APPLICATION OF HIGH DYNAMIC MICROFLUIDIZATION TO IMPROVE SOME QUALITY PARAMETERS AND STABILITY OF ORANGE JUICE

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ABSTRACT

APPLICATION OF HIGH DYNAMIC MICROFLUIDIZATION TO IMPROVE SOME QUALITY PARAMETERS AND STABILITY OF ORANGE JUICE

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The aim of current research is to analyze the effect of microfluidization on the stability and some quality characteristics of orange juice with respect to treatment pressure and cycle. Orange juice was microfluidized with four different pressures (34, 69, 103 and 138 MPa) and three different cycles (1, 2 and 3) at 18 ± 2 ^{0}C. Physical and chemical properties of microfluidized juices were compared with non-microfluidized freshly squeezed orange juice.

Microfluidization made orange juice brighter and decreased redness and yellowness. There was a huge difference between non-microfluidized juice and microfluidized juice in terms of particle size. Microfluidization decreased the volume weighted mean (VWM) of orange juice between 90 % and 97 %. The results of total phenol content and antioxidant activity experiments showed that treatment pressure affected them positively; however cycle had not a significant effect on total phenol content and antioxidant property of orange juice ($p<0.05$).

Our current research also includes effect of microfluidization on stability of orange juice. The broken down of aggregated structure and reduction in particle size due to treatment were observed by the scanning electron and light microscopes. Therefore, it was observed that treated orange juice could be homogeneous and opaque for 14 days at 4 ^{0}C. Cloud stability of juice showed that both pressure and cycle had important effect on the cloud stability ($p<0.05$). Microfluidization made the juice very stable but increase in pressure and cycle resulted in less stable juice. It was also measured that pectin methyl esterase activity was increased due to treatment of microfluidization.

Keywords: High Dynamic Microfluidization, Orange Juice, Phenol and Antioxidant Activity, Pectin Methyl Esterase Activity

ÖZ

PORTAKAL SUYUNUN BAZI KALİTE PARAMETRELERİNİN VE STABİLİTESİNİN ARTMASI İÇİN YÜKSEK DİNAMİK MİKROFLUDİZASYONUN UYGULANMASI

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Bu çalışmanın amacı mikrofludizasyonun portakal suyunun bazı kalite özelliklerine ve stabilitesine etkisini işlem basıncına ve cihazdan geçiş sayısına (devir) göre analiz etmektir. Portakal suyu 4 farklı basıç (34, 69, 103 and 138 MPa) ve 3 farklı devir ile (1, 2 and 3) 18 ± 2 ^{0}C de mikrofludizasyonlandı. İşlenmiş meyve sularının fiziksel ve kimyasal özellikleri işlenmeyen taze sıkılmış portakal suyu ile kıyaslandı.

Mikrofludizasyon portakal suyunu daha parlak hale getirdi ve kırmızılığını ve sarılığını azalttı. Parça boyutu bakımından işlenmiş ve işlenmemiş portakal suyu arasında çok büyük bir fark elde edildi. Mikrofluidizasyon portakal suyunun VWM

(volume weighted mean) değerini 90 % ile 97 % arasında azalttı. Toplam fenol miktarı ve antioksidan aktivitesi deney sonuçları, işlem basıncının bu özellikleri olumlu yönde etkilediğini ancak devir sayısının portakal suyunun toplam penol miktarı ve antioksidan özelliği üzerinde önemli bir etkisinin olmadığını gösterdi ($p<0.05$).

Çalışmamız mikrofludizasyonun portakal suyunun stabilitesine etkisini de içermektedir. İşlemin topaklanmış yapıyı parçalaması ve parça boyutunu azaltması ışık ve taramalı elektron mikroskopları ile incelendi ve işlenmiş portakal suyunun 14 gün boyunca 4 ^{0}C de homojen ve opak olduğu gözlemlendi. Meyve suyu bulanık stabilitesi hem basıncın hem de devir sayısının bulanık stabilite üzerinde etkisi olduğunu gösterdi ($p<0.05$). Basınç ve devir sayısı arttıkça daha stabil meyve suyu elde edildi. Diğer taraftan, pektin metil esteraz aktivitesinin mikrofluidizasyon işleminden dolayı arttığı ölçüldü.

Anahtar Kelimeler: Yüksek Dinamik Mikrofluidizasyon, Portakal Suyu, Fenol ve Antioksidan Aktivitesi, Pektin Metil Esteraz Aktivitesi

TO MY FAMILY...

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INTRODUCTION

1.1 Microfluidization Mechanism and Applications in Food Industry

Many applications have been introducing to replace conventional treatments as an alternative in the food processing. Some example of these applications can be high hydrostatic pressure, pulsed electric field application, micro-wave heating, ultrasonication and microfluidization. The common aim of these applications is to preserve and enhance the quality of the products. Microfluidization is a very new technology gaining popularity nowadays in the food industry and it has been looking into to obtain products with high quality with the help of the high pressures up to 200 MPa. Microfluidization combines the impact of extremely high velocity, high frequency vibrations, pressure decrease, high shear, high pressure in a very short time (about 5 seconds) and the continuous treatment (Liu et al., 2009).

Microfluidizer contains a chamber where fluid flows. Initially, the fluid is forced to split and flow into two microstreams. Then, these two microstreams are clashed at each other extremely high speeds by the help of the air compression and as a result of the clash cavitations, turbulence and shear are formed (Cook &Lagace, 1987). Inside these microstreams the fluid is not only subjected to high shear rate but also subjected to the striking

coerces which resulted in the broken of large particles into the fine particles (McCrae, 1994). Therefore, microfluidization treatment made the fluid more homogenized and can be used as a homogenization technology in the food processes. As a new application microfluidization has been trialed for themilk homogenization and it was found that microfluidization made the milk fat more homogenized (Strawbridge et al., 1995, Hardham et al., 2000).

There are lots of researches basing on the microfluidization treatment in the food industry. Lebeuf et al. (1998) studied the effect of microfluidizated whey proteins on the cheddar cheese properties and Ciron et al. (2010) compared the high-pressure microfluidized and conventional homogenized milk in terms of the particle size, water retention and texture of yoghurts made from these milks. Diane et al. (2005) studied the effect of microfluidization on rheology and melting characteristics of cheese made from microfluidized milk and observed that fat globes became smaller after microfluidization and this affected characteristics of cheese mostly. Moreover, high pressure microfluidization has been widely researched as a treatment for preparation of nanoemulsions (Jafari et al., 2007), decrease of microbial load (Giancarlo et al., 1996). Liu et al. (2006) studied the effect of microfluidization on solubility and rheological properties of soybean dietary fibers and observed the improvement of dietary fibers.

As shown there are many studies dealing with the application of microfluidization and these studies showed that microfluidization can be used as an alternative treatment for the homogenization of the systems. Therefore, orange juice can be processed with the microfluidization to obtain more homogenized orange juice for consumers those do not like orange juice with high pulp content.

1.2 Characteristics of Orange Juice

In the beverage processing industry orange juice is the most common juice in all over the world. Annually, over 63 million tons of oranges are produced throughout the world (FAOSTAT, 2004). Conventional production produces safe juices but it may cause some changes in the sensory and nutritional properties of juice. However, consumers want to get high quality products which are freshly prepared properties such as color, texture, and flavor. Consumer also tries to consume functional products which supply antioxidant, vitamins and special nutritive and functional compounds.

1.2.1. Color

Color of juice is a significant element playing role for the acceptance of the consumers. Carotenes and Xanthophylls are main color carotenoids responsible for the color of orange juice (Shewfelt, 1986). Carotene amount increases with increase in chlorophyll levels in citrus peels but xanthophylls amount is high in green mature fruit (Kale et al., 1995).

Color of juices is generally determined by measuring Hunter color parameters and calculating the total color difference by using these values (Choi et al., 2001). Lightness is pointed by L, yellowness and blueness is indicated by positive (yellow) and negative (blue) value of a, and greenness and redness is indicated by positive (red) and negative (green) value of b (Rocha et al., 2003). The difference is sorted out analytically according to value of total color difference as very distinct for larger than 3.0, distinct for value between 1.5 and 3.0 and small difference small than 1.5 (Dr. Lange, 1999).

Lee et al. (2001) informed that CIE parameters (L, a, b) may be used as an indicator for determination of ripeness of orange juice and found that increment in the level of ripeness resulted in increase in the yellowness of orange juice. They also showed that chance in b (Δb) and a (Δa) have a positive value for totally ripened fruit juices and the correlation between the carotenoid content and color parameters is important. Labuza et al. (1992) observed a decrease in lightness of fruits. They concluded their research like that there is an association between the decline of L value and browning of fruits.

1.2.2. Ascorbic Acid Content

Nutritional quality of orange juice majorly associated with the ascorbic acid concentration of juice (Zerdin et al., 2003). Ascorbic acid is known as the most important water soluble antioxidant. Recommended Daily Acceptance suggests consuming 100-120 mg ascorbic acid in a day and range of ascorbic acid content in orange juice is between 150-450 mg/l juice so the consumption of a glass of orange juice (200 ml) can provide nearly 30-80 % of recommended intake of ascorbic acid (Gleszczynska-Swinglo et al., 2004).

Ascorbic acid is affected most of the processes because it is highly sensitive. The mechanism of loss of ascorbic acid depends on the treatment conditions (Vieira et al., 2000). Temperature, oxygen, amount of soluble solid content, acidity, enzymes of juice, microbial load and degree of the protection of container are substantial factors affecting the ascorbic acid degradation in orange juice (Tannebaum et. al., 1985). Firstly ascorbic acid is degradated to

dehydroascorbic acid with first order reaction rate with respect to ascorbic acid concentration if oxygen is present (Khan et al., 1967).

Degradation of ascorbic acid may be used an indicator of other unwanted chances such as formation of brown pigments. Solomon et al. (1995) studied relationship between the browning of orange juice and loss of ascorbic acid and they found that there is a correlation between them.

1.2.3. Phenolic Compound Content and Antioxidant Activity

There is a rising care has been paying to the significant role of bioactive compounds in citrus juice due to the potential health protecting properties of these bioactive compounds. The intake of these compounds appears to be related to the decrease the risk of many cancers like colorectal, esophageal, stomach and gastric by having antioxidant properties (Tripoli et al., 2007). Phenolic compounds are one the most important bioactive compounds; they are present in many seeds, vegetables, fruits. Among them orange juice may compass a big part due to relatively high consumption and including high amount of vitamin C and polyphenolic compounds. Hyrodxycinnamic acids and flavonoids are the dominant phenolic compounds of oranges and orange juices. Inside the orange juice hyrodxycinnamic acids present as esters of *p*-coumaric, sinapic, ferrulic and caffeic acids. Among flavanoids, flavanones are the most dominant compounds and they are presents as glycosides. Naringenin, aglycons, hesperetin, eriodictyol and isokuranetin are other common flavonoids of orange juices but their amount is smaller than glycosides (Klimezak et al., 2006).

Polyphenols are hydrogen donors and their structural configuration,

arrangement of hydroxyl groups, number of hydroxyl groups, and presence of electron-donor and electron-acceptance pairs in the ring structure affects the antioxidant property of these compounds (Rice-Evans et al., 1995).Figure 1.1 represents two phenolic compound of orange juice and shows as an example of ring structure and hydroxyl groups of phenolics.

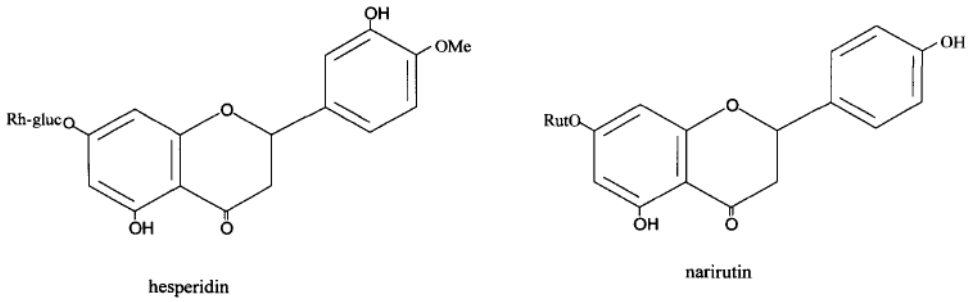


Figure 1.1 Phenolic compounds (hesperidin and narirutin) in orange juice

The main capacity of antioxidant of a fruit could attribute to the compounds like vitamins, carotenes, and phenolics (Kaur et al., 2001). Orange juice is one of the important reservoirs of antioxidant carotenoids like beta-carotene, zeaxanthin, lutein due to the presents of that kind of compounds and their high compositions. These compounds have role on the reducing of heart disease, cancer and some other diseases due to the free-radical scavenging functions of them (Temple, 2000). It is assumed that free radicals damaged to proteins, lipids even nucleic acids so function of antioxidants is the neutralization of these free radicals and prevention of these diseases (Rice-Evans et al., 1993).

Orange juice contains high amount of vitamin C which is an important antioxidant. Antioxidant property of Vitamin C has been studying and it was

found that it can forestall the improvement of cataracts (Mares-Prelman, 1997), can decrease the risk of Alzheimer disease (Engelhart et al., 2002) so antioxidant property of the orange juice may have very important role on the functions of the body by decreasing the free radicals of medium. Miller et al. (1997) studied the contribution of ascorbic acid to antioxidant activity of fruit juices and found that the most significant contribution was from ascorbic acid with 87 %. Another research showed that antioxidant activity of orange juice mostly because of ascorbic acid (Gardner et al., 2000).

In order to measure antioxidant activity of a fruit different studies have being conducted. These studies have being applied different assays including DPPH, FRAP, ABTS. On the other hand, different results and tendency have been found (Nilsson et al., 2005, Thaipong et al., 2006).

The interaction of antioxidant property and total phenol content was studied by many researchers. Garner et al. (2000) studied the effect of total phenol content on antioxidant potential of fruit juices and found that phenolic content and ascorbic content are correlated with antioxidant capacity of fruit juices. Furthermore, Kalt et al. (1999) researched the influence of total phenol content of some fruits like strawberry, blackberry on the antioxidant capacity and observed that there is a linear relationship between antioxidant capacity and total phenol and anticynanine content of these fruits.

