EFFECT OF HIGH HYDROSTATIC PRESSURE TREATMENT ON SOME QUALITY PROPERTIES, SQUEEZING PRESSURE EFFECT AND SHELF LIFE OF POMEGRANATE (*Punica granatum)* JUICE AGAINST THERMAL TREATMENT

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ABSTRACT

EFFECT OF HIGH HYDROSTATIC PRESSURE TREATMENT ON SOME QUALITY PROPERTIES, SQUEEZING PRESSURE EFFECT AND SHELF LIFE OF POMEGRANATE (*Punica granatum)* JUICE AGAINST THERMAL TREATMENT

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The aim of this study was to investigate the effect of high hydrostatic pressure (HHP) treatment (200, 300, 400 MPa; 5°C, 15°C, 25°C; 5 and 10 minutes) on some quality properties of two different squeezing pressure processed pomegranate juices against traditional thermal treatment at 85°C/10 min. Among these combinations, for 100 psi squeezed, 400 MPa 15°C 5 min and for 150 psi, 400 MPa 5°C 10 min were chosen the best. Antioxidant activity, total phenolic content, total monomeric anthocyanin concentration, ascorbic acid content, mannitol content, color values (ΔE) and other routine quality properties as °Brix, pH, titrable acidity besides microbial analyses as total meshopilic areobic bacteria count and total yeast and mould count were investigated. HHP combinations around 400 MPa at 10 min at every temperature were sufficient to decrease the microbial load around 4.0 log cycles for both squeezed juices. All HHP treatments showed no significant decrease at antioxidant activity, total phenolic content and monomeric anthocyanin pigment concentrations while there was a significant decrease ($p < 0.05$) for thermal treated. Ascorbic acid increased with 5 min HHP treatments but decreased with 10 min. ΔE values were smaller with HHP treatments for all combinations for both squeezed juices. HHP treatments gave lower mannitol content. In shelf life study during 30 days, antioxidant and ascorbic acid levels stayed more stable than control and pasteurized ones. Sensory evaluations, odor and appearance, HHP treatments gave highest results then the others as well as the smallest ∆E values. For all combinations, there was no significant difference for °Brix, pH and titrable acidity values between HHP and thermal treatments.

Keywords: high hydrostatic pressure, pomegranate juice, shelf life, quality, non-thermal fruit juice processing

ÖZ

YÜKSEK HİDROSTATİK BASINCIN ISIL İŞLEME KARŞI NAR (*Punica granatum)* SUYUNUN BAZI KALİTE ÖZELLİKLERİ, SIKMA BASINCI VE RAF ÖMRÜ ÜZERİNE ETKİSİ

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Bu çalışmanın amacı, yüksek hidrositatik basınc (YHB) uygulamasının (200, 300, 400 MPa ; 5ᵒC, 15ᵒC, 25ᵒC; 5 ve 10 dakika), iki farklı presleme basıncında sıkılan nar suyunun bazı kalite özellikleri üzerine etkisi ve sonuçların geleneksel ısıl işlem (85ᵒC/10) uygulanmış nar suyu ile kıyaslanmasıdır. Uygulanan tüm kombinasyonlar içerisinde, 100 psi ile sıkılmış nar suyu için, 400 MPa 15ᵒC 5 dakika ve 150 psi ile sıkılmış için 400 MPa 5ᵒC 10 dakikalık uygulamalar en iyi seçilmiştir. Antioksidan aktivite, toplam fenolik madde, toplam monomerik antosiyanin konsantrasyonu, askorbik asit içeriği, mannitol içeriği, renk değerleri (ΔE) ve ᵒBrix, pH, titrasyon asitliği gibi diğer rutin kalite özellikleri yanı sıra toplam mezofilik aerobik bakteri ve toplam maya küf sayısı gibi mikrobiyal analizler incelenmiştir. Her sıcaklık derecesinde, 400 MPa/ 10 dakikalık YHB kombinasyonları her iki pres derecesinde sıkılmış nar suyunda mikrobiyal yükü 4.0 log azaltmaya yeterli olmuştur. Uygulanan hiç bir YHB uygulaması antioksidan aktivitesi, toplam fenolik madde içeriği, ve toplam monomerik antosiyanin konsantrasyonlarında önemli bir değişime yol açmazken, ısıl işlem uygulanmış nar suyundaki azalış önemli bulunmuştur ($p > 0.05$).

Askorbik asit içeriğinde, 5 dakikalık YHB uygulamasıyla artış, 10 dakikalık uygulamayla düşüş görülmüştür. Her iki pres derecesinde sıkılmış nar sularında, ΔE değerleri tüm YHB kombinasyonlarında, ısıl işleme tabi tutulan nar suyu ΔE değerine gore daha düşük bulunmuştur. YHB uygulamalarında daha düşük mannitol içeriğine rastlanılmıştır. 30 gün boyunca süren raf ömrü çalışmasında, antioksidan ve askorbik asit seviyeleri kontrol ve pastörize nar sularına göre daha stabil kalmıştır. Koku ve renkten oluşan duyusal değerlendirme sonuçlarına göre YHB uygulanmış nar suları, en düşük ΔE değerlerini vermesi gibi, en yüksek değerleri almıştır. YHB kombinasyonları uygulanmış ve ısıl işlem yapılmış nar sularında ^{o}Brix, pH, titrasyon asitliği değerlerindeki değişim önemli bulunmamıştır.

Anahtar Kelimeler: yüksek hidrostatik basınç, nar suyu, raf ömrü, kalite, ısısal olmayan meyve suyu işlemesi

Dedicated To My Beloved

Family

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LIST OF ABBREVIATIONS

AIJN: European Fruit Juice Association

ANOVA: Analysis of Variance

$C_2H_3NaO_2$: sodium acetate

CFU: Colony Forming Units

DF: Dilution Factor

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DPPHH: 2,2-diphenyl-1-picrylhydrazine

FC: Folin-Ciocalteau

HHP: High Hydrostatic Pressure

HPLC: High Performance Liquid Chromatography

TA: Titratable Acidity

TMAB: Total Mesophilic Aerobic Bacteria

TYM: Total Yeast and Mould

PCA: Plate Count Agar

RSC: Radical Scavenging Capacity

YGCA: Yeast Extract Glucose Chloramphenicol Agar

CHAPTER 1

INTRODUCTION

1.1 A trendy fruit: *Punica granatum* L., Punicaceae, Pomegranate

Pomegranate (*Punica granatum* L., Punicaceae) is an ancient fruit; it has been widely consumed in various cultures for thousands of years. It is native from the area of Iran to the Himalayas in northern India, and has been cultivated and naturalized over the entire Mediterranean region since ancient times (Meerts et al., 2009). Since ancient times, the pomegranate has been regarded as a "healing food" with numerous beneficial effects in several diseases (Vidal et al., 2003). As a result, the field of pomegranate research has experienced tremendous growth (Mart' ınez et. al., 2006; Jaiswal and others 2010).

Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of pomegranates have been reported over years by various researchers (Aviram et al., 2000; Mirdehghan & Rahemi 2007; Davidson et al., 2009; Tezcan et al., 2009). About 50% of the total fruit weight corresponds to the peel, which is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ETs), and proanthocyanidin compounds (Li et al., 2006), minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium (Mirdehghan & Rahemi 2007), and complex polysaccharides (Jahfar et al., 2003). A study by Gil et al. (2000) showed there are much higher hydrolysable tannins present in the fruit peel. This could account for primarily higher antioxidant activity of commercial juices compared to the experimental ones. In industry, pomegranate fruit is processed into juice by hydraulic press and the pressurization value directly affects the tannin and antioxidant content of the pomegranate juice.

1.2 Processing and the Consumption of Pomegranate Fruit in Turkey

There has been a virtual explosion of interest in the pomegranate as a medicinal and nutritional product because of its multi functionality and its great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction. As a result, the field of pomegranate research has experienced tremendous growth (Mart´ınez et al., 2006; Jaiswal et al., 2010).

Pomegranate is native from the area of Iran to the Himalayas in northern India, and has been cultivated and naturalized over the entire Mediterranean region since ancient times (Meerts et al., 2009). Turkey, being located in the pomegranate fruit's homeland boundaries, has a rich variety of pomegranate cultivars. Pomegranate can be cultivated in all tropical and subtropical geographies. It is also grown in warm and temperate regions limitedly. Due to special climate necessities, pomegranate cultivation in Turkey is done according to areas as Mediterranean region (% 61.8), Aegean region (% 23.3) and South-East of Anatolia (% 9.1) (Gültekin et al., 2007). Incredible raising interest of consumers in Turkey as well as of other countries led a great pomegranate necessity at the market and gave the opportunity for wide cultivation. The numbers of both, production and income from exportation getting bigger year by year as seen in Table 1.1 and Table 1.2.

Table 1.1 Pomegranate productions in Turkey according to years (1000 Tones) (Prime Ministry Republic of Turkey, Turkish Statistical Institute)

PRODUCTS	2005	2006	2007	2008	2009	2010
Pomegranates	80	90	107	128	170	209

Table 1.2 Pomegranate exportation of Turkey according to years (Quantity: Ton, Value: US $1000) (Prime Ministry Republic of Turkey, Turkish Statistical Institute)

	2008		2009		2010	
PRODUCTS	Q	V	Q	V	Q	V
Pomegranates	28.788	27.669	40.820	39.104	63.011	59.302

The edible parts of pomegranate fruits are consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, sauce and paste and also for flavoring and coloring beverage products (Fadavi et al., 2005; Mousavinejad et al., 2009). In addition, it is widely used in therapeutic formulas, cosmetics, and food seasonings.

1.3 High Hydrostatic Pressure Processing (HHP) Technology

In recent years, there is a great interest of natural food without additives. However, natural and non-treated foods have quite limited shelf life due to the risk of undesired microbial content (Buzrul et al., 2008). Traditional heat treatment causes a high vitamin C loss in orange juice (Farnworth et al., 2001), lower antioxidant retention (Polydera et al., 2004, Scalzo et al., 2004) and undesired color and anthocyanin losses (Patras et al., 2010). Because of this situation there had been a popular tendency to non-thermal food processing methods. With these methods, microbial count could be controlled under a spoilage leading levels during the shelf life period besides protecting the heat susceptible sensory and nutritional compounds. Therefore there is a need for alternate methods of processing which can increase microbiological stability and will aid in preserving nutritional characteristics. Non-thermal processing methods such as high hydrostatic pressure processing (HHP) could potentially fill this role. HHP uses water as a medium to transmit pressures from 0 to 800 MPa (Patras et al., 2009). One of the main advantages of this process is the almost instantaneous isostatic pressure transmission to the product, independent of size, shape and food composition yielding highly homogeneous products (Patterson et al., 1996). Food treated in this way has been shown to keep its original freshness, flavor, taste and color changes are minimal (Dede et al., 2007).

While the structure of high-molecular-weight molecules, such as proteins and carbohydrates, can be altered by high pressure processing, smaller molecules such as volatile compounds, pigments, vitamins, and other compounds connected with the sensory, nutritional, and health promoting are unaffected (Cheftel, 1992; Oey et al., 2008). High pressure treatment in comparison with those of traditional thermal processing results in better retention of levels of bio-active compound groups (Patras et al., 2008), increasing microbiological stability (Meyer et al., 2000) and decreasing enzyme activity (Weemaes et al., 1999). The microbiological results showed that HHP treatment at or over 350 MPa for 150 s resulted in a reduction of the microbial load around 4.0 log cycles and were sufficient to keep microbial populations investigated below the detection limit during the whole storage period in pomegranate juice (Meyer et al., 2000). Phenolic content increased significantly ($p<0.05$) between 3.38 and 11.99 % for treated samples with 350 and 550 MPa at day 0. The ΔE values, which are an indicator of total color difference, showed that there were significant differences ($p<0.05$) in color between untreated and treated samples (Varela-Santos et al., 2011). There is no study about the HHP on vitamin C content of pomegranate juice yet. In orange juice, just after 350 MPa/30°C/2.5 min treatment, juice had the same levels of vitamin C compounds compared to untreated juices (Polydera et al., 2005). Also, these results confirm those reported by Donsi et al. (1996) and Van den Broeck et al. (2000) about the stability of ascorbic acid in orange juice when pressurized at mild temperatures.

1.4 Earlier High Hydrostatic Pressure Studies in Turkey

Fruit juice’s long and qualified storage periods are effected by storage time and temperature, storage and packaging conditions, the first quality level of the product and microbial load besides environmental conditions. Using HHP technology on processing the fruit into the fruit juice is a brand new and developing phenomenon in Turkey. Alpas et al. (2000) demonstrated that the HHP treatment affected the pressure resistant and resistless food pathogens. In the same study, the effect of HHP treatment increases with increasing the process temperature and decreasing pH is determined. Due to being a low pH food, HHP process is more efficient on fruit juices to microbial purification. The effect of thermal treatment on color and total phenolic compound content of food systems are investigated and found these two quality factor are negatively affected by thermal treatment (Alper et al., 2005). On the contrary, HHP process helped to protect the stability of color and total phenolic compounds. In their study on carrot and tomato juices, Dede et al. (2007) reported that; through the storage period, HHP- treated juices were judged to be of superior quality than the conventional, thermally processed ones in terms of microbiological stability, ascorbic acid retention and antioxidant activity.

1.5 Aim of the Study

The objective of this research was divided into two main parts. At the first part, the aim is to evaluate the effects of HHP treatment on physical and chemical quality parameters such as pH, ^{o}Brix, titrable acidity, color values (ΔE), antioxidant activity, total phenolic compounds, total monomeric anthocyanin, mannitol, ellagic acid and vitamin C contents besides microbial load and stability as total mesophilic aerobic bacteria and total yeast and mould content of two different hydraulic pressure squeezed (100 and 150 psi) pomegranate juice with a comparison of traditional thermal treated one against untreated (raw-control) sample. As HHP parameters, different pressure, temperature and time combinations (200, 300, 400 MPa; 5, 15, 25^{o}C; 5 and 10 minutes) was be carried out and the best combinations for two different squeezed samples was proposed.

In the second part, the best combinations was applied as 400 MPa at 15^{o}C for 5 minutes for 100 psi squeezed juice and 400 MPa at 5^{o}C for 10 minutes for 150 psi squeezed juice. The HHP treated, thermal treated and untreated samples were stored at 4^{o}C in the dark during 30 days and evaluated for TMAB, TYM, pH, color, RSA, ascorbic acid and sensory property alterations.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Supplying the Samples

Fresh pomegranate fruit (Punica *granatum L*. c*v*. Hicaznar) is made order from a main wholesaler from Antalya. Pomegranates were harvested in the late-season of 2010. Just after the transportation of pomegranates to Ankara, fruits are immediately processed.

2.1.2 Sample Processing

Just after the transformation of 40 kg pomegranate from Antalya to Ankara, pomegranates were immediately taken under squeezing process in the pilot food processing plant of Ankara University Food Engineering Department. Primarily pomegranates were washed with compressed tap water, then cut into four pieces, processed with pilot plant press (Bucher-Guyer, Niederweningen, Switzerland) and the juice was extracted by applying a gauge pressure of 8.4 kg/cm^2 (≈100 psi) and 11.2 kg/cm^2 (≈150 psi) and juice obtained with approximately 43 % efficiency. No clarification was applied for both 100 and 150 psi squeezed pomegranate juices. Than all the juice was packed in 330 mL polyethylene flexible bottles and stored at -18^{o}C until experiments.

2.1.3 Reagents

All chemicals used were of analytical grade. The chemicals and biologic materials not specified were purchased from Merck, Germany. All equipment used was sanitized prior to usage with 60 % ethanol (Merck, Germany), followed by sterile water rinse.

2.2 Methods

2.2.1 Treatments

2.2.1.1 High Hydrostatic Pressure Application

Deeply frozen at -18^{o}C and stored at -35^{o}C packed samples were taken out off freezer and placed in to 4^{o}C conditions for controlled dissociation. Pomegranate juices were refilled into 20 mL plastic scintillation bottles (LP Italiana SPA) and placed into pressuration vessel. HHP treatment was performed with 760.01 laboratory type high pressure equipment supplied by SITEC-Sieber Engineering AG, Zurich, Switzerland. The vessel had a volume of 100 mL with ID 24 mm and length is 153 mm. Ethylene glycol was used as a cooling / heating agent that was circulated around the jacketed pressure vessel. The maximum design pressure was 700 MPa at an operating temperature of -10^{o} to 80^{o}C. A built-in cooling / heating system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control the required temperature which is measured by a thermocouple type K. It was fitted through the upper plug to measure the inner temperature of the vessel during the pressure treatment. The vessel was filled with a pressure transmitting medium consisting of distilled water. Pressure come up and release times were less than 20 seconds for each.

Pressurization time reported in this study did not include the pressure increase and release times. Temperature increases due to adiabatic heating was reduced to 4-5^{o}C during the time period of pressurization upto 400 MPa. Reported temperature is the actual process temperature during hold time at reported pressure levels. HHP conditions were chosen as 200, 300 and 400 MPa at 5, 15 and 25^{o}C for 5 and 10 minutes for this study.

2.2.1.2 Heat Treatment

Thermal treatment process was conducted in water bath for 10 minutes at 85^{o}C. These conditions were chosen according to industrial pasteurization application.

Heat stable glass tubes were filled with the same amount of (10 mL) sample and sealed with an appropriate cover. One tube also filled with 10 mL pomegranate juice was used to control the inner temperature by the help of a thermocouple. Tubes are settled down in a rack and rack was submerged in already heated up water bath. Samples were hold under these conditions during treatment time while monitoring the inner temperature of samples. At the end of the holding time, samples were taken out of the water bath and submerged into ice-cold (≈ 0^{o}C) water immediately for cooling down. After approximately 3 minutes of holding time, inner temperature of pomegranate juice cooled down below 4^{o}C. Freshly pasteurized samples were analyzed and excess amount of the samples were stored at -18^{o}C until further requirement.

Both thermal and HHP treatments were carried out for 100 and 150 psi squeezed pomegranate juices. After the treatments, all microbiological, physical and chemical analyses were performed within 1 day. All experiments and measurements were replicated three times.

For shelf life analysis, thermally and HHP treated samples (400 MPa at 15^{o}C for 5 minutes for 100 psi squeezed juice and 400 MPa at 5^{o}C for 10 minutes for 150 psi squeezed juice) were kept at 4^{o}C in the dark during 30 days. The samples were taken at 3-days intervals during the first 3 weeks besides a last experiment day as day 30. New tubes and bottles were opened for each experiment day. Untreated samples were used as controls.

2.2.2 Analyses

2.2.2.1 Microbiological Analyses

All samples were analyzed as colony-forming unit per mL (cfu mL^{-1}) of total mesophilic aerobic bacteria (TMAB) and total yeast and mould (TYM). For enumeration of total mesophilic aerobic bacteria, spread plate technique was used with non selective Plate Count Agar (PCA; Merck, Darmstadt, Germany). After incubation period at 37°C for 48 h, plates with 25-250 colonies were considered. In order to enumerate total yeast and mould, spread plate technique was used with selective Yeast Extract Glucose Chloramphenicol Agar (YGCA; Merck, Darmstadt, Germany). Total yeast and mould incubation lasted 5 days at 26°C and at the end of this duration plates with 25-250 colonies were considered. Microbial data were transformed into logarithms of colony-forming units ($\log_{10}$ cfu mL^{-1}). When no colonies were detected, value of 1 $\log_{10}$ cfu mL^{-1} is used intending to obtain $\log_{10}1=0$.

2.2.2.2 Physical and Chemical Analyses

2.2.2.2.1 pH, Titrable Acidity and °Brix

Total soluble solids content (°Brix) of samples were determined at 20 °C using Atago hand refractometer (London, England) and pH of the samples were determined at room temperature by using pH meter, Mettler-Toledo MP220, Schwerzenbach, Switzerland. For titratable acidity determination, 1 mL of pomegranate juice is diluted with 9 mL distilled water and the dilution was titrated with 0.1 N NaOH to an endpoint of pH 8.1. Results were expressed gram citric acid per liter (g citric acid/L).

2.2.2.2.2 Color Measurement

Color values of the samples were analyzed by Avantes spectrophotometer (Avantes, Avaspec-2048, The Netherlands) with a light source set on D65. L*, a* and b* values are measured and ΔE values are calculated with the formula below (Billmeyer and Saltzman, 1981). L_0, a_0 and b_0 values in the formula indicate the control (raw) pomegranate juice's values for both 100 and 150 psi squeezed juices. Distilled water is used as reference.

$$\Delta E^2 = \{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2\}$$

2.2.2.2.3 Total Phenolic Content

Total phenolic content was determined for the pomegranate juice by the Folin–Ciocalteau method with modifications (Singleton & Rossi, 1965; Coseteng et al., 1987; Spanos et al., 1990). From the dilution 1:5, 20 µL pomegranate juice, 1.58 mL distilled water, 100 µL Folin–Ciocalteau reagent and 300 µL of Na_2CO_3 (75 g/L) solution were added and mixed well. After 2 h incubation at ambient temperature, absorbance was measured in UV-Visible Spectrophotometer (Analytic Jena SPECORD 50, Germany) at 765 nm and compared to a gallic acid equivalent (GAE) calibration curve (see Appendix A). Results were expressed as gallic acid meswg/mL.

2.2.2.2.4 Total Monomeric Anthocyanin Concentration

Total monomeric anthocyanin content of samples was determined by the pH differential method (Lee et al., 2005; Giusti et al., 2001; Wrolstad et al., 2004).

Potassium chloride pH 1.0 buffer (0.025 M) and sodium acetate pH 4.5 buffer (0.4M) were used as buffer solutions. 0.1 mL sample was diluted with 3.9 mL pH 1.0 and pH 4.5 buffer solutions in different couvettes and after 30 min absorbance was measured at 515 and 700 nm. According to know-how in literature and verification of some assumptions, distilled water was read as blank versus diluted samples in both wavelengths (Cemeroğlu, 2010).

Total monomeric anthocyanin content which was expressed as mg/L cyanidin-3-glucoside for pomegranate juice with this equation;

$$[Total\ Monomeric\ Anthocyanins, mg/L] = \frac{(A)(MW)D_f(1000)}{\varepsilon(\gamma)}$$

In this equation, **A** denotes $(A_{520} - A_{700})$ pH 1.0 – $(A_{520} - A_{700})$ pH 4.5, A_{520} is the absorbance at 520 nm and A_{700} is the absorbance at 700 nm. **MW** denotes molecular weight of cyanidin-3-glucoside which is 449.2 g/mol. $\mathbf{D_f}$ denotes dilution factor, which is 40. $\boldsymbol{\varepsilon}$ denotes molar extinction coefficient, which is 26 900 L mol-1cm-1 for cyanidin-3-Glucoside. $\boldsymbol{\gamma}$ denotes path length of couvettes in cm, which is 1 cm. 1000 is the conversion factor of g to mg.

