) color coordinates. Chroma (C^*) and color tone (hue angle, $h°$) were calculated from a^* and b^* color coordinates using the following equations:

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2}$$

$$h° = \arctan\left(b^*/a^*\right)$$

During color measurements, a specular component was included with C illuminant and 10° observer angle. The concentrate samples were diluted with distilled water to juice °Brix (ca. 16°Bx). The diluted samples were then transferred to 1 cm path length quartz cuvettes with a 10-mL capacity. Measurements were replicated three times.

HPLC Separation of ACNs

Extraction. ACNs were extracted following the method described by Lee and Wrolstad (2004). PJC (1.25 g) was mixed with 10 mL aqueous acetone (30:70, v/v, 70%) and homogenized at 9,500 rpm for 2 min (Heidolph Silent-Crusher M). The acetone extract was filtered on a Buchner funnel using Whatman No. 1 filter paper. ACNs on the homogenizer blade were washed with first 10 mL 100 acetone and then with 10 mL 70% acetone. The resulting acetone extract was filtered through a Buchner funnel and combined with the previous acetone extract. The filter cake was also extracted with 70% acetone until the solution became colorless. The filtrates were combined and partitioned with chloroform (1:2 acetone : chloroform, v/v) in a separatory funnel and left overnight at 4C for complete separation of organic and aqueous phases. The red-colored aqueous phase containing ACNs was collected and transferred to a rotary evaporator (Heidolph Laborota 4003) to remove residual acetone at 40C. The aqueous extract was dissolved in purified water (containing 0.01% HCl, v/v), and the final volume was brought to 10 mL with purified water. The resulting extract was filtered through a 0.45-μm PVDF filter (Millipore) directly to an amber-colored bottle. Three extracts were prepared from each sample.

ACN Purification. The ACNs were purified on a C-18 cartridge (Waters Co., Milford, MA) using vacuum manifold system (Waters Co.). Prior to sample load, the cartridge was activated with 5 mL ethyl-acetate followed by 5 mL methanol containing (0.01% HCl, v/v) and 2 mL aqueous 0.01% HCl (v/v). After loading of 1 mL ACN extract, the cartridge was washed with 2 mL aqueous 0.01% HCl to remove compounds not adsorbed by the column, such as sugars and organic acids. The cartridge was then dried under a stream of nitrogen for 10 min. Non-ACN polyphenols were removed from the cartridge by rinsing with 5 mL ethyl-acetate. Elution of ACNs was carried out by rinsing the cartridge with 2 mL methanol (containing 0.01% HCl, v/v). The methanol extract containing ACNs was then evaporated to dryness in a water bath (Memmert WB 14, Schwabach, Germany) at 35 ± 0.1C under a stream of nitrogen, and ACNs were dissolved in aqueous 0.01% HCl. The resulting extract was filtered through a 0.22-μm PVDF filter (Sartorious AG, Goettingen, Germany) directly to an amber-colored autosampler vial, and the filtered extract was immediately injected into HPLC.

Instrumentation and Chromatography. Separation and quantification of ACNs were performed using HPLC (Agilent 1200 series, Waldbronn, Germany) with a binary pump, a photodiode array (PDA) detector, a thermostatted autosampler, a degasser and a thermostatted column compartment. Chromatographic data were recorded and processed on an Agilent 1200 series ChemStation rev.B.02.01 software. ACNs were separated on a C_{18} (5 μm) column (250 × 4.6 mm) (Phenomenex, Los Angeles, CA) with a C_{18} (5 μm) guard column (4 × 3 mm, 5 μm) (Phenomenex). The eluents used were (A) 100% acetonitrile and (B) *O*-phosphoric acid, acetic acid, acetonitrile, water (1:10:5:84; v/v/v/v) with a flow rate of 1 mL/min.

Separation was performed with gradient elution using a modification of the elution profile described by Skrede *et al.* (2000). The linear gradient program for the separation of pomegranate ACNs was as follows: from 0% to 12% A in 10 min, from 12% to 22% in 10 min, and holding at 22% A for 5 min. The sample injection volume was 50 μL, the column temperature was 25C and the detector was set at 520 nm. Identification of ACNs was carried out by comparing retention times and absorption spectra of unknown peaks with external reference standards. Quantification of ACNs was carried out using calibration curves of the following external reference standards: cyanidin 3-*O*-glucoside ($R^2 = 0.9995$), cyanidin 3,5-*O*-diglucoside ($R^2 = 0.9834$), delphinidin 3-*O*-glucoside ($R^2 = 0.9749$), pelargonidin 3-*O*-glucoside ($R^2 = 0.9944$) and pelargonidin 3,5-*O*-diglucoside ($R^2 = 0.8980$). The calibration curves for each ACN standard contained seven data points. Quantification of total ACNs by HPLC was calculated based on cyanidin 3-*O*-glucoside.

