

EFFECTS OF PROCESSING TECHNIQUES AND COLD STORAGE ON ELLAGIC ACID CONCENTRATION AND SOME QUALITY PARAMETERS OF POMEGRANATE JUICE

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Received / Geliş tarihi: 07.07.2011

Received in revised form / Düzeltilerek geliş tarihi: 14.09.2011

Accepted / Kabul tarihi: 19.09.2011

Abstract

The purpose of this study was the comparative assessment of the alterations in ellagic acid concentration and some quality parameters (soluble solid (°Brix), pH and colour) of pomegranate juices throughout different conventional processing techniques and storage at 4 °C for 150 days. Three different conventional methods were used to process pomegranates into juice. In the first method, a unclarified juice was produced. In the second method, a clear juice was obtained by addition of fining agents. In the third method, a clear juice was obtained by reconstitution from the concentrate at 65-70 °Brix produced by thermal evaporation. A significant increase was observed in ellagic acid concentration during thermal processing and storage period which is likely due to depolymerization of the high molecular weight ellagitannins. Variations during storage were more significant in clear juice compared to unclarified pomegranate juice, which may be due to the contribution of free ellagic acid to the development of insoluble sediments in pomegranate juice during storage.

Keywords: Pomegranate juice, ellagic acid, ellajitannen, clarification, concentration, storage

ÜRETİM TEKNİKLERİ VE SOĞUKTA DEPOLAMANIN NAR SUYUNDAKİ ELLAJİK ASİT MİKTARI VE BAZI KALİTE PARAMETRELERİ ÜZERİNE ETKİLERİ

Özet

Bu çalışmanın amacı, farklı işleme teknikleri ile üretilen nar sularının işleme ve 4 °C'de 150 gün depolama boyunca ellajik asit içeriğinde ve diğer bazı kalite parametrelerinde (renk, suda çözünür kuru madde (°Briks), pH) meydana gelen değişimlerin karşılaştırmalı olarak incelenmesidir. Narlar meyve suyuna üç farklı geleneksel yöntemle işlenmiştir. İlk yöntemde, bulanık meyve suyu üretilmiştir. İkinci yöntemde, durultma yardımcı maddelerin ilavesiyle berrak nar suyu üretilmiştir. Üçüncü yöntemde ise termal

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evaporasyonla üretilen 65-70 °Briksteki konsantreden rekonstitüsyonla berrak nar suyu elde edilmiştir. Isıl işlemler ve depolama boyunca ellajik asit konsantrasyonunun, yüksek molekül ağırlıklı ellajitanenlerin depolimerizasyonuna bağlı olarak, önemli oranda azaldığı gözlenmiştir. Depolama boyunca ellajik asit içeriğindeki azalmanın, berrak nar sularında bulanık olanlara kıyasla, daha belirgin olduğu görülmüştür. Bu durumun nar suyunda serbest ellajik asidin depolama süresince suda çözünmeyen sediment oluşumuna katılmasından kaynaklandığı düşünülmektedir.

Anahtar kelimeler: Nar suyu, ellajik asit, ellajitanen, berraklaştırma, konsantrasyon, depolama

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important fruit crop of many tropical and subtropical regions of the world and generally consumed as fresh (seeds) or juice (1). It is a very rich source of polyphenols including ellagitannins (ETs), such as punicalagin [2,3-(*S*)-hexahydroxydiphenoyl-4,6-(*S,S*)-gallagyl-D-glucose] with proven antioxidant and antiatherosclerotic activity. These ETs are extracted in significant levels into the juice during industrial hydrostatic processing methods of the whole fruits (2-3). Ellagitannins are water-soluble high molecular weight phenolic compounds in which hexahydroxydiphenic acid forms diesters with sugars (most often α -D-glucose). These polymers can be hydrolyzed with acids or bases and the hexahydroxydiphenic acid spontaneously rearranges into the water-insoluble ellagic acid (EA), a plant phenolic compound, which has been shown to be a potent anticarcinogenic agent (4). Recent studies have indicated ellagic acid to possess antimutagenic, antioxidant and anti-inflammatory activity in bacterial and mammalian systems (5).