2.2.2.2.5 Antioxidant (Free Radical Scavenging) Activity

The antioxidant activities of the pomegranate juices were determined by reaction with the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St Louis, Missouri) radical, according to the Brand-Williams method (Brand-Williams et al., 1995). After adding 0.025 g/L DPPH solution to 1:35 diluted with ethanol and distilled water pomegranate samples in a glass tube, reaction medium was mixed well with vortex (DG-800, Donglin, Beijing, China) and left in a dark place for 1 h at room temperature. The absorbance was measured at 517 nm, using UV-Visible

Spectrophotometer (Analytic Jena SPECORD 50, Germany). The spectrophotometer was equilibrated with methanol. Control sample was prepared without adding pomegranate juice into the same reaction medium. Ethanol and methanol were obtained from Merck, Darmstadt, Germany. Total antioxidant activity was expressed as the percentage inhibition of the DPPH radical using the standard DPPH curve (see Appendix B).

2.2.2.2.6 Ascorbic acid

Ascorbic acid content of pomegranate juice was determined using the modified version by Cemeroğlu 2010, of the spectrophotometric method advised by Anonymous 1951 and Freed 1966. Inhibition of 2,6-dichlorophenolindophenol (Merck) by ascorbic acid and extraction of inhibited color substance by xylene was read in UV-Visible Spectrophotometer (Analytic Jena SPECORD 50, Germany). Absorbance of extracted solution was measured at 500 nm, using UV-Visible Spectrophotometer (Analytic Jena SPECORD 50, Germany) against pure xylene. Control sample was prepared without adding pomegranate juice into the same reaction medium. Ascorbic acid content of pomegranate juice was calculated using standard ascorbic acid curve (Supelco) (see Appendix C) and the results expressed as mg/L ascorbic acid.

2.2.2.2.7 Mannitol

Mannitol content determination was carried out by Middle East Technical University Central Laboratory, using high performance liquid chromatography (HPLC). Samples of 1 ml of pomegranate juice were filtered through a 0.45 μm GHP Acrodisc filter and injected directly. An aliquot then was injected into the chromatographic column. The chromatographic system (Varian ProStar, Palo Alto, CA, USA) consisted of a quaternary pump, a vacuum degasser, a Rheodyne 25 μl injection loop, a Refractive Index Detector. A Carbohydrate Ca (300 mm X 6.5 mm) column with a flow rate of 0.5 ml/min was used. Results were calculated as mg mannitol per ml of pomegranate juice. Each sample was prepared and analyzed in triplicate.

2.2.2.3 Sensory Analyses

Sensory evaluations of the samples were conducted by 3 women and 3 men, total 6 laboratory trained panelists. Panelists used 1-9 hedonic scales consumer test to evaluate the pomegranate samples for odor and color properties (O'Mahony, 1988). At the end of the evaluations, the grades given by the panelists according to the hedonic scale are used to calculate the sensorial aspect of the samples.

2.2.2.4 Statistical Analyses

The results of thermal and HHP treatments were evaluated statistically using SPSS 15 for Windows (SPSS Inc., Chicago, IL, USA). In the first part of the study, Univariate General Linear Model was used with pressure, temperature and time as factors; in the second part of the study, one-way analyses of variance (ANOVA) was used with storage period as a factors to determine the significant differences ($p < 0.05$). Tukey test was used as a post-hoc test if a factor had a significant effect and if the factor had 3 or more groups.

CHAPTER 3

RESULTS AND DISCUSSION

Pomegranate is one the most complex fruit among all due to its constituent variety and their excessive amounts. Not the constituent number but their amounts chance from type to type and according to the growing region or harvest time so much. The results obtained during this study were compared with other studies to make comments about HHP treatment of fruit juices like tomato, carrot, orange juice (Dede et al., 2007; Polydera et al., 2004). The effects of pressure treatment on each type of fruit are quite different, not just the other fruits but even within the varieties of pomegranate (Varela-Santos et al., 2011).

3.1 Assessment of pressure, temperature and time combinations

3.1.1 Effects on microbial values of pomegranate juice

High hydrostatic pressure (HHP) processing has been introduced as an alternative non-thermal technology that causes inactivation of microorganisms (Linton et al., 1999; Parish, 1998a; Reyns et al., 2000; Teo et al., 2001; Zook et al.,1999). HHP inactivates microorganisms by interrupting cellular functions responsible for reproduction and survival (Norton et al., 2008; Torres et al., 2008). In industry, pomegranate fruit is processed into juice by squeezing the whole fruit. Pomegranate peel is very convenient for mould and yeast growth from the time of harvest to transportation and to final storage. Processing the whole fruit causes undesirable yeast and mould transfer into the juice. However, for academic researches pomegranate is generally separated into arils by hand or squeezed with a home type fruit juicer. Due to this situation there is no much data about freshly squeezed whole pomegranate's microbial load. Examining the studies about pomegranate peels, microbial load give some ideas. The initial microbiological analysis of pomegranate peel showed a low microbial count (65 cfu/g), before the washing and after being washed with chlorinated water and treated with antioxidant solution, mesophilic aerobic count decreased to 10 cfu/g and, on moulds and yeast, a decrease from 185 cfu/g to 5 cfu/g was observed (Sepulveda et al., 1998). In another study, pomegranate peels obtained from a commercial company were cleaned, hot air dried at 60°C for 7 h. The initial mean populations of the total plate and total fungal counts for pomegranate peel powder were found 3.2×10^3 and 1.8×10^3 cfu/g; respectively (Mali et al., 2011).

In Figure 3.1 and Figure 3.2, effects of HHP and thermal treatments on total mesophilic aerobic bacterial load of 100 and 150 psi squeezed pomegranate juices are given, respectively. Initial microbial loads of 100 and 150 psi squeezed pomegranate juice are 3.85 and 3.93 log cfu/mL, respectively. As seen from the bar diagram 10 minute treatments at 300 MPa, 5 and 10 minutes treatments at 400 MPa give desired log reduction as 3.85 cfu/mL for 100 psi squeezed one in Figure 3.1. In Figure 3.2, 10 minutes treatments at 400 MPa at every temperature inactivated the entire initial load of 3.93 cfu/mL for 150 psi squeezed pomegranate juice.

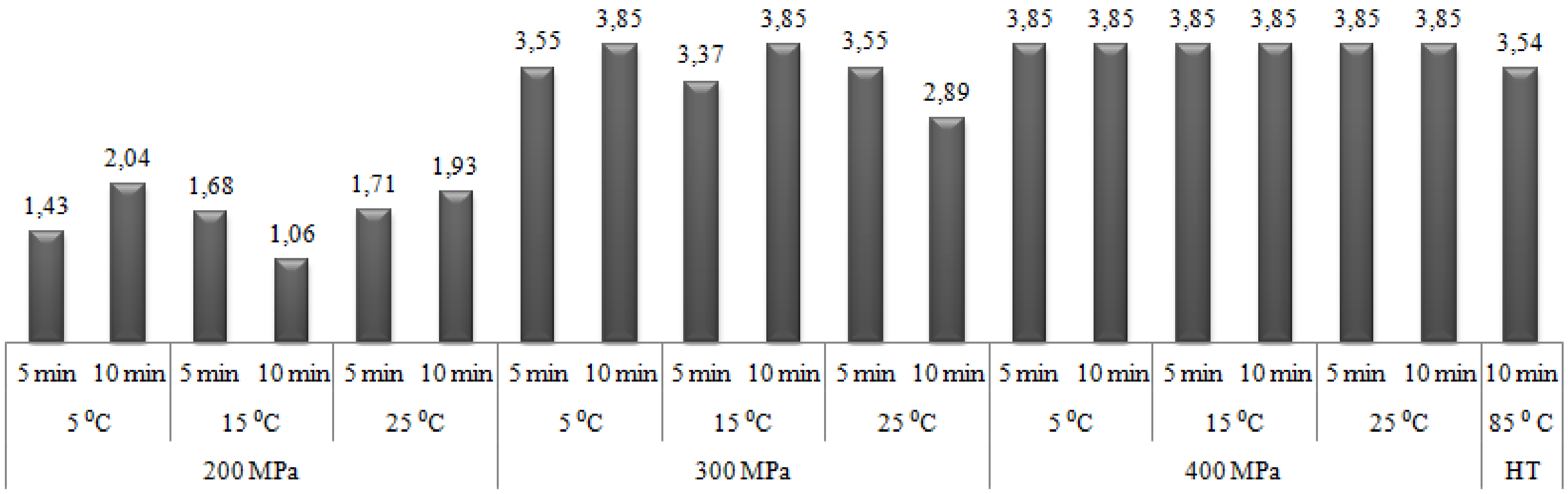

Figure 3.1 Mean total mesophilic aerobic bacteria reduction (log cfu/mL) of high pressure and traditional thermal treatments for 100 psi squeezed pomegranate juice. Initial microbial load is 3.85 log cfu/mL.

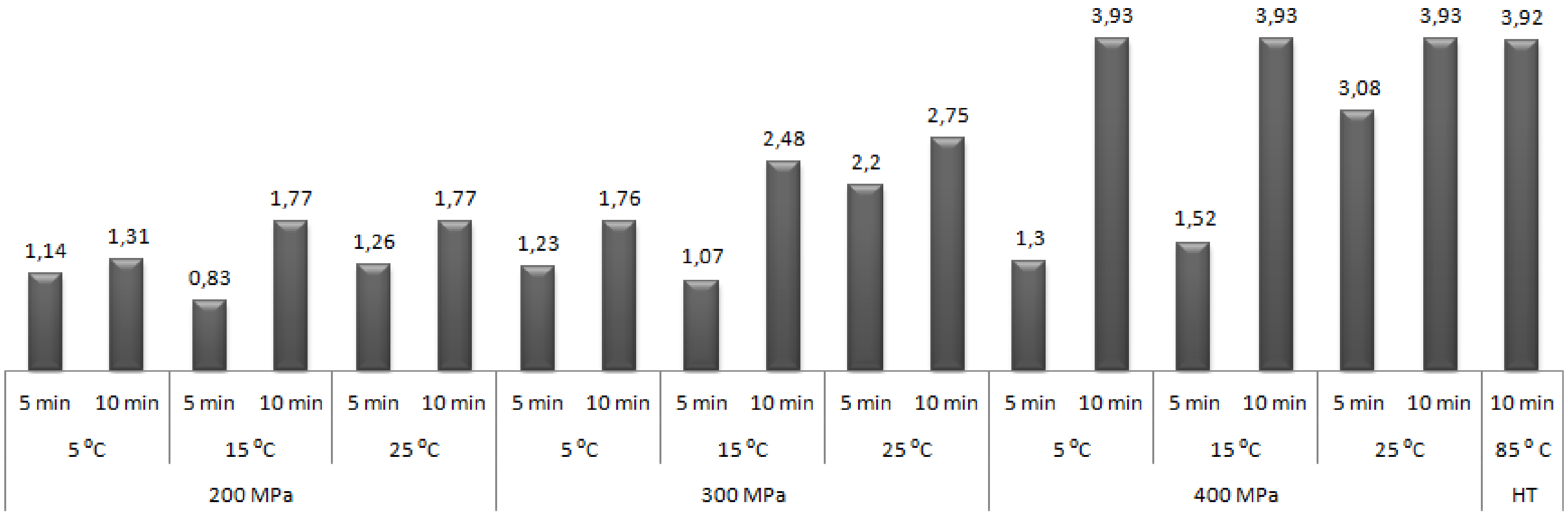

Figure 3.2 Mean total mesophilic aerobic bacteria reduction (log cfu/mL) of high pressure and traditional thermal treatments for 150 psi squeezed pomegranate juice. Initial microbial load is 3.93 log cfu/mL.

Figure 3.3 and Figure 3.4 show log reduction of total yeast and mould counts as cfu/mL for 100 and 150 psi squeezed pomegranate juices, respectively. In Figure 3.3, targeted microbial reduction was achieved (initial load 4.24 cfu/mL) for total yeast and mould content at every temperatures and time of 400 MPa for 100 psi squeezed pomegranate juice. Lastly, Figure 3.4 shows the results for 150 psi squeezed pomegranate juice and desired log reduction (4.15 cfu/mL) is obtained with only 10 minutes treatment at 400 MPa for every temperature. Consequently, 150 psi squeezed juice has much more microbial load than 100 psi squeezed but with some combinations, all the initial microbial level of both juice was inactivated.

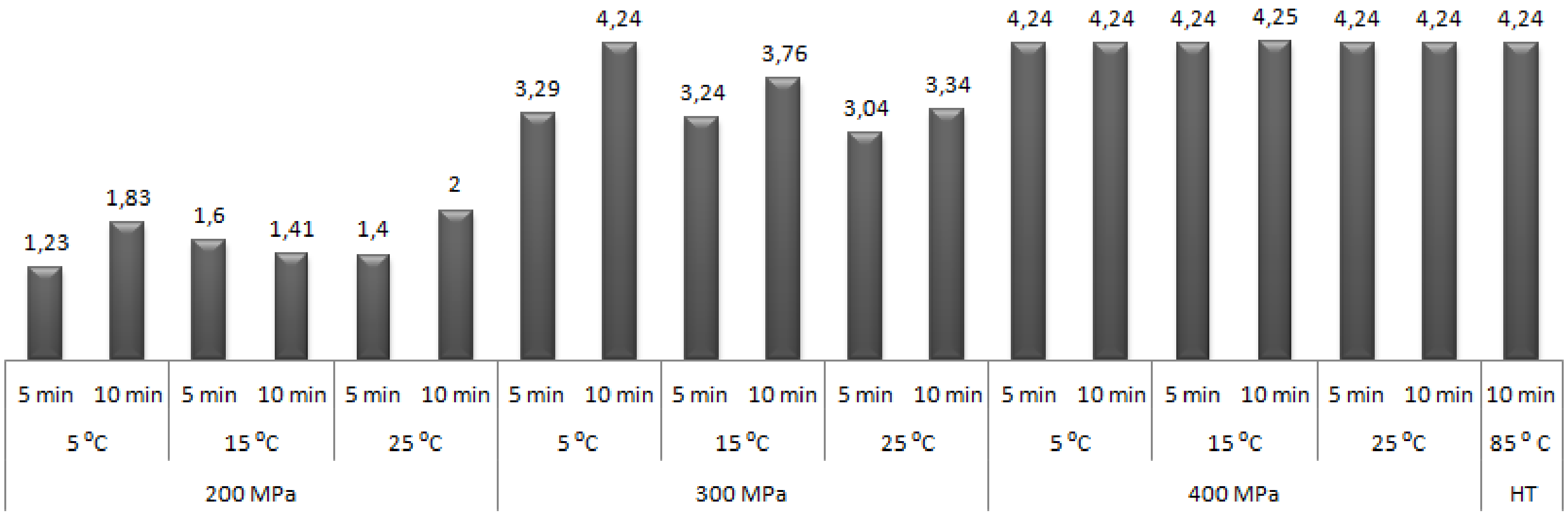

Figure 3.3 Mean total yeast and mould reduction (log cfu/mL) of high pressure and traditional thermal treatments for 100 psi squeezed pomegranate juice. Initial microbial load is 4.24 log cfu/mL.

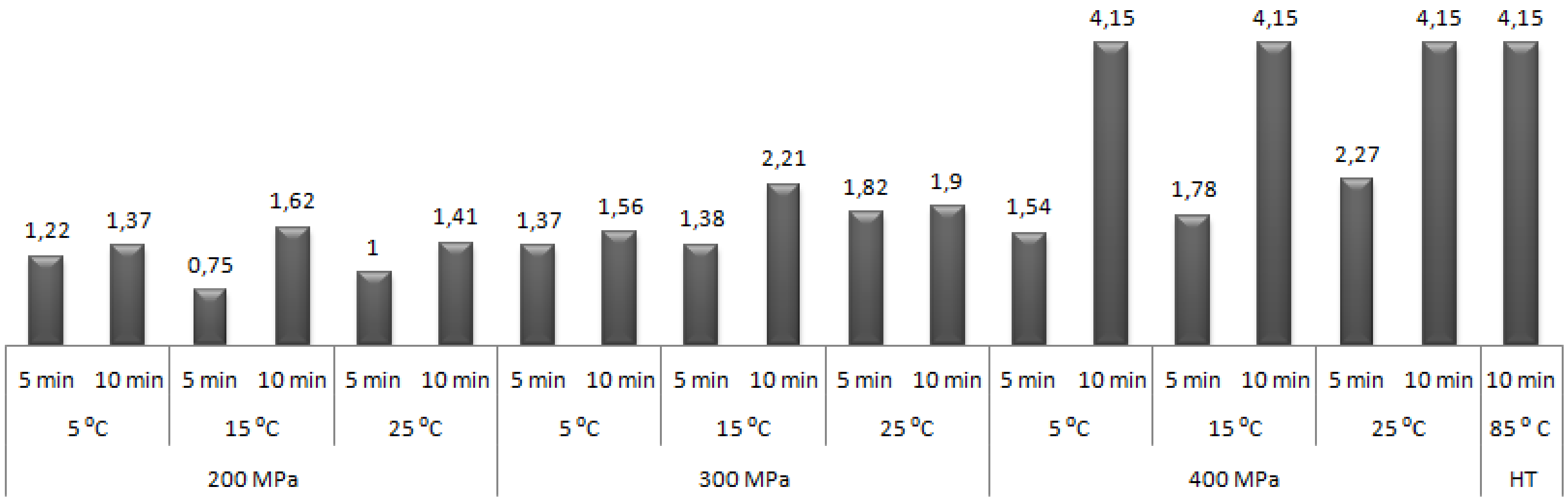

Figure 3.4 Mean total yeast and mould reduction (log cfu/mL) of high pressure and traditional thermal treatments for 150 psi squeezed pomegranate juice. Initial microbial load is 4.15 log cfu/mL.

3.1.2 Effects on Physical and Chemical Quality Parameters

3.1.2.1 pH, Titrable Acidity and ^{o}Brix

Between all three groups; P, T, t combinations of HHP treated samples, thermally treated and untreated pomegranate juice samples for both 100 and 150 psi squeezed juices, there is no significant pH, ^{o}Brix and titratable acidity differences ($p > 0.05$). pH, ^{o}Brix and titrable acidity values of untreated pomegranate juice are found as 3.27 ± 0.05; 16.36 ± 0.20; 12.51± 0.88, respectively. Titratable acidity is g/L citric acid. pH, ^{o}Brix and titrable acidity values of samples just after HHP treatment did not show significant differences ($p > 0.05$) for treated juices at 350, 450 and 550 MPa for 30 s, 90 s and 150 s, respectively but after this storage time (15 days), the samples showed significant differences ($p < 0.05$) in pH, soluble solids and titratable acidity (Varela-Santos et al., 2011). Borochov-Neori et al. (2009) and Poyrazoglu et al. (2002) showed in their studies that pH, ^{o}Brix and titrable acidity did change through the pomegranate cultivars, ripening degree etc. González-Molina et al. (2009) working with pomegranate juices using high pressure treatment reported that there were no significant differences over the 70 days of storage at 4^{o}C in the quality parameters (pH, ^{o}Brix and titrable acidity) in the mixtures and control pomegranate juices (pH=3.60 ± 0.25, Titrable acidity = 0.34 ± 0.09 and ^{o}Brix = 16.99 ± 0.11).

3.1.2.2 Color Measurement

ΔE values show overall color differences containing L*, a* and b* values relative to the untreated pomegranate juice. Pérez-Vicente (2004) reported that the color of pomegranate juices became browner with the use of high temperatures. This situation can be obviously seen for 100 psi squeezed juice’s ΔE value as 12.62 ± 0.11. For 150 psi squeezed juice, ΔE value is relatively smaller than 100 psi’s ΔE as 5.21 ± 0.25, in Table 3.1.

Table 3.1 The effect of HHP and time on ΔE value of pomegranate juice squeezed at 100 psi and 150 psi pressure by hydraulic press just after HHP treatment [1, 2, 3].

	5 minutes ^{x}			10 minutes ^{y}		
	5°C	15°C	25°C	5°C	15°C	25°C
100 psi						
200 MPa	9.25 ± 0.30 $^{A\,a}$	9.41 ± 0.19 $^{A\,b}$	9.09 ± 0.16 $^{A\,b}$	9.77 ± 0.04 $^{A\,c}$	10.42 ± 0.08 $^{A\,d}$	9.89 ± 0.26 $^{A\,d}$
300 MPa	9.12 ± 0.10 $^{B\,a}$	10.20 ± 0.55 $^{B\,b}$	9.56 ± 0.49 $^{B\,b}$	9.02 ± 0.30 $^{B\,c}$	11.23 ± 0.51 $^{B\,d}$	10.64 ± 0.31 $^{B\,d}$
400 MPa	8.25 ± 0.19 $^{B\,a}$	9.65 ± 0.36 $^{B\,b}$	9.09 ± 0.53 $^{B\,b}$	10.71 ± 0.70 $^{B\,c}$	10.29 ± 0.63 $^{B\,d}$	12.06 ± 0.30 $^{B\,d}$
150 psi						
200 MPa	5.19 ± 0.25 $^{C\,a}$	5.26 ± 0.43 $^{C\,b}$	6.57 ± 0.32 $^{C\,c}$	8.99 ± 0.38 $^{C\,d}$	9.10 ± 0.17 $^{C\,e}$	8.15 ± 0.18 $^{C\,f}$
300 MPa	3.46 ± 0.06 $^{D\,a}$	4.99 ± 0.13 $^{D\,b}$	5.32 ± 0.22 $^{D\,c}$	8.52 ± 0.21 $^{D\,d}$	9.01 ± 0.34 $^{D\,e}$	8.80 ± 0.16 $^{D\,f}$
400 MPa	4.26 ± 0.23 $^{E\,a}$	5.67 ± 0.09 $^{E\,b}$	6.92 ± 0.06 $^{E\,c}$	8.12 ± 0.19 $^{E\,d}$	8.72 ± 0.10 ^{Ee}	9.04 ± 0.12 ^{Ef}

1 All ΔE values are the mean ± standard deviation of three replicates (n=3).

2 For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$

3 Thermally treated (85°C/10 min) samples ΔE value for 100 psi squeezed is 12.62 ± 0.11 while for 150 psi squeezed is 5.21 ± 0.25.