As mentioned above, five out of six ACNs in PJCs were identified by comparing retention times and absorption spectra of unknown peaks with external reference standards. Because the commercial standard for delphinidin-3,5-diglucoside was not available at the time of analysis, mass spectrophotometric detection was carried out for delphinidin-3,5-diglucoside on the same HPLC system used for the identification of ACNs in PJC. The mass/charge (*m/z*) ratio of 627 for delphinidin-3,5-diglucoside was used (Brito *et al.* 2007). The DAD detector was interfaced with a mass spectrometer (Agilent 1200 Series HPLC system) with an ESI source operating in positive ionization mode. Nitrogen was used at a flow rate of 12 L/min and a pressure of 35 psig, both as a drying and a nebulizing gas. The nebulizer temperature was set at 250C, and a potential of 2,000 V was used on the capillary. The mobile phase consisted of 100% acetonitrile (eluent A) and 1% formic acid (v/v, eluent B). The other chromatographic conditions were the same as described above for the HPLC separation of ACNs in PJC.

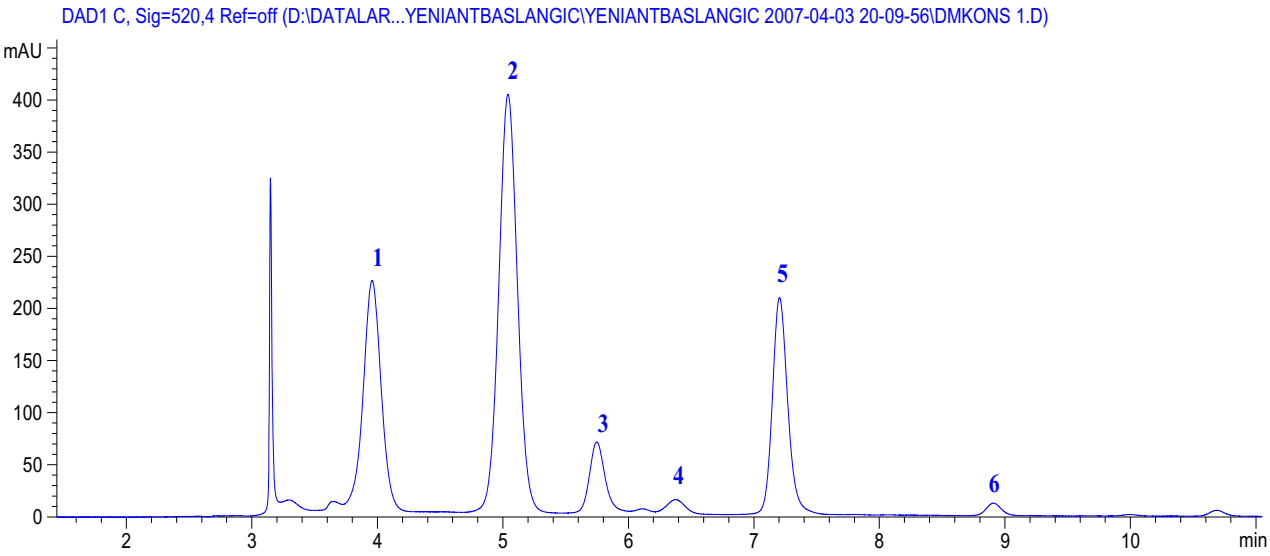

FIG. 2. HPLC CHROMATOGRAM FOR THE ANTHOCYANINS OF POMEGRANATE JUICE CONCENTRATE
1: Dp-3,5-diglu, **2:** Cy-3,5-diglu, **3:** Dp-3-glu, **4:** Pg-3,5-diglu, **5:** Cy-3-glu, **6:** Pg-3-glu.

Recovery Assays. Recoveries were determined by adding a known amount of the external standard of cyanidin-3,5-diglucoside, delphinidin-3-glucoside, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside and pelargonidin-3-glucoside to the PJC samples before extraction of ACNs. For recovery assays, 1 ± 0.001 g of PJC was weighed in test tubes, and the external standards were spiked into concentrate samples at the same or half of the concentration in original sample. Recovery assays were carried out in duplicate. The amount extracted for each spiked level was calculated from the calibration curves of ACN standards. Recoveries and reproducibilities were calculated according to the equations given by Li *et al.* (2002).

Determination of Limit of Detection and Quantification. The limit of detection (LOD) and limit of quantification (LOQ) for ACNs were determined based on the signal to noise (S/N) ratio. According to ICH guideline for validation of analytical procedures, an acceptable S/N is 3:1 (or 2:1) for estimating the LOD and 10:1 for estimating the LOQ (Li *et al.* 2002).

Statistical Analyses

Results from ACN content of PJC samples were analyzed by three-way analysis of variance using the Minitab statistical software, version 14 (Minitab Inc., State College, PA). Clarification and storage times at each storage temperature were considered as the main effects. Statistical differences among means were determined by Duncan's multiple range test at a significance level of 5%.

RESULTS AND DISCUSSION

Compositional Measurements

There were very slight differences in pH, titratable acidity and soluble solid content (°Brix) of the samples after clarification and during storage (data not shown). For example, the pH, titratable acidity and soluble solid contents of PJC samples during storage ranged from 3.22 to 3.38, 5.81 to 6.23 g/100 g (as anhydrous citric acid) and 67.9 to 69.4°Bx , respectively. Similarly, Rommel *et al.* (1992) observed slight changes in the compositional measurements in red raspberry and blackberry juices during processing and storage. The results from compositional analyses showed that clarification did not have important effects on the organic acid and sugar contents of PJCs.