As in other clear juice manufacturing processes, clarification is a fundamental step in the pomegranate juice production. The purpose of clarification is to remove the substances causing turbidity in the freshly pressed juice and to prevent the eventual development of haze and sediment during storage or after reconstitution of the juice concentrate (6-7). In addition, the taste of the product is improved by clarification, thus, the bitter taste due to the high tannin content can be avoided. In general, the presence of cell fragments and insoluble pectin has been found to be responsible for the immediate turbidity in freshly pressed fruit juices, whereas the development of haze may result from prior

polymerization or condensation leading to the formation of polymeric complexes between polysaccharides, sugars, metal ions, and proteins. The protein-phenol haze forms via hydrogen and/or hydrophobic bonding between so-called haze-active proteins and phenolic compounds can be dispelled by warming but to obtain a bright, clear product, the pomegranate juice must be clarified by fining agents. Clarification typically involves addition of fining agents. Bentonite is effective for protein stabilization. However, a very little effect of bentonite on polyphenol removal has also been reported (8). Gelatin is positively charged in the low pH range of fruit juices and reacts with negatively charged phenolics. When added in solution, gelatin forms insoluble gel lumps which are allowed to sediment slowly; both the gelation and the sedimentation processes further capture additional insoluble and particulate material to further reduce the immediate turbidity.

So far, studies on pomegranate juice processing have mainly focused on technological aspects of membrane filtration systems (9-11). Nevertheless, the literature has little information on the product's quality during conventional processing of pomegranate into juice including clarification by using fining agents and thermal concentration. There is a lack of a comparison of processing technologies regarding the influence of storage on quality of resulting pasteurized pomegranate juices. Accordingly, the objective of our research is to evaluate the changes in ellagic acid concentration and some quality parameters (soluble solid ($^{\circ}$ Brix), pH and colour) of pomegranate juices throughout different processing techniques and cold storage.

MATERIALS AND METHODS

Materials

The sweet pomegranates used in this study were obtained from a local market in Ankara, Turkey. Gelatin and bentonite were obtained from Aldrich (Milwaukee, WI). All solvents were of chromatographic grade and purchased from Merck (Germany). Ellagic acid standard and all other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA).

Determination of Soluble solid (°Brix), pH and colour

Soluble solid (°Brix) values were analyzed by Bausch & Lomb Abbe-3L type refractometer at 20 °C. Colour measurements were performed using the $L^*a^*b^*$ colour space (CIE LAB space) with Minolta Spectrophotometer CM-3600d by placing the juice in a transparent plastic cell of 10 mm path length and by using a black plate as the background to standardize the measurements. All measurements are taken under the conditions of standard illuminant D65 and 10° observer. The L^* value indicates lightness, the a^* and b^* values are the chromaticity coordinates (a^* , from green to red, b^* , from blue to yellow).

The pH measurements were carried out with a Suntex glassy electrode/pH meter (Suntex SP-701, Taipei, Taiwan). The reported values are the means of three replicates.

Preparation of pomegranate juice

Pomegranates were stored at 4 °C before preparation of juice samples. They were washed with water to reduce surface dirt and microbial flora. Then fruits were cut from the middle into two pieces, pressed with a laboratory type press to obtain unclarified juice with a yield of 35-40% (w/w). Three types of pomegranate juices were produced by different treatments. The details are given below:

Juice I (Unclarified pomegranate juice): The unclarified pomegranate juice was bottled in amber bottles and pasteurized at 80 °C for 25 min.

Juice II (Clear pomegranate juice): Clear juice was obtained after filtering the unclarified juice through two layers of cheesecloth following flocculation with 300 mg/L of gelatin and 300 mg/L of bentonite at 50 °C for 1 hour. The clear

pomegranate juice was bottled and pasteurized at 80 °C for 25 min.

Juice III (Clear pomegranate juice from concentrate): Clear juice was concentrated using a rotary evaporator (Heidolph, Germany) at 65 °C. Before analysis, clear juice reconstituted from the concentrate and the soluble solid was adjusted to 17 °Brix with an Abbe type refractometer. Reconstituted juice samples were pasteurized at 80 °C for 25 minutes.

The prepared pomegranate juices were analyzed immediately after processing. The juice samples prepared by different processing techniques were stored at 4 °C for 150 days in amber glass bottles. Triplicate solutions were prepared for each experiment and the mean values are reported in each case.

Determination of Ellagic Acid

HPLC Equipment

The quantification of ellagic acid was performed by an Agilent 1100 HPLC system (Waldbronn, Germany). The analytical separation was performed on an ACE 5 C18 column (250 x 4.6 mm, 5 µm). HP Chem-Station for LC (rev. A08.01) was used for data processing.