Ferrari et al. (2010) working with pomegranate juice reported that levels of pressure higher than 500 MPa caused important variations in color (brown color) but till this value, there is an increase on ΔE values. Pressure causes hydro soluble color pigments transmission into the juice. Also higher squeezing values lead the pigment transmission, too. At the first glimpse, it could be seen that ΔE values of 150 psi squeezed juices are smaller than 100 psi one. This could be due to the presence of already transmitted pigments during the squeezing pressure. There is a positive correlation between anthocyanin and color values examined in this study.

For 100 psi squeezed pomegranate, all P-T and t combinations show statistically important smaller ΔE values than thermally treated one ($p < 0.05$). At constant P and T; ΔE value increases with increasing time. At constant P and t, there is no significant ΔE value difference between 15 and 25°C treatments while 5°C treatment gives the smallest ΔE value. At constant T and t, smaller P gives the smaller ΔE value ($p > 0.05$). For 150 psi squeezed pomegranate juice, all P, T and t combinations show statistically important smaller ΔE values than thermally treated one. At constant P and T, ΔE value increases with increasing time. At constant P and t, there are significant differences between all T values and at constant T and t, there is significant difference between all P values. For smaller ΔE values relatively smaller P, T, t combinations could be chosen.

3.1.2.3 Total Phenolic Content

Primarily, there is an obvious total phenolic content difference between the 100 and 150 psi squeezed pomegranate juices. Higher squeezing pressure leads higher phenolic content ($p < 0.05$). Polyphenols are the major class of pomegranate fruit phytochemicals, including flavonoids (anthocyanins), condensed tannins pro-anthocyanidins and hydrolysable tannins (ellagitannins and gallotannins) (Gil et al., 2000). They are extracted into the juice upon commercial processing of the whole fruits from the husk (Fischer et al., 2011). Polyphenols are important constituents regarding the organoleptic properties of pomegranate arils and juices as they impart the appealing red color and provide mild astringency that is characteristic of pomegranate flavor (Fischer et al., 2011). The phenolic constituents of pomegranates give color, astringency and bitterness to the juice (Rouseff 1990; de Simon *et al*. 1992). These compounds are also responsible for the formation of cloudy appearance of fruit juices during concentration and storage (Macheix *et al*., 1990; Spanos et al., 1992). These situations shows higher phenolic content is not a deserved factor despite enhancing organoleptic values until a limit. From Table 3.2, heat treated 100 psi pomegranate juice have higher phenolic content than both untreated and HHP treated samples. However, HHP treated samples show similar phenolic values with untreated one ($p > 0.05$). Among the HHP treatment, while T and t constant, phenolic content shows differences with different pressures according to means and standard deviations and the phenolic content is the highest at 300 MPa. At constant P and t, 15^{o}C gives the lowest phenolic value at 5 min and 25^{o}C at 10 min. For 150 psi pomegranate juice, there is no significant difference among t and P but at constant t and P, treatment at 25^{o}C has the lowest phenolic content for both 5 and 10 min. No significant effect was found between treatment time at constant P and T according to independent samples t-test with $p > 0.05$.

Table 3.2 The effect of HHP and time on Total Phenolic Content (gallic acid mdg/mL) of pomegranate juice squeezed at 100 psi and 150 psi pressure by hydraulic press just after HHP treatment [1, 2, 3].

	5 minutes ^{x}			10 minutes ^{x}		
	5°C	15°C	25°C	5°C	15°C	25°C
100 psi						
200 MPa	504.16 ± 2.51 ^{Aa}	476.30 ± 29.60 ^{Ab}	537.73 ± 14.71 ^{Ac}	514.26 ± 9.78 ^{Ad}	521.31 ± 17.12 ^{Ad}	489.87 ± 15.72 ^{Ad}
300 MPa	540.10 ± 18.50 ^{Ba}	507.80 ± 10.80 ^{Bb}	537.50 ± 34.80 ^{Bc}	550.11 ± 15.36 ^{Bd}	499.6 ± 23.70 ^{Bd}	512.73 ± 9.29 ^{Bd}
400 MPa	507.50 ± 9.61 ^{Ca}	398.93 ± 6.55 ^{Cb}	536.54 ± 7.86 ^{Cc}	462.97 ± 8.03 ^{Cd}	525.6 ± 26.50 ^{Cd}	436.50 ± 20.20 ^{Cd}
150 psi						
200 MPa	682.50 ± 33.60 ^{Da}	707.00 ± 83.40 ^{Db}	623.50 ± 19.60 ^{Dc}	645.83 ± 8.37 ^{Dd}	702.30 ± 38.70 ^{Dd}	636.07 ± 4.69 ^{Dd}
300 MPa	668.20 ± 29.10 ^{Ea}	673.45 ± 7.23 ^{Eb}	674.16 ± 10.34 ^{Ec}	668.20 ± 29.00 ^{Ed}	659.10 ± 78.20 ^{Ed}	638.00 ± 36.90 ^{Ed}
400 MPa	681.24 ± 12.59 ^{Fa}	683.90 ± 67.10 ^{Fb}	605.83 ± 11.98 ^{Fc}	670.12 ±10.93 ^{Fd}	638.20 ± 30.70 ^{Fd}	614.20 ± 19.30 ^{Fd}

1 All phenolic content values are the mean ± standard deviation of three replicates (n=3).

2 For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$

3 Untreated (raw) and thermally treated (85°C/10 min) samples phenolic content values for 100 psi squeezed are 509.40 ± 6.07 and 543.90 ± 30.40 respectively while for 150 psi squeezed are 705.60 ± 36.60 and 699.4 ± 40.10 respectively.

3.1.2.4 Total Monomeric Anthocyanin Concentration

The presence of anthocyanins is responsible for the appealing bright red color of juice and other products of pomegranate fruit. Anthocyanin concentrations of pomegranate juice generally vary between 10 and 700 mg/L depending on the pomegranate cultivar. Nutritionists recommend to preserving these compounds during fruit juice processing, because they exert health protective effects for human (Vardin et al., 2003). It was observed that all heat treatment processes decreased the color parameters (L, a, and b values) of pomegranate juice significantly and the products turned reddish brown (Maskan, 2006). The extent of color degradation increased with soluble solids content. Sugar and sugar degradation products have been found to be effective on accelerating anthocyanin (pomegranate pigment) breakdown and enhance non-enzymatic browning during thermal processing (Cemeroğlu et al., 1994; Suh et al., 2003). In Table 3.3, anthocyanin concentration is drastically decreased with thermal treatment compared with the untreated pomegranate juice from 332.31 ± 5.21 to 263.84 ± 6.84 ($p < 0.05$). On the other hand, results of HHP treatments are closer to raw pomegranate juice values. Even if the raw anthocyanin concentrations of 100 and 150 psi show similarities, 150 psi showed better anthocyanin retention than 100 psi after HHP treatment ($p < 0.05$). At constant t and T, there is no significant effect of P values on anthocyanin concentrations for both 100 and 150 psi. Temperature and time have a significant effect on anthocyanin concentrations while pressure is ineffective for 100 psi squeezed juice. In addition, there is a significant difference only between 5 and 25^{o}C treated samples at constant P and t for 150 psi juice while all T values differences are important for 100 psi squeezed juice ($p < 0.05$). Treatments for 5 minutes enhance better retention than 10 minutes and treatments at 15^{o}C give the highest anthocyanin concentration rather than other T values for both 100 and 150 psi squeezed juices ($p < 0.05$).

Table 3.3 The effect of HHP and time on Total Monomeric Anthocyanin Pigment Concentration (mg/L cyanidin-3-glucoside) of pomegranate juice squeezed at 100 psi and 150 psi pressure by hydraulic press just after HHP treatment [1, 2, 3].

	5 minutes ^{x}			10 minutes ^{y}		
	5ºC	15ºC	25ºC	5ºC	15ºC	25ºC
100 psi						
200 MPa	292.73 ± 7.53^{Aa}	337.65 ± 13.00^{Bb}	285.05 ± 3.98^{Cc}	273.69 ± 3.69^{Dd}	264.01 ± 7.41^{Ee}	280.21 ± 11.27^{Ff}
300 MPa	298.57 ± 7.41^{Aa}	322.62 ± 2.30^{Bb}	280.87 ± 3.62^{Cc}	280.37 ± 4.94^{Dd}	266.85 ± 8.24^{Ee}	260.17 ± 5.81^{Ff}
400 MPa	304.59 ± 6.09^{Aa}	327.97 ± 11.82^{Bb}	280.71 ± 3.22^{Cc}	275.53 ± 7.28^{Dd}	265.34 ± 11.82^{Ee}	267.35 ± 5.98^{Ff}
150 psi						
200 MPa	311.43 ± 7.99^{Aa}	320.4 ± 17.5^{Bb}	304.59 ± 10.71^{Ac}	337.32 ± 13.29^{Cd}	308.26 ± 1.61^{Dd}	305.10 ± 18.1^{Ce}
300 MPa	310.26 ± 2.52^{Aa}	310.10 ± 4.10^{Bb}	298.58 ± 12.31^{Ac}	308.43 ± 12.78^{Cd}	323.46 ± 3.69^{Dd}	312.10 ± 5.21^{Ce}
400 MPa	318.61 ± 3.91^{Aa}	317.78 ± 3.41^{Bb}	306.42 ± 3.52^{Ac}	314.27 ± 12.09^{Cd}	291.90 ± 21.10^{Dd}	300.25 ± 7.54^{Ce}

1 All anthocyanin pigment concentration values are the mean ± standard deviation of three replicates (n=3).

2 For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.

3 Untreated (raw) and thermally treated (85ºC/10 min) samples anthocyanin concentration values for 100 psi squeezed are 332.31 ± 5.21 and 263.84 ± 6.84 respectively while for 150 psi squeezed are 323.50 ± 19.10 and 246.98 ± 2.18 respectively.

In a few experiments and studies, negative effect of HHP on enzymes is reported (Hendrickx et al., 1998; San-martin et al., 2002; Lopez-Malo et al., 1998; Park et al., 2006). One of those enzymes, polyphenol oxidase enzyme activity has a negative effect on anthocyanin stability of pomegranate juice (Jaiswal et al., 2009). Polyphenol oxidase enzyme denaturation due to HHP treatment may also protect anthocyanin concentration stability. Further investigations are needed to prove this situation.

3.1.2.5 Antioxidant (Radical Scavenging) Activity (RSA)

At constant P and T, there is a significant difference ($p < 0.05$) between HHP treatment times; 10 minutes treatment show a slight decrease on % RSA compared with 5 minutes treatment and at constant T and t, there is no statistical difference between treatment pressures for both 100 and 150 psi squeezed juices. Results are shown in Table 3.4. 5 minutes treatments give higher RSA than 10 minutes treatment but importance of time is higher for 100 psi than 150 psi. At constant P and t, there is a significant RSA decrease with rising T ($p < 0.05$). The highest RSA could be obtained with the lowest temperature for both 100 and 150 psi squeezed juices. At the end of these evaluations, for 100 psi squeezed pomegranate, at any pressure value, 5 minutes with 5°C and for 150 psi squeezed pomegranate, at any pressure value, 10 minutes and 5°C treatments could be the best proposal for RSA.

The increase in antioxidant activity value detected during pomegranate juice processing could be due to the extraction of some of the hydrolysable tannins, present in the fruit rind, and/or related to the increase in ellagic acid, ellagic structures polymerized into ellagitannins, and/or anthocyanin polymers formed during the storage period (Pérez-Vicente et al., 2004). In another study, HHP treated samples at 450 and 550 MPa exhibited higher antioxidant capacity (IC50 is 11–13 mg/mL), than the control sample (IC50 is 14 mg/mL) (The smaller IC50 value the higher antioxidant activity) (Santos et al., 2011).

Table 3.4 The effect of HHP and time on Free Radical Scavenging Activity (RSA, % DPPH) of pomegranate juice squeezed with 100 psi and 150 psi pressure by hydraulic press just after HHP treatment [1,2,3].

	5 minutes ^{x}			10 minutes ^{y}		
	5°C	15°C	25°C	5°C	15°C	25°C
100 psi						
200 MPa	123.04 ± 3.83 ^{Aa}	121.77 ± 3.92 ^{Bb}	127.35 ± 15.18 ^{Cc}	126.57 ± 3.69 ^{Dd}	116.08 ± 7.55 $^{E\ e}$	92.64 ± 1.39 ^{Ff}
300 MPa	126.37 ± 2.98 ^{Aa}	118.33 ± 1.77 ^{Bb}	116.47 ± 5.27 ^{Cc}	114.80 ± 4.12 ^{Dd}	117.94 ± 4.60 ^{Ee}	115.00 ± 5.57 ^{Ff}
400 MPa	125.88 ± 3.35 ^{Aa}	116.67 ± 1.77 ^{Bb}	125.20 ± 5.69 ^{Cc}	117.94 ± 9.18 ^{Dd}	118.73 ± 5.05 ^{Ee}	123.34 ± 6.50 ^{Ff}
150 psi						
200 MPa	168.14 ± 3.79 ^{Aa}	169.91 ± 6.58 ^{Bb}	165.89 ± 0.45 ^{Cc}	180.99 ± 0.88 ^{Dd}	160.89 ± 7.02 ^{Ee}	165.01 ± 1.11 ^{Ff}
300 MPa	162.16 ± 4.33 ^{Aa}	163.83 ± 1.19 ^{Bb}	166.67 ± 2.09 ^{Cc}	172.26 ± 2.50 ^{Dd}	165.89 ± 5.34 ^{Ee}	168.73 ± 3.97 ^{Ff}
400 MPa	165.50 ± 4.06 ^{Aa}	163.44 ± 1.62 ^{Bb}	165.89 ± 3.48 ^{Cc}	162.36 ± 5.75 ^{Dd}	166.58 ± 2.33 ^{Ee}	170.89 ± 5.30 ^{Ff}

1 All RSA values are the mean ± standard deviation of three replicates (n=3).

2 For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.

3 RSA values for untreated (raw) 100 and 150 psi squeezed pomegranate juices accepted 100 %. Thermally treated (85°C/10 min) samples % RSA value for 100 psi squeezed is 73.53 ± 9.42 % and for 150 psi squeezed is 82.93 ± 2.37 %.

3.1.2.6 Ascorbic Acid

There is no study in literature about the HHP treatment effect on ascorbic acid content of pomegranate juice yet. In orange juice, just after 350 MPa/30 °C/2.5 min HHP treatments, juice had the same levels of ascorbic acid compared to untreated juices (Polydera et al., 2005). Also, the previously mentioned results confirm those reported by Donsi et al. (1996) and Van den Broeck et al. (2000) about the stability of ascorbic acid in orange juice when pressurized at mild temperatures. Beside these, in another study, HHP treatment increased the ascorbic acid content in a more complex food structure, egg yolk (Sancho et al., 1999). In our study, vitamin C content is protected as in untreated juice with every temperature and pressure value studied but only 5 minute application. Around 10 minute application there is a sharp decrease in vitamin C level in pomegranate juice approximately 40-50 % of the initial value. The exact effect of time on ascorbic acid level should be investigated to find the best HHP application time.

In Table 3.5, it could be seen that thermally treated samples show a lower ascorbic acid content than untreated juice ($p < 0.05$). At constant P and T, there is significant difference ($p < 0.05$) between HHP treatment time; 5 minutes treatment leads to a higher ascorbic acid content than untreated pomegranate juice but as a result of 10 minutes treatment, ascorbic acid content shows a sharp decrease for both 100 and 150 psi squeezed juices. Treatment time is the only effective parameter on ascorbic acid content for 150 psi squeezed juices. But 15°C treatment causes lower ascorbic acid content than 5 and 25°C treatments for 100 psi squeezed juices. There is no statistical difference among treatment pressures for both 100 and 150 psi squeezed juices ($p > 0.05$). According to these results, for better ascorbic acid retention, precisely 5 minutes treatment with 5 or 25°C at any pressure for 100 psi and for 150 psi squeezed juice, precisely 5 minutes treatment at any pressure and temperature could be proposed.

Table 3.5 The effect of HHP and time on Ascorbic Acid content (mg / L) of pomegranate juice squeezed with 100 psi and 150 psi pressure by hydraulic press just after HHP treatment [1,2,3].

	5 minutes ^{x}			10 minutes ^{y}		
	5°C	15°C	25°C	5°C	15°C	25°C
100 psi						
200 MPa	98.41 ± 5.21 ^{Aa}	92.73 ± 0.19 ^{Bb}	106.57 ± 3.09 ^{Ca}	66.21 ± 2.21 ^{Dc}	65.18 ± 1.60 ^{Ed}	65.07 ± 3.14 ^{Fc}
300 MPa	107.48 ± 2.12 ^{Aa}	94.09 ± 0.70 ^{Bb}	101.36 ± 3.28 ^{Ca}	58.84 ± 0.68 ^{Dc}	57.14 ± 7.66 ^{Ed}	66.09 ± 2.89 ^{Fc}
400 MPa	102.26 ± 4.82 ^{Aa}	95.34 ± 1.93 ^{Bb}	102.60 ± 6.29 ^{Ca}	61.67 ± 2.08 ^{Dc}	59.86 ± 9.94 ^{Ed}	62.92 ± 1.70 ^{Fc}
150 psi						
200 MPa	108.50 ± 4.45 ^{Aa}	102.49 ± 5.17 ^{Ba}	114.05 ± 0.39 ^{Ca}	57.93 ± 2.51 ^{Db}	59.18 ± 5.28 ^{Eb}	57.25 ± 0.39 ^{Fb}
300 MPa	106.23 ± 2.26 ^{Aa}	109.75 ± 2.57 ^{Ba}	113.49 ± 1.37 ^{Ca}	56.69 ± 4.28 ^{Db}	50.67 ± 3.87 ^{Eb}	53.85 ± 2.05 ^{Fb}
400 MPa	101.70 ± 5.48 ^{Aa}	117.23 ± 3.98 ^{Ba}	106.91 ± 3.42 ^{Ca}	60.31 ± 2.83 ^{Db}	53.97 ± 2.26 ^{Eb}	49.43 ± 3.75 ^{Fb}

1. All ascorbic acid values are the mean ± standard deviation of three replicates (n=3).
2. For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.
3. Untreated (raw) and thermally treated (85°C/10 min) samples ascorbic acid values for 100 psi squeezed are 97.14 ± 0.71 and 86.61 ± 1.19 respectively while for 150 psi squeezed are 110.61 ± 2.12 and 97.49 ± 1.53 respectively.

3.1.2.7 Mannitol

One of the basic criteria used for the definition of fruit juices is certainly Brix degree. According to European Fruit Juice Association (AIJN) proposal, the minimum Brix degree of pomegranate juice should be 14.0 (Anonymous, 2008). At a total sugar concentration of 16 °Brix, pomegranate juice contains characteristic sugars including mannitol at > 0.3 g/100 mL. Ratios of glucose to mannitol of 4−15 and of glucose to fructose of 0.8−1.0 are also characteristic of pomegranate juice (Zhang et al., 2009).

Any mannitol criterion for pomegranate juice is not determined by AIJN or other authority yet. But due to being the highest sugar alcohol in pomegranate juice, mannitol content must be considered on determining the authenticity. Mannitol level could be more specific on this determination for pomegranate juice.

To adjust the astringent taste of poor-quality juice or peel extract, addition of non-pomegranate sugars is a commonly detected adulteration method (Zhang et al., 2009). Due to the fact that, determining the sugar alcohol ratios/levels, mainly mannitol, are quite important to determine any authenticity. Moreover, many researches and studies on the changes that occur in pomegranate juice during processing and storage have been published (Alper et al., 2005, Bayındırlı et al., 1994, Maskan, 2006 and Tabur et al., 1987). However, it was expressed that sorbitol/xylitol is not unique to pomegranate but it can also be formed by the microbial reduction of fructose in pomegranate juice (Jones and Silveira, 2004). In addition, it is also speculated that processes or changes, such as heating, enzyme addition and fermentation may cause an increase in the mannitol/sorbitol/xylitol content of pomegranate juice.

Mannitol content of pomegranate juice is also so important due to the anaphylaxis caused by mannitol in pomegranate. As little as 0.25 mL of pomegranate juice (derived from ~0.4 g pomegranate fruit), containing mannitol at a concentration of 0.22 mM, caused subjective and objective symptoms of immunoglobulin E (IgE)-mediated allergy in *double-blind placebo-controlled food challenge* (*DBPCFC*) (Hedge et al., 2002). Excessive amounts of mannitol in pomegranate juice, occurred due to so many factors, are crucial especially for hypersensitive people.

In this study; mannitol content of raw, thermally treated and HHP treated samples squeezed with two different presses were investigated. Only 5 minutes HHP treated samples are examined as time option. As seen in the Table 3.6, mannitol content totally depends on the pressure and temperature for HHP treatment for both squeezing pressures. At 100 psi squeezed juice, only the difference between 300 MPa at 25°C and the raw pomegranate juice is insignificant. For 150 psi, only the difference between raw sample and 400 MPa at 5°C is insignificant ($p > 0.05$). Except these, mannitol content increases with squeezing pressure and thermal treatment. Some of the other HHP combinations give lower and some of them give higher mannitol contents. As a best option, the combination gives the lower results could be proposal for both squeezed pomegranates.

Table 3.6 The effect of HHP on Mannitol content (mg/mL) of pomegranate juice squeezed with 100 psi and 150 psi pressure as HHP treatment for 5 min[1,2,3].

	5ᵒC	15ᵒC	25ᵒC
100 psi			
200 MPa	2.92 ± 0.03 [Aa]	3.27 ± 0.01 [Ab]	3.29 ± 0.01 [Ac]
300 MPa	3.35 ± 0.02 [Ba]	3.14 ± 0.008 [Bb]	3.06 ± 0.01 [Bc]
400 MPa	2.94 ± 0.01 [Ca]	3.29 ± 0.02 [Cb]	3.13 ± 0.01 [Cc]
150 psi			
200 MPa	3.88 ± 0.03 [Aa]	3.94 ± 0.01 [Ab]	3.46 ± 0.01 [Ac]
300 MPa	3.55 ± 0.01 [Ba]	3.68 ± 0.002 [Bb]	3.56 ± 0.02 [Bc]
400 MPa	3.36 ± 0.05 [Ca]	3.77 ± 0.001 [Cb]	3.43 ± 0.003 [Cc]

1. All mannitol content values are the mean ± standard deviation of three replicates (n=3).
2. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.
3. Untreated (raw) and thermally treated (85ᵒC/10 min) samples mannitol content for 100 psi squeezed are 3.05 ± 0.05 and 3.13 ± 0.01 respectively while for 150 psi squeezed are 3.34 ± 0.02 and 3.59 ± 0.01 respectively.