Characterization of ACNs in PJC

The separation of ACNs by HPLC is shown in Fig. 2. Among the 6 ACNs detected, cyanidin-3,5-diglucoside was the major ACN in PJC with 47.9% of total peak area, followed by delphinidin-3,5-diglucoside (23.2%), cyanidin-3-glucoside (18.5%), delphinidin-3-glucoside (7.7%), pelargonidin-3,5-diglucoside (1.7%) and pelargonidin-3-glucoside (1%). Studies showed that the major ACN of pomegranates depends on variety. The major ACN was cyanidin-3-glucoside in "Wonderful" variety (American variety) (Gil *et al.* 2000), cyanidin-3-glucoside (40%) and cyanidin-3,5-diglucoside (38%) in "Mollar" variety (Spanish variety) (Marti *et al.* 2002), delphinidin-3-glucoside in "Assaria" variety (Portuguese variety) (Miguel *et al.* 2004) and

TABLE 1. CHANGES IN ACN CONTENTS AND POLYMERIC COLOR OF POMEGRANATE JUICE CONCENTRATE SAMPLES DURING STORAGE

Concentrate samples	Temperature (C)	Time (days)	ACN content* (mg/kg) pH-differential	ACN content* (mg/kg) HPLC	Polymeric color (%)
From unclarified juice (control)					
	–23, 5, 12, 20	0	1,091 ± 14.0a	1,005	38 ± 0.18d
	–23	334	1,018 ± 18.0b (7%)†	1,357	31 ± 0.00e
	5	167	631 ± 6.40c (37%)	399 (60%)	46 ± 0.00c
	12	101	445 ± 9.77d (59%)	446 (56%)	50 ± 0.00b
	20	73	189 ± 0.70e (83%)	189 (81%)	61 ± 0.54a
From clarified juice					
	–23, 5, 12, 20	0	863 ± 5.87a	839	39 ± 0.97d
	–23	334	862 ± 4.91a	1,148	37 ± 0.18e
	5	167	420 ± 6.83b (51%)	315 (63%)	52 ± 0.01c
	12	101	317 ± 2.12c (63%)	357 (57%)	57 ± 0.09b
	20	73	132 ± 1.60d (85%)	130 (85%)	66 ± 0.21a

* Expressed as cyanidin-3-*O*-glucoside, and values are expressed in mean ± SE ($n = 3$).

† Values in parentheses are ACN losses.

a–e: Different letters in the same column are significantly different at 5% level.

delphinidin-3,5-diglucoside in Sweet Aalak, Sooleghan, Malase Ardestan, Saveh Sweet White Leather, Malase Ashkezar, Saveh Black Leather, Ardestan Black Leather and Ostokhanie Tabas varieties (Iranian varieties) (Mousavinejad *et al.* 2009).

Difference between Total ACN Contents from Two Methods

Total ACN contents of PJC samples were determined by both pH-differential (spectrophotometric) and HPLC methods (Table 1). Although there were differences between total ACN contents obtained by both methods, there was high correlation ($r = 0.997$) between the amounts of ACNs found in samples by both methods. Similarly, Lee *et al.* (2002) also indicated that total ACN contents of blueberry juices obtained by the pH differential method were different from those obtained by HPLC. The differences in ACNs from two methods are attributed to several factors. First, the ACN spectral characteristics may be influenced by the different solvent systems utilized for HPLC and pH differential methods (Lee *et al.* 2002). Second, the ACN analyses were carried out at different wavelengths, i.e., 520 and 512 nm by HPLC and pH differential methods, respectively. Third, the interference of polymeric pigments may have occurred during ACN analyses. In fact, polymeric pigments may be retained in the HPLC column and not included in HPLC measurements, whereas they might have contributed to the results from the pH differential method (Lee *et al.* 2002).

Changes in Total ACN Content during Clarification

Total ACN content of pomegranate juice decreased substantially after clarification. The PJC obtained from clarified juice had much lower ACN content than the PJC obtained from unclarified juice (Table 1). The loss of ACNs was more than 20% in PJC obtained from clarified juice as compared with PJC obtained from unclarifed juice. The apparent decrease in ACN content was caused by the interaction between ACNs and gelatin-tannin flocks formed during clarification. Similarly, previous report also showed that there were 18% decreases in ACN contents of blackberry juices after clarification (Rommel *et al.* 1992).

Changes in Total ACN Content during Storage

The effects of storage on ACNs in PJC samples were studied at −23, 5, 12 and 20C. Analysis of kinetic data suggested first-order model ($R^2 = 0.973$ in unclarified juice and $R^2 = 0.999$ in clarified juice; figure not shown) for the degradation of ACNs during the storage of PJCs at 68−69°Bx. Our results agree with those from the previous studies, reporting a first-order reaction model for the degradation of ACNs from various sources (Kirca and Cemeroğlu 2003; Kirca *et al.* 2007). The first-order reaction rate constants (k) and half-lives ($t_{1/2}$), i.e., the time needed for 50% degradation of ACNs, were calculated by the following equations:

$$\ln(C_t/C_0) = -k \times t,$$

$$t_{1/2} = -\ln 0.5 \times k^{-1},$$

where C_0 is the initial ACN content and C_t is the ACN content after t minutes heating at a given temperature.