Miller et al. (1997) found that hesperidin and narirutine are the soluble phenolic compounds presenting in the orange juice but main antioxidant compound of orange is ascorbic acid. However, Rapisarda et al. (1999) studied the influence of phenolic compounds of freshly squeezed orange juice on antioxidant activity of juice and found that antioxidant activity mostly depends on the total phenolic content of juice and ascorbic acid has less significant role on the antioxidant activity.

1.2.4. Particle Size

High pressure treatments for homogenization of citrus juices usually applied to provide cloudy juice and to improve the color of the juices in the citrus juice industry (Betoret et al., 2009). Homogenization makes the big coarse particles as smaller particles so the fraction of the particles smaller than 2 μm, which are the main contribution to juice stability, increases (Baker et al., 1999). Cloud particles of orange juice distributed from 400 to 5000 nm (Buslig et al., 1974). Therefore, to increase the quality of orange juice by increasing the cloudiness microfluidization can be applied. Most of the studies revealed that microfluidization decrease the particle size of the samples. Ciron et al. (2010) researched the effect of microfluidization on particle size of fat globes of milk and found that after treatment fat globes became smaller and more uniform. Moreover, oil in water emulsion production with microfluidization was studied and small emulsion droplets with narrow distributions were gotten after the treatment (Jafari et al., 2007). Therefore microfluidization should have decreased the particle size of orange juice by pulling the pulpy structure into pieces. Decrease in particle size also influence the efficiency and yield of process and other characteristics of the treated juice.

1.2.5. Stability and Pectin Methyl Esterase Activity

Cloudiness is very important indicator of quality of orange juice because cloud stability have important role on flavor, color and mouth feel. Therefore, most of the consumers try to get more cloudy juice. The clouds of citrus juices include organelles and membranes of cells, oil droplets, cell wall components like pectin, cellulose, complex mixture of proteins and lipids (Baker et al., 1999).

Pectin methyl esterase is an enzyme presents in plants and causes the deesterification of pectin in the cell wall. PME (EC 3.1.1.11) may be named as pectinoesterase, pectin methoxylase, pectase, pectinoesterase and pectinesteraze (Tiwari et al., 2009). Pectin methyl esterase activity is one of the most important issues related with the orange juice production because many food properties such as viscosity, cloud stability are influenced by function of pectic enzymes (Espachs-Barroso et al., 2006). Freshly squeezed orange juice losses it's cloudy within a few days due to the activity of pectin methyl esterase (Nianaber et al., 2001). PME lets to undesired cloud instability in citrus juices. It causes the hydrolysis of methyl ester groups of pectin and formation of calcium pectate gel which is responsible for cloud loss of juice.

There are heat-stable and heat-labile isomers of PME. Both of them are presents inside the orange juice so they are commercially important for processing of orange juice (Verteeg et al., 1980). Opalescence problem is mainly due to TS-PME (thermal stable pectin methyl esterase) activity in the citrus juice production (Cameron et al., 1998). The application of heat treatments on the citrus juices is aimed to inactivate the PME. According to the study conducted by Reynolds (1963) the sufficient heat treatment for inactivation of enzyme may have resulted in loss of fresh-like characteristics of juice and caused of the non-enzymatic browning. As a result, non- thermal technologies are researched for novel methods to provide products with more stable and have characteristics similar to those of non-processed orange juice by decreasing the pectin methyl esterase activity.

Application of pressure as a process parameter has been increasing for preservation of natural properties of foods and the influence of pressure on biopolymers like proteins and enzymes. According to study of Cheftel

(1992) high pressure application may have influenced the protein structure reversibly or irreversibly but the chance of enzyme activity may depend on type of enzyme and processing conditions like temperature and time.

Several studies have showed that static pressurization can be used to inactivate the PME in orange juice (Goodner et al., 1998). However, continuous pressurization is more preferred for food industrial applications. Recently dynamic high pressure applications may have been using to inactivate PME and get more stable orange juice mainly processed with pressurization instead of pasteurization. (Lacroix et al., 2005, Clark et al., 1993). Dynamic high pressure application is different from microfluidization. In dynamic high pressure treatment referring to homogenization process liquid is forced to flow a very narrow orifice at high pressure resulting in physical chances in the processed product (Lacroix et al., 2005). On the other hand, in microfluidization fluid is forced to flow inside a chamber for a short time. Therefore both of the operations make the orange juice more stable but they may affect the juice properties differently.

1.3 Objective of the Study

Microfluidization may be a potential cold treatment application for fruit juice processing with high nutritionality and quality, short treatment time and a continuous process. This research concentrates on the influence of microfluidization on the physical, chemical and morphological properties of orange juice by hypothesizing that microfluidization may enhance the stability and quality of orange juice. The effect of microfluidization on the color, nutrition content, total phenol content and antioxidant activity and

mainly the stability of orange juice are aimed to be measured and observed.

Another objective of this study is to observe the influence of microfluidization conditions, which are the combinations of pressure and cycle, to desired characteristics of orange juice. A favorable consumer attitude towards the orange juice processed by microfluidization is necessary to achieve the success of microfluidized orange juice in global market.

CHAPTER 2

MATERIALS AND METHODS

2.1. Sample and Application

2.1.1. Orange

Oranges were purchased from the local market in Ankara (Turkey). The variety of the orange was Washington. They were stored at refrigerator (4 ^{0}C) until the treatment.

2.1.2. Juice Processing and Treatment

A home type fruit processor was used to juice the oranges (Arzum, Turkey). Colloid mill (Thomas-Wiley Laboratory Mill, Artur H. Thomas Company, Model 4, USA) was used to prior to the microfluidization treatment in order to prevent blockage of microfluidizer with the pulp structure of orange juice. After the treatments, samples were put into the refrigerator (4 ^{0}C) and the measurements were done within 1 hour.

The samples were treated in a designed and constructed lab-scale unit microfluidizer (M-110Y, Microfluidics, USA). They were pressurized at four different pressures (34, 69, 103 and 138 MPa) and three different cycles (1, 2 and 3). Unpressurized freshly squeezed orange juices were used as control.

Inside the microfluidizer the liquid is firstly divided into two microstreams, then they are forced to flow inside microchannels and finally they leave the microfluidizer by contacting of the liquids with each other.

2.2. Physical Analysis

2.2.1. Color

Color of the juices were measured by Avantes spectrophotometer (Avantes, model:Avaspec 2048, Netherlands). The light source of the spectrophotometer was D65. Reference white color was prepared by dissolving 0.05 g TiO_2 into 100 ml distillated water (Dede, 2005). The brightness (L), redness (a) and yellowness (b) values of juices were measured and total color chance (ΔE) was calculated based on the below equation (Billmeyer et al., 1981).

$$\Delta E = \sqrt{[(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2]} \quad (2.1)$$

where

L_0: brightness of reference white solution

a_0: redness of reference white solution

b_0: yellowness of reference white solution

2.2.2. Particle Size

Particle size was measured by using Malvern Mastersizer (Malvern Instruments Limited, U.K, 2000 Model). 1.52 and 1.33 values were used for particle refractive

index and dispersant refractive index, respectively. The absorption index was 0.1 for equipment during the measurements.

The equipment determined the size of particles according to laser diffraction technique and gave particle size distribution based on volume percent. The particle size was described as WolumeWeighted Mean Diameter, D[4,3]. It was calculated according to the below equation.

$$D[4,3] = (\sum n_i * d_i^4) / (\sum n_i * d_i^3) \quad (2.2)$$

where

di: diameters of particles

ni: number of particles

2.2.3. Brix

Total soluble solid content of the juices was measured using a refractometer (Atago N1, Japan). Measurements were performed at room temperature.

2.2.4. Browning Index

Browning was determined by the method of Meydav et al. (1977). Sample of juices were centrifuged at 760 *g* for 10 minutes at room temperature (25^0C). 5 ml supernatant was mixed with 5 ml ethanol (Riede, Germany). The mixture was again centrifuged at 760 *g* for 10 minutes at room temperature. After the centrifugation, absorbance of samples was measured at 420 nm in spectrophotometer.

2.3. Chemical Analysis

2.3.1. Ascorbic Acid Content

Amount of ascorbic acid inside the orange juices was determined by a titrimetric method by using 2,6-dicholoroindophenol (Cemeroğlu, 2007).This method basically depends on the reduction of ascorbic acid by the dye 2,6-dicholoroindophenol a oxidation-reduction indicator. Reduction of ascorbic acid results in a colorless solution. Therefore, at the end point, excess dye gives a rose pink color.

After the sample was diluted with 3 % Meta-Phosphoric acid (Riedel-de Haen, Germany), it was titrated with the dye 2,6-dicholoroindophenol (Merck, Germany) solution and the volume of titration was used in equation below to determine ascorbic acid (Carlo Erba Reagent, Italy) of the juices.

Amount of ascorbic acid = V*D*f (2.3)

where;

V: volume of dye used for titration, ml

D: dilution factor

f: factor represents amount of ascorbic acid of 1 ml dye 2,6-dicholoroindophenol solution

f factor is determined by titration of solution containing 1 mg ascorbic acid (Merck, Germany) with the 2,6-dicholoroindophenol solution. The value of f factor is determined by the equation given below.

f factor = 1/volume of dye solution, ml (2.4)

2.3.2. Reducing Sugar Content

Reducing sugar amount of juices were determined with the method descript in the book of Cemeroğlu (2007) with some modifications. This method depends on the Fehling method or namely Lane-Eynon method. First of all Fehling solutions and standard sugar solutions were prepared. 69.3 g $CuSO_4.5H_2O$ (Sigma-Aldrich, C2284) was dissolved in distillated water and the final volume was made 1 liter by addition of distillated water to prepared Fehling-I solution. 346 g potassium sodium tartrate tetrahydrate (Sigma-Aldrich, S2377) and 100 g KOH (Sigma-Aldrich, P5958) were dissolved and made the final volume 1 liter with distillated water to prepared Fehling-II solution. Standard sugar solution was prepared by dissolving of 0.5 g glucose (Sigma-Aldrich, G8270) into 100 ml distillated water.

Standard sugar solution was used for standardization of Fehling solution which was mixing of Fehling solutions in a 1:1 ratio just before the experiment. For standardization 25 ml Fehling solution and 25 ml sugar solution were mixed and heated to boiling point. After reaching to boiling point 2 minutes were waited during boiling of mixing. Methylene-blue indicator solution (2-3 drops) was added during this period. After 2 minutes mixture was titrated with standard sugar solution in 1 minute during boiling to determine the factor representing amount of reducing sugar to titrate 25 ml Fehling solution.

After finding the factor of Fehling solution, the amount of reducing sugar content of juices was determined. Firstly sample was diluted. 25 ml Fehling solution, 15 ml standard sugar solution and 10 ml diluted sample solution were mixed, heated and boiled for 2 minutes and titrated with sugar solution within 1 minute. The amount of reducing sugar was determined by standardization factor and reducing sugar content of added standard sugar solution.

2.3.3. Total Phenol Content

Total phenol content of the samples was determined by Folin-Ciocalteu method (Singleton and Rossi, 1965). This method depends on the color change due to reduction of Folin-Ciocalteu reagent by phenols produced in the presence of the sodium carbonate.

After the sample was diluted, 60 µl of sample was placed in a tube and 4.74 ml of distillated water and 300 µl of by Folin-Ciocalteu phenol reagent (Merck, Germany) were added, respectively. After mixing well, the contents waited for 8 min 30 sec. Then 900 µl of 20 % sodium carbonate (Merck, Germany) solution was added into the tubes, the mixture was mix well by vortex and incubated at 40 ^{0}C for 30 min. At the end of the incubation the samples were put into the water batch containing water-ice mixture for 5 min to decrease the temperature. The absorbance was measured at 765 nm in spectrophotometer (Analytic Jena SPECORD 50, Germany).

Total phenol content was given as gallic acid (Merck, Germany) equivalent using the standard curve which is located at the appendix part (Fig A.1).

The relationship between the absorbance and gallic acid (mg gallic acid/liter sample) found as;

A_{765}=0.0011*[gallic acid] (R^2= 0,9998) (2.5)

Where A is absorbance at 765 nm

Ascorbic acid affects the result of the Folin-Ciocalteu assay (Asami et al., 2003). Reducing sugars also may interfere with the total phenol reagent if sugar content of the product is high (Slinkard et al., 1977). Therefore correction factors for ascorbic acid and reducing sugar were determined.

2.3.3.1. Correction of Ascorbic Acid

Ascorbic acid interferes with the total phenol analysis by Folin-Ciocalteu reagent. (Asami et al., 2003). A correction factor was determined to correct the total phenol content of the samples. The correction factor represents weight to weight ratio of gallic acid absorbance versus ascorbic acid absorbance prepared by Folin-Ciocalteu reagent and measured at 765 nm. Therefore same amount of ascorbic acid and gallic acid solutions were prepared according to the Folin-Ciocalteu method. The absorbance was measured at 765 nm. These values were used to draw the curve of gallic acid versus ascorbic acid as shown in the Figure A.2. The correction factor, slope of the curve, was determined as 0.7544.

The correction factor was multiplied with the ascorbic acid concentration of the sample measured by titrimetric method. That value represents the effect of ascorbic acid concentration on reagent of Folin-Ciocalteu. Therefore, it was deducted from the total phenol values obtained and calculated from spectrophotometer.

2.3.3.2. Correction of Reducing Sugar

Reducing sugar also contributes to the Folin-Ciocalteu reagent so this can be compensated with a standard correction (Asami et al., 2003). The correction factor was found with the same approach of ascorbic acid correction. Figure A.3 shows the effect of reducing sugar concentration on the reagent of Folin-Ciocalteu. The correction factor to correct the reducing sugar effect on the total phenol amount was found as 0.0891. The correction factor was multiplied with the reducing sugar content of the sample measured titration method. That value represents the effect of reducing sugar on the reagent of Folin-Ciocalteu. Therefore, it was deducted from the total phenol values obtained and calculated from spectrophotometer and corrected with the ascorbic acid correction.