Analysis of Ellagic Acid by HPLC

Samples were diluted (1:3) with methanol and filtered through 0.45 µm Millipore filter and injected onto HPLC. HPLC elution was carried out at room temperature (20±2 °C). The following solvent system and elution profiles were used: solvent A, the mixture of formic acid and water (5:95, v/v), and as solvent B, methanol. The elution profile was 15% solvent B isocratic for 5 min followed by a 15–30% linear gradient for 15 min and 30–50% linear gradient for 10 min with solvent B and holding with 50% solvent B for an additional 10 min, and finally followed by a 50–15% linear gradient with solvent B for 10 min (12). The flow rate was 0.7 mL/min. Chromatograms were recorded at 254 nm with spectra (200–600 nm) taken continuously throughout the elution for confirmation.

Statistical Analysis

Statistical examinations of data were performed by using an SPSS software (9.05 for Windows) package. The differences in examined quality

Table 1. Influence of processing methods and storage on some quality parameters of pomegranate juices^a.

	°Brix, %	pH	Juice color					
			L	a	b	hue	chroma	
Juice I	Raw juice	15.4±0.0	3.18±0.04	27.1±0.1	12.2±0.1	3.6±0.1	0.29±0.00	12.76±0.10
	After pasteurization & cooling	15.4±0.0	3.19±0.12	27.8±0.2	11.8±0.1	2.5±0.3	0.21±0.02	12.04±0.17
	After storage	15.9±0.1	3.14±0.02	30.7±0.1	14.4±0.6	0.1±0.0	0.01±0.00	14.45±0.65
Juice II	Raw juice	16.0±0.0	3.24±0.00	26.1±0.1	9.2±0.1	2.2±0.0	0.23±0.00	9.43±0.10
	After clarification & filtration	16.0±0.0	3.27±0.00	26.5±0.1	10.6±0.1	3.0±0.1	0.28±0.00	11.03±0.09
	After pasteurization & cooling	16.0±0.0	3.29±0.00	26.8±0.1	9.9±0.1	2.6±0.1	0.26±0.00	10.26±0.06
	After storage	15.2±0.0	3.32±0.02	26.3±0.1	9.0±0.1	2.3±0.1	0.25±0.00	9.26±0.10
Juice III	Raw juice	17.0±0.0	3.54±0.01	26.1±0.1	6.6±0.1	0.3±0.1	0.05±0.01	6.57±0.03
	After clarification & filtration	17.0±0.0	3.53±0.01	28.1±0.2	9.6±0.2	0.9±0.3	0.10±0.03	9.68±0.22
	After concentration	NA	3.61±0.01	28.2±0.1	7.1±0.1	1.6±0.1	0.23±0.00	7.27±0.10
	After pasteurization & cooling	NA	3.52±0.04	30.3±1.3	7.0±1.2	2.8±0.5	0.39±0.00	7.53±1.29
	After storage	NA	3.61±0.01	28.2±0.1	5.5±0.1	2.6±0.1	0.45±0.00	6.14±0.12

^a Abbreviations: NA, not applicable. Each value is expressed as a mean ± SD; n = 3.

parameters were compared using one-way ANOVA. When analysis of variance revealed a significant effect ($P < 0.05$), data means were compared with the least significant difference test.

RESULTS AND DISCUSSION

Effects of processing methods and storage on soluble solid, pH and colour values of pomegranate juices

The effects of processing methods and storage at 4 °C for 150 days on soluble solid (°Brix), pH and colour of pomegranate juices are shown in Table 1. Soluble solid (°Brix) contents of pomegranate juices obtained by three different processing methods varied significantly ($P < 0.05$). The effect of pasteurization was also found to be significant ($P < 0.05$). The variation in pH of pomegranate juices after pasteurization was not significant with coefficient of variation of 4.34%. However, pH values of pomegranate juices after different processing methods varied significantly ($P < 0.05$). There was an increase in lightness (L) value of each juice after pasteurization. Pasteurization also resulted in significant decreases in redness (a) values of juices which could be attributed to the loss of anthocyanins, in accordance with previous reports regarding red fruits (13). In Juice III, concentration process was resulted in an increase in lightness (L) and a

decrease in redness (a) values and the same trend continued throughout the pasteurization process. The most significant colour loss resulting in undesirable brown colour was observed in clear juice obtained from concentrate. The decrease of a* values can be attributed to the degradation or polymerization of anthocyanins at high temperatures. The colour of clear pomegranate juices were slightly lighter and more red after clarification processes compared to unclarified juice, as expected. Storage at 4 °C for 150 days resulted in a 5% decrease in the °Brix of clear pomegranate juice (Juice II) which may be due to formation of insoluble sediments, in agreement with previous studies (14). However, a slight increase was observed in soluble solid values of unclarified pomegranate juices after 150 days storage at 4 °C. A slight, but not significant ($P > 0.05$) increase in pH was found after storage in almost all treatments. Considerable alterations in lightness (L value) and redness (a value) were observed in clear juices (Juice II and Juice III) throughout the storage, showing a general decrease, which was higher for those juices concentrated in rotary evaporator. There was a slight increase in L and a values of unclarified pomegranate juice (Juice I) after storage. The low stability of anthocyanins has been reported in processed and stored pomegranate juices (13). Loss of anthocyanin pigments during storage is probably due to oxidation. Finally, a considerable alteration was observed in yellowness (b value) for all juices after 150 days storage at 4 °C.