The stability of ACNs was significantly affected by storage temperature ($P < 0.05$). As expected, higher storage temperatures increased the rate of ACN degradation in PJCs (Table 2). For example, the calculated $t_{1/2}$ values for ACN deg-

TABLE 2. KINETIC PARAMETERS FOR THE ACN DEGRADATION AND POLYMERIC COLOR FORMATION OF POMEGRANATE JUICE CONCENTRATE DURING STORAGE

Concentrate samples	Temperature (C)	ACN degradation				Polymeric color formation		
		$-k \times 10^3$ (day^{-1})	$t_{1/2}$ (days)	Q_{10} (5–20C)	E_a (kJ/mol)	$-k \times 10^3$ (day^{-1})	Q_{10} (5–20C)	E_a (kJ/mol)
From unclarified juice (control)								
	5	3.22 *(0.973)*[a]	215			1.15 *(0.994)*		
	12	8.75 *(0.999)*	79	3.79	90	2.76 *(0.970)*	3.08	76
	20	23.72 *(0.999)*	29			6.22 *(0.995)*		
From clarified juice								
	5	4.15 *(0.986)*	167			1.84 *(0.856)*		
	12	9.90 *(0.998)*	70	3.36	82	4.15 *(0.985)*	2.52	62
	20	25.56 *(0.990)*	27			7.37 *(0.951)*		

a Numbers in parentheses are the determination coefficients (R^2).
Values in italics are the determination coefficients (R^2). R^2 takes on values between 0 and 1. The higher the value, the more useful the model.

radation at 5, 12 and 20C were, respectively, 167, 70 and 27 days in PJC obtained from clarified juice. Previous studies showed that the $t_{1/2}$ values of ACNs of sour cherry juice concentrate (at 71°Bx) and blood orange juice concentrate (at 69°Bx) at 20C were 38 days (Cemeroğlu *et al*. 1994) and 0.74 days (Kirca and Cemeroğlu 2003), respectively. These $t_{1/2}$ values indicated that ACNs in PJC have higher stability than the ACNs in blood orange juice concentrate, but they have lower stability than the ACNs in sour cherry juice concentrate. The comparatively low stability of the ACNs of pomegranates can be attributed to the nonacylated and nonmethoxy composition of pomegranate ACNs. The chromatogram of ACNs in PJCs showed that no acylated and no methoxylated ACNs were present in PJCs (Fig. 2). Studies showed that the acylated ACNs considerably higher half-lives than the nonacylated ACNs. In fact, the ACNs from black carrot (Kirca *et al*. 2007), red-fleshed potato and red radish (Rodriguez-Saona *et al*. 1999) have much higher stability during storage than the ACNs from pomegranates. The high stabilities of the ACNs from these sources can be attributed to the acylation of their ACNs.

Contrary to lower stability of nonacylated ACNs, they have much higher bioavailability than the acylated ACNs (Oh *et al*. 2008). Nonacylated ACNs showed an 8- to 10-fold higher recovery in plasma and an 11- to 14-fold higher recovery in urine than acylated ACNs in a clinical feeding study (Kurilich *et al*. 2005). Results from these studies clearly showed that acylated ACNs have higher stability during storage, but nonacylated ACNs have higher health benefits. Therefore, while the ACNs of pomegranates have lower storage stability, from the nutritional point of view, they have more health benefits compared to acylated ACNs from other sources.

The rate of ACN degradation was slower in unclarified PJC at all storage temperatures than that of the clarified PJC (Table 2). This may be due to the differences between polyphenol and ACN contents of PJC samples. The concentrates obtained from unclarified pomegranate juices had 34% more polyphenols (Güzel 2010) and 21% more ACNs than those obtained from clarified juices. According to Del Pozo-Insfran *et al*. (2004), the rate of ACN degradation during storage was significantly affected by sources, molar ratios among reactants (ACNs and/or polyphenols), non-ACN polyphenol concentration, secondary free radical formation and other oxidative reactions such as *O*-quinone formation involving with polyphenols and ACNs. Moreover, the degradation of ACNs depends on the specific composition of ACNs as well as the characteristics and composition of food matrix such as the micellar systems, soluble solids and polyphenols (Ruenroengklin *et al*. 2009).

The stability of ACN color can be improved by copigmentation, where the ACN molecule reacts with especially flavonoids, and also alcoloids and amino acids directly or through weak interactions, resulting in an enhanced and stabilized color (Talcott *et al*. 2003). ACN copigmentation gives brighter, stronger and more stable colors than what would be expressed by an intact ACN molecule. Rein (2005) demonstrated that the polyphenol enrichment improved and stabilized berry juice color during storage due to copigmentation effects. Since the clarification with gelatin leads the substantial loss in polyphenols, much lower color stability of gelatin-clarified PJC occurred than the color stability of unclarified PJC during storage.

The ACNs of PJC stored at −23C almost unchanged during 334 days of storage (Table 1). On the contrary, significant losses in ACNs occurred in PJCs stored at 20C. There were 83–85% losses in ACN contents in PJC samples stored at 20C for 73 days. The adverse effects of temperature rise at pH 2–4 (pH of PJCs was 3.3) were shown to induce the loss of the glycosyl moieties of the ACNs by the hydrolysis of glycosidic bond (Adams 1973). This leads to further loss of ACN color since the aglycones are much less stable than their glycosidic forms.

