EFFECT OF HIGH PRESSURE HOMOGENIZATION (MICROFLUIDIZATION) ON THE QUALITY OF OTTOMAN STRAWBERY (*F.ananassa*) JUICE

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ABSTRACT

EFFECT OF HIGH PRESSURE HOMOGENIZATION (MICROFLUIDIZATION) ON THE QUALITY OF OTTOMAN STRAWBERY (*F.ananassa*) JUICE

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High pressure homogenization (microfluidization) is a non-thermal process that is commonly used in food processing. Microfluidization provides convenience because it does not require any chemical. Also, the quality of juice is affected minimally.

In this study, the effect of high-pressure homogenization (microfluidization) on the physical and chemical properties of Ottoman strawberry juice was evaluated. Two different pass numbers and two different pressures were used for the homogenization treatment. Effect of homogenization process was investigated by conducting antioxidant capacity, total phenolic content, particle size distribution, total soluble solid content, color analyses and Nuclear Magnetic Resonance measurements on the strawberry juices. Besides microfluidization, effect of enzyme (depectinization process) on the strawberry juice quality was also investigated. Different types of strawberry juices were compared in terms of color, antioxidant capacity and total phenolic content.

Microfluidization at high pressure increased the total phenolic content and antioxidant capacity of the Ottoman strawberry juice. 600 bar treatment caused an increase in the T_2 values and 1000 bar treatment caused an increase in the T_1 values

of the juice. Particle size decreased when the pass number increased. Furthermore, the pass number and pressure change affected, significantly, the anthocyanin content which caused an alteration in the redness value of Ottoman strawberry juice.

Homogenization process also increased the total phenolic content of ordinary strawberry juice. Enzyme addition gave rise to significant changes on the quality of different strawberry juices.

Keywords: Microfluidization, depectinization, color, strawberry juice, phenolic content

ÖZ

YÜKSEK BASINÇLI HOMOJENİZASYON (MİKRO-AKIŞKANLAŞTIRMA) İŞLEMİNİN OSMANLI ÇİLEĞİ (*F.ananassa)* SUYU ÜZERİNDEKİ ETKİSİ

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Yüksek basınçlı homojenizasyon (mikro-akışkanlaştırma) gıda işlemede yaygın olarak kullanılan ısıl olmayan bir işlemdir. Mikro-akışkanlaştırma, herhangi bir kimyasal gerektirmediğinden kolaylık sağlar. Aynı zamanda, meyve suyu kalitesi minimum seviyede etkilenir.

Bu çalışmada, yüksek basınçlı homojenizasyonun (mikro-akışkanlaştırma) Osmanlı çileği suyunun fiziksel ve kimyasal özellikleri üzerindeki etkisi değerlendirilmiştir. Homojenizasyon işlemi sırasında, iki farklı geçiş sayısı ve iki farklı basınç kullanılmıştır. Homojenizasyon işleminin çilek suları üzerindeki etkisi antioksidan kapasite, toplam fenolik içeriği, parçacık boyutu dağılımı, toplam çözünebilir kuru madde içeriği, renk analizleri ve Nükleer Manyetik Rezonans ölçümü yapılarak araştırılmıştır. Mikro-akışkanlaştırmanın yanı sıra, enzimin çilek suyu kalitesi üzerindeki etkisi (depektinizasyon işlemi) araştırılmıştır. Farklı tip çilek suları renk, antioksidan kapasite ve toplam fenolik içeriği açısından karşılaştırılmıştır.

Yüksek basınçta mikro-akışkanlaştırma, Osmanlı çileği suyunun toplam fenolik içeriğini ve antioksidan kapasitesini arttırmıştır. 600 bar işlemi T_2 değerlerinde artışa sebep olmuştur ve 1000 bar işlemi çilek suyunun T_1 değerlerinde artışa sebep olmuştur. Geçiş sayısı arttıkça parçacık boyutu azalmıştır. Ayrıca, geçiş sayısı ve

basınç değişimi, Osmanlı çileği suyunun kırmızılık değerinde değişime sebep olan antosiyanin içeriğini önemli ölçüde etkilemiştir.

Homojenizasyon işlemi aynı zamanda normal çilek suyunun toplam fenolik içeriğini arttırmıştır. Enzim ekleme işlemi, farklı çilek sularının kalitesi üzerinde önemli değişikliklere sebep olmuştur.