3.1.3 Selection Criteria for the Optimum Combination

According to the aforementioned results, for 100 psi squeezed juice, as a common pressure-temperature-time combination could be proposed for both TMAB and TYM as 400 MPa at 15ᵒC for 5 minutes. This combination is primarily chosen for microbial stability and secondly the best retention of other factors as ascorbic acid and antioxidant activity. For 150 psi squeezed juice, primary microbial stabilization is possible for only 400 MPa for 10 minutes. Temperature could be chosen as 5ᵒC. These values could be not proper for especially ascorbic acid content but microbial validity limits do not let another option. Microbial stability is the number one prerequisite for foods. Addition to aforementioned, HHP treatment has an important effect on ΔE values of pomegranate juice samples. Smaller treatment time has an enormous positive effect on ascorbic acid content while this does not affect antioxidant activity. Relative to heat treatment, HHP treatments give better results in the aspects of total phenolic content and anthocyanin pigment concentration. Referring the results, this study must be maintained with the shelf life examination in order to give a more reliable expression about the effects of HHP on pomegranate juice quality factors against the thermal treatment over time.

3.2 Shelf Life Study for Optimum Combinations

3.2.1 Total Mesophilic Aerobic Bacteria and Total Yeast and Mould During Shelf Life

All group samples are evaluated to determine the total mesophilic aerobic bacteria (TMAB) levels as log colony forming unit/mL during the shelf life period (30 days). The TMAB levels of the control samples started from 3.46 and 4.09 log cfu/mL and reached 4.41 and 5.48 log cfu/mL for 100 and 150 psi squeezed juices respectively. For thermally treated and HHP treated samples, no detectable colony (above the detection level, 25 cfu/mL) was observed during the shelf life. This means both thermal and HHP treatment are provided the microbial stability for TMAB during 30 days. Results can be followed from Table 3.7.

All group samples are also evaluated to determine the total yeast and mould (TYM) levels as log colony forming unit/mL during the shelf life period (30 days). As seen at the table 3.7, the TYM levels of the control samples started from 4.05 and 4.36 log cfu/mL and reached 5.12 and 5.77 log cfu/mL for 100 and 150 psi squeezed juices respectively. For thermally treated and HHP treated samples, no detectable colony (above the detection level, 25 cfu/mL) was observed during the shelf life. This means both thermal and HHP treatments are provided the microbial stability for TYM during 30 days.

Table 3.7 The effect of storage at 4°C on Total Mesophilic Aerobic Bacteria (TMAB) and Total Yeast and Mould (TYM) content (log cfu/ml) of pomegranate juice squeezed with 100 psi and 150 psi pressure for HHP treated and pasteurized samples against control.

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 30
TMAB									
100 psi									
Raw (Control)	3.46	3.51	3.86	3.87	3.91	3.99	4.07	4.21	4.41
Pasteurized*	ND***	ND	ND	ND	ND	ND	ND	ND	ND
HHP Treated**	ND	ND	ND	ND	ND	ND	ND	ND	ND
150 psi									
Raw (Control)	3.97	4.05	4.07	4.09	4.10	4.28	4.43	4.91	5.12
Pasteurized*	ND	ND	ND	ND	ND	ND	ND	ND	ND
HHP Treated**	ND	ND	ND	ND	ND	ND	ND	ND	ND
TYM									
100 psi									
Raw (Control)	4.09	4.16	4.22	4.65	4.73	5.22	5.34	5.59	5.48
Pasteurized*	ND	ND	ND	ND	ND	ND	ND	ND	ND
HHP Treated**	ND	ND	ND	ND	ND	ND	ND	ND	ND
150 psi									
Raw (Control)	4.35	4.36	4.44	4.49	5.11	5.52	5.59	5.70	5.77
Pasteurized*	ND	ND	ND	ND	ND	ND	ND	ND	ND
HHP Treated**	ND	ND	ND	ND	ND	ND	ND	ND	ND

*Pasteurization condition is 85°C for 10 minutes.

**HHP treatment conditions are 400 MPa at 15°C during 5 min for 100 psi squeezed and 400 MPa at 5°C during 10 min for 150 psi squeezed pomegranate juice.

*** ND: Not Detected

3.2.2 pH During Shelf Life

It is determined that the pH of all the group samples was not affected by the treatment type during the storage period. pH differences were not found statistically significant ($p > 0.05$) and accepted stable during the shelf life (See Appendix D).

3.2.3 Color Measurement During Shelf Life

L*, a* and ΔE values were evaluated during shelf life (Table 3.8). 150 psi squeezed juice have lower L* and a* values since day 0 to day 30 than 100 psi squeezed juice for all groups. This means 100 psi squeezed ones are brighter and redder than the others. HHP treated samples are brighter and more red, higher L* and a* values, than the other groups, even the control at the day 0.

It is a fact that the color of the pomegranate juice becomes browner with the use of high temperatures (Perez-Vicente et al., 2004). Furthermore, thermally treated samples showed the highest ΔE values during the storage for every group of 100 psi squeezed juices and the first 12 days for 150 psi squeezed juices. The highest ΔE value for 150 psi squeezed one belongs to the control group. The final product at the end of storage of HHP treatment is the brightest and the reddest one among all for both squeezing groups. It demonstrates the HHP provides better color value retention than thermal treatment.

For 150 psi squeezed pomegranate juice, control sample turned into an unpleasant appearance more than the pasteurized one. For 100 psi squeezed one, control sample results are closer to HHP treated one and the thermally treated has the lowest values.

During the storage period, some days has better or the same color values with the previous one. The color differences of juices are another way of correcting the antioxidant activity change. Color is affected by ascorbic acid and other antioxidant compounds oxidation (Dede et al., 2007). It can be seen that there is also a direct proportion with the color values and the antioxidant and vitamin C content. Fluctuations around day 12 and day 15 of L* and a* values for all groups can be caused by the fluctuations of ascorbic acid and antioxidant values at those days. Also higher ascorbic acid and antioxidant amounts of HHP treated samples could be caused higher color stability.

Color properties of each group during the storage period also showed a correlation with the sensory analyses. The highest color evaluation grades were given to the HHP treated samples even at the day 30 by the panelists.

Table 3.8 The effect of storage at 4°C on L*, a* and ΔE values of pomegranate juice squeezed with 100 psi and 150 psi pressure for HHP treated and pasteurized samples against control. [1,2]

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 30
L*									
100 psi									
Raw (Control)	25.00 ± 0.08 ^{Aa}	23.93 ± 0.02 ^{Ab}	21.00 ± 0.06 ^{Ac}	22.75 ± 0.04 $^{A d}$	21.76 ± 0.14 ^{Ad}	20.76 ± 0.10 ^{Af}	20.05 ± 0.48 ^{Af}	19.12 ± 0.16 $^{A h}$	18.70 ± 0.70 $^{A i}$
Pasteurized*	24.06 ± 0.11 $^{B a}$	23.45 ± 0.03 ^{Bb}	20.39± 0.04 $^{B c}$	19.53 ± 0.02 $^{B d}$	22.53 ± 0.01 $^{B d}$	20.16 ± 0.40 ^{Bf}	20.47 ± 0.09 $^{B f}$	19.11 ± 0.08 $^{B h}$	18.09 ± 0.07 $^{B i}$
HHP Treated**	26.10 ± 0.12 $^{C a}$	24.90 ± 0.41 $^{C b}$	22.78 ± 0.10 $^{C c}$	22.91 ± 0.23 $^{C d}$	21.41 ± 0.00 $^{C d}$	20.73 ± 0.07 ^{Cf}	20.70 ± 0.03 $^{C f}$	19.22 ± 0.09 ^{Ch}	18.89 ± 0.03 $^{C i}$
150 psi									
Raw (Control)	22.36 ± 0.25 $^{A a}$	20.01 ± 0.06 $^{A b}$	17.26 ± 0.09 $^{A c}$	15.70 ± 0.22 $^{A d}$	15.82 ± 0.07 $^{A d}$	14.21 ± 0.07 $^{A f}$	14.68 ± 0.01 $^{A g}$	13.47 ± 0.07 $^{A h}$	11.60 ± 0.13 $^{A i}$
Pasteurized*	22.54 ± 0.20 $^{B a}$	20.15 ± 0.03 $^{B b}$	18.95 ± 0.05 $^{B c}$	18.21 ± 0.05 $^{B d}$	18.22 ± 0.07 $^{B d}$	17.74 ± 0.02 $^{B f}$	18.31 ± 0.11 $^{B g}$	16.85 ± 0.03 $^{B h}$	14.62 ± 0.62 $^{B i}$
HHP Treated**	23.43 ± 0.09 ^{Ca}	21.17 ± 0.09 $^{C b}$	19.94 ± 0.15 $^{C c}$	18.30 ± 0.05 $^{C d}$	17.81 ± 0.06 $^{C d}$	16.63 ± 0.37 $^{C f}$	16.83 ± 0.06 $^{C g}$	15.70 ± 0.04 $^{C h}$	14.77 ± 0.06 $^{C i}$
a*									
100 psi									
Raw (Control)	49.17 ± 0.04 $^{A a}$	48.66 ± 0.18 $^{A b}$	45.61 ± 0.12 $^{A c}$	47.04 ± 0.03 $^{A c}$	45.29 ± 0.27 $^{A d}$	44.28 ± 0.12 $^{A e}$	42.82 ± 0.07 $^{A f}$	41.56 ± 0.30 $^{A g}$	40.43 ± 0.47 $^{A i}$
Pasteurized*	44.74 ± 0.12 $^{B a}$	43.57 ± 0.07 $^{B b}$	41.95 ± 0.15 $^{B c}$	40.09 ± 0.05 $^{B c}$	43.03 ± 0.02 $^{B d}$	41.32 ± 0.12 $^{B e}$	41.24 ± 0.36 $^{B f}$	38.86 ± 0.10 $^{B g}$	36.57 ± 0.15 $^{B i}$
HHP Treated**	50.01 ± 0.11 $^{C a}$	49.05 ± 0.10 $^{C b}$	47.67 ± 0.07 $^{C c}$	47.51 ± 0.33 $^{C c}$	44.85 ± 0.32 $^{C d}$	44.18 ± 0.14 $^{C e}$	43.65 ± 0.09 $^{C f}$	41.35 ± 0.17 $^{C g}$	40.28 ± 0.24 $^{C i}$
150 psi									
Raw (Control)	47.28 ± 0.32 $^{A a}$	45.73 ± 0.07 $^{A b}$	41.64 ± 0.08 $^{A c}$	38.73 ± 0.57 $^{A d}$	38.42 ± 0.14 $^{A d}$	36.02 ± 0.05 $^{A e}$	36.15 ± 0.19 $^{A e}$	34.77 ± 0.15 $^{A f}$	32.54 ± 0.48 $^{A g}$
Pasteurized*	43.51 ± 0.28 $^{B a}$	41.89 ± 0.08 $^{B b}$	40.76 ± 0.12 $^{B c}$	39.77 ± 0.12 $^{B d}$	39.80 ± 0.12 $^{B d}$	38.90 ± 0.04 $^{B e}$	40.00 ± 0.19 $^{B e}$	37.63 ± 0.08 $^{B f}$	35.90 ± 0.03 $^{B g}$
HHP Treated**	46.90 ± 0.18 $^{C a}$	45.60 ± 0.73 $^{C b}$	45.62 ± 0.20 $^{C c}$	42.49 ± 0.41 $^{C d}$	42.72 ± 0.08 $^{C d}$	40.02 ± 0.30 $^{C e}$	39.30 ± 0.19 ^{Ce}	38.65 ± 0.04 $^{C f}$	37.51 ± 0.03 ^{Cg}

1. All values are the mean ± standard deviation of three replicates (n=3).
2. For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.

*Pasteurization condition is 85°C for 10 minutes.

**HHP treatment conditions are 400 MPa at 15°C during 5 min for 100 psi squeezed and 400 MPa at 5°C during 10 min for 150 psi squeezed pomegranate juice.

Table 3.8 Cont'd

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 30
ΔE									
100 psi									
Raw (Control)		1.56 ± 0.11 A a	8.01 ± 0.23 A b	4.96 ± 0.16 A c	6.76 ± 0.56 A bc	8.05 ± 0.17 A d	10.63 ± 0.16 A e	12.34 ± 0.60 A f	14.08 ± 0.43 A g
Pasteurized*		9.50 ± 0.21 B a	13.88 ± 0.26 B b	16.19 ± 0.12 B c	10.99 ± 0.05 B bc	13.24 ± 0.23 B d	13.62 ± 0.46 B e	16.79 ± 0.17 B f	19.78 ± 0.11 B g
HHP Treated**		0.23 ± 0.07 C a	4.53 ± 0.27 C b	3.75 ± 0.34 C c	7.46 ± 0.23 C bc	8.29 ± 0.28 C d	9.27 ± 0.17 C e	12.63 ± 0.30 C f	13.63 ± 0.20 C g
150 psi									
Raw (Control)		3.61 ± 0.12 A a	8.96 ± 0.09 A b	13.51 ± 0.84 A c	13.00 ± 0.27 A c	16.19 ± 0.05 A d	15.80 ± 0.33 A d	17.38 ± 0.20 A e	21.07 ± 0.26 A f
Pasteurized*		8.79 ± 0.48 B a	9.53 ± 0.20 B b	11.75 ± 0.20 B c	11.76 ± 0.18 B c	12.22 ± 0.05 B d	10.92 ± 0.23 B d	14.08 ± 0.14 B e	17.10 ± 0.01 B f
HHP Treated**		2.64 ± 1.01 C a	2.94 ± 0.25 C b	7.24 ± 0.20 C c	7.61 ± 0.09 C c	10.07 ± 0.49 C d	10.88 ± 0.29 C d	12.18 ± 0.06 C e	13.95 ± 0.02 C f

1. All antioxidant and ascorbic acid values are the mean ± standard deviation of three replicates (n=3).
2. For treatment time, similar letters demonstrate no statistical difference at $p < 0.05$. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$.

*Pasteurization condition is 85°C for 10 minutes.

**HHP treatment conditions are 400 MPa at 15°C during 5 min for 100 psi squeezed and 400 MPa at 5°C during 10 min for 150 psi squeezed pomegranate juice.

3.2.4 Antioxidant (Free Radical Scavenging) Activity and Ascorbic Acid During Shelf Life

At the first glimpse, HHP treated samples showed similar % radical scavenging activity (RSA) with control (raw) samples whereas thermal treatment causes nearly 10 % loss of RSA at the day 0 (Table 3.9). At the end of the shelf life period, % RSA retention and stability is the highest one relative to untreated control and thermally treated samples. During the shelf life period there is an increase of % RSA values for all treatment groups around day 15. This situation can be supported by the increase of the ascorbic acid content between day 15 and day 18. There could be so many reasons of this increase like the inactivation of some inhibitor compounds or formation of some promoter compounds for antioxidant or/and ascorbic acid. An antioxidant activity could happen during the storage period for pomegranate juice due to anthocyanin polymers formation (Pérez-Vicente et al 2004).

Santos et al (2011) also reported similar results for antioxidant capacity of pomegranate juice during shelf life period of 350, 450 and 550 MPa with 30, 90, 150 s treatments. An increasement was observed after a day-by-day decrease of antioxidant capacity between day 15 and day 20.

Antioxidant capacity differences were found to be statistically significant ($p < 0.05$) among treatment groups for the same days during storage period. Within a group, there is a significant alteration for RSA during storage. This alteration is generally a small decrease for the first 10 days followed by an increase around day 15 and then again a decrease till the end of the shelf life period for HHP treated 100 psi squeezed pomegranate juice. The decrease for thermally treated one is quite sharp relative to HHP treated ones while control samples show an alteration as better than the thermally treated, worse than the HHP treated. The overall % RSA changes are 69.11, 59.33 and 86.44 for the control, pasteurized and HHP treated samples, respectively.

For 150 psi squeezed juice the increasement are seen in the day 3, day 12 for all groups and day 30 except for thermally treated one. The overall % RSA changes are 95.27, 75.07 and 108.01 for the control, pasteurized and HHP treated samples, respectively. As a result, it can been said that, 150 psi squeezed pomegranate juice has higher RSA than 100 psi squeezed one, for each group, during shelf life period ($p < 0.05$).

Prior to shelf life study, while examining the effects of 10 minutes treatments with all pressure and temperature combinations for HHP treatment, showed a statistically significant decrease of ascorbic acid content relative to untreated sample. Even this decrease was found sometimes a half, for some combinations. Here, for shelf life study, this aforementioned decrease can be seen again for HHP treatment relative to control sample for 150 psi squeezed juice. But during the storage, stability of ascorbic acid is much better than for both thermally treated and control samples. 100 psi squeezed juice has nearly the same amount of ascorbic acid content with the control sample but thermal treated has a much lower level. For 100 psi squeezed juice, the ascorbic acid content changes, decrease during the storage period ($p < 0.05$) but between day 15 and day 18 an increasement can be seen. For 150 psi squeezed juice, the ascorbic acid content changes, decrease during the storage period ($p < 0.05$) but around day 9 and day 12, a sharp increasement can be seen. To explain this situation, the same case for the antioxidant capacity could be said as the formation or/and inhibition of some compounds. At the end of the storage period HHP treated samples shows a higher ascorbic acid level than the other groups, even higher than the value of day 0 of the control sample for 150 psi squeezed juice. Thermally treated sample’s values are always the lowest.

In literature, no study is done about the HHP treatment effect on ascorbic acid content of pomegranate juice yet. But in the study of Kulkarni et al (2005) decreases and increasement of ascorbic acid content were seen during the shelf life period for pomegranate arils concomitant with the same alterations of antioxidant activities and the reason for this situation was expressed with buildup of anthocyanins.

Table 3.9 The effect of storage at 4°C on Antioxidant Activity (%) and Ascorbic Acid Content (mg / L) of pomegranate juice squeezed with 100 psi and 150 psi pressure for HHP treated and pasteurized samples against control. [1,2]

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 30
Antioxidant									
100 psi									
Raw (Control)	100 ± 0.00 Aa	87.11 ± 2.04 Ab	87.72 ± 0.31 Ac	88.05 ± 11.05 Ad	80.01 ± 1.74 Ae	88.19 ± 2.47 Af	79.70 ± 1.23 Ag	78.81 ± 3.54 Ah	69.11 ± 1.22 Ai
Pasteurized*	92.59 ± 1.11 Ba	76.39 ± 2.60 Bb	79.09 ± 3.03 Bc	76.39 ± 2.03 Bd	67.89 ± 2.94 Be	76.39 ± 1.11 Bf	72.68 ± 3.87 Bg	64.18 ± 0.73 Bh	59.33 ± 2.43 Bi
HHP Treated**	101.3 ± 3.70 Ca	92.37 ± 2.44 Cb	92.24 ± 7.15 Cc	91.30 ± 1.82 Cd	82.66 ± 1.41 Ce	93.45 ± 2.80 C f	92.64 ± 3.14 Cg	88.12 ± 2.69 Ch	86.44 ± 3.40 Ci
150 psi									
Raw (Control)	100 ± 0.00 Aa	102.39 ± 1.28 Ab	100.62 ± 3.88 Ac	107.94 ± 1.13 Ad	120.63 ± 2.18 Ae	106.18 ± 2.11 Af	103.77 ± 3.11 Ag	93.11 ± 2.87 Ah	95.27 ± 1.31 Ai
Pasteurized*	88.20 ± 1.34 Ba	95.32 ± 1.32 Bb	85.04 ± 2.68 Bc	94.88 ± 6.03 Bd	102.20 ± 2.75 Be	96.71 ± 3.50 Bf	91.92 ± 2.25 Bg	79.42 ± 1.90 Bh	75.07 ± 0.89 Bi
HHP Treated**	101.06 ± 2.09 Ca	107.88 ± 0.76 Cb	107.88 ± 1.07 Cc	112.55 ± 1.39 Cd	123.02 ± 6.71 Ce	114.31 ± 2.07 Cf	107.25 ± 6.29 Cg	99.30 ± 1.53 Ch	108.01 ± 1.53 Ci
Ascorbic Acid									
100 psi									
Raw (Control)	121.90 ± 0.84 Aa	107.34 ± 4.70 Ab	100.94 ± 6.73 Ac	97.95 ± 10.22 Ad	88.83 ± 5.65 Ae	82.31 ± 1.65 Af	84.62 ± 5.54 Ag	80.40 ± 4.70 Ah	74.68 ± 3.23 Ai
Pasteurized*	105.76 ± 7.17 Ba	84.07 ± 1.63Bb	79.31 ± 3.56 Bc	87.75 ± 1.46 Bd	77.41 ± 3.79 Be	71.29 ± 1.24 Bf	79.04 ± 2.89 Bg	75.91 ± 1.78 Bh	69.92 ± 2.72 Bi
HHP Treated**	121.22 ± 6.74 Ca	97.68 ± 3.32 Cb	99.58 ± 5.61 Cc	108.29 ± 2.09 Cd	103.39 ± 3.67 Ce	99.58 ± 3.24 Cf	101.62 ± 1.47 Cg	97.81 ± 1.02 Ch	93.33 ± 0.84 Ci
150 psi									
Raw (Control)	111.24 ± 4.45 Aa	109.79 ± 1.77 Ab	105.02 ± 2.46 Ac	132.78 ± 3.47 Ad	128.70 ± 1.70 Ae	93.46 ± 0.81 Af	92.78 ± 5.77 Ag	89.24 ± 2.71 Ah	93.87 ± 2.85 Ai
Pasteurized*	83.67 ± 1.86 Ba	95.50 ± 3.89 Bb	94.01 ± 2.86 Bc	105.16 ± 3.87 Bd	107.88 ± 3.12 Be	83.12 ± 1.69 Bf	73.19 ± 14.62 Bg	64.48 ± 1.47 Bh	68.16 ± 11.04 Bi
HHP Treated**	94.69 ± 0.82 Ca	100.81 ± 1.77 Cb	98.36 ± 1.63 Cc	116.05 ± 4.39 Cd	120.13 ± 2.71 Ce	112.78 ± 3.47 Cf	113.87 ± 1.47 Cg	114.68 ± 5.20 Ch	118.90 ± 3.46 Ci

1 All antioxidant and ascorbic acid values are the mean ± standard deviation of three replicates (n=3).

2 For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$. To examine the effect of days, see Table E.1 and E .2

*Pasteurization condition is 85°C for 10 minutes.