2.3.4. Antioxidant Activity

Antioxidant activity of each sample determined according assay described by Brand-Williams et al. in 1995. The assay involves the color change due to reduction of free radicals by phenolic compounds.

Different researchers determined different reaction times for their studies. The reaction time was determined according to time to reach a plateau with a phenolic compound, gallic acid. Gallic acid has an intermediate reaction rate (Sanchez-Moreno et al., 1998). Therefore, different concentrations of gallic acid solutions (50 mg/L, 25 mg/L and 5 mg/L) were prepared. These solutions were used as samples for the antioxidant activity assay. Absorbance of the samples was measured at equal interval time, 15 min until reach a plateau. Plateau time of smallest concentration, 5 mg gallic acid/L, was determined as 45 min. As a result, the reaction time was selected as 1 hour to eliminate the antioxidant compounds have smaller reaction rate than gallic acid.

1 ml juice sample was mixed with 9 ml methanol (Merck, Germany) to dissolve the antioxidants. 0.05 ml of this methanol-juice mixture and 1.95 ml of 25 mg/L DPPH (Sigma, D 9132) solution were mixed. After holding the mixture for 1 hour in a dark room, the absorbance was measured by spectrophotometer (Analytic Jena SPECORD 50, Germany) at 518 nm against a blank of methanol.

The standard curve was shown in the figure A.1.4. The relationship between the absorbance and DPPH (mg DPPH / liter methanol) found as

$$A_{517}=0.0298*[DPPH] \qquad (R^2= 0,998) \qquad (2.6)$$

A is absorbance at 518 nm determined by scanning of the spectrum of spectrophotometer (Figure A.4).

Antioxidant activity was expressed according to the percentage of reduction of DPPH. Percentage of reduction DPPH was calculated as

$$\% \text{ DPPH}_{\text{reduction}} = ([\text{DPPH}]_0 - [\text{DPPH}]_t)*100 / [\text{ DPPH}]_0 \quad (2.7)$$

where;

$[\text{DPPH}]_t$: Concentration of DPPH 1 hour later

$[\text{DPPH}]_0$: Initial concentration of DPPH

2.3.5. Cloud Appearance

After treatment samples, microfluidized with different pressure and cycle and non-microfluidized orange juices, were placed into plastic centrifugal tubes and stored in a cold room (4 ^{0}C) to measure time of serum separation and observe the changes of juices compared to control sample (non-microfluidized orange juice). They were stored in cold room for 14 days due to safety of juices in terms of microbial load.

2.3.6. Cloud Stability

Cloud stability of samples was determined by measuring the loss of opalescence of juices with method descripted in the book of Cemeroğlu (2007). 10 ml of juice was centrifuged (Sigma 2-16 PK, Germany) at 370 g for 10 min at room temperature. Percent transmission of upper portion of juices was measured using spectrophotometer at 650 nm against blank of distilled water. The results were analyzed as follows: 0-24 % no loss opalescence, 24-35 % slight loss opalescence, 36-60 % pronounced loss opalescence and 61-100 % extreme loss opalescence (Cemeroğlu, 2007).

2.3.7. Pectin Methyl Esterase (PME) Activity

Activity of PME was determined by titrimetric procedure (Kimball, 1991). Previously pectin-salt solution was prepared by mixing 10 grams pectin (Sigma-Aldrich, P8471) and 15.3 grams NaCl (Sigma-Aldrich, S6191) into one liter distillated water. 10 ml of sample and 40 ml of pectin-salt solution were mixed into the beaker, a magnetic stirring bar was added and temperature of contents of beaker was made 30 ^{0}C by heating on heater (Wisestir, WSH-20A). Then a pH electrode (Mettler Toledo, MP 220) was inserted into the beaker.

While stirring and maintaining constant temperature, pH of the solution was made 7.0 by adding of 2.0 N NaOH (Sigma-Aldrich, 221465) from a disposable Pasteur pipette. While adding 0.01 N NaOH from another disposable Pasteur pipette pH of the mixing was made between 7.6 and 7.8 and pH was recorded. Then 0.2 ml of 0.1 N NaOH was added and timer was started to measure time to take regain recorded pH. Each test was performed in triplicate. Finally activity of PME was calculated according to below equation.

PEU=rate=(volume titrated)*(NaOH Normality) / (reaction time)*(sample volume)

(2.8)

PEU = rate = (0.2)*(0.1) / reaction time*(10)

(2.9)

2.4. Microscopic Analysis

2.4.1. Microscopic Analysis

Pulpy structure of orange juice was examined with a visible light microscope (Leica DM 3000, Meyer Instruments, Germany). A drop of sample was placed to microscope and observations were done at magnification of 100*.

Chances in the morphology of orange juice and decrease in particle size of suspended particles of juice were also analyzed by scanning electron microscopy (SEM QUANTA 400F Field Emission). After squeezed and processed, 15 ml juice was centrifuged at 8700 *g* for 10 minutes and the supernatant was removed. 10 ml distillated water was added to the remaining solid particles to wash and remove soluble compounds and it was centrifuged 8700 *g* for 10 min again. The washing procedure was duplicated. After washing, the insoluble suspended particles of orange juice were dried at 37 ^{0}C for 48 hours in incubator (Electro-mag, M 420B). The dried particles were analyzed by SEM at different magnifications between 5,000* and 30,000*.

2.5. Statistical Analysis

The analysis of microfluidization on orange juice characteristics was conducted by a two way Analysis of Variance (ANOVA) with pressure and cycle as factors. Statistical evaluation was conducted with Minitab 15 Statistical Software after providing the necessary assumptions; normality test and equal variance assumption (Barlett test). Significant differences between the variables were tested by Tukey test whose probability level was $p<0.05$. An example of statistical evaluation was in the appendix part (Appendix C).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Physical Properties

3.1.1. Effect of Microfluidization on Color

The color of a food is a very important factor for consumer by having a noteworthy impression on acceptance. Therefore the influence of microfluidization on color was studied. The effect of microfluidization on CIE parameters (Lab) and change of total color (ΔE) of orange juice were represented respectively in the Table 3.1.

The comparison of microfluidized and non-microfluidized orange juices showed that there was a minor decrease in the brightness (L) of the orange juice microfluidized with low pressure (34 MPa). On the other hand, rising of pressure and cycle made the orange juice brighter. Moreover, it was measured that microfluidization caused to decrease of redness (a) and yellowness (b) of the orange juice. However, increment in pressure and cycle of treatment induced the enhancing of redness and yellowness of orange juice. As a result, product of microfluidized orange juice became brighter but less yellow and red color. The reason of this decrease in yellowness and redness may have been collection of smallest particles and sedimentation of them. Betoret et al. (2009) studied the effect of homogenization on the color of citrus juices and found the similar results. Therefore, decrease in particle size and interaction between the particles for the collection of them may have played an important role on the color of the orange juice.

Table 3.1 Influence of microfluidization on CIE-Lab parameters and ΔE of orange juice

	L	**a**	**b**
Fresh juice (control)	74.28±0.30	2.69±0.15	41.33±0.96

	1 cycle				2 cycles				3 cycles			
Pressure (MPa)	**L**	**a**	**b**	**ΔE**	**L**	**a**	**b**	**ΔE**	**L**	**a**	**b**	**ΔE**
34	72.46±0.21	0.15±0.09	33.22±0.43	8.68±0.43	72.71±0.11	0.12±0.07	33.40±0.47	8.47±0.17	73.15±0.14	0.12±0.04	33.49±0.26	8.23±0.22
69	75.10±0.19	0.13±0.02	35.01±0.19	6.87±0.11	75.98±0.09	0.59±0.09	36.10±0.06	5.90±0.10	76.53±0.24	1.08±0.11	37.28±0.30	4.92±0.02
103	76.92±0.31	1.11±0.03	37.20±0.23	5.17±0.09	78.26±0.15	1.47±0.04	38.70±0.10	4.95±0.14	79.29±0.02	1.77±0.06	39.68±0.12	5.39±0.05
138	78.98±0.04	1.43±0.12	39.19±0.11	5.34±0.05	80.52±0.32	2.30±0.04	41.40±0.28	6.28±0.04	81.42±0.19	2.78±0.14	42.50±0.40	7.26±0.15

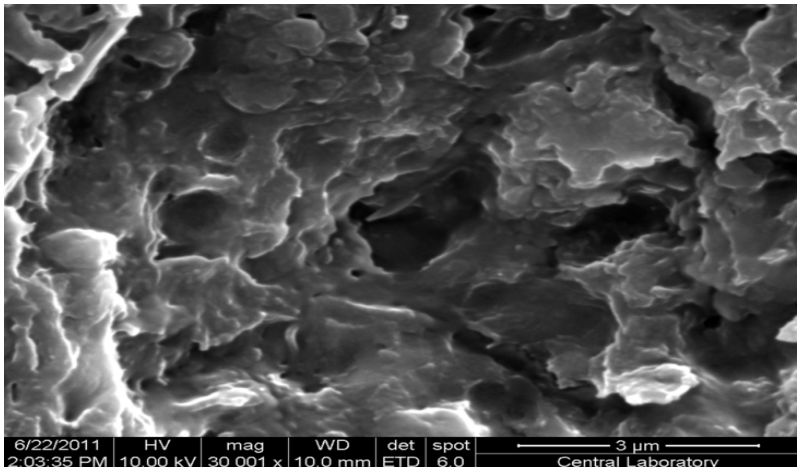
Total color chance (ΔE) was calculated based on the non-microfluidized, freshly squeezed juice. Frances and Clydesdale (1975) determined the lower limit in visual perception for ΔE as 2. Lee et. al. (2003) also studied on pasteurized orange juice and fresh orange juice and found the same result. All the juices treated by microfluidized had a higher value than 2 and the difference was statistically significant ($p<0.05$). This clearly showed that microfluidization changed the color of orange juice. Color of orange juice primarily depends on carotenoid pigments and the change is in relation with the technological treatments and processing conditions (Vikram et al., 2005, Lee et al., 2003). Although there is too little information about the effect microfluidization treatment on food systems, it may have influenced on the carotenoid pigments as occurred in phenol. Therefore, it may have changed the color of orange juice by affecting the carotenoid pigments presenting in juice.

The other contribution to color change of juices may have been the absence of pulp. Microfluidization had very important influence on the pulpy structure of juices represented in the images of Scanning Electron Microscopy (Fig 3.1). Low pulp juices differed significantly in terms of total color change because of homogenization (Betoret et al. 2009). The study conducted by Frances et. al. (1975) pointed that color of orange juice is pulp content dependent. Therefore, microfluidization may have affected the color of orange juice by influencing the pulp content of orange juice by homogenization.

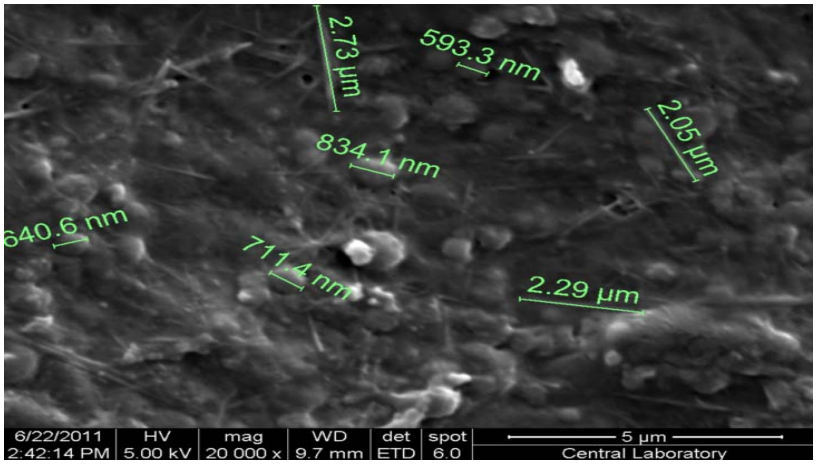
To decide the significance effect of microfluidization parameters (pressure & cycle) on color of orange juice samples analysis of variance was carried on. The analysis pointed that both pressure and cycle had very significant effect on the color parameters (L, a and b) and total color difference (ΔE) ($p<0.05$) and all orange juice samples were found significantly different from each other.

3.1.2. Effect of Microfluidization on Particle Size

Images of the scanning electronic microscopy evidently showed that microfluidization broke the fibrous structure of the orange juice into micro fibrils.



a- Freshly squeezed orange juice 30 000*



b- Microfluidized orange juice at 138 MPa 20 000*

Figure 3.1 SEM images of orange juices

The size of the non-processed orange juice solids could not be measured but the processed orange juice at 138 MPa and 3 cycles had very small particulates and fibrils as seen in the Figure 3.1.

Table 3.2 Volume Weighted Mean D[4,3] (um) of different orange juices

Fresh juice (control)	710.2±26.74		
Pressure (MPa)	**cycle 1**	**cycle 2**	**cycle 3**
34	77.6±1.40	59.5±0.11	53.7±0.45
69	48.5±0.40	40.3±0.32	34.3±0.29
103	36.2±0.25	30.7±0.52	26.1±0.21
138	37.5±0.70	33.3±0.62	23.2±0.52

Table 3.2 shows the influence of microfluidization pressure and cycle on the particle size of the orange juices. Variance of analysis with confidence level 95 % was carried on to observe the effects of parameters. Both pressure and cycle had very important influence on the size of the particles of the orange juice ($p<0.05$). When the comparison was done between the microfluidized and non-microfluidized orange juices, there was a great difference between them. Microfluidization decreased the volume weighted mean of orange juice between 90 % and 97 %. These results may probably have showed that microfluidization may have exchanged suspended pulpy structure to the colloidal pulp and may have changed the structure of the particles by broken them from different parts. The effect of microfluidization on particle size distribution can be analyzed in detail by looking at the percent volume of particle size versus particle size curves.

Figure 3.2 summarized the effect of treatment pressure on the particle size distribution of orange juices. Increase in the pressure led to reduction of the size of suspended particles inside the orange juice. Although harsh treatment contributed to the reduction of particle size of orange juice, it could not have been necessary to get a high quality product in terms of smaller particle size because the smallest pressure and cycle combination of microfluidization treatment of our research (34 MPa & 1 cycle) performed very well (90% reduction).