Anahtar Kelimeler: Mikro-akışkanlaştırma, depektinizasyon, renk, çilek suyu, fenolik içeriği

To My Family…

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INTRODUCTION

1.1 Strawberry Fruit

Strawberry cultivation started in 1970s and it has rapidly increased in recent years in Turkey (Gündüz & Özdemir, 2012a). The production of strawberry plants rose from 130,000 tons to 300,000, becoming the second largest strawberry producer in the world in 2010 (Whidden, Guan, & Wu, 2012). Moreover, the export policy reached 30,000 tons in 2010 in Turkey (Gündüz & Özdemir, 2012a). The majority of the production in Turkey is made in the regions of Mediterranean (62 %), Marmara (20 %) and Aegean (12 %), respectively (Gündüz & Özdemir, 2012a).

Strawberry plants, which are both wild and cultivated, belong to the Rosacea family and *Fragaria* genus (Aubert, 2004). The most common commercially used variety is *F. ananassa*. The cultivated variety (*F.ananassa*) is a hybrid from *F. chilosensis* and *F. virginiana* (Aubert, 2004). It is grown in temperate, grassland, Mediterranean, taiga and subtropical climates (Hancock & Luby, 2014). *F. ananassa* contributes to the U.S economy to a great extent as United States is the world's leading producer (Hancock & Luby, 2014). From 2000 to 2010, the production of strawberry was around 1.1 million metric tons on 20,904 ha and was worth $ 1.5 billion annually in the U.S (Yue et al., 2014).

In Turkey, *Fragaria ananassa* is cultivated in "Karadeniz Eregli" that is on the West Black Sea Region of Anatolia. It has a special local name that is known as the "Ottoman Strawberry". Ottoman strawberry is a unique fruit with its pale pink color, oval seem, rich aroma and excellent smell. Sürücü (2010) found out that Ottoman strawberry had higher aroma than *Seyhun* and *Camarosa* strawberry cultivars. *Seyhun* strawberry cultivars are obtained by the hybridization of *Camarosa* and

Ottoman strawberry cultivars at controlled conditions (Sürücü, 2010). *Camarosa* is one of the varieties with the highest concentrations of anthocyanin compounds, that is, it has more intense pigments in the inner tissues of the fruit (Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010). Strawberries have a highly preferable flavor and taste and are mostly desirable edible spring and summer fruits (Kosar, Kafkas, Paydas, & Baser, 2004). Quality criteria for strawberry fruits are fruit size, fruit flesh firmness, fruit shape, total soluble solid content, the proportion of dry material to acid, total sugar content (fructose, glucose, sucrose) and acidity (Darbellay, Luisier, Villettaz, Amado, & Azodanlou, 2004; Gündüz & Özdemir, 2012b). Glucose, fructose and sucrose have an important role as soluble components in strawberries, and act as precursors for flavor compounds, and are primarily used as an energy source in the ripening process (Darbellay et al., 2004). The other important quality criterion for strawberry is color. Color is used as quality criterion for the determination of strawberry maturation time and anthocyanins have an important role in the formation of color (Kosar et al., 2004).

There are many other factors that can change the taste quality of a strawberry and these factors include ripeness level, maturity, cultivar, irrigation and fertilization (Kosar et al., 2004). Strawberry plants are highly sensitive to variation in environmental conditions and quality is affected by factors such as day and night time temperatures, water availability, and intensity of daylight (Wang & Camp, 2000).

Strawberry aroma is composed of the combination of numerous volatile compounds at different concentrations (Sürücü, 2010). In strawberry fruits, the metabolic activity is very high and aroma substances can rapidly change after the harvest. Therefore, aroma analysis should be performed as quickly as possible (Sürücü, 2010).

The antioxidant capacity varies between different plants and also is affected strongly from some factors such as cultivar, condition of cultivation, and ripeness (Artmann, Atz, Ndlauer, Ietrich, & Udwig, 2008). Natural antioxidants, which are present in all parts of plants, include carotenoids, vitamins, phenols, flavonoids, dietary glutathionine, and endogenous metabolites (Wang & Lin, 2000). Plant-derived antioxidants have some functions such as oxygen quenchers, free radical scavengers,