**HHP treatment conditions are 400 MPa at 15°C during 5 min for 100 psi squeezed and 400 MPa at 5°C during 10 min for 150 psi squeezed pomegranate juice.

3.2.5 Sensory Analyses during Shelf Life

All groups of samples were evaluated for odor and color properties during the storage period. According to the results of the panelist's grading, especially color evaluation has corroboration with the color measurement of the samples with the colorimetric equipment during the storage.

As a results of the sensory evaluations (Table 3.10), HHP treated samples were preferred very much during the first 15 days for both in the aspects of color and odor. Pressurized samples did not loose their bright color and fresh-fruit-like odor during the first three weeks of storage. This freshness and brightness were higher than the untreated samples at the day 0. The color and odor stability are higher in 150 psi squeezed juice than 100 psi squeezed one for all groups. It is probably because of higher antioxidant, ascorbic acid and phenolic compound content of 150 psi squeezed juice.

Untreated control samples odor and color properties were strong and stable just only for 6 days. Then, color started to turn to brown as the pasteurized juice and odor started to turn into a rotten smell. This is due to the increasing microbial load during the storage.

Thermally treated samples color was quite dark and the smell was bitter-astringent since the day 0. These properties did not change over time so much. At the day 30, astringent smell was a bit stronger and color was darker than the first day. The pasteurized samples were referred as the most unpleasant group among all from the beginning by the panelists.

Table 3.10 The effect of storage at 4ºC on Sensory Properties as Odor and Color of pomegranate juice squeezed with 100 psi and 150 psi pressure for HHP treated and pasteurized samples against control. [1,2]

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 30
Odor									
100 psi									
Raw (Control)	8.16 ± 0.68 Aa	7.41 ± 0.37 Aa	7.25 ± 0.41 Aa	6.50 ± 0.44 Ab	5.83 ± 0.25 Ac	4.66 ± 0.40 Ad	3.75 ± 0.41 Ae	2.75 ± 0.61 Af	2.75 ± 0.61 Af
Pasteurized*	5.83 ± 0.75 B a	5.50 ± 0.44 Ba	4.75 ± 0.61 Bb	4.00 ± 0.54 Bc	3.91 ± 0.20 Bc	3.50 ± 0.44 Bd	2.50 ± 0.54 Be	2.50 ± 0.54 Be	2.50 ± 0.54 Be
HHP Treated**	8.83 ± 0.25 Ca	8.75 ± 0.27 Ca	8.58 ± 0.37 Ca	7.58 ± 0.49 Ca	7.08 ± 0.37 Ca	6.91 ± 0.37 Ca	6.83 ± 0.25 Cb	6.75 ± 0.27 Cb	6.75 ± 0.27 Cb
150 psi									
Raw (Control)	8.75 ± 0.41 Aa	8.41 ± 0.49 A	8.25 ± 0.27 A	8.00 ± 0.31 A	7.33 ± 0.40 A	5.08 ± 0.49 A	4.00 ± 0.63 A	3.00 ± 0.31 A	1.83 ± 0.51 A
Pasteurized*	6.58 ± 0.49 Ba	6.41 ± 0.37 B	6.16 ± 0.25 B	5.25 ± 0.27 B	5.08 ± 0.37 B	4.33 ± 0.25 B	4.08 ± 0.37 B	3.66 ± 0.40 B	2.75 ± 0.61 B
HHP Treated**	9.00 ± 0.00 Ca	9.00 ± 0.00 Ca	8.91 ± 0.20 Ca	8.83 ± 0.25 Ca	8.66 ± 0.40 Ca	8.41 ± 0.37 Ca	7.91 ± 0.37 Cb	7.91 ± 0.37 Cb	7.66 ± 0.25 Cb
Color									
100 psi									
Raw (Control)	7.83 ± 0.25 Aa	7.58 ± 0.37 Aa	7.16 ± 0.40 Aa	6.00 ± 0.63 Ab	4.40 ± 0.37 Ac	4.33 ± 0.25 Ac	4.16 ± 0.25 Ac	3.66 ± 0.40 Ad	3.14 ± 0.23 Ad
Pasteurized*	4.58 ± 0.66 Ba	4.50 ± 0.63Ba	4.41 ± 0.49 Ba	4.08 ± 0.37 Bb	4.08 ± 0.37 Bb	3.50 ± 0.31 Bc	3.33 ± 0.40 Bc	3.21 ± 0.78 Bc	2.87± 0.72 Bd
HHP Treated**	9.00 ± 0.00 Ca	9.00 ± 0.00 Ca	8.66 ± 0.40 Ca	7.91 ± 0.37 Cb	7.25 ± 0.27 Cb	7.25 ± 0.27 Cb	7.16 ± 0.25 Cb	7.16 ± 0.25 Cb	6.91 ± 0.20 Cb
150 psi									
Raw (Control)	8.50 ± 0.44 Aa	8.33 ± 0.40 Aa	8.33 ± 0.40 Aa	7.91 ± 0.20 Ab	7.08± 0.37 Ac	4.33 ± 0.25 Ad	5.75 ± 0.41 Ae	3.50 ± 0.44 Af	3.25 ± 0.27 Ag
Pasteurized*	5.66 ± 0.60 Ba	5.58 ± 0.49 Ba	5.25 ± 0.41 Ba	5.08 ± 0.20 Ba	4.75 ± 0.27 Bb	4.41 ± 0.37 Bb	3.66 ± 0.51 Bc	3.66 ± 0.51 Bc	2.83 ± 0.68 Bd
HHP Treated**	9.00 ± 0.00 Ca	9.00 ± 0.00 Ca	9.00 ± 0.00 Ca	8.91 ± 0.20 Ca	8.75 ± 0.27 Ca	8.16 ± 0.40 Ca	7.25 ± 0.27 Cb	7.16 ± 0.25 Cb	7.00 ± 0.00 Cb

1. All values are the mean ± standard deviation of six replicates (n=6). Values ranged between1-9, 1: completely disliked, 9: liked very much.
2. For each column, similar capital letters demonstrate no statistical difference at $p < 0.05$. For each row similar small letters demonstrate no statistical difference at $p < 0.05$. To examine the effect of days, see Table E.3 and E.4.

*Pasteurization condition is 85ºC for 10 minutes.

**HHP treatment conditions are 400 MPa at 15ºC during 5 min for 100 psi squeezed and 400 MPa at 5ºC during 10 min for 150 psi squeezed pomegranate juice.

CHAPTER 4

CONCLUSION

Non thermal processing techniques are a rising trend all around the world against traditional thermal treatment methods. In this study, evaluating the effect of high hydrostatic pressure treatment on two different pressure squeezed pomegranate juice quality factors relative to traditional thermal treatment with untreated sample as control and investigating the shelf life period was aimed. The main goal was to prove superiority of HHP treatment to thermal treatment. Using two different hydraulic pressure squeezed pomegranate juice was the bonus for this study as to show the squeezing pressure effect for pomegranate juice and analyze the HHP effect on this situation. In the first part of this study, chosen HHP combinations 200, 300, 400 MPa; 5°C, 15°C, 25°C; 5 and 10 minutes were applied. Most of these combinations gave better results than thermal treatment: % RSA of 200 MPa/15°C/5 min treatment was 121.77 versus of thermal treatment was % 73.53 for 100 psi; Ascorbic Acid content (mg / L) of 300 MPa/25°C/5 min treatment was % 113.49 versus of thermal treatment 97.49 for 150 psi. According to the measurements and experiments for specified quality factors, 400 MPa at 15°C during 5 minutes for 100 psi squeezed, 400 MPa at 5°C during 10 minutes for 150 psi squeezed pomegranate juice are chosen as the best combinations. In the second part, shelf life analyses were performed to samples which were treated with specified conditions. Both sensory and chemical analyses gave the best results for HHP among all three: control, thermal treated and HHP treated. For instance ΔE values of HHP treated sample was 13.63 while thermal treated was 19.78 at the end of day 30 for 100 psi; %RSA of HHP treated was % 108.8 while thermal treated was 75.07 for 150 psi. Furthermore, increasing the squeezing pressure increases the shelf life stability due to higher amounts of antioxidant compounds. The situation is the same for sensory evaluation as well.

In brief, with HHP treatment-a cold pasteurization technique, pomegranate juice can be processed and stored at 4^{o}C with protecting its quality constituents much more than thermal treatment. Higher squeezing pressure leads higher amounts of quality factors besides extended the shelf life stability than the juice squeezed at lower pressures.

CHAPTER 5

RECOMMENDATION

This study's issue was chosen to create a general idea about the HHP treatment effects on chosen quality parameters of pomegranate juice. These quality elements have been referred as the most important ones in literature. The exact mechanisms of the effect of HHP combinations on chosen quality parameters require further and more extensive studies. For example, while 5 min HHP treatment causes better ascorbic acid content, 10 min treatment make it quite worse. In the industry, sometimes filtration or clarification is applied to pomegranate juice prior to bottling. The effects of these kinds of physical treatments to pomegranate juice could give different quality results after HHP treatment. To propose a more reliable judgment about the processed pomegranate juice for the market, further investigation could be done in terms of different pre-processing steps and/or other pressure-temperature-time combinations. Despite all positive effects and results of HHP treatment, economical aspects of the technique and processing equipments are also extremely important. Managing the economical extent, further academic studies for other food structures and sharing all results with manufacturers will increase the use and extent of non-thermal food treatments among the community. Consequently, HHP treatment seem to be a much better option for food processing and can be used as a perfect alternative of thermal pasteurization.

REFERENCES

Alpas H., Kalchayanand N., Bozoglu F. and Ray B. (2000). Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology* 60, 33-42.

Alper H., Bahceci K., Acar J. (2005). Influence of processing and pasteurisation on colour values and total phenolic compounds of pomegranatejuice. *Journal of Processing and Preservation*, 29 , pp. 357–368.

Anonymous. (1951). Methods of Vitamin Assay. *Association of Vitamin Chemists*, Interscience Publisher, New York, NY, USA.

Anonymous. (2008). Provisional reference guideline for pomegranatejuice, AIJN, Brussels.

Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Coleman R., Hayek T., Presser D., Fuhrman B. (2000). Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clinl Nutr 71:1062–76.

Bayındırlı L., Şahin S., Artık N. (1994). The effect of clarification methods of pomegranatejuice quality. *Fruit Processing*, 9, pp. 264–270.

Billmeyer Jr. F.W., Saltzman M. (1981). Principles of Color Technology. (2nd ed.)Wiley-Interscience, New York (1981), p. 50.

Borochov-Neori H., Judeinstein S., Tripler E., Harari M., Greenberg A., Shomer I., Holland D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (Punica granatum L.) fruit. *Journal of Food Composition and Analysis*, 22, 189–195.

Brand-Williams W., Cuvelier M.E., Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und -Technologie/Food Science and Technology*, 28, 25-30.

Buzrul S., Hami A., Largeteau A., Demazeau G. (2008). Inactivation of Escherichia coli and Listeria innocua in kiwifruit and pineapple juices by high hydrostatic pressure. *International Journal of Food Microbiology, 124,* 275–278.
.
Cemeroğlu B., Velioğlu S., Işık S. (1994). Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *Journal of Food Science*, 59, 1216–1218.

Cemeroğlu, *B.* (Ed.). (2010). *Gıda Analizleri*. Ankara: Gıda Teknolojisi Derneği Yayınları.

Cheftel J.C. (1992). Effect of high hydrostatic pressure on food constituents: An overview, High-Pressure and Biotechnology, vol. 224, John Libbey Eurotext Ltd, UK, London, pp. 195–209.

Coseteng M.Y., Lee C.Y. (1987). *Journal of the Science,* 52, 985.

Davidson M. H., Maki K. C., Dicklin M. R., Feinstein S. B., Witchger M. S., Bell M., McGuire D. K., Provos J. C., Liker H., Aviram M. (2009). Effects of consumption of pomegranate juice on carotid intima-media thickness in men and women at moderate risk for coronary heart disease. Ame J Cardiol 104(7):936–42.

De Simon B.F., Perez-Ilzarbe J., Hernandez T., Gomezcordovez C., Esrtella I. (1992). Importance of phenolic compounds for the characterization of fruit juices. J. Agric. Food Chem. *38*, 1565–1571.

Dede S., Alpas H., Bayindirli A. (2007). High hydrostatic pressure treatment and storage of carrots and juices: Antioxidant activity and microbial safety. *Journal of the Science of Food and Agriculture, 87,* 773–872.

Donsi G., Ferrari G., Di Matteo M. (1996). High pressure stabilization of orange juice: Evaluation of the effects of process conditions *Italian Journal of Food Sciences, 8 (2),* pp. 99–106.

Fadavi A, Barzegar M, Azizi MH, Bayat M. (2005). Physicochemical composition of ten pomegranate cultivars (*Punica granatum* L.) grown in Iran. Food Sci Technol Int 11:113–9.

Farnworth E.R.., Lagace M., Couture R. (2001). Thermal processing, storage conditions, and the composition and physical properties of orange juice. *Food Research International,* 34 (1) pp. 25–30.

Ferrari G., Maresca P., Ciccarone R. (2010). The application of high hydrostatic pressure for the stabilization of functional foods: Pomegranate juice. *Journal of Food Engineering, 100,* 245–253.

Fischer U. A., Carle R, Kammerer D.R. (2011). Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. *Food Chemistry,* 127, 807–821.

Freed M. (1966). Methods of Vitamin Assay. 3rd ed., *Interscience Publishers,* New York, NY, USA.

Gil M. I., Tomas-Barberan F. A., Hess-Pierce B., Holcroft D. M., Kader A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry,* 48(10), 4581–4589.

Giusti M.M., Wrolstad R.E. (2001). Unit F1.2. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. R.E. Wrolstad, S.J. Schwartz (Eds.), *Current protocols in food analytical chemistry,* Wiley, New York, pp. 1–13.

González-Molina E., Moreno D. A., García-Viguera C. (2009). A new drink rich in healthy bioactives combining lemon and pomegranate juices. *Food Chemistry,* 115, 1364–1372.

Gültekin M, Özçoban D, Karaali A. (2007). Antioksidan kaynağı bir içecek: Nar suyu. Dünya *GIDA*, Temmuz, 85- 87.

Hegde V.L.V, Mahesh P.A., Venkatesh Y.P. (2002). Anaphylaxis Caused by Mannitol in Pomegranate (*Punica granatum*). Allergy & Clinical Immunology International - Journal of the World Allergy Organization, Vol 14 , No. 1.

Hendrickx M., Ludikhuyze L., Van den Broeck I., Weemaes C. (1998). Effects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology,* pp 197–203.

Jahfar M., Vijayan K. K., Azadi P. (2003). Studies on a polysaccharide from the fruit rind of *Punica granatum*. *Res J Chem Environ*, 7:43–50.

Jaiswal V, DerMarderosian A, Porter JR. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). Food Chem 118:11–6.

Jones R., Silveira M.M. (2004). Sorbitol canbo produced not only ennemiedily but also biotechnologically. *Applied Biochemistry and Biotechnology,* 118, pp. 321–336.

Lee J., Durst R.W, Wrolstad R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal Association of Official Analytical Chemists International*, 88 (5), pp. 1269–1278.

Li Y., Guo C., Yang J., Wei J., Xu J., Cheng S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry,* 96(2):254–60.

Linton M., McClements J.M.J., Patterson M.F. (1999). Inactivation of *Escherichia coli* O157:H7 in orange juice using a combination of high pressure and mild heat *Journal of Food Protection, 62 (3)* pp. 277–279.

López-Malo A., Palou E., Barbosa-Cánovas G.V., Welti-Chanes J., and Swanson B.G. (1998). Polyphenoloxidase activity and color changes during storage in high hydrostatic pressure treated avocado puree. *Food Res. Int. Submitted for publication.*

Macheix J.J., Fleuriet A., Billiot J. (1990). Fruit Phenolics, pp. 24–31, 295–342, CRC Press, Boca Raton, FL.

O' Mahony. (1988). Sensory difference and preference testing: The use of signal detection measures. *Applied Sensory Analyses of Food* (H.R. Moskowitz, ed) pp. 145-175. CRC Press, Boca Raton, FL.

Mali A.B., Khedkar K., Lele S.S. (2011). Effect of Gamma Irradiation on Total Phenolic Content and *in Vitro* Antioxidant Activity of Pomegranate (*Punica Granatum* L.) Peels. *Food and Nutrition Sciences*, 2, 428-433.

Mart´ınez JJ, Melgarejo P, Hern´andez F, Salazar DM, Mart´ınez R. (2006). Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. Sci Hortic 110:241–6.

Maskan M. (2006). Production of pomegranate (Punica granatum L.) juice concentrate by various heating methods: colour degradation and kinetics. *Journal of Food Engineering,* 72 218–224.

Meerts IATM, Verspeek-Rip CM, Buskens CAF, Keizer HG, Bassaganya-Riera J, Jouni ZE, van Huygevoort AHBM, van Otterdijk FM, van de Waart EJ. (2009). Toxicological evaluation of pomegranate seed oil. Food Chem Toxicol 47(6):1085–92.

Meyer R.S., Cooper K.L., Knorr D., Lelieveld H.L.M. (2000). High pressure sterilization of foods *Food Technology*, 54, pp. 67–72.

Mirdehghan S. H., Rahemi M. (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. Sci Hort 111(2):120– 7.

Mousavinejad G, Emam-Djomeh Z, Rezaei K, Khodaparast MHH. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem 115:1274–8.

Norton T., Sun D.-W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food Bioprocess Technology*, 1(1), 2–34.

Oey I., Van der Plancken I., Van Loey A., Hendrickx, M. (2008). Does high pressure processing influence nutritional aspects of plant based food systems? *Trends in Food Science and Technology*, 19, 300–308.

O'Mahony M. (1988). Sensory difference and preference testing: The use of signal detection measures. *Applied Sensory Analyses of Food* (H.R. Moskowitz, ed) pp. 145-175.

Parish M.E. (1998). High pressure inactivation of *Saccharomyces cerevisiae*, endogenous microflora and pectinmethylesterase in orange juice *Journal of Food Safety, 18 (1),* pp. 57–65.

Park S.-J., Lee J.-I., Park J. (2006). Effects of a Combined Process of High-Pressure Carbon Dioxide and High Hydrostatic Pressure on the Quality of Carrot Juice. *Journal of Food Science,* pp. 1827–1834.

Patras A., Brunton N. P., Da Pieve S., Butler F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies*, 10, 308–313.

Patras A., Brunton N. P., O'Donnell C., Tiwari B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; Mechanisms and kinetics of degradation. *Trends in Food Science and Technology,* 21, 3–11.

Patras A., Brunton N., Butler F., Gerard D. (2008). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative food science and emerging technologies.*

Patterson M.F., Quinn M., Simpson R., Gilmour A. (1996). High pressure inactivation in foods of animal origin. *High Pressure Bioscience and Biotechnology*, 13 pp. 267–272.

Pérez-Vicente A., Serrano P., Abellán P., García-Viguera C. (2004). Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. *Journal of the Science of Food and Agriculture*, 84, pp. 639–644.

Polydera A. C., Stoforos N. G., Taoukis P. S. (2005). Quality degradation kinetics of pasteurized and high pressure processed fresh Navel orange juice: nutritional parameters and shelf life. *Innovative Food Science and Emerging Technologies,* 6, 1e9.

Polydera A.C., Stoforos N.G., Taoukis P.S. (2004). Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice. *Food Chemistry (in press).*

Poyrazoğlu, E., Gökmen, V., Artık, N. (2002). Organic acid and phenolic compounds in pomegranates (Punica granatum L.) grown in Turkey. *Journal of Food Composition and Analysis, 15*(5), 567-575.

Reyns K.M.F.A., Soontjens C.C.F.,Cornelis K, Weemaes C.A., Hendrickx M.E., Michiels C.W. (2000). Kinetic analysis and modelling of combined high-pressure–temperature inactivation of the yeast *Zygosaccharomyces bailii. International Journal of Food Microbiology*, 56 (2–3), pp. 199–210.

Rouseff R.L. (1990). Bitterness in foods and beverage. *In Developments in Food Science*, Vol 25 (R.L.Rouseff, ed.) pp. 1–12.

Sancho F., Lambert Y., Demazeau G., Largeteau A., Bouvier J.M, Narbonne J.F. (1999). Effect of ultrahigh hydrostatic pressure on hydrosoluble vitamins *Journal of Food Engineering, 39,* pp. 247–253.

SanMartín M.F., Barbosa-Cánovas V., Swanson B.G. (2002). Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition*, 42 (1) pp. 627–645.

Scalzo R.L., Iannoccari T., Summa C., Morelli R., Rapisarda P. (2004). Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. *Food Chemistry,* 85: 41–47.

Sepulveda E., Galletti L., Saenz C., Tapia M. (1998). Minimal processing of pomegranate var. Wonderful. *CIHEAM – Options Mediterraneennes*, 42: 237–242.

Singleton V. B., Rossi J. A. (1965). Colorimetry of total phenolics with phoshomolybdic-phosphotungustic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Spanos G.A., Wrolstad R.E. (1992). Phenolics of apple, pear and white grape juices and their changes with processing and storage – a review. *Journal of Agricultural and Food Chemistry, 40*, 1478–1487.

Spanos G.A., Wrolstad R.E. (1990). *Journal of Agricultural and Food Chemistry*, 38,1565.

Suh H. J., Noh D. O., Kang C. S., Kim J. M., Lee S. W. (2003). Thermal kinetics of color degradation of mulberry fruit extract. *Nahrung*, 47, 132–135.

Tabur D., Bakkal G., Yurdagel Ü. (1987). Nar suyunun durultma işlemi ve depolama süresince meydana gelen değişmeleri üzerine araştırmalar. *Gıda*, 12 (5), pp. 305–311.

Teo A.Y.L., Ravishankar S., Sizer C.E. (2001). Effect of low-temperature, high-pressure treatment on the survival of *Escherichia coli*O157:H7 and *Salmonella* in unpasteurized fruit juices *Journal of Food Protection, 64 (8)*, pp. 1122–1127.
Tezcan F., Gültekin-Özgüven M., Diken T., Özçelik B., Erim F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, 115(3):873–7.

The Summary of Agricultural Statistics. (2010). TÜİK.

Torres, J. A., Velazquez, G. (2008). Hydrostatic pressure processing of foods. In S. Jun & J. Irudayaraj (Eds.), *Food processing operations modeling: design and analysis*. Boca Ratón, FL: CRC Press Inc. pp. 173–212.