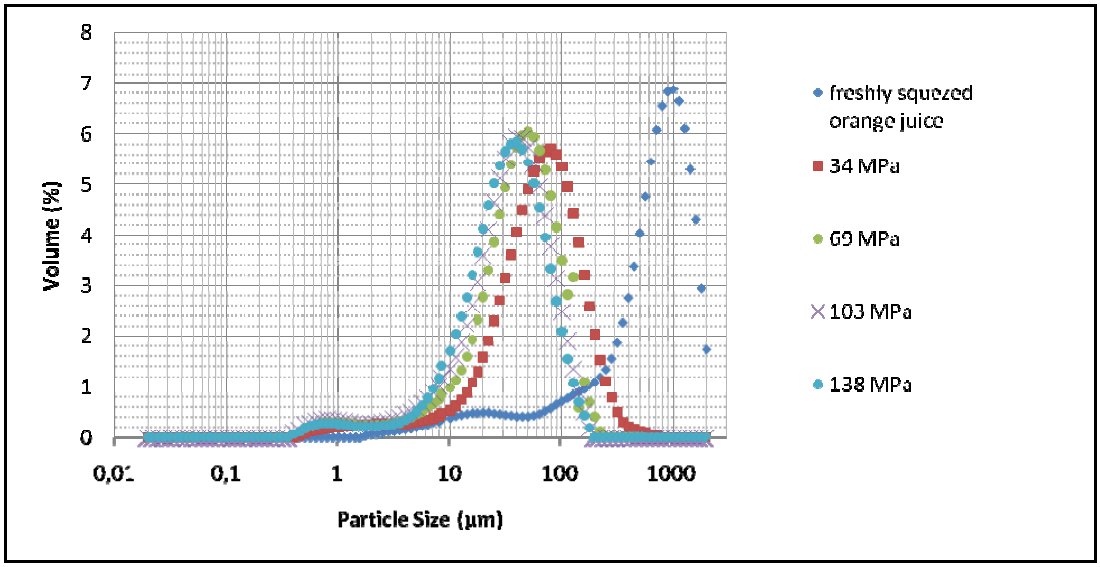


Figure 3.2 Influence of microfluidization pressure on the particle size distribution of orange juice

On the other hand, increment in the passes number of orange juice through the microfluidizer caused the broken down of aggregaration structure and decrement of the size of particles. Figure 3.3 displays the influence of cycle on the particle size distribution of orange juice at a constant pressure (34 MPa).

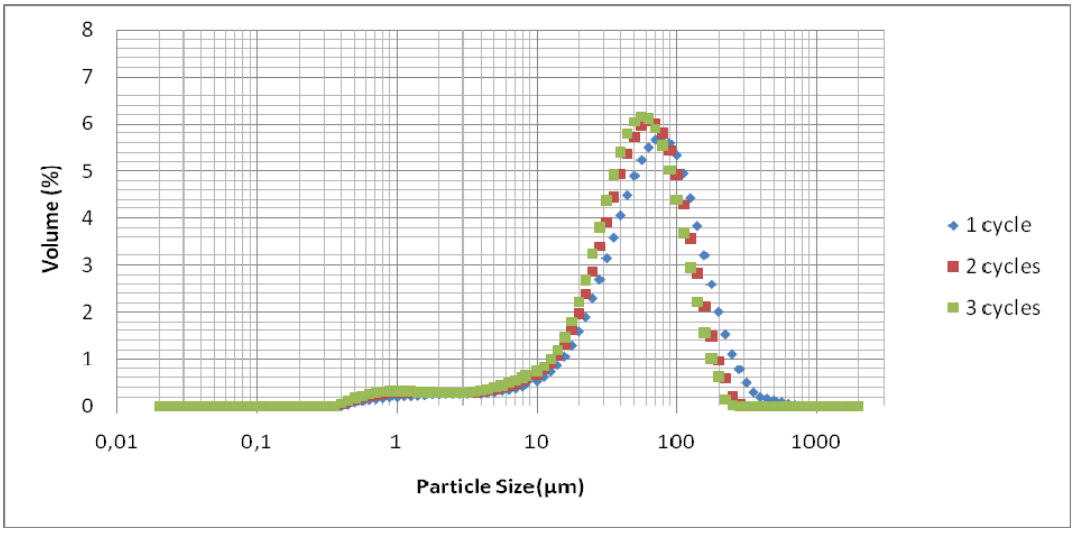


Figure 3.3 Effect of cycle on the particle size distribution of orange juice microfluidized at 34 MPa

This decrease in the particle size due to mechanism of microfluidization may have probably imparted the slowing the clarification of orange juices. The more microfluidized orange juice, the more reducing the particle size of juice and the slower of turning from opaque to transparent was observed. Therefore, microfluidization increase the stability of the orange juice by reducing the size of the suspended particles and effecting the distribution of the particles, resulting in high quality because cloudiness is an important quality factor for the citrus products. Baker and Cameron (1999) studied the cloud of citrus juices and emphasized that cloud stability is mainly relation with size of particles. Since low particle size microfluidization can be applied as an alternative process for this type of products for consumers do not like high pulp content orange juice to get a high quality product.

3.1.3 Effect of Microfluidization on Brix

Brix indicating the percentage of soluble solid content of a solution is one of the most important physical properties of the citrus juice because it is used to grade the quality of the citrus juices. Microfluidization pressure had a significant effect on the brix of the orange juice ($p<0.05$). Microfluidized orange juices gave higher brix after processing. Brix of the orange juice was increased about 4 % by microfluidization. Table 3.3 shows the effect of microfluidization on the brix of the orange juices.

Table 3.3 Brix of orange juices

	Brix (%)
Fresh orange juice	9.3±0.42
Lest microfluidized orange juice(34 MPa & 1 cycle)	13.4±0.57
Most microfluidized orange juice(138 MPa & 3 cycle)	12.5±0.71

3.1.4 Effect of Microfluidization on Browning Index

Browning index which is absorbance of orange juice is related to the change of color to brown. Browning is important in most of the products because browning reactions result in unwanted off-taste and off-color (Handwerk, 1988). Therefore, influence of microfluidization on the browning index of orange juices was measured. Statistical evaluation showed that microfluidization pressure had a significant role on browning index however cycle not. The results of different

pressure applications were presented in the Figure 3.4. As seen, increase in pressure caused to increase in the absorbance of orange juice.

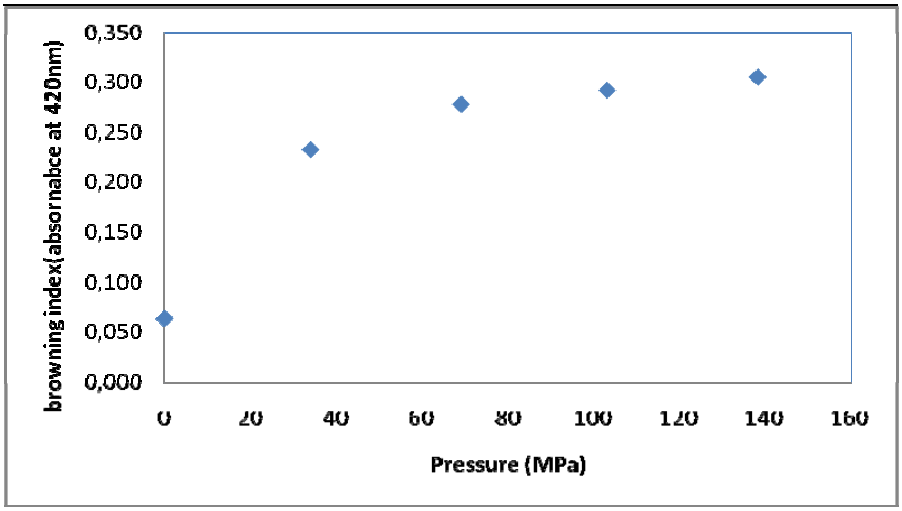


Figure 3.4 Effect of microfluidization on browning index of orange juice

Browning happens many fruits or vegetables like apple, banana, and potato possible due to the low composition of ascorbic acid (Saper & Miller, 1995). However, orange juice contains high amount of ascorbic acid preventing their browning and also microfluidization did not decrease the amount of ascorbic acid. The indicator of non-enzymatic browning reaction is the reducing sugar degradation. However, it was found that reducing sugar amount of processed and non-processed juices were same. Therefore, the expected result was that microfluidization would not have caused of browning of orange juice. However, there was a huge difference between microfluidized and non-microfluidized orange juices. This increase of absorbance may have related with the decrease in particle size not related with the browning of orange juice. Before the measurement of absorbance, orange juices were centrifuged very slowly in very short time to remove the coarse particles.

Microfluidization broke these particles so the processed juices contained these particles with small sizes and the power of the centrifugation might have afforded to remove them. The particles could have remained in suspense so the absorbance (browning index) increased not indicating the browning of orange juices.

3.2 Chemical Properties

3.2.1 Effect of Microfluidization on Ascorbic Acid Content

Orange juices are a rich source of ascorbic acid and the amount of the ascorbic acid in the orange juice is a significant indicator of nutritional quality. Ascorbic acid is sensitive to the many processes like storing, cooking and the loss of ascorbic acid indicates the negative effects of the process because ascorbic acid can be broken easily by oxidation (Cemeroğlu, 2007). Therefore, in most of the production processes the concentration of ascorbic acid is analyzed to ensure the high nutritional product and efficient process. There are many factors let to decrease the ascorbic acid in the orange juice like oxygen, temperature, pH, initial amount of ascorbic acid, concentration of microorganisms inside the juice and sugar and salt concentration (Tannebaum, 1985). However in this research the mentioned factors were kept constant. Microfluidization resulted in the rise of the temperature of the juices during the process. In order to eliminate this problem water-ice mixture was circulated during the microfluidization so that the temperature of the operation was kept constant about $18 \pm 2\ ^{0}C$. pH values of the samples were measured and it was seen that their pH values were not effected by the process. The other factors were mostly related to the source of the juice. All the samples were taken from the same juices and microfluidized. Although many factors kept constant, it could have been possible that microfluidization may speed the oxidation process of the ascorbic acid with the effect of pressure. However the results showed that ascorbic acid amount did not change with the process of microfluidization.

In all the juices, microfluidized with different pressures and cycles, analyzed for this research, the ascorbic acid content of the juices was found to be similar. Statistical evaluation indicated that both pressure and cycle did not affect the ascorbic acid content of the orange juices ($p<0.05$). Therefore, ascorbic acid amount did not vary with microfluidization treatment and it kept the same quantity as in freshly squeezed juice.

Table 3.4 represents the ascorbic acid content of the freshly squeezed juice as control, 34 MPa & 1 cycle microfluidized orange juice as the lest processed juice and 138 MPa & 3 cycle microfluidized orange juice as the most processed juice.

Table 3.4 Amount of ascorbic acid of the orange juices

	mg ascorbic acid/ liter juice
Fresly squeezed juice	455.7 ± 27.02
34 MPa & 1 cycle microfluidized juice	446.2 ± 20.66
138 MPa & 3 cycle microfluidized juice	450.5 ± 30.10

The non-microfluidized orange juice included 455.7±27.02 mg ascorbic acid/liter juice. There is a good understanding between our results and those in the literature (Topuz et al., 2005). The lest and most microfluidized juices containing 446.2±20.66 mg ascorbic acid/liter juice and 450.5±30.10 mg ascorbic acid/liter juice, respectively indicating clearly that microfluidization did not effect the nutritional quality of the orange juices. Suárez-Jacobo et al. (2011) studied on the

impact of ultra high pressure homogenization on vitamin C in apple juice and found that the content of ascorbic acid did not vary with different pressure applications.

On the other hand the most important problem with the ascorbic acid is the loss of it during the storage. However our study focused on only the effect of mechanism of the microfluidization to the amount of ascorbic acid as an indicator of nutritional quality.

Although ascorbic acid quantity primarily related with the nutritionary of the orange juice, it is also crucial to find total phenol content of the juices. Ascorbic acid gives reaction with Folin-Ciocalteu phenol reagent (Asami, 2003). Therefore, an ascorbic acid correction was done to find the total phenol content of the juice. On the other hand, ascorbic acid could have played an important role on the antioxidant capacity of the juices. However, the results showed that role would be same for all the juices microfluidized or not.

3.2.2 Effect of Microfluidization on Reducing Sugar Content

The importance of determination of reducing sugar for our research was the correction of the total phenol content. Reducing sugars may have impact on the Folin-Ciocalteu phenol reagent (Silinkard et. al., 1977). As a result, in determination of the total phenol a correction had done to get the accurate outcome. Although reducing sugar had a very minor effect on the Folin-Ciocalteu phenol reagent, a correction factor was determined to adjust the final results of total phenol.

The reducing sugar of the juice was determined 5.9±0.14 g/100 ml juice for the fresh juice. There is a good correlation between our result and those in the literature (Topuz, 2005). It was also found there was no difference between the juices whether processed or not, showing that microfluidization did not impact on the reducing sugar content of the orange juice as seen in the Table 3.5.

Table 3.5 Amount of reducing sugar of the orange juices

	g reducing sugar/100 ml juice
Fresh orange juice	5.9 ± 0.14
Lest microfluidized juice (34 MPa & 1 cycle)	5.9 ± 0.07
Most microfluidized juice (138 MPa & 3 cycle)	5.8 ± 0.14

The most important problem with the reducing sugar is sugar-amino acid reaction causing of browning of the orange juice (Kaanane, 1988). However, it was discovered that below pH 6, browning because of the sugar and amino acids was not important (Ashoor, 1984). pH of our orange juices varied from 3.9 to 4.1. Therefore, there was no difference between the reducing sugar content of the fresh juice and microfluidized juices.