peroxide decomposers, enzyme inhibitors, and synergists (Wang & Lin, 2000). Strawberries have high level of antioxidant compounds that provide the protection against harmful free radicals (Zheng, Wang, Wang, & Zheng, 2007). Wang et al. (2002) indicated that strawberries (*Fragaria x ananassa* Duch.) have high oxygen radical absorbance activity against peroxyl radicals ($ROO^{\bullet}$), superoxide radicals ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$). Previous studies showed that strawberries had 1.3 times the antioxidant activity of oranges, twice that of red grapes, five times that of apples and bananas, and thirteen times that of honeydew (Oszmiański & Wojdyło, 2008). Strawberries which are produced under organic culture conditions have significantly higher antioxidant capacity than those produced under conventional culture conditions (Crecente-Campo, Nunes-Damaceno, Romero-Rodríguez, & Vázquez-Odériz, 2012). Data from epidemiological studies have shown that the consumption of fruits reduce the risk of several important diseases such as ophthalmological, cardiovascular, and gastrointestinal, neurodegenerative disorders, and some types of cancer (Crecente-Campo et al., 2012). Furthermore, fruits have protective role in stroke and also provide the prevention of coronary heart disease, chronic obstructive pulmonary disease, cataract formation, and hypertension (Crecente-Campo et al., 2012). Wang et al. (2000) indicated that eating fruits decreases blood pressure, enhance the immune system, detoxifies contaminants and pollutants and decreases inflammation. Anthocyanins have antioxidant activity and this also plays an important role in the prevention of diseases (Boranbayeva, Karadeniz, & Yılmaz, 2014). That is why, interest in anthocyanins has increased, recently and they are used as colorants in food and pharmaceutical industries (Boranbayeva et al., 2014). However, anthocyanin stability is easily affected from pH, temperature, oxygen, light, enzymes and the amount of anthocyanin found in the food can decrease during processing and storage as temperature increases (Boranbayeva et al., 2014).

Strawberry is soft juicy and also an important bio-resource which has good processing potential (Cao et al., 2012). Not only strawberries are consumed as fresh fruits, but they are also used in processed products such as liquor, syrup, jam, juice, ice cream, and concentrated flavor preparations (Aubert, 2004). Strawberries contain antioxidative compounds, which are mainly composed of phenolic compounds and

anthocyanins (Cao et al., 2012). The main anthocyanins existed in strawberry fruits are pelargonidin-3-glucoside and cyanidin-3-glucoside (Zabetakis, Leclerc, & Kajda, 2000). Other minor anthocyanins have acylated derivatives with the following organic acids: malic, malonic, succinic, or acetic acids (Cerezo et al., 2010). Anthocyanins also condense with other phenolic compounds to form oligo- and polymers. Thus, distinguishable changes of anthocyanins are visible to the naked eye during processing and storage of strawberry products (Artmann et al., 2008). Antioxidant activity of strawberries can be correlated with the content of polyphenolic compounds and anthocyanins since the antioxidant capacity of plant tissues is mostly based upon to some phytochemicals such as phenolics, anthocyanins, ascorbic acid and other flavonoids (Wang & Lin, 2000, Oszmiański & Wojdyło, 2008). Because of attractive color, excellent aroma and sweet-sour mouthfeel, the juice obtained from strawberry is considered as one of the most popular fruit juice and the juice can be preferred as a natural antioxidant drink (Cao et al., 2012).

In fruit juice processing and storage, Maillard reaction can become important. The Maillard reaction occurs between α-amino groups and reducing sugars and this is the reason of browning in fruit juice during storage (Boranbayeva et al., 2014). This reaction can be affected from many factors such as temperature, time, reactant concentration and initial pH. Moreover, Maillard reactions give rise to losses in nutritional value of foods, and form undesirable compounds such as furfural, hydroxy methyl furfural and brown pigments (Boranbayeva et al., 2014).

Fruits are main source of polyphenols, a group of phytochemicals recognized as the most abundant antioxidants (Crecente-Campo et al., 2012). Phenolic compounds, which are secondary metabolites of the plant, are important components of plants' natural defense against pathogens (Karlund et al., 2014). Phenolic compounds are one of the main groups of phytochemicals among the bioactives exist in strawberries and affect quality, enhancing sensorial-organoleptic properties and health properties (Álvarez-fernández, Hornedo-Ortega, Cerezo, Troncoso, & García-Parrilla, 2014). Phenolic substances which are commonly found in strawberry fruits are hydroxybenzoic acids (gallic and ellagic acids), hydroxycinnamic acids (p-cumaric),

hydrolysable tannins (ellagitannins), flavonols (quercetin, kaempferol and myricetin), flavan-3-ols (catequins, epicatechins), and anthocyanins (Pineli et al., 2011). These substances are very unstable and expose to destruction during fruit transformation, especially during the production processes of the juice and the nectar (Oszmiański & Wojdyło, 2008). The polyphenol content affects the nutritional quality of strawberry. In processed strawberry, diversity and content of polyphenols can decrease during storage that depends on conditions such as time, temperature and oxygen content (Oliveira et al., 2015).