Van den Broeck I., Ludikhuyze L.R, Van Loey A.M., Hendrickx M.E. (2000). Inactivation of orange pectinesterase by combined high pressure and temperature treatments: A kinetic study *Journal of Agricultural and Food Chemistry, 48 (5)*, pp. 1960–1970.

Vardin H, Fenercioğlu H. (2003). Study on the development of pomegranate juice processing technology: Clarification of pomegranate juice. *Nahrung, 47*, 300-303.

Varela-Santos E., Ochoa-Martinez A., Tabilo-Munizaga G., Reyes J. E., Pérez-Won M., Briones-Labarca V., Morales-Castro J. (2011). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, oi:10.1016/j.ifset.2011.10.009.

Vidal A., Fallarero A., Pena B. R., Medina M. E., Gra B., Rivera F., Gutierrez Y., Vuorela P. M. (2003). Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. J Ethnopharmacol 89:295–300.

Weemaes C., Ludikhuyze L., Van den Broeck I., Hendrickx M. (1999). Kinetic study of antibrowning agents and pressure inactivation of avocado polyphenoloxidase *Journal of Food Science*, 64 (5) pp. 823–827.

Wrolstad R.E. (2004). Anthocyanin pigments – bioactivity and coloring properties. *Journal of Food Science*, 69 (5), pp. 419–421.

Zhang Y., Krueger D., Durst R., Lee R., Wang D., Seeram N., Heber D. (2009). International Multidimensional Authenticity Specification (IMAS) Algorithm for Detection of Commercial Pomegranate Juice Adulteration. *Journal of Agricultural Food Chemistry, 57* (6), pp 2550–2557.

Zook C.D., Parish M.E., Braddock R.J., Balaban M.O. (1999). High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juices. *Journal of Food Science*, 64 (3), pp. 533–535.

APPENDIX A

STANDARD GALLIC ACID CURVE FOR TOTAL PHENOLIC CONTENT CALCULATION

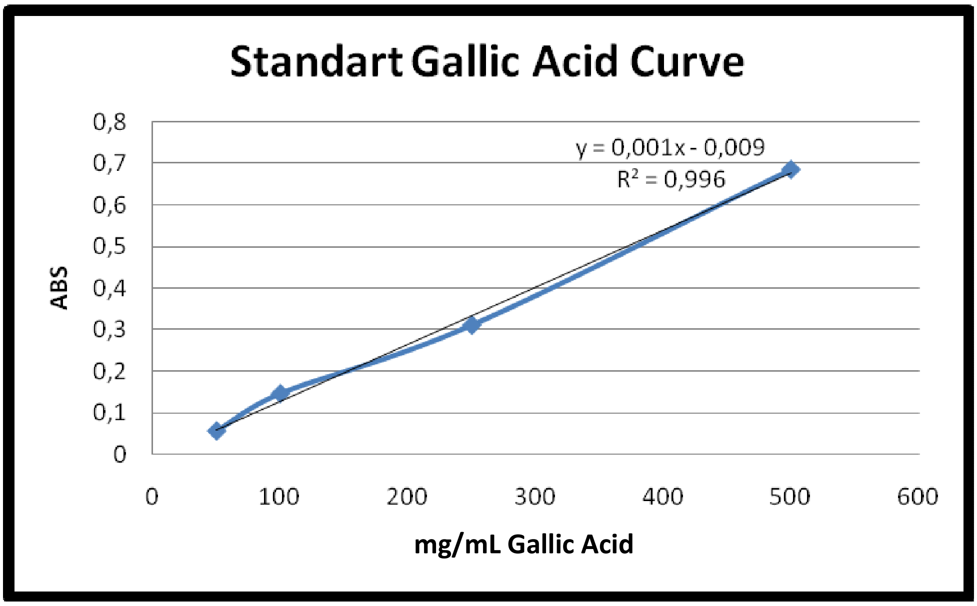

Figure A.1 The standard gallic acid curve for Singleton & Rossi Method

APPENDIX B

STANDARD DPPH CURVE FOR ANTIOXIDANT ACTIVITY CALCULATION

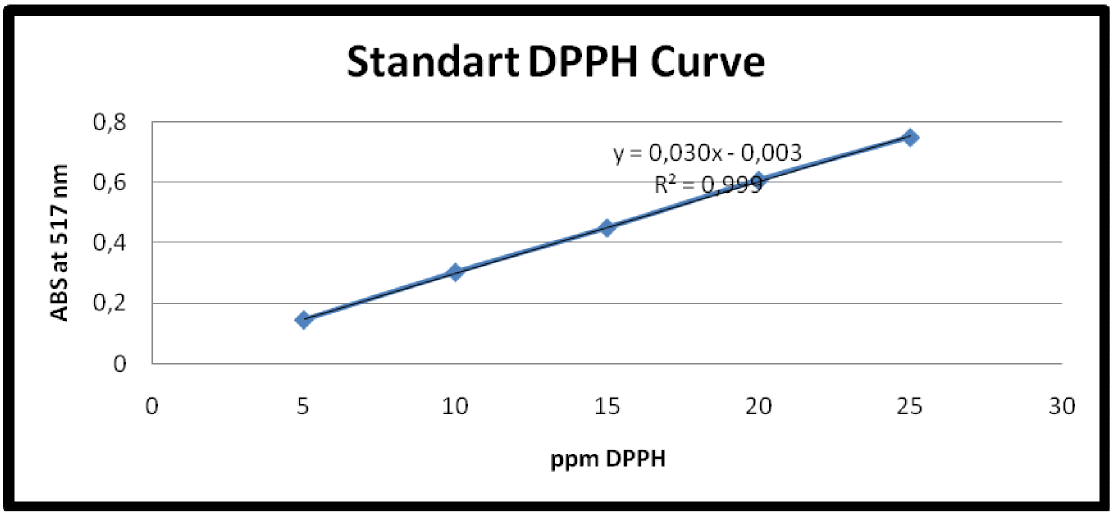

Figure B.1 The standart curve for Brand-Williams Method

APPENDIX C

STANDARD CURVE FOR ASCORBIC ACID CALCULATION

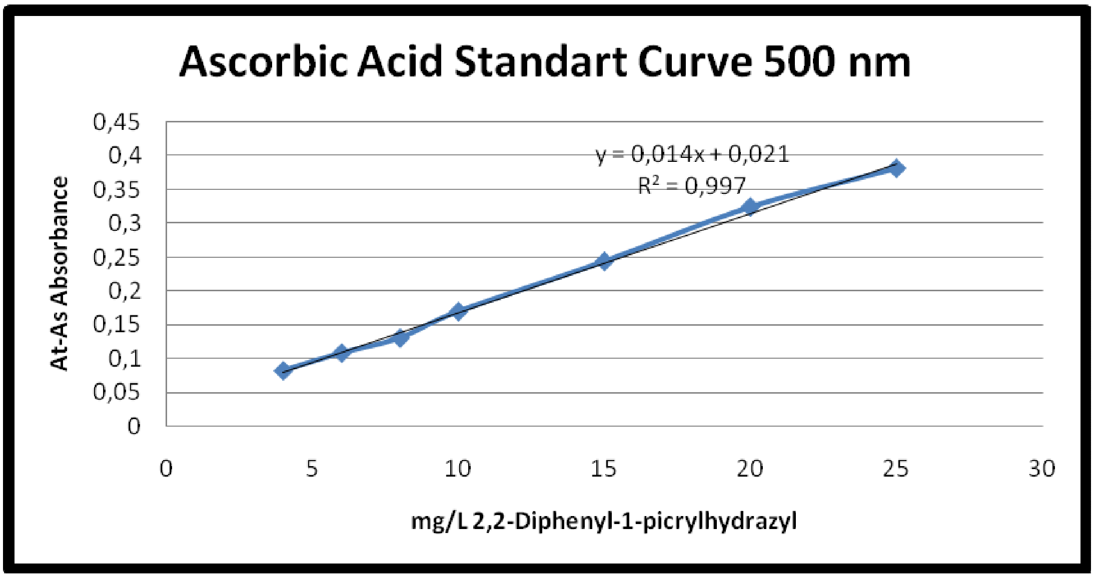

Figure C.1 The standard curve for Cemeroğlu Method

APPENDIX D

pH

DURING SHELF LIFE

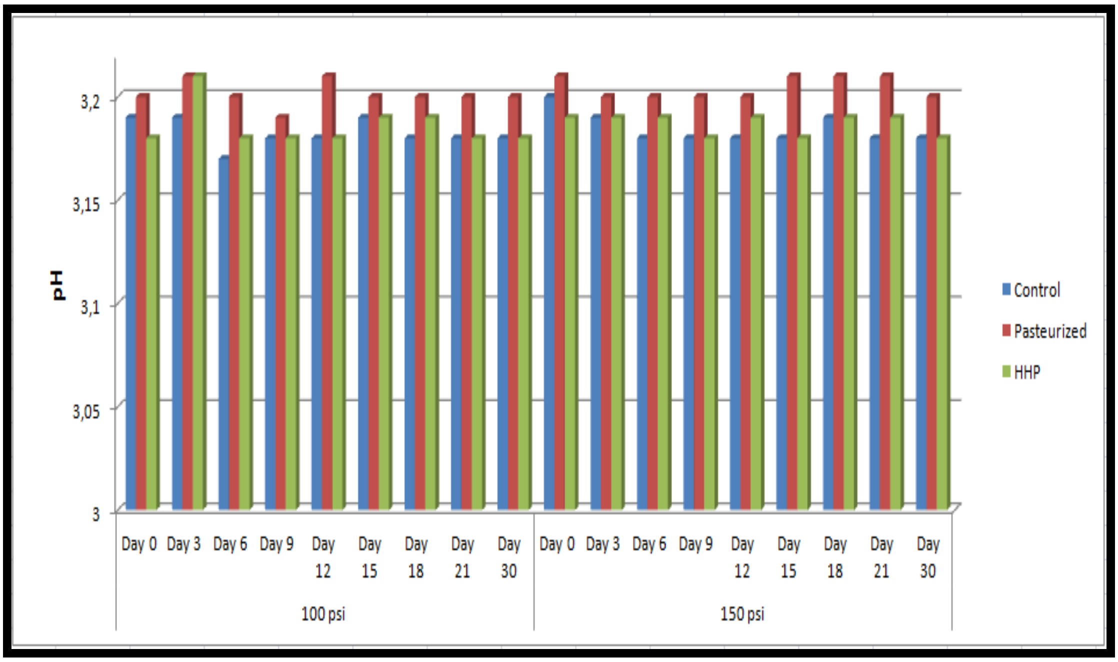

Figure D.1 pH During Shelf Life Period

APPENDIX E

TUKEY TEST RESULTS OF DAYS FOR SHELF LIFE

Table E.1 Tukey test results day by day for Ascorbic Acid

Multiple Comparisons

Dependent Variable: Asc100
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
,00	3,00	19,9291*	2,01890	,000	13,4066	26,4517
	6,00	23,0147*	2,01890	,000	16,4921	29,5372
	9,00	18,2958*	2,01890	,000	11,7732	24,8183
	12,00	26,4158*	2,01890	,000	19,8932	32,9383
	15,00	31,9002*	2,01890	,000	25,3777	38,4228
	18,00	27,8658*	2,01890	,000	21,3432	34,3883
	21,00	31,5836*	2,01890	,000	25,0610	38,1061
	30,00	36,9813*	2,01890	,000	30,4588	43,5039
3,00	,00	-19,9291*	2,01890	,000	-26,4517	-13,4066
	6,00	3,0856	2,01890	,837	-3,4370	9,6081
	9,00	-1,6333	2,01890	,996	-8,1559	4,8892
	12,00	6,4867	2,01890	,052	-,0359	13,0092
	15,00	11,9711*	2,01890	,000	5,4486	18,4937
	18,00	7,9367*	2,01890	,007	1,4141	14,4592
	21,00	11,6544*	2,01890	,000	5,1319	18,1770
	30,00	17,0522*	2,01890	,000	10,5297	23,5748
6,00	,00	-23,0147*	2,01890	,000	-29,5372	-16,4921
	3,00	-3,0856	2,01890	,837	-9,6081	3,4370
	9,00	-4,7189	2,01890	,339	-11,2414	1,8037
	12,00	3,4011	2,01890	,753	-3,1214	9,9237
	15,00	8,8856*	2,01890	,002	2,3630	15,4081
	18,00	4,8511	2,01890	,303	-1,6714	11,3737
	21,00	8,5689*	2,01890	,003	2,0463	15,0914
	30,00	13,9667*	2,01890	,000	7,4441	20,4892
9,00	,00	-18,2958*	2,01890	,000	-24,8183	-11,7732
	3,00	1,6333	2,01890	,996	-4,8892	8,1559
	6,00	4,7189	2,01890	,339	-1,8037	11,2414
	12,00	8,1200*	2,01890	,005	1,5975	14,6425
	15,00	13,6044*	2,01890	,000	7,0819	20,1270
	18,00	9,5700*	2,01890	,001	3,0475	16,0925
	21,00	13,2878*	2,01890	,000	6,7652	19,8103
	30,00	18,6856*	2,01890	,000	12,1630	25,2081
12,00	,00	-26,4158*	2,01890	,000	-32,9383	-19,8932
	3,00	-6,4867	2,01890	,052	-13,0092	,0359
	6,00	-3,4011	2,01890	,753	-9,9237	3,1214
	9,00	-8,1200*	2,01890	,005	-14,6425	-1,5975
	15,00	5,4844	2,01890	,166	-1,0381	12,0070
	18,00	1,4500	2,01890	,998	-5,0725	7,9725
	21,00	5,1678	2,01890	,228	-1,3548	11,6903
	30,00	10,5656*	2,01890	,000	4,0430	17,0881
15,00	,00	-31,9002*	2,01890	,000	-38,4228	-25,3777
	3,00	-11,9711*	2,01890	,000	-18,4937	-5,4486
	6,00	-8,8856*	2,01890	,002	-15,4081	-2,3630
	9,00	-13,6044*	2,01890	,000	-20,1270	-7,0819
	12,00	-5,4844	2,01890	,166	-12,0070	1,0381
	18,00	-4,0344	2,01890	,551	-10,5570	2,4881
	21,00	-,3167	2,01890	1,000	-6,8392	6,2059
	30,00	5,0811	2,01890	,247	-1,4414	11,6037
18,00	,00	-27,8658*	2,01890	,000	-34,3883	-21,3432
	3,00	-7,9367*	2,01890	,007	-14,4592	-1,4141
	6,00	-4,8511	2,01890	,303	-11,3737	1,6714
	9,00	-9,5700*	2,01890	,001	-16,0925	-3,0475
	12,00	-1,4500	2,01890	,998	-7,9725	5,0725
	15,00	4,0344	2,01890	,551	-2,4881	10,5570
	21,00	3,7178	2,01890	,655	-2,8048	10,2403
	30,00	9,1156*	2,01890	,001	2,5930	15,6381
21,00	,00	-31,5836*	2,01890	,000	-38,1061	-25,0610
	3,00	-11,6544*	2,01890	,000	-18,1770	-5,1319
	6,00	-8,5689*	2,01890	,003	-15,0914	-2,0463
	9,00	-13,2878*	2,01890	,000	-19,8103	-6,7652
	12,00	-5,1678	2,01890	,228	-11,6903	1,3548
	15,00	,3167	2,01890	1,000	-6,2059	6,8392
	18,00	-3,7178	2,01890	,655	-10,2403	2,8048
	30,00	5,3978	2,01890	,182	-1,1248	11,9203
30,00	,00	-36,9813*	2,01890	,000	-43,5039	-30,4588
	3,00	-17,0522*	2,01890	,000	-23,5748	-10,5297
	6,00	-13,9667*	2,01890	,000	-20,4892	-7,4441
	9,00	-18,6856*	2,01890	,000	-25,2081	-12,1630
	12,00	-10,5656*	2,01890	,000	-17,0881	-4,0430
	15,00	-5,0811	2,01890	,247	-11,6037	1,4414
	18,00	-9,1156*	2,01890	,001	-15,6381	-2,5930
	21,00	-5,3978	2,01890	,182	-11,9203	1,1248

Based on observed means.

*. The mean difference is significant at the ,05 level.

Multiple Comparisons

Dependent Variable: Asc150
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
,00	3,00	-5,5011	2,17110	,239	-12,5154	1,5132
	6,00	-2,5978	2,17110	,954	-9,6121	4,4165
	9,00	-21,4633*	2,17110	,000	-28,4776	-14,4491
	12,00	-22,3711*	2,17110	,000	-29,3854	-15,3568
	15,00	,0789	2,17110	1,000	-6,9354	7,0932
	18,00	3,2520	2,17110	,852	-3,7623	10,2663
	21,00	7,0633*	2,17110	,047	,0491	14,0776
	30,00	2,8889	2,17110	,917	-4,1254	9,9032
3,00	,00	5,5011	2,17110	,239	-1,5132	12,5154
	6,00	2,9033	2,17110	,915	-4,1109	9,9176
	9,00	-15,9622*	2,17110	,000	-22,9765	-8,9479
	12,00	-16,8700*	2,17110	,000	-23,8843	-9,8557
	15,00	5,5800	2,17110	,223	-1,4343	12,5943
	18,00	8,7531*	2,17110	,005	1,7388	15,7674
	21,00	12,5644*	2,17110	,000	5,5502	19,5787
	30,00	8,3900*	2,17110	,008	1,3757	15,4043
6,00	,00	2,5978	2,17110	,954	-4,4165	9,6121
	3,00	-2,9033	2,17110	,915	-9,9176	4,1109
	9,00	-18,8656*	2,17110	,000	-25,8798	-11,8513
	12,00	-19,7733*	2,17110	,000	-26,7876	-12,7591
	15,00	2,6767	2,17110	,945	-4,3376	9,6909
	18,00	5,8498	2,17110	,174	-1,1645	12,8641
	21,00	9,6611*	2,17110	,001	2,6468	16,6754
	30,00	5,4867	2,17110	,242	-1,5276	12,5009
9,00	,00	21,4633*	2,17110	,000	14,4491	28,4776
	3,00	15,9622*	2,17110	,000	8,9479	22,9765
	6,00	18,8656*	2,17110	,000	11,8513	25,8798
	12,00	-,9078	2,17110	1,000	-7,9221	6,1065
	15,00	21,5422*	2,17110	,000	14,5279	28,5565
	18,00	24,7153*	2,17110	,000	17,7011	31,7296
	21,00	28,5267*	2,17110	,000	21,5124	35,5409
	30,00	24,3522*	2,17110	,000	17,3379	31,3665
12,00	,00	22,3711*	2,17110	,000	15,3568	29,3854
	3,00	16,8700*	2,17110	,000	9,8557	23,8843
	6,00	19,7733*	2,17110	,000	12,7591	26,7876
	9,00	,9078	2,17110	1,000	-6,1065	7,9221
	15,00	22,4500*	2,17110	,000	15,4357	29,4643
	18,00	25,6231*	2,17110	,000	18,6088	32,6374
	21,00	29,4344*	2,17110	,000	22,4202	36,4487
	30,00	25,2600*	2,17110	,000	18,2457	32,2743
15,00	,00	-,0789	2,17110	1,000	-7,0932	6,9354
	3,00	-5,5800	2,17110	,223	-12,5943	1,4343
	6,00	-2,6767	2,17110	,945	-9,6909	4,3376
	9,00	-21,5422*	2,17110	,000	-28,5565	-14,5279
	12,00	-22,4500*	2,17110	,000	-29,4643	-15,4357
	18,00	3,1731	2,17110	,868	-3,8412	10,1874
	21,00	6,9844	2,17110	,052	-,0298	13,9987
	30,00	2,8100	2,17110	,929	-4,2043	9,8243
18,00	,00	-3,2520	2,17110	,852	-10,2663	3,7623
	3,00	-8,7531*	2,17110	,005	-15,7674	-1,7388
	6,00	-5,8498	2,17110	,174	-12,8641	1,1645
	9,00	-24,7153*	2,17110	,000	-31,7296	-17,7011
	12,00	-25,6231*	2,17110	,000	-32,6374	-18,6088
	15,00	-3,1731	2,17110	,868	-10,1874	3,8412
	21,00	3,8113	2,17110	,710	-3,2029	10,8256
	30,00	-,3631	2,17110	1,000	-7,3774	6,6512
21,00	,00	-7,0633*	2,17110	,047	-14,0776	-,0491
	3,00	-12,5644*	2,17110	,000	-19,5787	-5,5502
	6,00	-9,6611*	2,17110	,001	-16,6754	-2,6468
	9,00	-28,5267*	2,17110	,000	-35,5409	-21,5124
	12,00	-29,4344*	2,17110	,000	-36,4487	-22,4202
	15,00	-6,9844	2,17110	,052	-13,9987	,0298
	18,00	-3,8113	2,17110	,710	-10,8256	3,2029
	30,00	-4,1744	2,17110	,602	-11,1887	2,8398
30,00	,00	-2,8889	2,17110	,917	-9,9032	4,1254
	3,00	-8,3900*	2,17110	,008	-15,4043	-1,3757
	6,00	-5,4867	2,17110	,242	-12,5009	1,5276
	9,00	-24,3522*	2,17110	,000	-31,3665	-17,3379
	12,00	-25,2600*	2,17110	,000	-32,2743	-18,2457
	15,00	-2,8100	2,17110	,929	-9,8243	4,2043
	18,00	,3631	2,17110	1,000	-6,6512	7,3774
	21,00	4,1744	2,17110	,602	-2,8398	11,1887

Based on observed means.

*. The mean difference is significant at the ,05 level.