The mechanism of ACN degradation appears to be temperature dependent. The dependence of the degradation of ACNs in PJCs on temperature was determined by calculating

the activation energy (E_a) and temperature quotient (Q_{10}) values from the following equations:

$$k = k_0\ e^{-Ea/RT},$$

$$Q_{10} = (k_2/k_1)^{10/(T1-T2)}$$

The E_a values for the degradation of ACNs in PJCs were between 77 and 90 kJ/mol at 5–20C (Table 2). Our results agree with previous study reporting that the E_a values for the degradation of ACNs below 40C were around 70 kJ/mol (Markakis 1974). Similar E_a values for the degradation of ACNs were reported by Kirca *et al.* (2007) for black carrot juice concentrates at 64°Bx and 4–37C (E_a = 86 kJ/mol), and by Kirca and Cemeroğlu (2003) for blood orange juice concentrates at 69°Bx and at the same temperature range (E_a = 81 kJ/mol).

The E_a value for the degradation ACNs in PJC obtained from unclarified (control) juice (E_a = 90 kJ/mol) was higher than that of PJC obtained from clarified juice (E_a = 82 kJ/mol) (Table 2). A higher E_a value implies that a smaller temperature change is needed for the degradation of ACNs. Although $t_{1/2}$ values at 5C, 12C and 20C of ACNs in PJC obtained from unclarified (control) juice were higher than those of ACNs in PJC obtained from clarified juice, ACNs in PJC obtained from unclarified (control) juice was more affected by change in temperature as compared with ACNs of PJC obtained from clarified juice. This was attributable to copigmentation effect in PJC obtained from unclarified (control) juice. Copigmentation provides high color stability at a given temperature, but a change in temperature affects the copigmentation bond intensity. This phenomenon was reported by Bakowska *et al.* (2003), Mazza and Miniati (1993) and Parisa *et al.* (2007) who found that an increase in temperature causes the decrease in copigmentation bond intensity and hyperchromic shift (ΔA), which means increase in color intensity.

Another way to express the influence of temperature on reaction rate is the use of Q_{10} values. Almost same Q_{10} values were obtained for the degradation of ACNs in PJC samples during storage (Table 2). Slightly lower Q_{10} values were found for the degradation of ACNs in clarified PJC than those from unclarified PJC. High Q_{10} values at 5–20C show that low storage temperatures are needed to prevent the degradation of ACNs in PJCs.

Changes in Polymeric Color during Clarification and Storage

The percent polymeric color is a measure of ACNs resistance to bisulfite bleaching and reflects the degree of ACN polymerization. Even right after pressing, the percent polymeric color values were very high (25% and 29%) for pomegranate juice obtained from arils and whole fruits, respectively (Turfan 2008). During concentration, the percent polymeric color value of PJC reached up to 38%. The percent polymeric color over 10% is usually the indication of storage of fruits and vegetables in unfavorable conditions. However, the pomegranates used in this study were stored only for a very short time after harvest (at 4C for 2 days). The high initial percent polymeric color of pomegranate juice may be attributable to the polymeric ACN-tannin pigments formed in the fruits before juice processing rather than inconvenient storage conditions. The possible mechanism for this polymerization reaction involves condensation of ACNs with other polyphenols present in pomegranates, especially in the rinds. These phenolics are called as hydrolyzable tannins (polymers of gallic or ellagic acid, producing gallotannins or ellagitannins, respectively) (Gil *et al.* 2000) and condensed tannins (also referred to as proanthocyanidins, oligomers and polymers of flavan-3-ols, e.g., catechins and epicatechins) (Pascual-Teresa *et al.* 2000; Shabtay *et al.* 2008). In fact, Gil *et al.* (2000) found 1,979 mg/L hydrolyzable tannins in single-strength pomegranate juice obtained from whole pomegranates. Shabtay *et al.* (2008) also found the fresh pomegranate peel contained condensed tannins as high as 50 mg/g dried matter.

There was insignificant ($P > 0.05$) change in polymeric color of PJC obtained from unclarified and clarified juices before storage. However, the percent polymeric color values of PJC samples increased from 38% to 46–52% after storage at 5C for 167 days, to 50–52% after storage at 12C for 101 days and to 61–66% after storage at 20C for 73 days (Table 1). Similarly, there were significant losses in ACNs of PJCs with storage. Losses of ACNs in PJC samples during storage were accompanied by the increase in the percent polymeric color values, indicating that ACNs were extensively polymerized during storage. There was a negative correlation ($r = -0.988$) between the percent polymeric color values and ACN contents during storage. Other than ACN polymerization during storage, ACN degradation products may have also contributed to polymeric color formed during storage. In fact, it is postulated that the formation of a chalcone is the first step in the thermal degradation of ACNs (Adams 1973). When temperature is increased, the formation of unstable chalcone form is favored, and the chalcone is further degraded to brown products (Jackman and Smith 1996).