1.2 Fruit Juice Production

In the industry, most beverages obtained from fruits, especially wine and fruit juices except for citrus juices, are clarified during the production processes so as to remove unwanted turbidity, sediments, and haze in the final products (Pinelo, Zeuner, & Meyer, 2010). The turbidity in a juice occurs due to two reasons. The immediate turbidity in freshly pressed fruit juices comes from the suspended pectin particles stemming from the disrupted cell walls, and cell materials. The second one is the development of turbidity during cold storage. This type of formation is considered as haze formation, and assumed to be caused by interactions between polyphenols and haze-active proteins that can form insoluble multi-molecular structures (Pinelo et al., 2010). Strawberry juice is also one of the products that require the clarification process. For strawberry juice, depectinization is an important step. The mash, which is obtained after the pressing step, contains large amount of pectin and cell wall fragments. Pectins are acidic polysaccharides that originate in the cell membrane structure of plants and make the clarification process hard because of their fibre-like structure (Alvarez, Alvarez, Riera, & Coca, 1998).

Fruit processing plants can show difference from a simple facility to a complex manufacturing facility (Lozano, 2006). A simplified characteristic flow diagram of a juice processing line is indicated in Fig. 1.1.

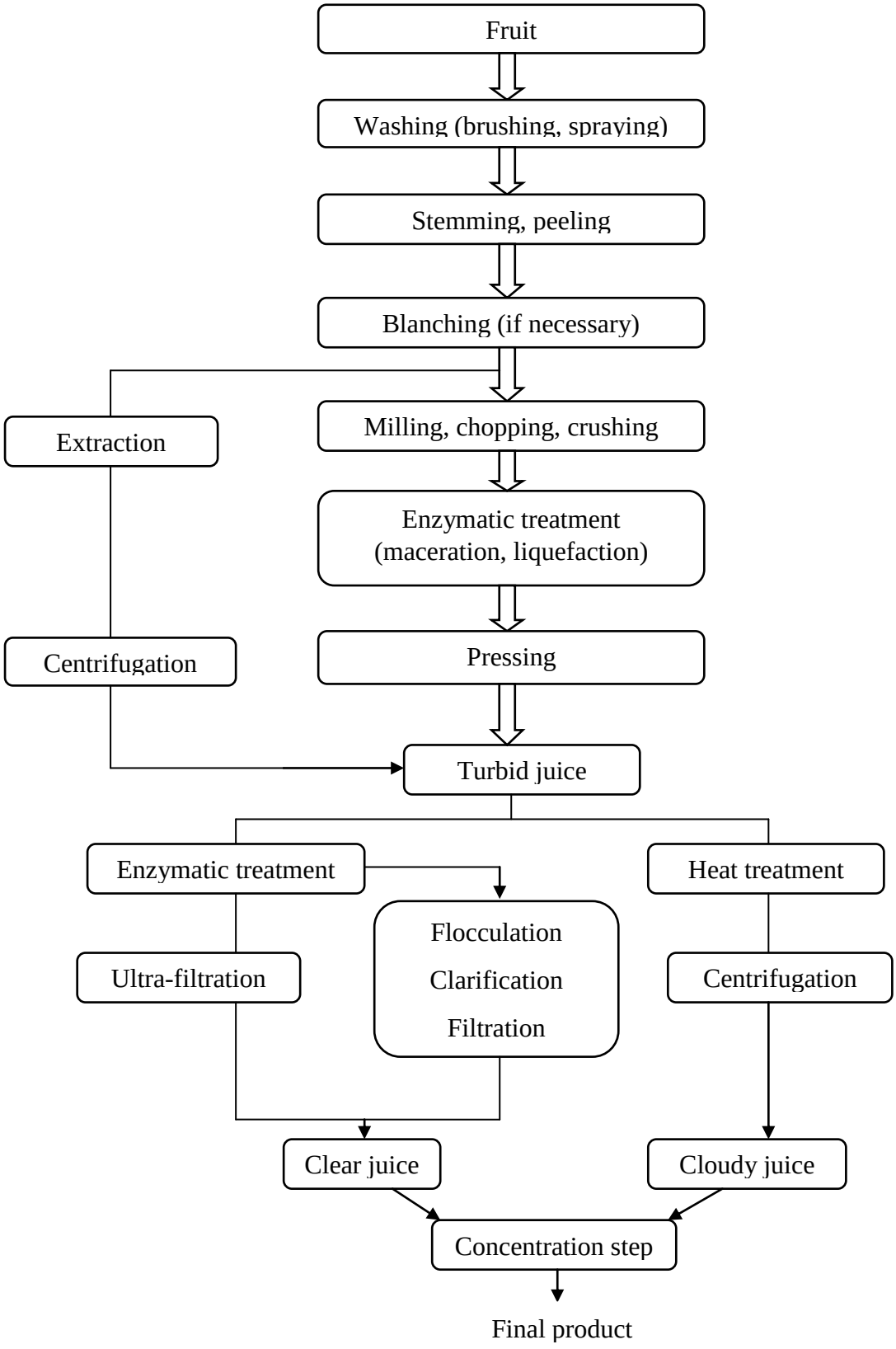

Figure 1.1 Typical fruit juice (clear or cloudy) processing line steps

Actually, enzymes are used to provide the extraction, clarification and modification of the juices from many fruits such as berries, grapes, apples and pears but when a cloudy juice (from oranges, pineapple or apricots) is preferred, clarification process is not used (Lozano, 2006).

Fruits are classified as pome fruits, stone fruits, berry fruits and citrus fruits (Yaralı, 2014). The most common used pome fruit is apple in the industry because the sugar-acid proportion of the apple is very convenient for the production of fruit juice. Also, pear has high sugar-acid content and pear juice provides sweet and silky mouthfeel (Yaralı, 2014). Stone fruits are appropriate for the production of nectar. Furthermore, citrus fruits are plentiful and they are used commonly in the juice industry. However, berry fruits have disadvantages. They can be easily damaged during the harvest and transportation because of their soft structure and decomposed by microorganisms (Yaralı, 2014). So, production line from raw material to final product has different steps because of the structure of fruits. Some fruits like peach, apricot and cherry are transferred to crushing unit after removing seeds. Crushing process show difference according to the type of fruit and next step of the production (Yaralı, 2014). After crushing step, the mash is obtained and the process which will be applied to the mash can change according to the properties of raw material and final product. Although heating mash is commonly used in berry fruits and stone fruits, it is not preferred in the production of clarified fruit juice produced from apple and pear (Yaralı, 2014). Heating mash treatment occur at 85-87 °C and fruits are hold for 3 min