Effect of processing methods and storage on ellagic acid content of pomegranate juices

Figure 1 represents the chromatograms of the ellagic acid standard and clarified pomegranate juice from concentrate (Juice III) after each processing step monitored at 255 nm as an example. Ellagic acid has been identified in pomegranate juice by comparing the retention times and spectral characteristics of the peaks against that of standard, as well as by spiking the samples with standard.

Significant changes in ellagic acid concentration were observed in each juice during storage, and were influenced by processing methods (Figure 2). In Juice I, a 4.5-fold increase was observed after pasteurization compared to raw juice. There was a 2-fold decrease in the ellagic acid concentration of Juice II after clarification process owing to the combined effect of gelatin and bentonite. However, the content of ellagic acid was increased 4-fold after pasteurization process. Similar trend observed in Juice III. The concentration process resulted in a 2-fold increase in the ellagic acid content of clarified juice and an additional 2-fold increase observed after pasteurization. Alper *et al.* (6) reported a 22.7% reduction in total phenolic content of pomegranate juice following conventional fining and pasteurization. Ellagic acid continued to increase during storage at 4°C for 150 days in Juice I and Juice II. Variations during storage were more significant in Juice II with a 97% increase compared to 57% increase in Juice I. In Juice III, a slight decrease observed in the content of free ellagic acid after storage with a ratio of 4.5%. The effect of thermal processing and storage on ellagic acid content agreed with previous studies. The concentration of ellagic acid in strawberry purees was almost doubled after 16 weeks storage at 22 °C (15). In raspberry jam, free ellagic acid increased steadily during jam cooking and storage at 20 °C (16). The increase observed during heating (concentration and pasteurization processes) and storage period is likely due to depolymerization of the high molecular weight ellagitannins. On the other hand, ellagic acid has been reported to have protein-binding activity with o-diphenol groups and has been demonstrated as one of the major phenolic compound responsible for the formation of insoluble sediments in muscadine juice,

blackberry juice, loganberry wine, and muscadine wine (17). Its concentration in sediments was significantly affected by the processing technologies. The content of ellagic acid increased following thermal treatment of Welder and Carlos grape juices but declined after appearance of sediment after 2 weeks of storage and continued to increase over time as ellagic acid decreased (17). Our results were in agreement with previous studies. During storage, a yellowish to red crystals appeared in Juice I and tended to increase throughout storage. Hence, the relatively slight increase in ellagic acid concentration of Juice I in comparison with Juice II and the slight decrease in ellagic acid concentration of Juice III after storage may be due to formation of insoluble ellagic acid sediments. Free ellagic acid increases with hydrolysis of its precursors including ellagic acid glycosides and ellagitannins after thermal treatments, hence contributes to the development of insoluble sediments in pomegranate during storage. Ellagic acid content decreased with a ratio of 56% in Juice II after clarification process. The clarification process resulted in a 60% decrease in free ellagic acid content of Juice III, but concentration process resulted in an additional increase in free ellagic acid content, thereby contributing more to sediment formation throughout storage. According to the work of Garrido *et al.*, (18), the formation of ellagic acid sediments in white muscadine juice was accelerated by increased storage temperature and following thermal pasteurization (100 °C for 10 min), which resulted in more sediment than sterile filtered juices after 8 months storage at 1.5 °C.

CONCLUSIONS

In this study, the effects of different pomegranate juice processing techniques and cold storage on the ellagic acid concentration and on some other quality parameters were investigated. Significant changes in ellagic acid concentration were observed in each juice during storage, and were influenced by processing methods. In general, clarification process resulted a 2-fold increase in the ellagic acid content compared to raw juice and ellagic acid continued to increase during thermal treatments as well as storage at 4 °C for 150 days in both unclarified and clarified pomegranate juice, which can be attributed to the depolymerization of the high molecular weight