Table E.2 Tukey test results day by day for Antioxidant Activity

Multiple Comparisons

Dependent Variable: Antiox100
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
,00	3,00	12,6701*	1,61508	,000	7,4522	17,8880
	6,00	11,6151*	1,61508	,000	6,3972	16,8330
	9,00	12,7167*	1,61508	,000	7,4987	17,9346
	12,00	21,1089*	1,61508	,000	15,8910	26,3268
	15,00	11,9533*	1,61508	,000	6,7354	17,1713
	18,00	16,2911*	1,61508	,000	11,0732	21,5090
	21,00	20,9222*	1,61508	,000	15,7043	26,1402
	30,00	26,3389*	1,61508	,000	21,1210	31,5568
3,00	,00	-12,6701*	1,61508	,000	-17,8880	-7,4522
	6,00	-1,0550	1,61508	,999	-6,2729	4,1629
	9,00	,0466	1,61508	1,000	-5,1714	5,2645
	12,00	8,4388*	1,61508	,000	3,2208	13,6567
	15,00	-,7168	1,61508	1,000	-5,9347	4,5012
	18,00	3,6210	1,61508	,395	-1,5969	8,8389
	21,00	8,2521*	1,61508	,000	3,0342	13,4700
	30,00	13,6688*	1,61508	,000	8,4508	18,8867
6,00	,00	-11,6151*	1,61508	,000	-16,8330	-6,3972
	3,00	1,0550	1,61508	,999	-4,1629	6,2729
	9,00	1,1016	1,61508	,999	-4,1164	6,3195
	12,00	9,4938*	1,61508	,000	4,2758	14,7117
	15,00	,3382	1,61508	1,000	-4,8797	5,5562
	18,00	4,6760	1,61508	,113	-,5419	9,8939
	21,00	9,3071*	1,61508	,000	4,0892	14,5250
	30,00	14,7238*	1,61508	,000	9,5058	19,9417
9,00	,00	-12,7167*	1,61508	,000	-17,9346	-7,4987
	3,00	-,0466	1,61508	1,000	-5,2645	5,1714
	6,00	-1,1016	1,61508	,999	-6,3195	4,1164
	12,00	8,3922*	1,61508	,000	3,1743	13,6102
	15,00	-,7633	1,61508	1,000	-5,9813	4,4546
	18,00	3,5744	1,61508	,412	-1,6435	8,7924
	21,00	8,2056*	1,61508	,000	2,9876	13,4235
	30,00	13,6222*	1,61508	,000	8,4043	18,8402
12,00	,00	-21,1089*	1,61508	,000	-26,3268	-15,8910
	3,00	-8,4388*	1,61508	,000	-13,6567	-3,2208
	6,00	-9,4938*	1,61508	,000	-14,7117	-4,2758
	9,00	-8,3922*	1,61508	,000	-13,6102	-3,1743
	15,00	-9,1556*	1,61508	,000	-14,3735	-3,9376
	18,00	-4,8178	1,61508	,092	-10,0357	,4002
	21,00	-,1867	1,61508	1,000	-5,4046	5,0313
	30,00	5,2300*	1,61508	,049	,0121	10,4479
15,00	,00	-11,9533*	1,61508	,000	-17,1713	-6,7354
	3,00	,7168	1,61508	1,000	-4,5012	5,9347
	6,00	-,3382	1,61508	1,000	-5,5562	4,8797
	9,00	,7633	1,61508	1,000	-4,4546	5,9813
	12,00	9,1556*	1,61508	,000	3,9376	14,3735
	18,00	4,3378	1,61508	,177	-,8802	9,5557
	21,00	8,9689*	1,61508	,000	3,7510	14,1868
	30,00	14,3856*	1,61508	,000	9,1676	19,6035
18,00	,00	-16,2911*	1,61508	,000	-21,5090	-11,0732
	3,00	-3,6210	1,61508	,395	-8,8389	1,5969
	6,00	-4,6760	1,61508	,113	-9,8939	,5419
	9,00	-3,5744	1,61508	,412	-8,7924	1,6435
	12,00	4,8178	1,61508	,092	-,4002	10,0357
	15,00	-4,3378	1,61508	,177	-9,5557	,8802
	21,00	4,6311	1,61508	,120	-,5868	9,8490
	30,00	10,0478*	1,61508	,000	4,8298	15,2657
21,00	,00	-20,9222*	1,61508	,000	-26,1402	-15,7043
	3,00	-8,2521*	1,61508	,000	-13,4700	-3,0342
	6,00	-9,3071*	1,61508	,000	-14,5250	-4,0892
	9,00	-8,2056*	1,61508	,000	-13,4235	-2,9876
	12,00	,1867	1,61508	1,000	-5,0313	5,4046
	15,00	-8,9689*	1,61508	,000	-14,1868	-3,7510
	18,00	-4,6311	1,61508	,120	-9,8490	,5868
	30,00	5,4167*	1,61508	,036	,1987	10,6346
30,00	,00	-26,3389*	1,61508	,000	-31,5568	-21,1210
	3,00	-13,6688*	1,61508	,000	-18,8867	-8,4508
	6,00	-14,7238*	1,61508	,000	-19,9417	-9,5058
	9,00	-13,6222*	1,61508	,000	-18,8402	-8,4043
	12,00	-5,2300*	1,61508	,049	-10,4479	-,0121
	15,00	-14,3856*	1,61508	,000	-19,6035	-9,1676
	18,00	-10,0478*	1,61508	,000	-15,2657	-4,8298
	21,00	-5,4167*	1,61508	,036	-10,6346	-,1987

Based on observed means.

*. The mean difference is significant at the ,05 level.

Multiple Comparisons

Dependent Variable: Antiox150
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
,00	3,00	-5,4444*	1,37197	,006	-9,8769	-1,0119
	6,00	-1,4278	1,37197	,980	-5,8603	3,0047
	9,00	-8,7044*	1,37197	,000	-13,1369	-4,2719
	12,00	-18,8644*	1,37197	,000	-23,2969	-14,4319
	15,00	-9,3144*	1,37197	,000	-13,7469	-4,8819
	18,00	-4,5600*	1,37197	,039	-8,9925	-,1275
	21,00	5,8078*	1,37197	,003	1,3753	10,2403
	30,00	3,6378	1,37197	,190	-,7947	8,0703
3,00	,00	5,4444*	1,37197	,006	1,0119	9,8769
	6,00	4,0167	1,37197	,105	-,4158	8,4492
	9,00	-3,2600	1,37197	,318	-7,6925	1,1725
	12,00	-13,4200*	1,37197	,000	-17,8525	-8,9875
	15,00	-3,8700	1,37197	,133	-8,3025	,5625
	18,00	,8844	1,37197	,999	-3,5481	5,3169
	21,00	11,2522*	1,37197	,000	6,8197	15,6847
	30,00	9,0822*	1,37197	,000	4,6497	13,5147
6,00	,00	1,4278	1,37197	,980	-3,0047	5,8603
	3,00	-4,0167	1,37197	,105	-8,4492	,4158
	9,00	-7,2767*	1,37197	,000	-11,7092	-2,8442
	12,00	-17,4367*	1,37197	,000	-21,8692	-13,0042
	15,00	-7,8867*	1,37197	,000	-12,3192	-3,4542
	18,00	-3,1322	1,37197	,370	-7,5647	1,3003
	21,00	7,2356*	1,37197	,000	2,8031	11,6681
	30,00	5,0656*	1,37197	,014	,6331	9,4981
9,00	,00	8,7044*	1,37197	,000	4,2719	13,1369
	3,00	3,2600	1,37197	,318	-1,1725	7,6925
	6,00	7,2767*	1,37197	,000	2,8442	11,7092
	12,00	-10,1600*	1,37197	,000	-14,5925	-5,7275
	15,00	-,6100	1,37197	1,000	-5,0425	3,8225
	18,00	4,1444	1,37197	,084	-,2881	8,5769
	21,00	14,5122*	1,37197	,000	10,0797	18,9447
	30,00	12,3422*	1,37197	,000	7,9097	16,7747
12,00	,00	18,8644*	1,37197	,000	14,4319	23,2969
	3,00	13,4200*	1,37197	,000	8,9875	17,8525
	6,00	17,4367*	1,37197	,000	13,0042	21,8692
	9,00	10,1600*	1,37197	,000	5,7275	14,5925
	15,00	9,5500*	1,37197	,000	5,1175	13,9825
	18,00	14,3044*	1,37197	,000	9,8719	18,7369
	21,00	24,6722*	1,37197	,000	20,2397	29,1047
	30,00	22,5022*	1,37197	,000	18,0697	26,9347
15,00	,00	9,3144*	1,37197	,000	4,8819	13,7469
	3,00	3,8700	1,37197	,133	-,5625	8,3025
	6,00	7,8867*	1,37197	,000	3,4542	12,3192
	9,00	,6100	1,37197	1,000	-3,8225	5,0425
	12,00	-9,5500*	1,37197	,000	-13,9825	-5,1175
	18,00	4,7544*	1,37197	,027	,3219	9,1869
	21,00	15,1222*	1,37197	,000	10,6897	19,5547
	30,00	12,9522*	1,37197	,000	8,5197	17,3847
18,00	,00	4,5600*	1,37197	,039	,1275	8,9925
	3,00	-,8844	1,37197	,999	-5,3169	3,5481
	6,00	3,1322	1,37197	,370	-1,3003	7,5647
	9,00	-4,1444	1,37197	,084	-8,5769	,2881
	12,00	-14,3044*	1,37197	,000	-18,7369	-9,8719
	15,00	-4,7544*	1,37197	,027	-9,1869	-,3219
	21,00	10,3678*	1,37197	,000	5,9353	14,8003
	30,00	8,1978*	1,37197	,000	3,7653	12,6303
21,00	,00	-5,8078*	1,37197	,003	-10,2403	-1,3753
	3,00	-11,2522*	1,37197	,000	-15,6847	-6,8197
	6,00	-7,2356*	1,37197	,000	-11,6681	-2,8031
	9,00	-14,5122*	1,37197	,000	-18,9447	-10,0797
	12,00	-24,6722*	1,37197	,000	-29,1047	-20,2397
	15,00	-15,1222*	1,37197	,000	-19,5547	-10,6897
	18,00	-10,3678*	1,37197	,000	-14,8003	-5,9353
	30,00	-2,1700	1,37197	,810	-6,6025	2,2625
30,00	,00	-3,6378	1,37197	,190	-8,0703	,7947
	3,00	-9,0822*	1,37197	,000	-13,5147	-4,6497
	6,00	-5,0656*	1,37197	,014	-9,4981	-,6331
	9,00	-12,3422*	1,37197	,000	-16,7747	-7,9097
	12,00	-22,5022*	1,37197	,000	-26,9347	-18,0697
	15,00	-12,9522*	1,37197	,000	-17,3847	-8,5197
	18,00	-8,1978*	1,37197	,000	-12,6303	-3,7653
	21,00	2,1700	1,37197	,810	-2,2625	6,6025

Based on observed means.

*. The mean difference is significant at the ,05 level.

Table E.3 Tukey test results day by day for Sensory Evaluation

Multiple Comparisons

Dependent Variable: Odor100
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
,00	3,00	,3889	,15383	,228	-,0961	,8739
	6,00	,7500*	,15383	,000	,2650	1,2350
	9,00	1,5833*	,15383	,000	1,0984	2,0683
	12,00	2,0000*	,15383	,000	1,5150	2,4850
	15,00	2,5833*	,15383	,000	2,0984	3,0683
	18,00	3,2500*	,15383	,000	2,7650	3,7350
	21,00	3,6111*	,15383	,000	3,1261	4,0961
	30,00	3,6111*	,15383	,000	3,1261	4,0961
3,00	,00	-,3889	,15383	,228	-,8739	,0961
	6,00	,3611	,15383	,322	-,1239	,8461
	9,00	1,1944*	,15383	,000	,7095	1,6794
	12,00	1,6111*	,15383	,000	1,1261	2,0961
	15,00	2,1944*	,15383	,000	1,7095	2,6794
	18,00	2,8611*	,15383	,000	2,3761	3,3461
	21,00	3,2222*	,15383	,000	2,7372	3,7072
	30,00	3,2222*	,15383	,000	2,7372	3,7072
6,00	,00	-,7500*	,15383	,000	-1,2350	-,2650
	3,00	-,3611	,15383	,322	-,8461	,1239
	9,00	,8333*	,15383	,000	,3484	1,3183
	12,00	1,2500*	,15383	,000	,7650	1,7350
	15,00	1,8333*	,15383	,000	1,3484	2,3183
	18,00	2,5000*	,15383	,000	2,0150	2,9850
	21,00	2,8611*	,15383	,000	2,3761	3,3461
	30,00	2,8611*	,15383	,000	2,3761	3,3461
9,00	,00	-1,5833*	,15383	,000	-2,0683	-1,0984
	3,00	-1,1944*	,15383	,000	-1,6794	-,7095
	6,00	-,8333*	,15383	,000	-1,3183	-,3484
	12,00	,4167	,15383	,155	-,0683	,9016
	15,00	1,0000*	,15383	,000	,5150	1,4850
	18,00	1,6667*	,15383	,000	1,1817	2,1516
	21,00	2,0278*	,15383	,000	1,5428	2,5128
	30,00	2,0278*	,15383	,000	1,5428	2,5128
12,00	,00	-2,0000*	,15383	,000	-2,4850	-1,5150
	3,00	-1,6111*	,15383	,000	-2,0961	-1,1261
	6,00	-1,2500*	,15383	,000	-1,7350	-,7650
	9,00	-,4167	,15383	,155	-,9016	,0683
	15,00	,5833*	,15383	,007	,0984	1,0683
	18,00	1,2500*	,15383	,000	,7650	1,7350
	21,00	1,6111*	,15383	,000	1,1261	2,0961
	30,00	1,6111*	,15383	,000	1,1261	2,0961
15,00	,00	-2,5833*	,15383	,000	-3,0683	-2,0984
	3,00	-2,1944*	,15383	,000	-2,6794	-1,7095
	6,00	-1,8333*	,15383	,000	-2,3183	-1,3484
	9,00	-1,0000*	,15383	,000	-1,4850	-,5150
	12,00	-,5833*	,15383	,007	-1,0683	-,0984
	18,00	,6667*	,15383	,001	,1817	1,1516
	21,00	1,0278*	,15383	,000	,5428	1,5128
	30,00	1,0278*	,15383	,000	,5428	1,5128
18,00	,00	-3,2500*	,15383	,000	-3,7350	-2,7650
	3,00	-2,8611*	,15383	,000	-3,3461	-2,3761
	6,00	-2,5000*	,15383	,000	-2,9850	-2,0150
	9,00	-1,6667*	,15383	,000	-2,1516	-1,1817
	12,00	-1,2500*	,15383	,000	-1,7350	-,7650
	15,00	-,6667*	,15383	,001	-1,1516	-,1817
	21,00	,3611	,15383	,322	-,1239	,8461
	30,00	,3611	,15383	,322	-,1239	,8461
21,00	,00	-3,6111*	,15383	,000	-4,0961	-3,1261
	3,00	-3,2222*	,15383	,000	-3,7072	-2,7372
	6,00	-2,8611*	,15383	,000	-3,3461	-2,3761
	9,00	-2,0278*	,15383	,000	-2,5128	-1,5428
	12,00	-1,6111*	,15383	,000	-2,0961	-1,1261
	15,00	-1,0278*	,15383	,000	-1,5128	-,5428
	18,00	-,3611	,15383	,322	-,8461	,1239
	30,00	,0000	,15383	1,000	-,4850	,4850
30,00	,00	-3,6111*	,15383	,000	-4,0961	-3,1261
	3,00	-3,2222*	,15383	,000	-3,7072	-2,7372
	6,00	-2,8611*	,15383	,000	-3,3461	-2,3761
	9,00	-2,0278*	,15383	,000	-2,5128	-1,5428
	12,00	-1,6111*	,15383	,000	-2,0961	-1,1261
	15,00	-1,0278*	,15383	,000	-1,5128	-,5428
	18,00	-,3611	,15383	,322	-,8461	,1239
	21,00	,0000	,15383	1,000	-,4850	,4850

Based on observed means.

*. The mean difference is significant at the ,05 level.

Multiple Comparisons

Dependent Variable: Color100
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
,00	3,00	,1111	,12801	,994	-,2925	,5147
	6,00	,3889	,12801	,068	-,0147	,7925
	9,00	1,1389*	,12801	,000	,7353	1,5425
	12,00	1,8944*	,12801	,000	1,4909	2,2980
	15,00	2,1111*	,12801	,000	1,7075	2,5147
	18,00	2,2500*	,12801	,000	1,8464	2,6536
	21,00	2,4167*	,12801	,000	2,0131	2,8202
	30,00	2,5000*	,12801	,000	2,0964	2,9036
3,00	,00	-,1111	,12801	,994	-,5147	,2925
	6,00	,2778	,12801	,431	-,1258	,6813
	9,00	1,0278*	,12801	,000	,6242	1,4313
	12,00	1,7833*	,12801	,000	1,3798	2,1869
	15,00	2,0000*	,12801	,000	1,5964	2,4036
	18,00	2,1389*	,12801	,000	1,7353	2,5425
	21,00	2,3056*	,12801	,000	1,9020	2,7091
	30,00	2,3889*	,12801	,000	1,9853	2,7925
6,00	,00	-,3889	,12801	,068	-,7925	,0147
	3,00	-,2778	,12801	,431	-,6813	,1258
	9,00	,7500*	,12801	,000	,3464	1,1536
	12,00	1,5056*	,12801	,000	1,1020	1,9091
	15,00	1,7222*	,12801	,000	1,3187	2,1258
	18,00	1,8611*	,12801	,000	1,4575	2,2647
	21,00	2,0278*	,12801	,000	1,6242	2,4313
	30,00	2,1111*	,12801	,000	1,7075	2,5147
9,00	,00	-1,1389*	,12801	,000	-1,5425	-,7353
	3,00	-1,0278*	,12801	,000	-1,4313	-,6242
	6,00	-,7500*	,12801	,000	-1,1536	-,3464
	12,00	,7556*	,12801	,000	,3520	1,1591
	15,00	,9722*	,12801	,000	,5687	1,3758
	18,00	1,1111*	,12801	,000	,7075	1,5147
	21,00	1,2778*	,12801	,000	,8742	1,6813
	30,00	1,3611*	,12801	,000	,9575	1,7647
12,00	,00	-1,8944*	,12801	,000	-2,2980	-1,4909
	3,00	-1,7833*	,12801	,000	-2,1869	-1,3798
	6,00	-1,5056*	,12801	,000	-1,9091	-1,1020
	9,00	-,7556*	,12801	,000	-1,1591	-,3520
	15,00	,2167	,12801	,750	-,1869	,6202
	18,00	,3556	,12801	,132	-,0480	,7591
	21,00	,5222*	,12801	,002	,1187	,9258
	30,00	,6056*	,12801	,000	,2020	1,0091
15,00	,00	-2,1111*	,12801	,000	-2,5147	-1,7075
	3,00	-2,0000*	,12801	,000	-2,4036	-1,5964
	6,00	-1,7222*	,12801	,000	-2,1258	-1,3187
	9,00	-,9722*	,12801	,000	-1,3758	-,5687
	12,00	-,2167	,12801	,750	-,6202	,1869
	18,00	,1389	,12801	,975	-,2647	,5425
	21,00	,3056	,12801	,300	-,0980	,7091
	30,00	,3889	,12801	,068	-,0147	,7925
18,00	,00	-2,2500*	,12801	,000	-2,6536	-1,8464
	3,00	-2,1389*	,12801	,000	-2,5425	-1,7353
	6,00	-1,8611*	,12801	,000	-2,2647	-1,4575
	9,00	-1,1111*	,12801	,000	-1,5147	-,7075
	12,00	-,3556	,12801	,132	-,7591	,0480
	15,00	-,1389	,12801	,975	-,5425	,2647
	21,00	,1667	,12801	,929	-,2369	,5702
	30,00	,2500	,12801	,579	-,1536	,6536
21,00	,00	-2,4167*	,12801	,000	-2,8202	-2,0131
	3,00	-2,3056*	,12801	,000	-2,7091	-1,9020
	6,00	-2,0278*	,12801	,000	-2,4313	-1,6242
	9,00	-1,2778*	,12801	,000	-1,6813	-,8742
	12,00	-,5222*	,12801	,002	-,9258	-,1187
	15,00	-,3056	,12801	,300	-,7091	,0980
	18,00	-,1667	,12801	,929	-,5702	,2369
	30,00	,0833	,12801	,999	-,3202	,4869
30,00	,00	-2,5000*	,12801	,000	-2,9036	-2,0964
	3,00	-2,3889*	,12801	,000	-2,7925	-1,9853
	6,00	-2,1111*	,12801	,000	-2,5147	-1,7075
	9,00	-1,3611*	,12801	,000	-1,7647	-,9575
	12,00	-,6056*	,12801	,000	-1,0091	-,2020
	15,00	-,3889	,12801	,068	-,7925	,0147
	18,00	-,2500	,12801	,579	-,6536	,1536
	21,00	-,0833	,12801	,999	-,4869	,3202

Based on observed means.

*. The mean difference is significant at the ,05 level.