3.2.3 Effect of Microfluidization on Total Phenol Content

Orange juices were processed at different pressures (34, 69,103 and 138 MPa) and cycles (1, 2 and 3). Their phenol contents were determined and compared with the control sample, freshly squeezed orange juice. Folin-Ciocalteu phenol reagent is interfered with ascorbic acid and reducing sugar content. Therefore, ascorbic acid and reducing sugar contents of the processed juices were determined and they were deducted. Finally total phenol content of non-microfluidized orange juice, control sample, was found as 536.96 ± 9.00 mg phenol/L juice after the corrections. All of the microfluidized juices had higher phenol content. On the other hand, statistical analysis showed that pressure had a significant effect on the total phenol content however cycle had not a significant effect. Moreover, Tukey Comparison Test represented that 69, 103 and 138 MPa pressures had same effect and they were not

significantly different from each other but 34 MPa was significantly different from them.

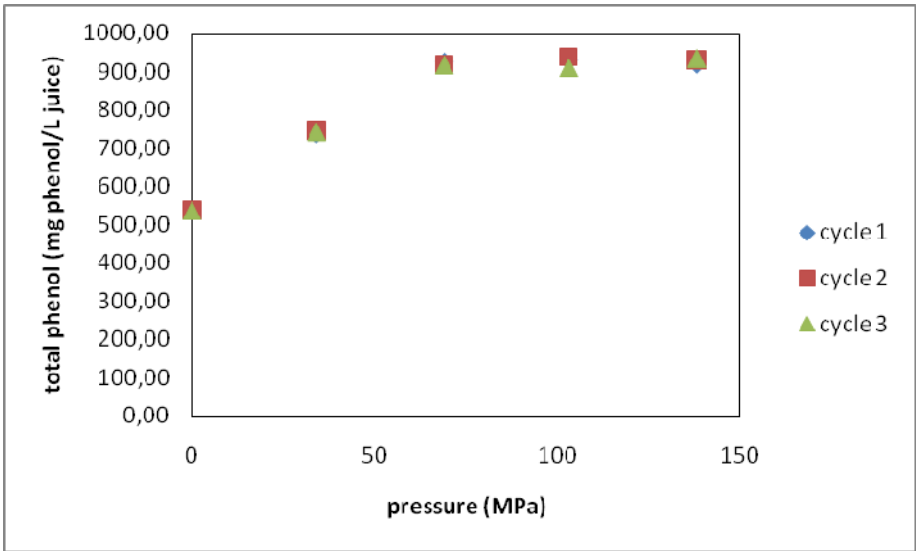


Figure 3.5 Effect of microfluidization pressure on phenol content

Figure 3.5 represents the effect of microfluidization on the total phenol content of the orange juice. As seen in the figure, microfluidization increased the phenol content of the orange juice. The increase of total phenol content may be correlated with the increase the extractability of some of phenolic compounds of the juice. 34 MPa pressure microfluidization increased the phenol content of the orange juice about 37 % while 69 MPa microfluidized orange juice had about 72 % higher phenol content than the control sample. It is also clear that increase in pressure led to increase in phenol content of the sample up to a point (about 70 MPa). After that point phenol content remained constant even juice was microfluidized more.

3.2.4 Effect of Microfluidization on Antioxidant Activity

Measured antioxidant activity represents the ability of sample to reduce the free radicals in the medium. Figure 3.6 shows the effect of microfluidization on the antioxidant activity. As seen in the figure, microfluidization made the juice more active to reduce the free radicals.

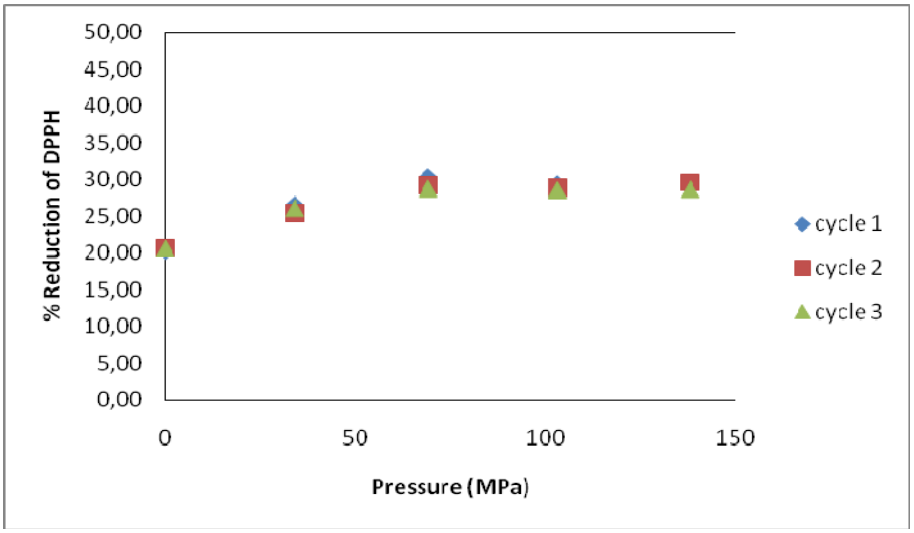


Figure 3.6 Effect of microfluidization pressure and cycle on antioxidant activity

According to statistical evaluation pressure was an effective factor but cycle was not. Increase in pressure led to increase the antioxidant activity up to a point as occurred in the total phenol change. Therefore, the increase of antioxidant activity seems related to phenolic content of the juice. This was not a surprising result because of the antioxidant actions of phenolic substances. Same results were achieved by research of Rapisarda et al in 1999, according to their study there was a direct correlation between the antioxidant effectiveness of orange juices and their total phenol contents and they found that phenolic compounds significantly contribute to the antioxidant capacity of orange juices.

The relationship between the phenolic compounds and antioxidant property depends on many factors like chemical structure of individual component, conditions of applied assays and interactions between the components (Huang et al., 2005). In our research the correlation analysis between the total phenol content and antioxidant activity revealed the importance role of the phenolic compounds as indicated in the Figure 3.7.

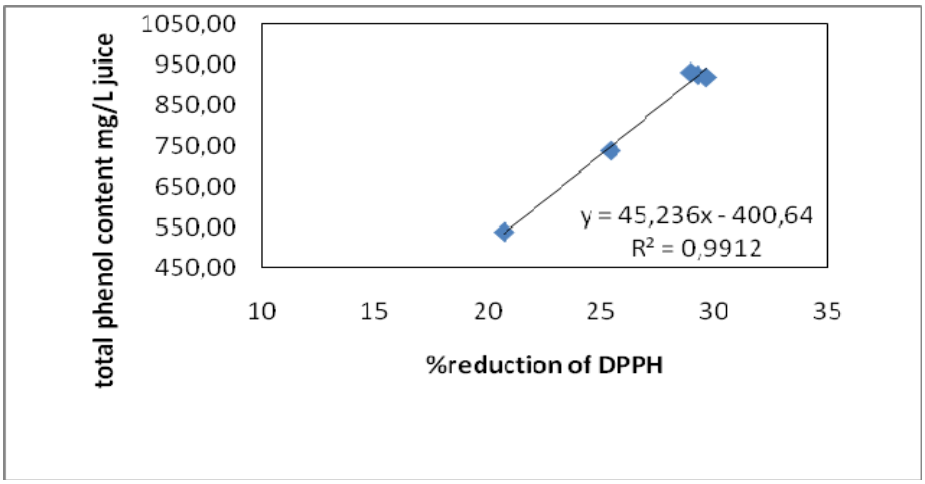


Figure 3.7 Correlation of total phenol content and antioxidant activity of orange juice

Moreover, microfluidization increased the soluble solid content about % 4 (34 MPa, 1 cycle). This may indicate that microfluidization increased the water soluble pigments by affecting the structure of them. Some research shows that water soluble pigments contribute to the antioxidant activity of the juices. (Rapisarda et al., 1999 and Arena et al., 2001).

The other important item for antioxidant capacity for the orange juices is the content of the ascorbic acid which is a very significant antioxidant (Miller et al, 1997). However, the outcomes of analysis of ascorbic acid content indicated that all the juices, microfluidized or not, had same quantity of ascorbic acid so antioxidant activity would be impressed with the same manner for all the juices.

3.2.5 Effect of Microfluidization on Stability

The cloudy appearance of both microfluidized at different pressures (34, 69,103 and 138 MPa) and different cycles (1, 2 and 3) and non-microfluidized orange juices were initially homogeneous and opaque (Fig 3.8). By simple inspection any difference was observed between them. They were stored in a cold room (4 ^{0}C) to observe serum separation showing the juice stability. After 7 hour storing, the non-microfluidized orange juice started turning from opaque to transparent because pectin methyl esterase and pectic substances inside the orange juice reacted, that resulted in loss of cloudy appearance (Haard, 1985). However, there was no serum separation at the microfluidized orange juices for 14 days (Fig 3.9 & Fig 3.11).

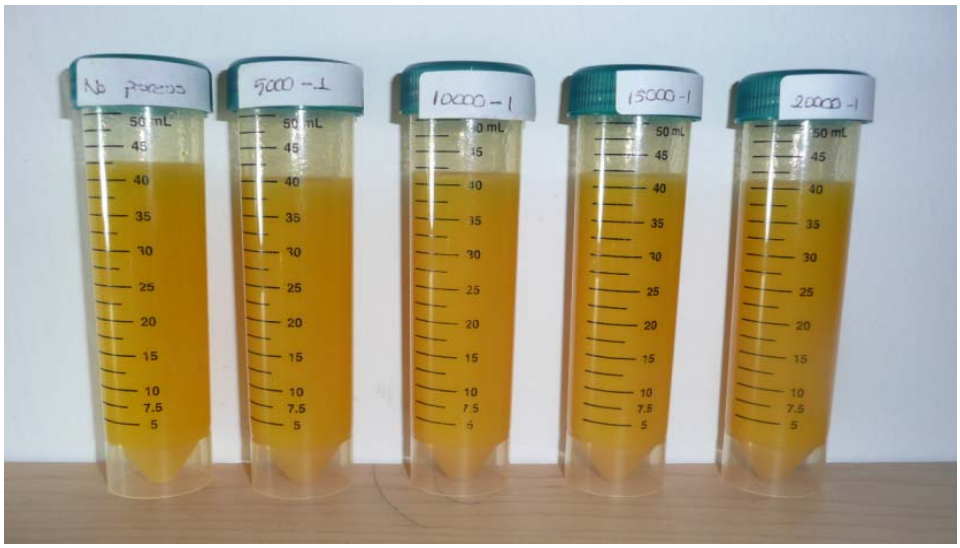


Figure 3.8 Stability of orange juices right after squeezed and microfluidized at different pressures

Figure 3.9 Stability of orange juices after 14 days storage at 4 ^{0}C after squeezed and microfluidized

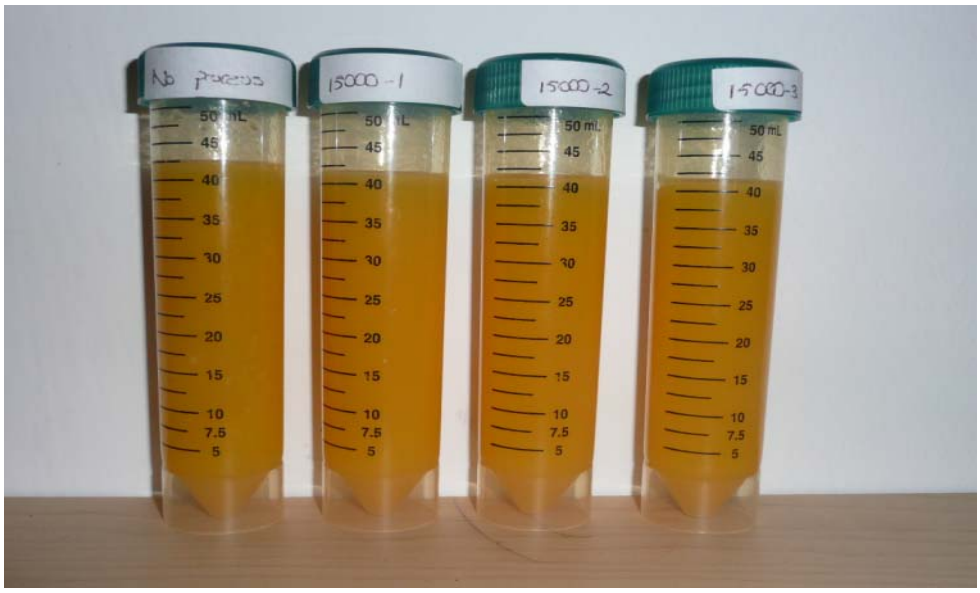


Figure 3.10 Stability of orange juices right after squeezed and microfluidized at 103 MPa (15000 PSI) and different cycles (1, 2 and 3)

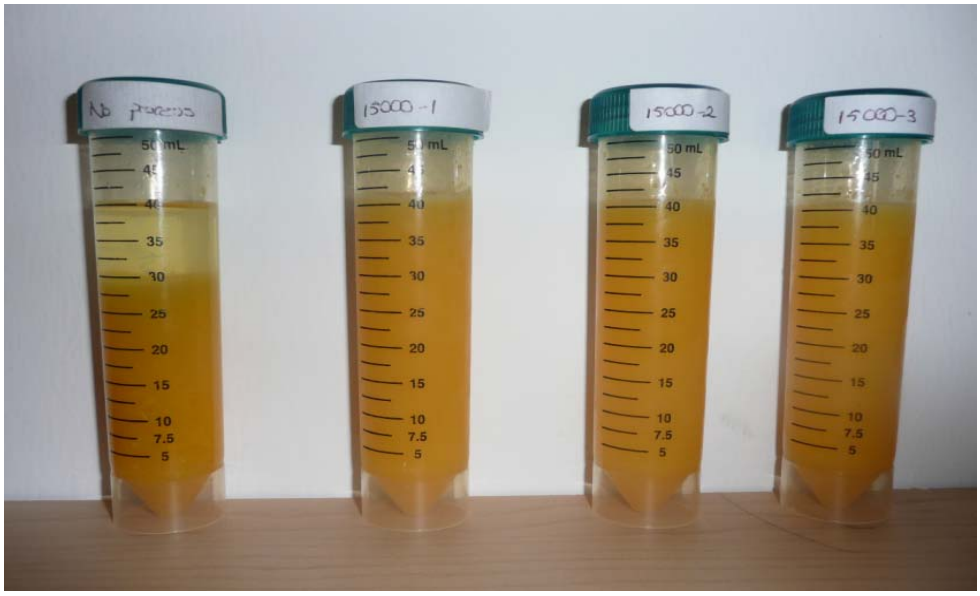


Figure 3.11 Stability of orange juices after 14 days storage at 4 ^{0}C after squeezed and microfluidized at 103 MPa (15000 PSI) and different cycles (1, 2 and 3)

To be stable for 14 days showed that microfluidization had an importance effect on the juice stability. No loss of cloud appearance may have been due to reduction of particle size of orange juice. The effect of microfluization on the enzyme activity of pectin methyl esterase also may have had an important role on the juice stability. Although there was no serum separation up to 14 days, storing in the cold room was ended because of the safety in terms of microorganisms.