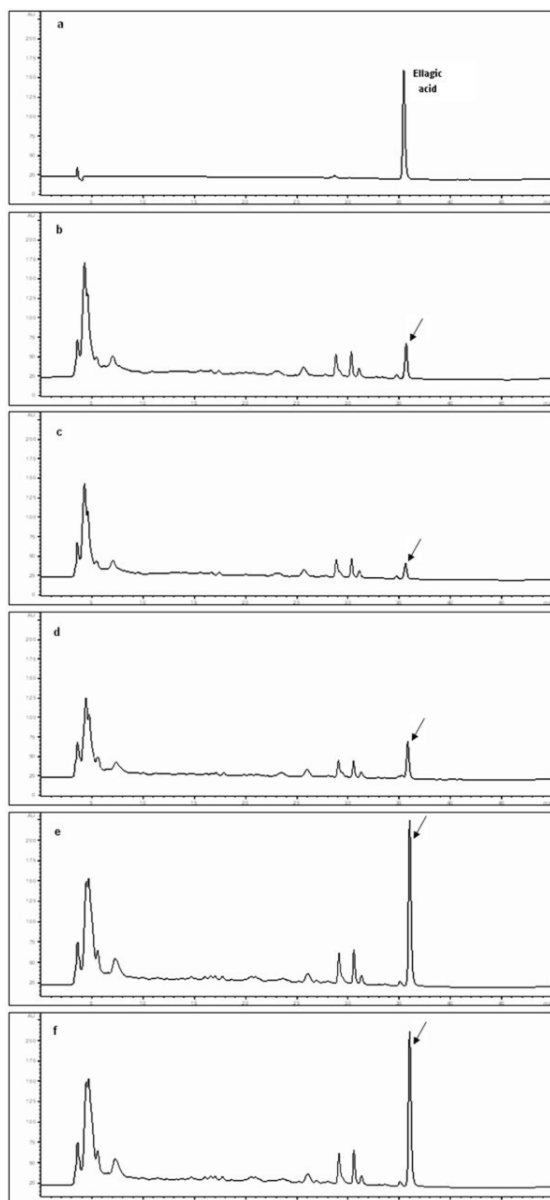


Figure 1. Chromatogram of ellagic acid in pomegranate juice from concentrate (Juice III) monitored at 255 nm: (a) ellagic acid standard solution (50 µg/mL); (b) raw juice; (c) after clarification & filtration (d) after concentration; (e) after pasteurization & cooling; (f) after storage.

ellagitannins. Variations were more significant in clear juice compared to the increase in unclarified pomegranate juice. These may be due to the contribution of free ellagic acid to the development of insoluble sediments in pomegranate juice during storage. The most significant colour loss was observed in clear juice obtained from concentrate due to the effect of additional thermal treatment.

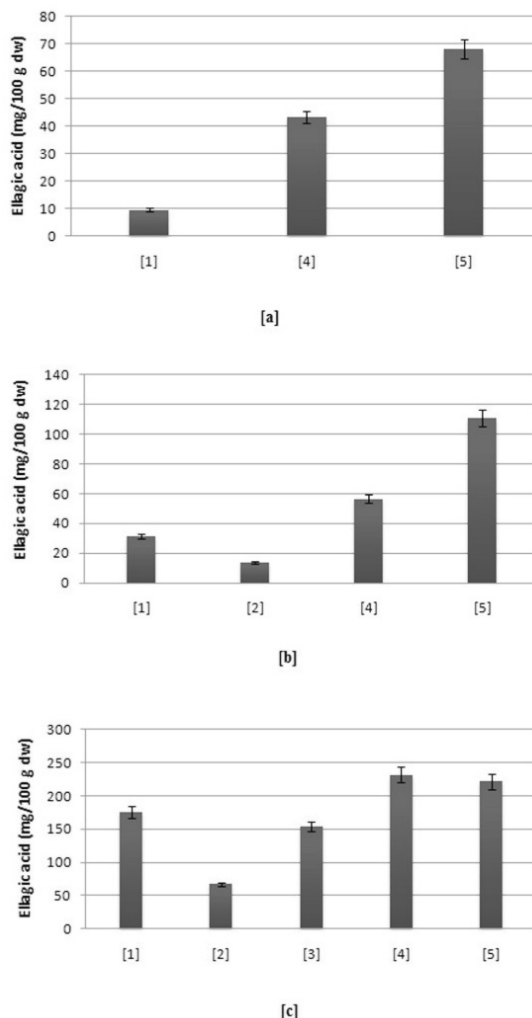


Figure 2. Ellagic acid content of: (a) unclarified pomegranate juice (Juice I); (b) clear pomegranate juice (Juice II); (c) clear pomegranate juice from concentrate (Juice III) throughout juice processing steps: [1] Raw juice; [2] After clarification and filtration; [3] After concentration; [4] After pasteurization & cooling; [5] After storage.

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