The formation of polymeric color during storage followed first-order kinetic model (R^2 = 0.964 in unclarified juice and R^2 = 1.000 in clarified juice; figure not shown). The rate constants (k) for polymeric color formation in unclarified PJC samples were 1.15, 2.76 and 6.22×10^{-3} days^{-1} at 5C, 12C and 20C, respectively (Table 2). The higher k values at higher storage temperatures clearly showed that the formation of polymeric color in PJC samples highly depended on storage temperatures. Similar increases in polymeric color were

TABLE 3. INDIVIDUAL ACN CONTENTS[a] POMEGRANATE JUICE CONCENTRATE SAMPLES DURING STORAGE

	Storage conditions		ACN content (mg/kg)[a]					
Concentrate samples	Temperature (C)	Time (days)	Dp-3,5-diglu[b]	Cy-3,5-diglu	Dp-3-glu	Pg-3,5-diglu	Cy-3-glu	Pg-3-glu
From unclarified juice (control)								
		0	15.8	40	9.6	1.8	15.1	1.3
	5	167	8.1 (49%)	16.3 (59%)	3.6 (63%)	0.6 (67%)	6.0 (60%)	0.3 (80%)
	12	101	8.6 (46%)	17.1 *(57%)*	4.0 (59%)	0.6 (67%)	7.5 (51%)	0.4 (70%)
	20	73	3.9 (75%)	7.7 *(81%)*	2.4 (75%)	0.4 (75%)	4.0 (74%)	0 (100%)
From clarified juice								
		0	10.6	39.6	5.0	1.6	12.3	1.3
	5	167	5.2 (51%)	14.4 *(64%)*	2.2 (56%)	0.7 (55%)	5.3 (57%)	0.3 (80%)
	12	101	5.7 (46%)	15.8 *(60%)*	2.4 (51%)	0.9 (45%)	5.9 (52%)	0.5 (60%)
	20	73	3.1 (71%)	5.9 *(85%)*	0.8 (85%)	0.3 (82%)	3.1 (75%)	0 (100%)

a Values were calculated taking into consideration the recovery rates.
b Delphinidin-3,5-diglucoside was expressed as cyanidin-3-glucoside.

observed during the storage of blackberry juice (Rommel *et al*. 1992).

The E_a and Q_{10} values for polymeric color formation in PJC samples obtained from unclarified and clarified juice at 5C to 20C were 76 and 62 kJ/mol, and 3.08 and 2.52, respectively (Table 2). Higher E_a and Q_{10} values indicated that the formation of polymeric color in PJC obtained from unclarified juices were more affected by temperature changes than PJC obtained from clarified juices. This may be due to higher tannin content of unclarified PJC than clarified juice.

Changes in ACN Profile during Clarification and Storage

The effects of clarification and storage on the stabilities of individual ACNs from PJC were compared by calculating the percent losses (Table 3) and half-life periods ($t_{1/2}$) (data not shown). Although there were slight differences in the three major ACNs (cyanidin-3,5-diglucoside, delphinidin-3,5-diglucoside and cyanidin-3-glucoside) of PJCs obtained from unclarified and clarified juices, the PJCs obtained from unclarifed juice contained more individual ACNs. For example, the losses of cyanidin-3,5-diglucoside were 59% and 64% at 5C after 167 days of storage for PJCs obtained from unclarified and clarified juices, respectively. Similar results were found for delphinidin-3,5-diglucoside and cyanidin-3-glucoside. The $t_{1/2}$ values of delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside and cyanidin-3-glucoside were 180, 137 and 137 days at 5C for PJC obtained from unclarified juice while they were 177, 120 and 150 days at the same temperature for PJC obtained from clarified juice, respectively (data not shown). The stability of individual ACNs depended on the storage temperature. For example, the $t_{1/2}$ values of cyanidin-3,5-diglucoside in PJC obtained from unclarified juice were 137, 77 and 31 days at 5C, 12C and 20C, respectively. Similar results were found for all six ACNs of PJCs during the storage at these three temperatures.

Among the major individual ACNs, delphinidin-3,5-diglucoside was the most stable followed by cyanidin-3-glucoside and cyanidin-3,5-diglucoside at 5–20C. The higher stability of delphinidin-3,5-diglucoside over cyanidin-3,5-diglucoside is mainly due to the increased hydroxylation of the aglycone portion of delphinidin-3,5-diglucoside. Moreover, the diglucosides of delphinidin, cyanidin and pelargonidin were more stable than the monoglucosides of the same ACNs during storage. Our results agree with previous reports (Hernandez *et al*. 1999; Gil *et al*. 2000).

Changes in Color Parameters during Clarification and Storage

CIE L^*, a^*, b^*, C^* and $h°$ values increased 11.6, 11.4, 18.7, 20.4 and 7.2 units after the clarification step, respectively (Table 4). The clarification with gelatin also resulted in 21% loss in total ACN content of PJCs. The similar increases in L^* and b^* values as well as decreases in ACN content were reported by Rommel *et al*. (1992) in blackberry juice and Fang *et al*. (2006) in bayberry juice after clarification. The authors attributed these increases in L^* and b^* values to the loss of ACNs. The $h°$ values increased from 33.55 to 40.75 in PJC obtained from unclarified and clarified juice, which indicates the color shift from red to more yellowish color after clarification. The increase in $h°$ values can indicate both ACN degradation and/or ACN loss. This increase in $h°$ values of clarified samples of PJC was believed to be due to the removal of ACNs

TABLE 4. COLOR PARAMETERS OF POMEGRANATE JUICE CONCENTRATE SAMPLES OBTAINED FROM UNCLARIFIED AND CLARIFIED JUICES

Concentrate samples	L^*	a^*	b^*	C^*	$h°$
From unclarified juice	17.37	44.80	29.71	53.76	33.55
From clarified juice	28.97	56.20	48.43	74.19	40.75

by gelatin-polyphenol flock rather than ACN degradation. Another color parameter that is related with ACN degradation and/or loss is a^* value. The higher a^* values were found for PJC obtained from clarified juice compared to PJC obtained from unclarified juice. This also indicates the ACN losses. The higher L^* values for PJC obtained from clarified juices indicates the removal of brown compounds from pomegranate juice samples during clarification.