3.2.6 Effect of Microfluidization on Cloud Stability

In citrus juices, an opaque appearance is considered as an important quality factor affecting acceptance of consumers. Storing in cold room gave information about the stability of the juices and showed that microfluidization made orange juice more stable. However, the difference between the samples operated different conditions could not be observed. Table 3.6 shows transmittance (cloud stability) related to stability of the juices processed at different pressures and cycles. Fresh juice had a transmission about % 45 meaning of definite cloud loss while the microfluidized samples had transmissions between % 2.5 and % 4.5, which means no cloud loss (Cemeroğlu, 2007). Moreover, for the quality control % 36 transmissions is used as an upper limit in the orange juices. (Bayındırlı et. al., 2006) The limit was satisfied with all of the processed juices.

Table 3.6 Cloud stability of orange juice % Transmission at 650 nm

Fresh squeezed orange juice (control) 45.18±0.64			
Pressure (MPa)	**cycle 1**	**cycle 2**	**cycle 3**
34	2.67±0.01	2.89±0.02	2.68±0.11
69	2.74±0.04	2.69±0.09	2.86±0.02
103	2.89±0.01	3.04±0.03	3.31±0.01
138	3.24±0.02	3.76±0.06	4.24±0.03

Microfluidization decreased the particle size of juice and made orange juice more homogenized. The cloud stability of the processed juices were very close to zero, thus the stability of juices were increased sharply by the microfluidization. Statistical values showed that both pressure and cycle had important effect on the cloud stability ($p<0.05$). As seen in the table, increase in pressure and cycle resulted in increase of transmission indicating loss of cloudiness. Although higher pressure and cycle caused of decrease in the orange juice stability, microfluidization had very important impact on the juice stability and increased it very sharply in general.

3.2.7 Effect of Microfluidization on Enzyme Activity

The main objective of most of the treatments in citrus juice production is to reduce activity of pectin methyl esterase, which is responsible for the loss of the stability. The best (34 MPa, 1 cycle) and worst (138 MPa, 3 cycle) cloud stability were found as 2.67 % transmission and 4.24 % transmission, respectively as shown in the Table 3.6. Table 3.7 shows effect of microfluidization on freshly squeezed juice as a

control and microfluidized best and worst stable juices. As seen in the table microfluidization made the enzyme more active.

Table 3.7 Pectin methyl esterase (PME) Activity (PEU)

Fresh orange juice (control)	$5.03*10^{-5}\pm0.06*10^{-5}$
34 MPa & 1 cycle microfluidized juice (best stable)	$6.41*10^{-5}\pm0.40*10^{-5}$
138 MPa & 3 cycle microfluidized juice (worst stable)	$8.03*10^{-5}\pm0.06*10^{-5}$

Increased in pressure and cycle caused the loss of cloudiness in orange juices because the pectin methyl esterase (PME) became more active. Microfluidization of orange juice at 34 MPa and 1 cycle increased PME activity 27 % and with same pressure but 3 cycles increased 33 %. Moreover 138 MPa, 1 and 3 cycles resulted in 49 % and 58 % enzyme activation, respectively as represented in the Figure 3.12.

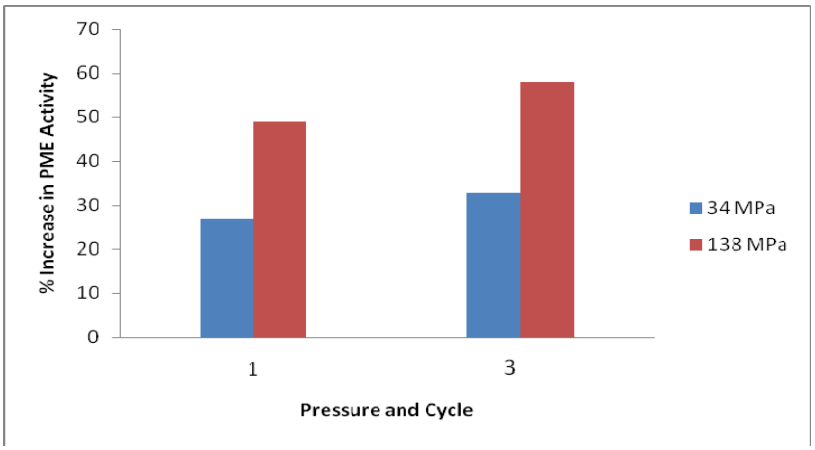


Figure 3.12 Pectin methyl esterase activity at 34 MPa 1 and 3 cycles and 138 MPa 1 and 3 cycles

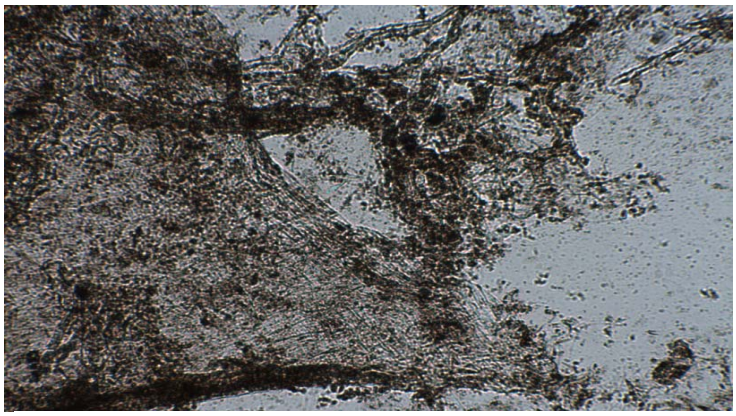
As a result, microfludization caused of the activation of PME and activity depends on the treatment pressure and cycle. Increase in pressure and cycle made the enzyme more active so it became easy for orange juice to turn from opaque to transparent, letting to decrease of juice stability. Liu et al. (2009) microfluidized the Chinese pear to observe the effect of microfluidization pressure and cycle on the polyphenol oxidase activity and found that both pressure and treatment pass had very important effect and they showed that as the treatment pressure and cycle was increased the enzyme activity was enhanced.

Cano et al. (1997) studied the effect of ultra high pressure treatment on the PME activity in the orange juice and obtained pressure treatments produced an activation of PME in the 200-400 MPa range. Many studies also showed that high pressure operations resulted in increasing of enzyme activity for some fruits (Asaka et al. 1991, Guerrero-Beltran et al. 2004). They focused on that high pressure would break isoenzymes and those isoenzymes could have reacted in the food system. Same phenomenon may have occurred at the microfluidization treatment due to the changing of structure and reducing of pulpy configuration as seen in the Figures 3.13, 3.14 and 3.15. Juice stability can be increased by microfluidization, even increase of PME activity because microfludization affected the structure mostly and reduced particle size inside juice.

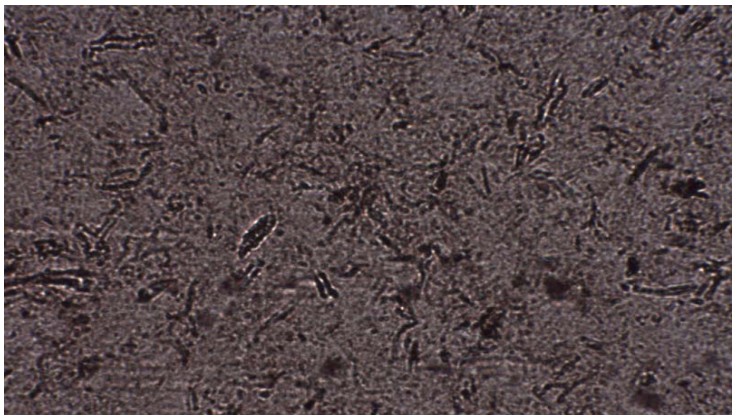
Lacroix et al. (2004) found that the opalescence stability, which does not entirely depends on PME activity but also on particle size reduction and structural changes of pectin, can be increased by the operation high pressure homogenization.

3.2.8 Effect of Microfluidization on Morphology

The aggregaration structure presents in the freshly squeezed orange juice was broken down during the microfluidization as seen in the images of light microscope (Fig 3.13) representing the impact of microfluidization on orange juice.



a. Freshly squeezed orange juice

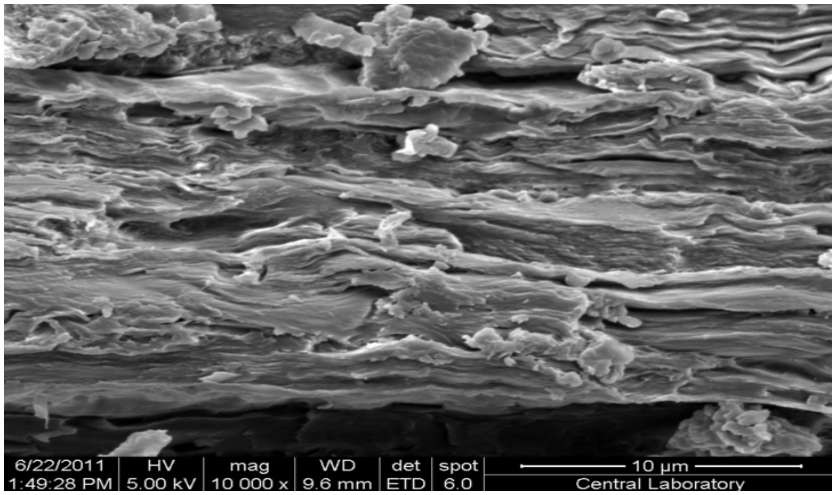


b. Microfluidized orange juice at 34 MPa & 1 cycle

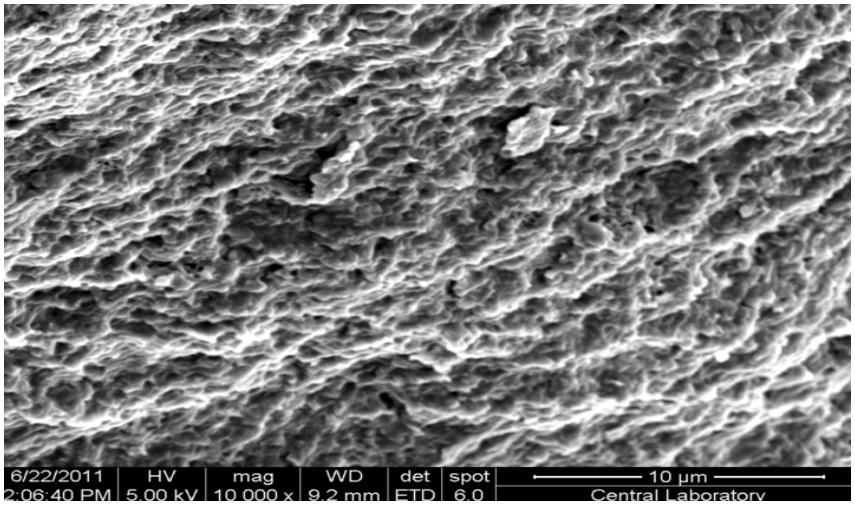
c. Microfluidized orange juice at 138 MPa & 1 cycle

Figure 3.13 Light microscope images 100* of orange juices

The images clearly indicate the effect of microfluidization on the morphology of orange juice. Microfluidization disintegrated the structure of material. As the pressure of microfluidization was elevated the structures were more dissociated.



a. Non-microfluidized orange juice

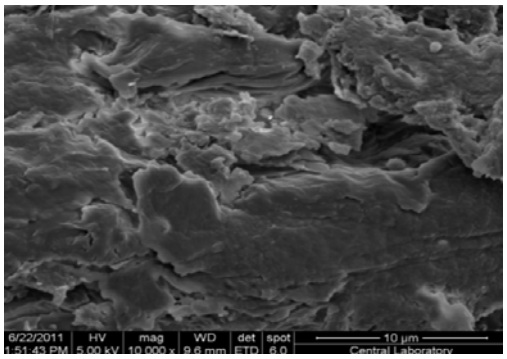


b. Orange juice microfluidized at 34 MPa & 2 cycles

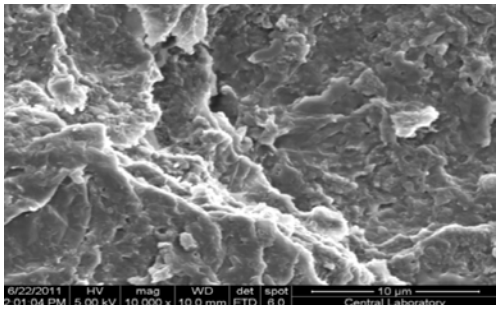
Figure 3.14 Side view of orange juices 10000*

In order to observe the influence of microfluidization in detail Scanning Electron Microscope (SEM) images of the orange juices were taken. Figure 3.14 represents the side view of non-processed and processed orange juices. The samples were washed to remove the sugar and other soluble ingredients. Then, they were centrifuged for accumulation and dried prior to the picturing; this may have caused to be heaped of the aggregarated structures and particles and rough plane appearance rather than fibrous structure. On the other hand, the images clearly showed that there was a huge difference in appearance between the non-microfluidized and microfluidized samples. Therefore, it was clearly realized that microfluidization influenced the structure of orange juice significantly.