at this temperature and cooled immediately. The purpose of this treatment is to provide the inactivation of enzymes naturally found in the fruits so, enzymatic reactions that have negative effects on color and flavor can be prevented. Moreover, microorganism load can be reduced with the help of this treatment (Yaralı, 2014). Other important process for the production of fruit juice is the pressing and this treatment is actually separation process which provides to separate the solid and liquid phases of the mash by applying pressure (Yaralı, 2014). When the viscosity of the juice decreases, pressing treatment can be applied easily. For some fruits such as strawberry, enzyme is added to the mash in order to ease the pressing process. In the fruit juice industry, the usage of decanter is common and it is a kind of centrifuge and it is used for lots of fruits such as apple, peach, pear, cherry,

grape, strawberry (Yaralı, 2014). Furthermore, filtration is an important step for the production of fruit juice. Filtration is a mechanical process and it makes the clarification easy by removing insoluble solids from a high-value liquid food, by the passage of most of the fluid through a porous barrier, which retards most of the solid particulates included in the food (Lozano, 2006). This process is used for all fruits during the fruit juice production.

1.3 Depectinization and Clarification

Pectin is formed by a backbone of galacturonic acid residues which is linked by α-1,4 glycosidic linkages (Xu et al., 2015). This backbone includes galactan, rhamnose and arabinan side chains and it is methyl-esterified to some extent (Xu et al., 2015). In order to obtain a clear strawberry juice, the hydrolysis of pectic substances is necessary. Hydrolysis is achieved by pectinases. Pectinases can be classified as two different groups: pectin esterases and depolymerases and depolymerases are classified as three types: polygalacturonase, pectate lyase and pectin lyase (Xu et al., 2015). Lyases break α-1, 4 bonds which hold galacturonic acid residues by trans elimination in order to produce 4,5-unsaturated galacturonide (Xu et al., 2015). Lyases affect different substrates. Pectin lyase affects highly methylated and non-esterified pectin and pectate lyase affects polygalacturonic acid (Xu et al., 2015). The enzymatic mix which include pectin methylesterase (PME), pectin lyase and endo-polygalacturonase is often used to get the clear juice (Hubert, Baron, Le Quere, & Renard, 2007). These pectolytic enzymes provide to hydrolyze the soluble pectin and partially solubilize the pectic fraction, which comes from suspended particles. Among these enzymes, polygalacturonase breaks α-1,4 glycosidic linkages of polygalacturonic acid chain and releases galacturonic acid residues (Dey, Adak, Bhattacharya, & Banerjee, 2014). Pectic enzymes are used in the processing of fruit in order to get better juice recovery, improve filtration rate and produce clear juices of high quality for concentration (Saxena, Sabikhi, Chakraborty, & Singh, 2014). The turbidity-causing pectin particles are retained in the suspension through the charge repulsion between particles and correspondingly, the turbidity-reducing effect of pectinases is mainly related to the electrostatic destabilization of suspended,