No systematic differences among the color parameters were found during the storage of PJCs. This may be attributable to the turbidity of the PJCs obtained from unclarified juice. Moreover, PJCs obtained from clarified juice also become turbid during storage as the storage temperature and time increased. Similar results were obtained for bayberry juice during storage (Fang *et al*. 2006). The authors attributed this phenomenon to the interference of the particles causing turbidity in juices.

CONCLUSIONS

Cold clarification of pomegranate juice with only gelatin resulted in very clear juice. Considerable losses in ACNs occurred during both clarification and storage. The PJC obtained from unclarified juice had significantly higher ACN content than the PJCs obtained from clarified before storage. Thus, gelatin dosage should be carefully determined and the excessive use of gelatin should be avoided. During storage at all storage temperatures, the rate of ACN degradation and polymeric color formation were slower in PJC obtained from unclarified juices than the PJC obtained from clarified juice. Therefore, unclarified pomegranate juice should be concentrated, instead of clarified juice. Meanwhile, higher storage temperatures increased the rates of ACN degradation as well as polymeric color formation in PJCs. The results from this study clearly showed that the PJC produced from unclarified juice should be stored at low temperatures, preferably subfreezing temperatures, to prevent ACN degradation and polymeric color formation. Before marketing the pomegranate juice, the PJC should be diluted to original juice °Brix and then cold-clarified with the minimum amount of gelatin.

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REFERENCES

ADAMS, J.B. 1973. Color stability of red fruits. Food Manuf. *78*, 19–20, 41.

BAKOWSKA, A., KUCHARSKA, A.Z. and OSZMIANSKI, J. 2003. The effect of heating, UV irradiation and storage on stability of anthocyanin–polyphenol copigment complex. Food Chem. *81*, 349–355.

BENK, E. 1971. Zur Kenntnis tropischer and subtropischer Früchte und Fruchtsaefte. Flüssiges Obst. *38*, 210–220.

BRITO, E.S., ARAUJO, M.C.P., ALVES, R.E., CARKEET, C., CLEVIDENCE, B.A. and NOVOTNY, J.A. 2007. Anthocyanins present in selected tropical fruits: Acerola, Jambolao, Jussara, and Guajiru. J. Agric. Food Chem. *55*, 9389–9394.

CEMEROĞLU, B., VELIOĞLU, S. and IŞIK, S. 1994. Degradation kinetics of anthocyanins in sour cherry juice and concentrate. J. Food Sci. *59*, 1216–1218.

DEL POZO-INSFRAN, D., BRENES, C.H. and TALCOTT, S.T. 2004. Phytochemical composition and pigment stability of Açai (*Euterpe oleracea Mart.*). J. Agric. Food Chem. *52*, 1539–1545.

EGE İNTERNET YAYINCILIK MERKEZI (AEGEAN INTERNET PUBLISHING CENTER). 2009. Narda Plansız Üretime Dikkat (Attention to unplanned production of pomegranate) http://www.tarimmerkezi.com/haber_detay.php?hid=12979 (accessed October 6, 2009).

FANG, Z., ZHANG, M., SUN, Y. and SUN, J. 2006. How to improve bayberry (*Myrica rubra* Sieb. Et Zucc) juice color quality: Effect of juice processing on bayberry anthocyanins and polyphenolics. J. Agric. Food Chem. *54*, 99–106.

GIL, M.I., TOMAS-BARBERAN, F.A., HESS-PIERCE, B., HOLCROFT, D.M. and KADER, A.A. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. *48*, 4581–4589.

GIUSTI, M.M. and WROLSTAD, R.E. 2005. Characterization and measurement of Anthocyanins by UV-visible spectroscopy. Unit F1.2. In *Handbook of Food Analytical Chemistry*, (R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz, C.F. Shoemaker, D.M. Smith and P. Sporns, eds.) pp. 19–31, John Wiley & Sons, New York, NY.

GÜZEL, N. 2010. *Changes in procyanidin contents of pomegranate juice concentrate during production*. Master Thesis, 64 pp., Ankara University, Ankara, Turkey.

HERNANDEZ, F., MELGAREJO, P., TOMAS-BARBERAN, F.A. and ARTES, A. 1999. Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. Eur. Food Res. Technol. *210*, 39–42.

IFU. 1968. Analysen. Nr: 3 (Determination of titratable acidity). Internationale Fruchtsaft Union Juris Verlag, Zurich, Switzerland.

JACKMAN, R.L. and SMITH, J.L. 1996. Anthocyanins and betalains. In *Natural Food Colorant*, (G.A.F. Hendry and J.D. Houghton, eds.) pp. 244–296, Blackie Academic & Professional, London, U.K.

KIRCA, A. and CEMEROĞLU, B. 2003. Degradation kinetics of anthocyanins in blood orange juice and concentrate. Food Chem. *81*, 583–587.