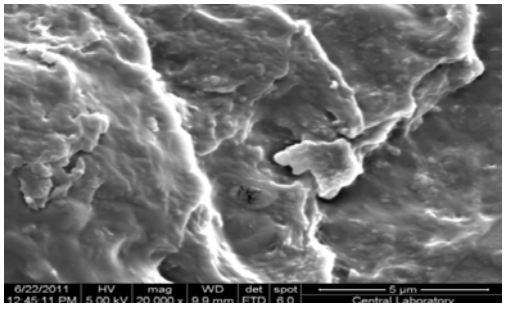
Figure 3.15 represents the effect of microfluidization pressure. As the microfluidization pressure was promoted fragments of the images became smaller and the orange juice solids got more dissociated. Clear porous fragments were presented inside the non-processed juice. This porous structure was also observed at low pressure microfluidized samples even this pressure (34 MPa) may have enhanced the porous appearance but increased in pressure made these structure more smooth at high pressure (138 MPa). Therefore, juices processed by microfluidization had a more homogeneous microstructure enhanced by the more distributed matrix.



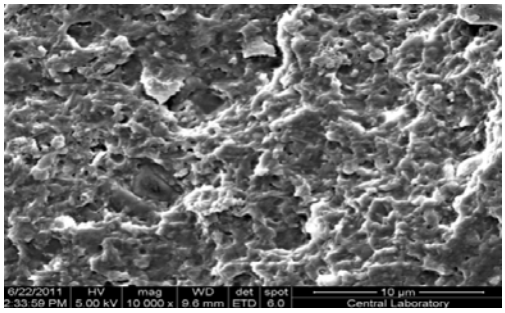
a. Non-microfluidized orange juice 10000*



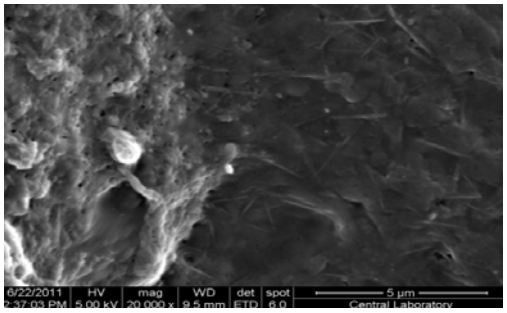
b.Microfluidized juice at 34 MPa 10000*



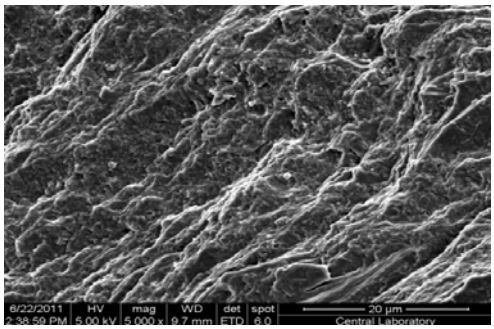
c- Microfluidized juice at 34 MPa 20000*



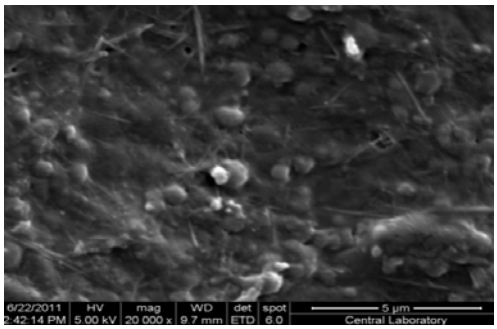
d-Microfluidized juice at 103 MPa 10000*



e- Microfluidized juice at 103 MPa 20000*



f-Microfluidized juice at 138 MPa 10 000*

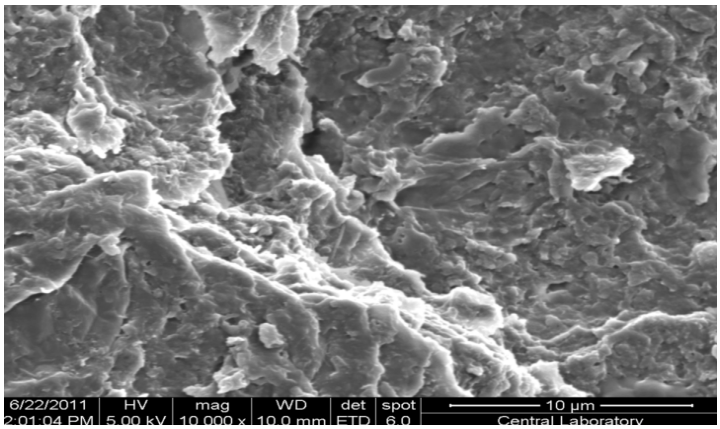


g- Microfluidized juice at 138 MPa 20000*

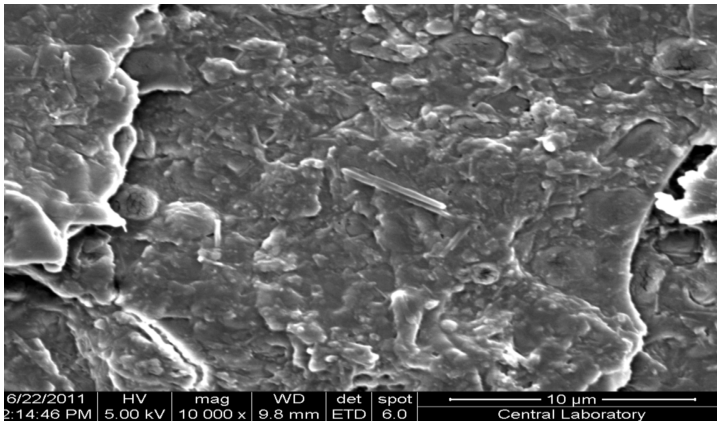
Figure 3.15 Impact of microfluidization pressure on orange juice

The other substantial consequence was the detection of micro fibrils. Fiber structure of orange juices could no be detected in the non-microfluidized juice and low microfluidized juices. However, at high pressures micro fibrils were clearly visible, indicating microfluidization broke the fibers into pieces (micro fibrils). Although the microstructure was shown in Figure 3.15.e, the quantity of these micro fibrils increased and the aggregated structure decreased with pressure as seen in Figure 3.15.g. This may explain the stability of orange juice because micro fibrils may have prevented fibers to come together and constitute the structure of aggregates.

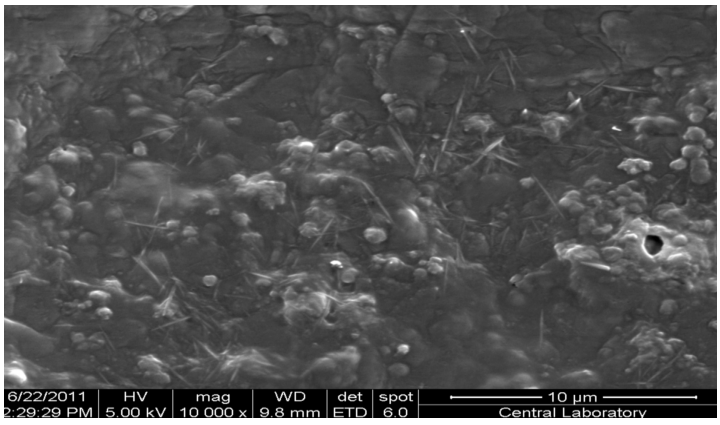
The effect of microfluidization cycle on the morphology of orange juice was very different. Figure 3.16 clearly showed the difference. Although the fibers could not have noticed in the orange juice microfluidized at 34 MPa and 1 cycle (Fig 3.16. a), as increase in the passes number (cycle) the fibers of orange juice was broken down and the amount of formed micro fibrils were increased as seen in the Figure 3.16.c. It was also clear that as the cycle and pressure were increased the size of suspended particles decreased (Fig 3.15 & Fig 3.16).



a. Microfluidized orange juice at 34 MPa & 1 cycle



b. Microfluidized orange juice at 34 MPa & 2 cycles



c. Microfluidized orange juice at 34 MPa & 3 cycles

Figure 3.16 Effect of microfluidization cycle on orange juice morphology 10000*

CHAPTER 4

CONCLUSION

The purpose of the current research is to study influence of microfluidization treatment on physical and chemical characteristics of orange juice. Stability and some quality characteristics of juices were analyzed with respect to microfluidization pressure and cycle. Juice was treated with the combination of 4 different pressures (34, 69, 103 and 138 MPa) and three different cycles (1, 2 and 3) at 18 ± 2 ^{0}C. The physical and chemical properties were analyzed and compared with non-microfluidized, freshly squeezed orange juice.

The examined physical properties were color, particle size, brix and browning index. The results showed that microfluidization caused in increase in brightness and decrease in yellowness and redness of orange juice. Moreover, total color difference also showed that both pressure and cycle changed the color of orange juice. The other important physical property was particle size and the distribution of particle size. Both microfluidization pressure and cycle had very important influence on the size of the particles of the orange juice ($p<0.05$) and microfluidization decreased the volume weighted mean of juice between 90 % and 97 %. The increase in brix about % 4 was measured and it was found that microfluidization pressure caused in increase in browning index. However, this increase could have not been correlated with browning of juice due to no decrease in reducing sugar and ascorbic acid amount of orange juice.

The analyzed chemical properties in this research were total phenol content, antioxidant property, stability and pectin methyl esterase activity. All of the microfluidized juices had higher phenol content compared to fresh juice. Statistical analysis showed that pressure had a significant effect on the total phenol content however cycle had not. On the other hand, it was measured that increase in pressure led to increase in phenol content of the sample up to a point (about 70 MPa). After that point phenol content remained constant even juice was microfluidized more. The antioxidant activity measurements also gave the same results. Increase in pressure led to increase the antioxidant activity up to a point as occurs in the total phenol change. Therefore, the increase of antioxidant activity seems related to phenol content of the juice. As a result, a good correlation between the antioxidant activity and total phenol content of orange juice was found.

The other important concept of our study was the stability of orange juice. It was clear that microfluidization broke down the aggregated structure of orange juice, clearly seen in the images of scanning electron and light microscopes. The microfluidized orange juices were stored at cold room (4 ^{0}C). After 7 hours storage, the non-microfluidized orange juice started turning from opaque to transparent but the microfluidized juices had still been opaque and homogenous for 14 days. Cloud stability results revealed that microfluidization made the orange juice more stable but increase in pressure and cycle resulted in increase of transmission indicating loss of cloudiness. Therefore, the effect of treatment on pectin methyl esterase activity was studied and it was measured that microfludization caused of activation of PME and activity depended on the treatment pressure and cycle. Increase in pressure and cycle made the enzyme more active so it became easy for orange juice to turn from opaque to transparent, letting to decrease of juice stability.

CHAPTER 5

RECOMMENDATION

The current study revealed that by the application of high pressure microfluidization, quality parameters such as color, nutrient content and antioxidant property, and stability of orange juice can be improved. It is advisable to concentrate on the effect of microfluidization on physical and chemical characteristics during the storage of orange juice. The other recommendation can be to analyze the influence of treatment on microbial load of orange juice due to effect of pressure on microorganisms.

The next recommendation is the treatment of other juices containing high amount of fiber or bioactive compounds with microfluidizer to improve the physical and chemical properties of them and enrichment of juice with micro fibrils and increase the function of bioactive compounds. For example, it can be used as a treatment for apricot juice for high amount of fiber and for tomato juice for high content of bioactive compounds. Therefore, microfluidization can be used for manufacturing for fruit and vegetable juices.