Table E.4 Tukey test results day by day for Sensory Evaluation

Multiple Comparisons

Dependent Variable: Odor150
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
,00	3,00	,1667	,12750	,928	-,2353	,5686
	6,00	,3333	,12750	,190	-,0686	,7353
	9,00	,7500*	,12750	,000	,3480	1,1520
	12,00	1,0833*	,12750	,000	,6814	1,4853
	15,00	2,1667*	,12750	,000	1,7647	2,5686
	18,00	2,7778*	,12750	,000	2,3758	3,1797
	21,00	3,2500*	,12750	,000	2,8480	3,6520
	30,00	4,0278*	,12750	,000	3,6258	4,4297
3,00	,00	-,1667	,12750	,928	-,5686	,2353
	6,00	,1667	,12750	,928	-,2353	,5686
	9,00	,5833*	,12750	,000	,1814	,9853
	12,00	,9167*	,12750	,000	,5147	1,3186
	15,00	2,0000*	,12750	,000	1,5980	2,4020
	18,00	2,6111*	,12750	,000	2,2091	3,0131
	21,00	3,0833*	,12750	,000	2,6814	3,4853
	30,00	3,8611*	,12750	,000	3,4591	4,2631
6,00	,00	-,3333	,12750	,190	-,7353	,0686
	3,00	-,1667	,12750	,928	-,5686	,2353
	9,00	,4167*	,12750	,036	,0147	,8186
	12,00	,7500*	,12750	,000	,3480	1,1520
	15,00	1,8333*	,12750	,000	1,4314	2,2353
	18,00	2,4444*	,12750	,000	2,0425	2,8464
	21,00	2,9167*	,12750	,000	2,5147	3,3186
	30,00	3,6944*	,12750	,000	3,2925	4,0964
9,00	,00	-,7500*	,12750	,000	-1,1520	-,3480
	3,00	-,5833*	,12750	,000	-,9853	-,1814
	6,00	-,4167*	,12750	,036	-,8186	-,0147
	12,00	,3333	,12750	,190	-,0686	,7353
	15,00	1,4167*	,12750	,000	1,0147	1,8186
	18,00	2,0278*	,12750	,000	1,6258	2,4297
	21,00	2,5000*	,12750	,000	2,0980	2,9020
	30,00	3,2778*	,12750	,000	2,8758	3,6797
12,00	,00	-1,0833*	,12750	,000	-1,4853	-,6814
	3,00	-,9167*	,12750	,000	-1,3186	-,5147
	6,00	-,7500*	,12750	,000	-1,1520	-,3480
	9,00	-,3333	,12750	,190	-,7353	,0686
	15,00	1,0833*	,12750	,000	,6814	1,4853
	18,00	1,6944*	,12750	,000	1,2925	2,0964
	21,00	2,1667*	,12750	,000	1,7647	2,5686
	30,00	2,9444*	,12750	,000	2,5425	3,3464
15,00	,00	-2,1667*	,12750	,000	-2,5686	-1,7647
	3,00	-2,0000*	,12750	,000	-2,4020	-1,5980
	6,00	-1,8333*	,12750	,000	-2,2353	-1,4314
	9,00	-1,4167*	,12750	,000	-1,8186	-1,0147
	12,00	-1,0833*	,12750	,000	-1,4853	-,6814
	18,00	,6111*	,12750	,000	,2091	1,0131
	21,00	1,0833*	,12750	,000	,6814	1,4853
	30,00	1,8611*	,12750	,000	1,4591	2,2631
18,00	,00	-2,7778*	,12750	,000	-3,1797	-2,3758
	3,00	-2,6111*	,12750	,000	-3,0131	-2,2091
	6,00	-2,4444*	,12750	,000	-2,8464	-2,0425
	9,00	-2,0278*	,12750	,000	-2,4297	-1,6258
	12,00	-1,6944*	,12750	,000	-2,0964	-1,2925
	15,00	-,6111*	,12750	,000	-1,0131	-,2091
	21,00	,4722*	,12750	,009	,0703	,8742
	30,00	1,2500*	,12750	,000	,8480	1,6520
21,00	,00	-3,2500*	,12750	,000	-3,6520	-2,8480
	3,00	-3,0833*	,12750	,000	-3,4853	-2,6814
	6,00	-2,9167*	,12750	,000	-3,3186	-2,5147
	9,00	-2,5000*	,12750	,000	-2,9020	-2,0980
	12,00	-2,1667*	,12750	,000	-2,5686	-1,7647
	15,00	-1,0833*	,12750	,000	-1,4853	-,6814
	18,00	-,4722*	,12750	,009	-,8742	-,0703
	30,00	,7778*	,12750	,000	,3758	1,1797
30,00	,00	-4,0278*	,12750	,000	-4,4297	-3,6258
	3,00	-3,8611*	,12750	,000	-4,2631	-3,4591
	6,00	-3,6944*	,12750	,000	-4,0964	-3,2925
	9,00	-3,2778*	,12750	,000	-3,6797	-2,8758
	12,00	-2,9444*	,12750	,000	-3,3464	-2,5425
	15,00	-1,8611*	,12750	,000	-2,2631	-1,4591
	18,00	-1,2500*	,12750	,000	-1,6520	-,8480
	21,00	-,7778*	,12750	,000	-1,1797	-,3758

Based on observed means.

*. The mean difference is significant at the ,05 level.

Multiple Comparisons

Dependent Variable: Color150
Tukey HSD

(I) Days	(J) Days	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
,00	3,00	,0833	,12340	,999	-,3057	,4724
	6,00	,1944	,12340	,816	-,1946	,5835
	9,00	,4167*	,12340	,026	,0276	,8057
	12,00	,8611*	,12340	,000	,4721	1,2501
	15,00	2,0833*	,12340	,000	1,6943	2,4724
	18,00	2,1667*	,12340	,000	1,7776	2,5557
	21,00	2,9444*	,12340	,000	2,5554	3,3335
	30,00	3,3611*	,12340	,000	2,9721	3,7501
3,00	,00	-,0833	,12340	,999	-,4724	,3057
	6,00	,1111	,12340	,993	-,2779	,5001
	9,00	,3333	,12340	,157	-,0557	,7224
	12,00	,7778*	,12340	,000	,3887	1,1668
	15,00	2,0000*	,12340	,000	1,6110	2,3890
	18,00	2,0833*	,12340	,000	1,6943	2,4724
	21,00	2,8611*	,12340	,000	2,4721	3,2501
	30,00	3,2778*	,12340	,000	2,8887	3,6668
6,00	,00	-,1944	,12340	,816	-,5835	,1946
	3,00	-,1111	,12340	,993	-,5001	,2779
	9,00	,2222	,12340	,682	-,1668	,6113
	12,00	,6667*	,12340	,000	,2776	1,0557
	15,00	1,8889*	,12340	,000	1,4999	2,2779
	18,00	1,9722*	,12340	,000	1,5832	2,3613
	21,00	2,7500*	,12340	,000	2,3610	3,1390
	30,00	3,1667*	,12340	,000	2,7776	3,5557
9,00	,00	-,4167*	,12340	,026	-,8057	-,0276
	3,00	-,3333	,12340	,157	-,7224	,0557
	6,00	-,2222	,12340	,682	-,6113	,1668
	12,00	,4444*	,12340	,013	,0554	,8335
	15,00	1,6667*	,12340	,000	1,2776	2,0557
	18,00	1,7500*	,12340	,000	1,3610	2,1390
	21,00	2,5278*	,12340	,000	2,1387	2,9168
	30,00	2,9444*	,12340	,000	2,5554	3,3335
12,00	,00	-,8611*	,12340	,000	-1,2501	-,4721
	3,00	-,7778*	,12340	,000	-1,1668	-,3887
	6,00	-,6667*	,12340	,000	-1,0557	-,2776
	9,00	-,4444*	,12340	,013	-,8335	-,0554
	15,00	1,2222*	,12340	,000	,8332	1,6113
	18,00	1,3056*	,12340	,000	,9165	1,6946
	21,00	2,0833*	,12340	,000	1,6943	2,4724
	30,00	2,5000*	,12340	,000	2,1110	2,8890
15,00	,00	-2,0833*	,12340	,000	-2,4724	-1,6943
	3,00	-2,0000*	,12340	,000	-2,3890	-1,6110
	6,00	-1,8889*	,12340	,000	-2,2779	-1,4999
	9,00	-1,6667*	,12340	,000	-2,0557	-1,2776
	12,00	-1,2222*	,12340	,000	-1,6113	-,8332
	18,00	,0833	,12340	,999	-,3057	,4724
	21,00	,8611*	,12340	,000	,4721	1,2501
	30,00	1,2778*	,12340	,000	,8887	1,6668
18,00	,00	-2,1667*	,12340	,000	-2,5557	-1,7776
	3,00	-2,0833*	,12340	,000	-2,4724	-1,6943
	6,00	-1,9722*	,12340	,000	-2,3613	-1,5832
	9,00	-1,7500*	,12340	,000	-2,1390	-1,3610
	12,00	-1,3056*	,12340	,000	-1,6946	-,9165
	15,00	-,0833	,12340	,999	-,4724	,3057
	21,00	,7778*	,12340	,000	,3887	1,1668
	30,00	1,1944*	,12340	,000	,8054	1,5835
21,00	,00	-2,9444*	,12340	,000	-3,3335	-2,5554
	3,00	-2,8611*	,12340	,000	-3,2501	-2,4721
	6,00	-2,7500*	,12340	,000	-3,1390	-2,3610
	9,00	-2,5278*	,12340	,000	-2,9168	-2,1387
	12,00	-2,0833*	,12340	,000	-2,4724	-1,6943
	15,00	-,8611*	,12340	,000	-1,2501	-,4721
	18,00	-,7778*	,12340	,000	-1,1668	-,3887
	30,00	,4167*	,12340	,026	,0276	,8057
30,00	,00	-3,3611*	,12340	,000	-3,7501	-2,9721
	3,00	-3,2778*	,12340	,000	-3,6668	-2,8887
	6,00	-3,1667*	,12340	,000	-3,5557	-2,7776
	9,00	-2,9444*	,12340	,000	-3,3335	-2,5554
	12,00	-2,5000*	,12340	,000	-2,8890	-2,1110
	15,00	-1,2778*	,12340	,000	-1,6668	-,8887
	18,00	-1,1944*	,12340	,000	-1,5835	-,8054
	21,00	-,4167*	,12340	,026	-,8057	-,0276

Based on observed means.

*. The mean difference is significant at the ,05 level.

APPENDIX F

MAIN EFFECTS, INTERACTIONS, RESIDUAL, PROBABILITY PLOTS AND EQUAL VARIANCES FOR Phenolic Compounds AS AN EXAMPLE, DECISION FOR OPTIMUM COMBINATIONS

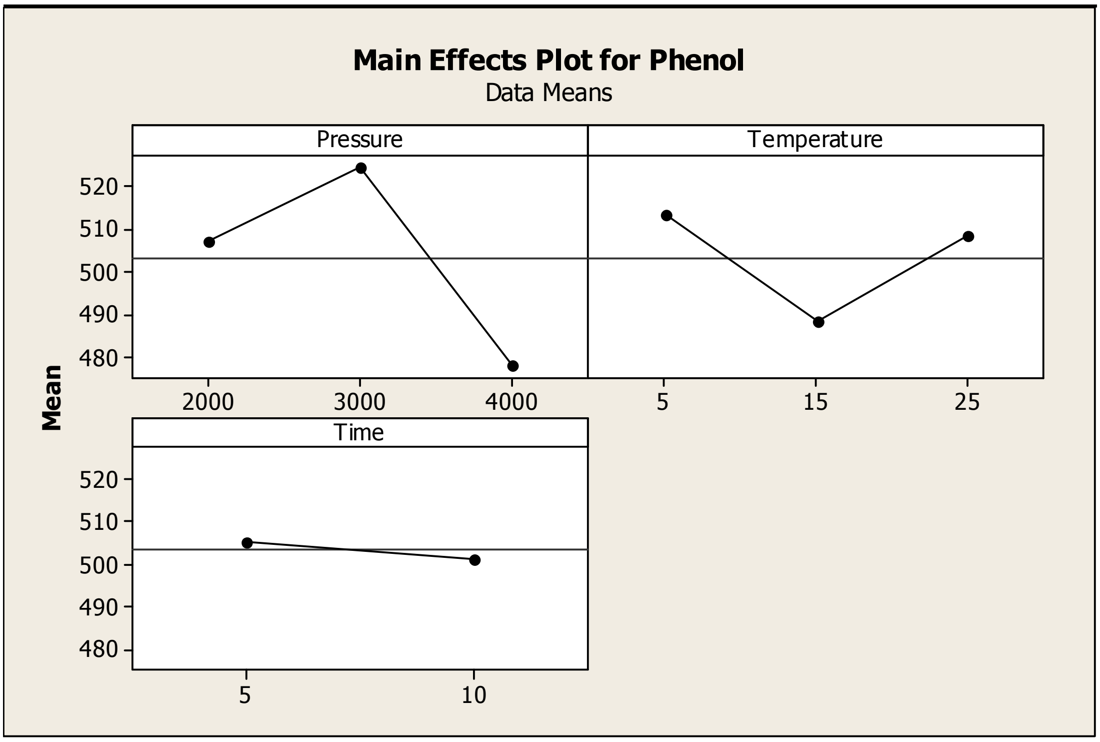

Figure F.1 Main Effects Plot for Phenol

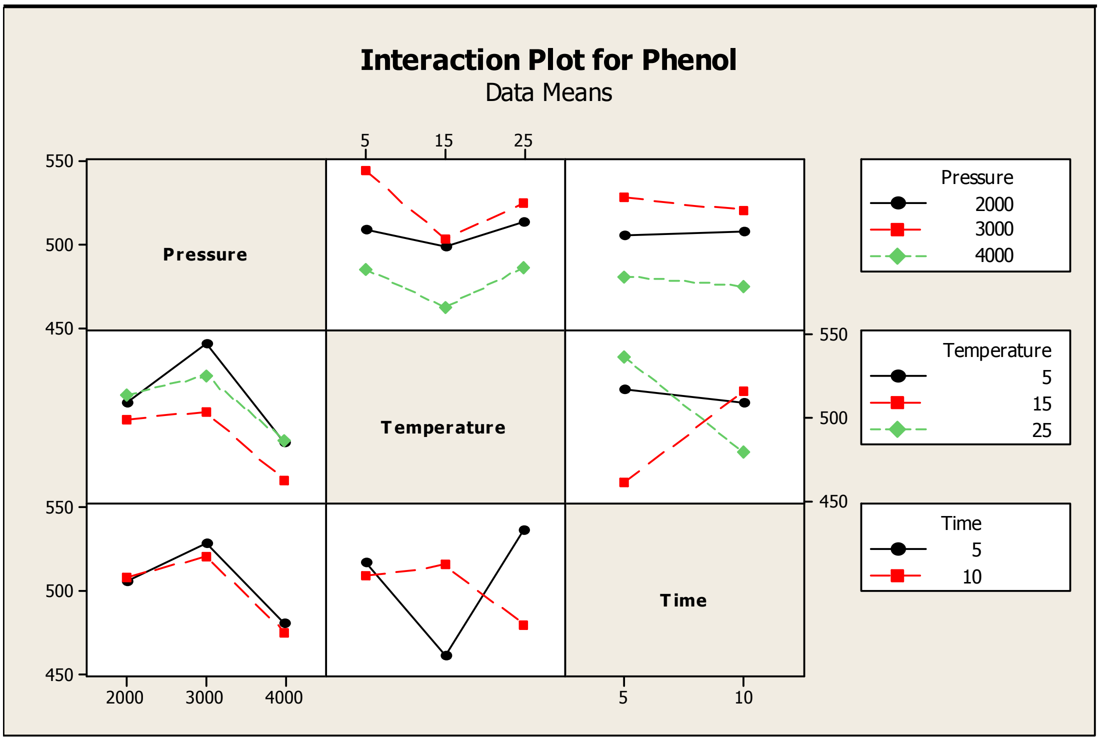

Figure F.2 Interaction Plot for Phenol

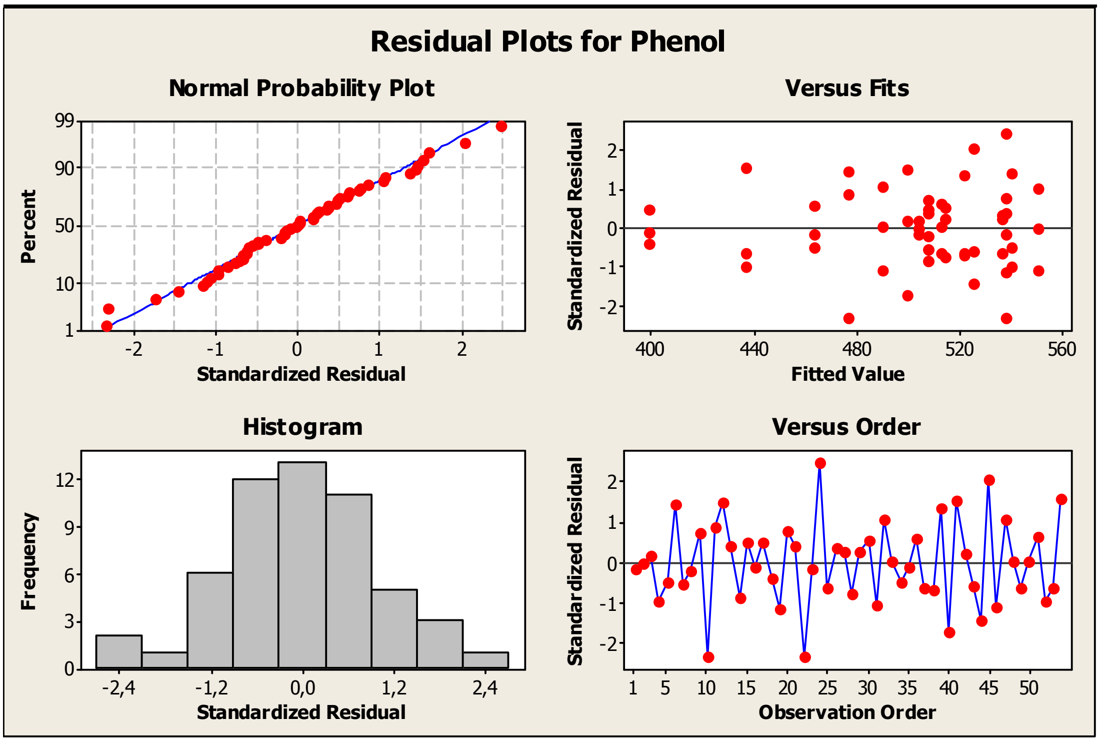

Figure F.3 Residual Plots for Phenol

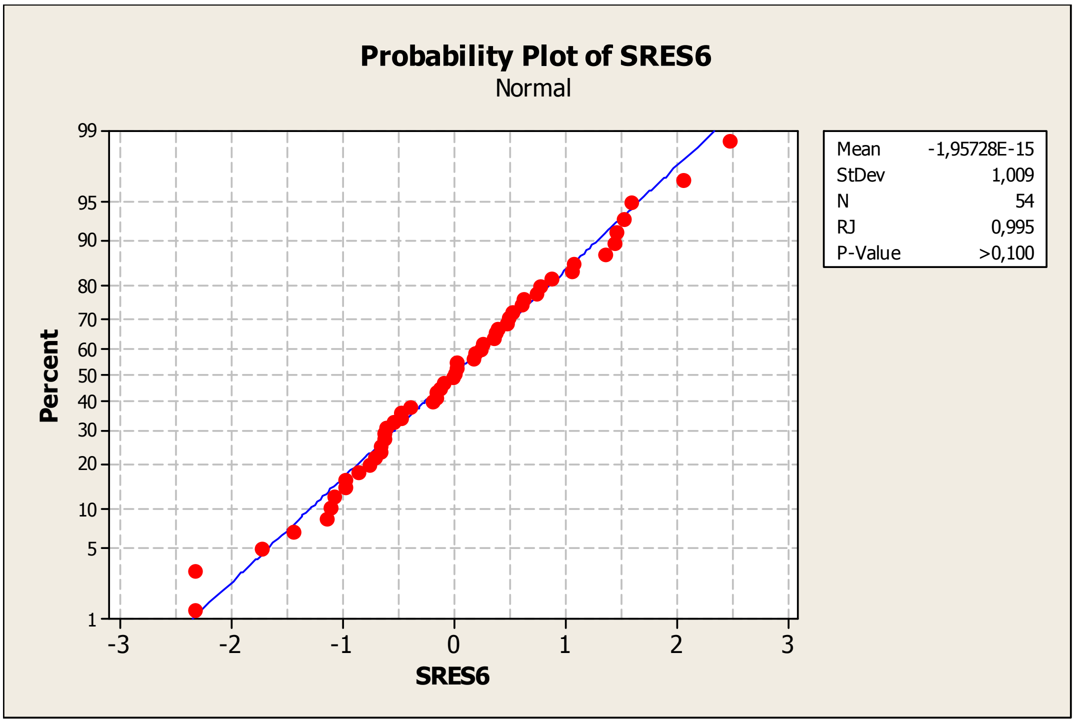

Figure F.4 Probability Plot of SRES6

H0: The errors are normally distributed.

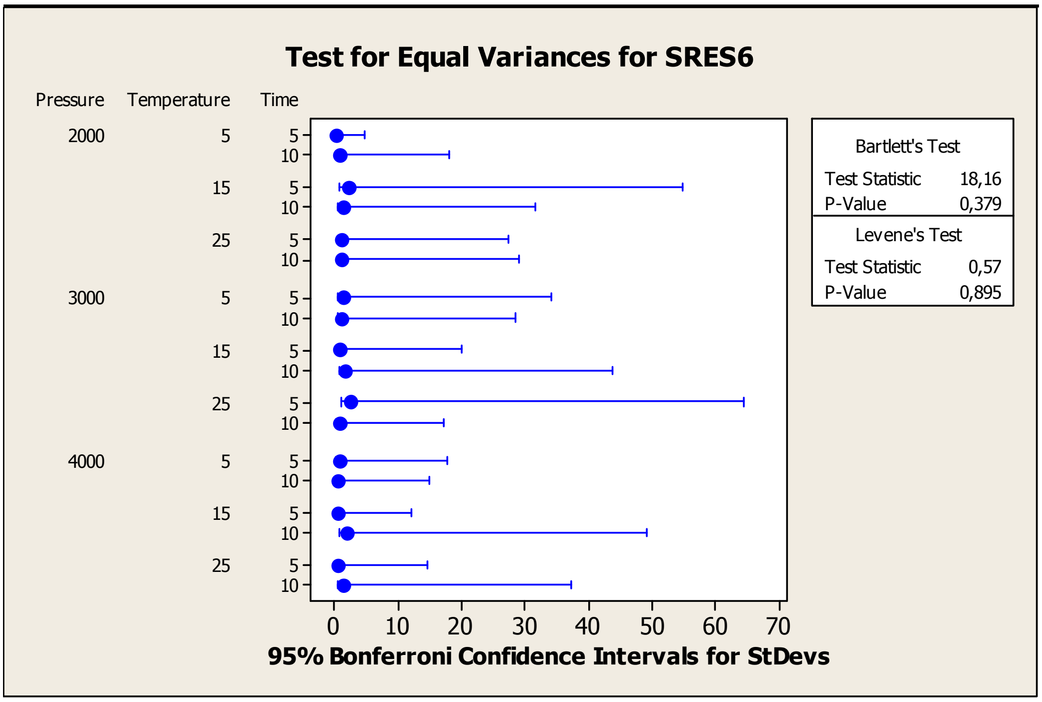

Figure F.5 Test for Equal Variances for SRES6

H0: Population of each treatment level have the same variance.

General Linear Model: Phenol versus Pressure; Temperature; Time

Factor	Type	Levels	Values
Pressure	fixed	3	2000; 3000; 4000
Temperature	fixed	3	5; 15; 25
Time	fixed	2	5; 10

Analysis of Variance for Phenol, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	2	20004,2	20004,2	10002,1	31,84	0,000
Temperature	2	6316,4	6316,4	3158,2	10,05	0,000
Time	1	187,9	187,9	187,9	0,60	0,444
Pressure*Temperature	4	1774,2	1774,2	443,5	1,41	0,250
Pressure*Time	2	261,5	261,5	130,7	0,42	0,663
Temperature*Time	2	28383,8	28383,8	14191,9	45,18	0,000
Pressure*Temperature*Time	4	21003,9	21003,9	5251,0	16,72	0,000
Error	36	11308,1	11308,1	314,1		
Total	53	89240,0				

S = 17,7233   R-Sq = 87,33%   R-Sq(adj) = 81,34%

Figure F.6 General Linear Model: Phenol versus Pressure; Temperature; Time