KIRCA, A., ÖZKAN, M. and CEMEROĞLU, B. 2007. Effect of temperature, solid content and pH on the stability of black carrot anthocyanins. Food Chem. *101*, 212–218.

KURILICH, A.C., CLEVEDENCE, B.A., BRITZ, S.J., SIMON, P.W. and NOVOTNY, J.A. 2005. Plasma and urine responses are lower for acylated versus nonacylated anthocyanins from raw and cooked purple carrots. J. Agric. Food Chem. *53*, 6537–6542.

LEE, J. and WROLSTAD, R.E. 2004. Extraction of anthocyanins and polyphenolics from blueberry processing waste. J. Food Sci. *69*, 564–573.

LEE, J., DURST, R.W. and WROLSTAD, R.E. 2002. Impact of juice processing on blueberry anthocyanins and polyphenolics: Comparison of two pretreatments. J. Food Sci. *67*, 1660–1667.

LI, T.L., CHUNG-WANG, Y.J. and SHIH, T.C. 2002. Determination and confirmation of chloramphenicol residues in swine muscle and liver. J. Food Sci. *67*, 21–28.

MAGERRAMOV, M.A., ABDULAGATOV, A.I., AZIZOV, N.D. and ABDULAGATOV, I.M. 2007. Effect of temperature, concentration, and pressure on the viscosity of pomegranate and pear juice concentrates. J. Food Eng. *80*, 476–489.

MARKAKIS, P. 1974. Anthocyanins and their stability in foods. CRC Crit. Rev. Food Technol. *4*, 437–456.

MARTI, N., PERZ-VICENTE, A. and GARCIA-VIGUERA, C. 2002. Influence of storage temperature and ascorbic acid addition on pomegranate juice. J. Sci. Food Agric. *82*, 217–221.

MAZZA, G. and MINIATI, E. 1993. *Anthocyanins in Fruits, Vegetables and Grain*, CRC Press, Boca Raton, FL.

MEYED (Turkish Fruit Juice Asssociation). 2011. Turkey fruit juice industry – statistical evaluation http://www.meyed.org.tr (accessed January 15, 2011).

MIGUEL, G., FONTES, C., ANTURES, D., NEVES, A. and MARTINS, D. 2004. Anthocyanin concentration of "Assaria" pomegranate fruits during different cold storage conditions. J. Biomed. Biotechnol. *5*, 338–342.

MOUSAVINEJAD, G., EMAM-DJOMEH, Z., REZAI, K. and KHODAPARAST, M.H.H. 2009. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem. *115*, 1274–1278.

OH, Y.S., LEE, S.H., YOON, C.H., CHOI, D.S., CHOE, E. and JUNG, M.Y. 2008. Characterization and quantification of anthocyanins in grape juices obtained from the grapes cultivated in Korea by HPLC/DAD, HPLC/MS, and HPLC/MS/MS. Food Chem. *73*, 378–389.

ÖZGEN, M., DURGAC, C., SERCE, S. and KAYA, C. 2008. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chem. *111*, 703–706.

PARISA, S., REZA, H., ELHAM, G. and RASHID, J. 2007. Effect of heating, UV irradiation and pH on stability of anthocyanin copigment complex. Pak. J. Biol. Sci. *10*, 267–272.

PASCUAL-TERESA, S., SANTOS-BUELGA, C. and RIVAS-GONZALO, J. 2000. Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. J. Agric. Food Chem. *48*, 5331–5337.

REIN, M.J. 2005. *Copigmentation reactions and color stability of berry anthocyanins*. Dissertation, EKT series 1331, 88 + 34 pp, University of Helsinki, Department of Applied Chemistry and Microbiology, Finland.

RODRIGUEZ-SAONA, L.E., GIUSTI, M.M. and WROLSTAD, R.E. 1999. Color and pigment stability of red radish and red-fleshed potato anthocyanins in juice model systems. J. Food Sci. *64*, 451–456.

ROMMEL, A., WROLSTAD, R.E. and HEATHERBELL, D.A. 1992. Blackberry juice and wine: Processing and storage effects on anthocyanin composition, color and appearance. J. Food Sci. *57*, 385–391.

RUENROENGKLIN, N., SUN, J., SHI, J., XUE, S.J. and JIANG, Y. 2009. Role of endogenous and exogenous phenolics in litchi anthocyanin degradation caused by polyphenol oxidase. Food Chem. *115*, 1253–1256.

SHABTAY, A., EITAM, H., TADMOR, Y., ORLOV, A., MEIR, A., WEINBERG, P., WEINBERG, Z.G., CHEN, Y., BROSH, A., IZHAKI, I. *et al*. 2008. Nutritive and antioxidant potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. J. Agric. Food Chem. *56*, 10063–10070.

SKREDE, G., WROLSTAD, R.E. and DURST, R.W. 2000. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). J. Food Sci. *65*, 357–364.

TALCOTT, S.T., BRENES, C.H., PIRES, D.M. and DEL POZO-INSFRAN, D. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. J. Agric. Food Chem. *51*, 957–963.

TURFAN, Ö. 2008. *Changes in anthocyanins of pomegranate juice concentrate during production and storage*. Master Thesis, 146 pp., Ankara University, Ankara, Turkey.