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APPENDIX A

FIGURES OF MATERIALS AND METHODS

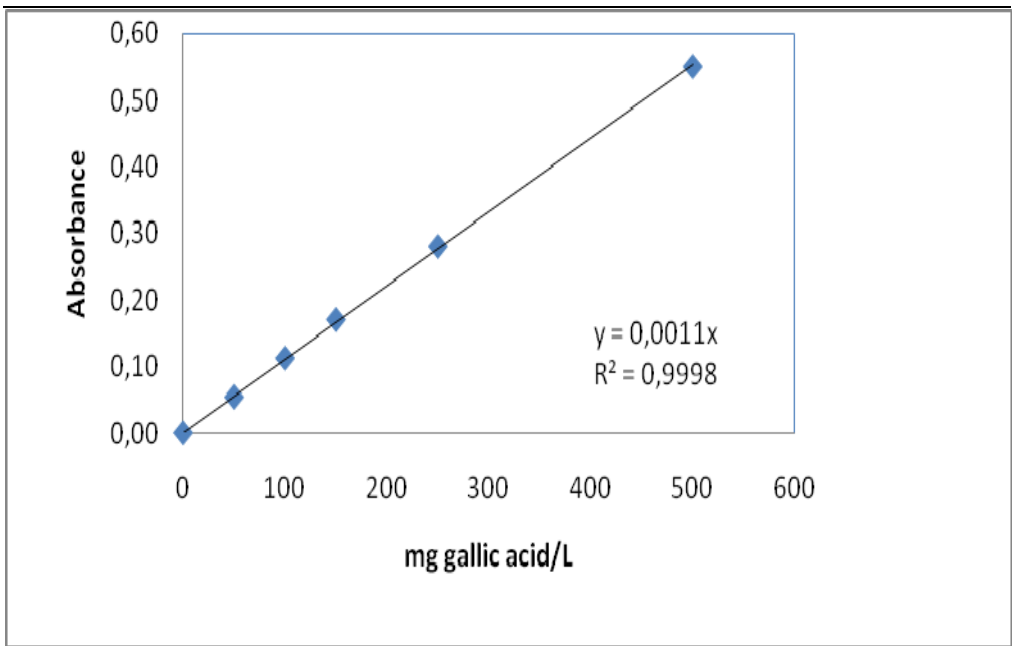

Figure A.1 Gallic acid standard curve for determination of total phenol content

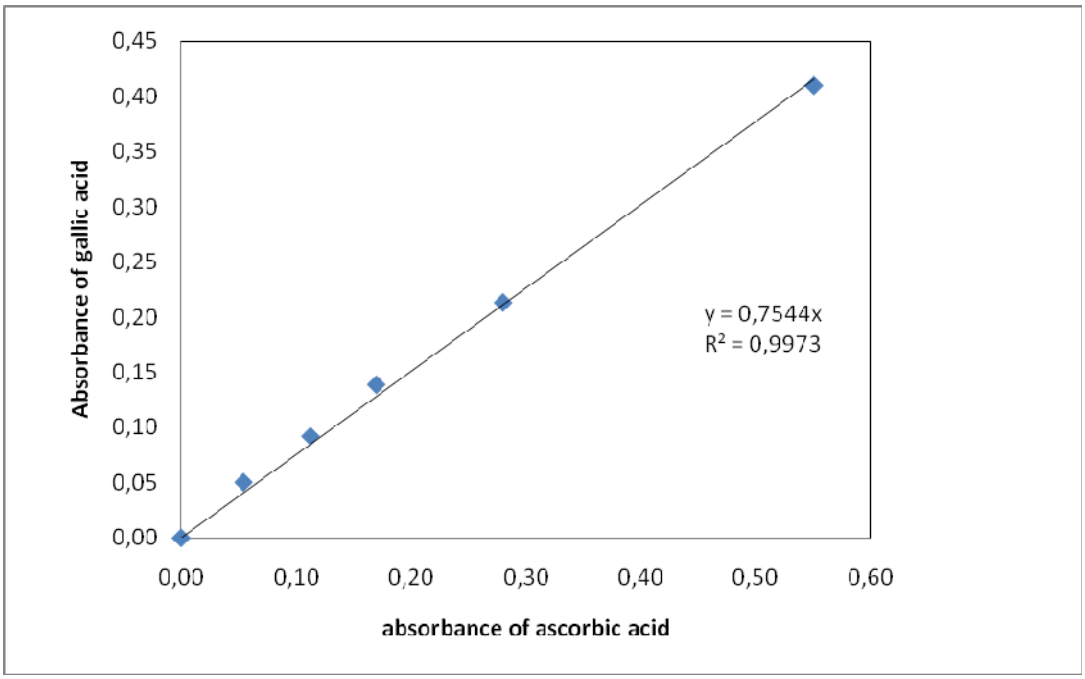

Figure A.2 Ascorbic acid correction of Folin-Ciocalteu reagent

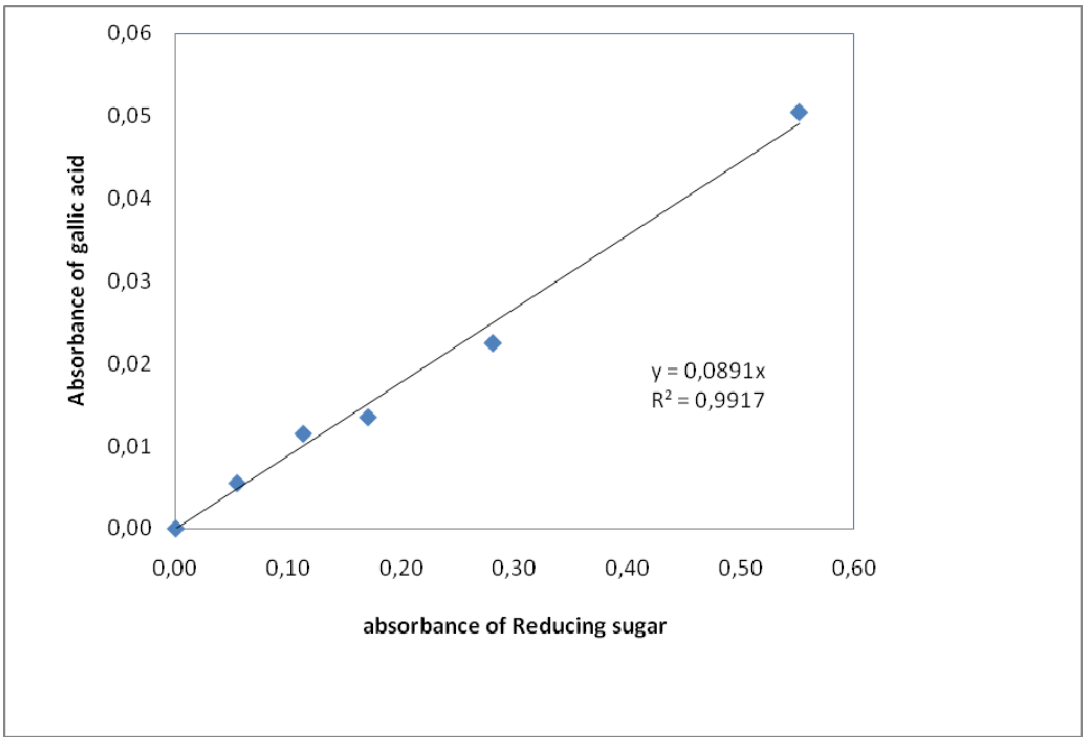

Figure A.3 Reducing sugar correction of Folin-Ciocalteu reagent

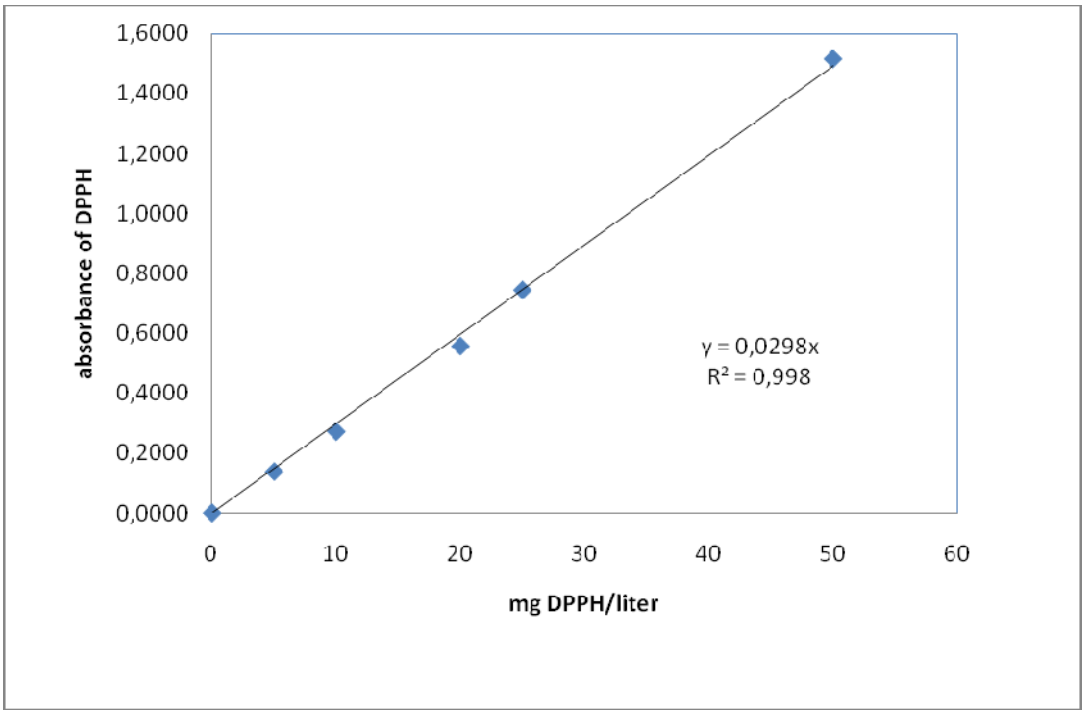

Figure A.4 DPPH standard curve

APPENDIX B

DETERMINATION OF ABSORBANCE WAVELENGTH OF ANTIOXIDANT ACTIVITY (DPPH)

Figure B.1 Absorbance wavelength of DPPH solution

APPENDIX C

STATISTICAL ANALYSIS OF EFFECT OF MICROFLUIDIZATION ON ANTIOXIDANT ACTIVITY

C.1 Normality test

Ho: error distributed normally

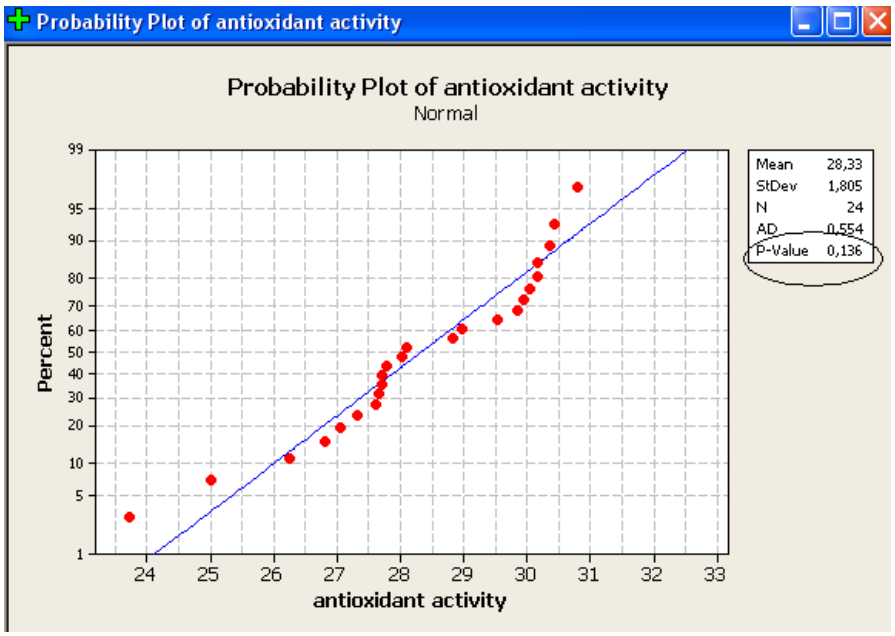

Figure C.1 Probability plot of results of antioxidant activity

p=0,136>0, 05 (accepting of the hypothesis)

C.2 Barlett test (equal variance assumption)

H_1: equal variance assumption is satisfied

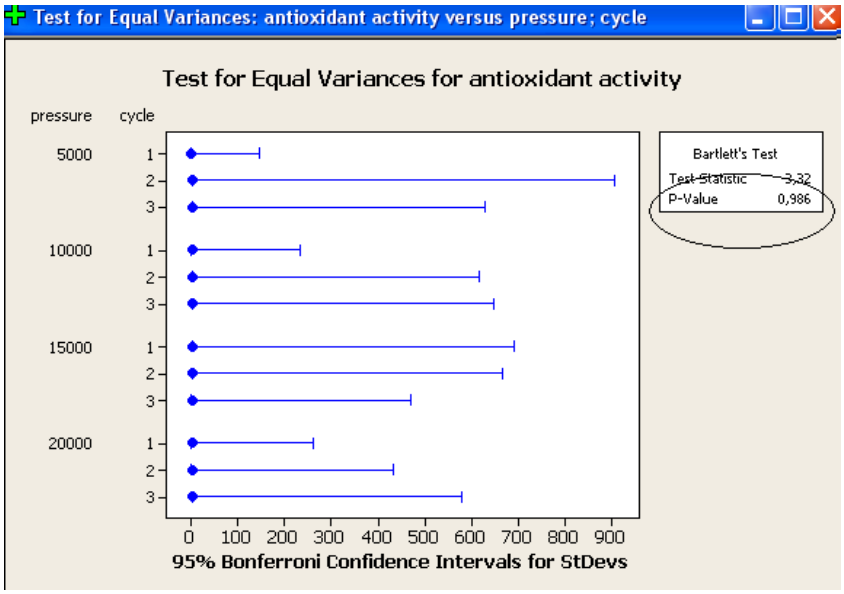

Figure C.2 Test for equal variances for results of antioxidant activity

p=0,986>0, 05 (accepting the hypothesis).

Two-way ANOVA: antioxidant activity versus pressure; cycle

Source	DF	SS	MS	F	P
pressure	3	43,6465	14,5488	6,71	0,007
cycle	2	0,8535	0,4268	0,20	0,824
Interaction	6	4,4568	0,7428	0,34	0,901
Error	12	26,0053	2,1671		
Total	23	74,9622			
Error	12	26,0053	2,1671		
Total	23	74,9622			

As a result, pressure had a significant effect on antioxidant activity (p=0.007<0.05); but cycle had not a significant effect (p=0.824>0.05). Therefore, only pressure is analyzed for the Tukey comparison test.

Comparison with Tukey Test

```
Tukey 95,0% Simultaneous Confidence Intervals
Response Variable antioxidant activity
All Pairwise Comparisons among Levels of pressure
pressure =  5000  subtracted from:

pressure   Lower  Center  Upper    -+---------+---------+---------+-----
10000     1,4337   3,457  5,480                    (-------*-------)
15000     0,7704   2,793  4,816                (-------*-------)
20000     0,9121   2,935  4,958                 (-------*-------)
                                -+---------+---------+---------+-----
                              -2,5       0,0       2,5       5,0


pressure = 10000  subtracted from:

pressure   Lower   Center  Upper    -+---------+---------+---------+-----
15000     -2,686  -0,6633  1,360    (-------*-------)
20000     -2,545  -0,5217  1,501     (-------*-------)
                                -+---------+---------+---------+-----
                              -2,5       0,0       2,5       5,0


pressure = 15000  subtracted from:

pressure   Lower  Center  Upper    -+---------+---------+---------+-----
20000     -1,881  0,1417  2,165       (--------*-------)
                                -+---------+---------+---------+-----
                              -2,5       0,0       2,5       5,0


Tukey Simultaneous Tests
Response Variable antioxidant activity
All Pairwise Comparisons among Levels of pressure
pressure =  5000  subtracted from:

          Difference       SE of           Adjusted
pressure    of Means  Difference  T-Value   P-Value
10000          3,457      0,7224    4,785    0,0006
15000          2,793      0,7224    3,866    0,0049
20000          2,935      0,7224    4,063    0,0031


pressure = 10000  subtracted from:

          Difference       SE of           Adjusted
pressure    of Means  Difference  T-Value   P-Value
15000        -0,6633      0,7224  -0,9182    0,7955
20000        -0,5217      0,7224  -0,7221    0,8871


pressure = 15000  subtracted from:

          Difference       SE of           Adjusted
pressure    of Means  Difference  T-Value   P-Value
20000          0,1417      0,7224   0,1961    0,9972
```