negatively charged, pectin particles (Pinelo et al., 2010). With the help of enzymatic pectin hydrolysis, proteinaceous core, which can be positively charged at the low pH of juices, undergoes a reaction with the negatively charged pectin (Pinelo et al., 2010). After this step, flocculation occurs and agglomerates can be removed by the way of centrifuge of the juice. With enzyme treatment, the must viscosity reduces, and the particles flocculate slowly (Hubert et al., 2007). Moreover, it increases the juice yield, sweetness, and shelf life of the product (Dey et al., 2014). The effect of pectinolytic enzymes, or pectinases, occur in different forms on their substrate, the pectin. The commercial pectinases can contain one or more types of microbial pectinolytic enzymes (Sandri, Fontana, Barfknecht, & da Silveira, 2011). Industrially useful polygalacturonases are obtained from different microorganisms such as *Penicillium sp.*, *Aspergillus sp.*, *Thermoascus aurantiacus*, *Trichoderma sp.*, *Lentinus edodes*, *Rhizopus sp.*, *Bacillus sp.* (Dey et al., 2014). Depectinization process can be combined with fining process with some proteins such as gelatin. At the pH of fruit juice, gelatin has positive charges and flocculation is achieved by the help of electrostatic interactions with the negative charges of remaining pectin in the particles (Hubert et al., 2007). Conventional clarification also involves the addition of bentonite. The behavior of bentonite is related to the adsorption capacity which especially affect proteins (Bagci, 2014).

1.4 High Pressure Homogenization

Process conditions of the strawberry juice are important in terms of the consumer preference because the treatment affects the juice quality considerably. Each applied process on the strawberry juice can change color, viscosity, flavor, texture, aroma, antioxidant capacity or the total phenolic content. Consumers, generally demands the minimal processed products so non-thermal applications have gained popularity (Cao et al., 2012). Previous studies had shown that strawberry juice quality is affected minimally from the applications of non-thermal techniques (Cao et al., 2012). One of the most used non-thermal processes on the food is the high pressure homogenization. This technology uses the combined forces of high-velocity impact, high-frequency vibration, instantaneous pressure drop, intense shear, cavitation, and

ultra-high pressures up to 200 Mpa with a short treatment time and continuous operation (Liu et al., 2009). During high pressure homogenization process, mechanical energy is transferred to fluid particles under high pressure. In the case of microfluidization (a high pressure homogenization technique) the solution is pumped and split into two microstreams which are impacted or collided against each other in a chamber, which is called as the interaction chamber where shear, turbulent and cavitation forces are generated (Kasaai, Charlet, Paquin, & Arul, 2003).

1.5 Applications of High Pressure Homogenization on Fruit Juice

Although high pressure homogenization is used for various processes, such as microbial reduction, preparation of nanoemulsions, and improvement of dietary fiber, etc., this "cold" treatment technology has started to be used in the fruit juice processing too. Lacroix et al., (2005) showed that the PME activity of orange juice decreased by 20% through homogenization at 170 MPa. In another study, the effect of high pressure homogenization (HPH) on the banana juice was evaluated. Calligaris et al., (2012) evaluated microbial load, temperature, pectate lyase activity, color, and viscosity changes of banana juice samples which were exposed to HPH. Effect of high-pressure homogenization (up to 100 MPa) on the physical stability of tomato juice was investigated by Kubo et al., (2013). According to this study, treatment changed the tomato juice pulp sedimentation behavior, particle size distribution (PSD), color, turbidity, and microstructure by disrupting the suspended pulp particles (Kubo et al., 2013). Silva et al., (2010) evaluated the effects of different homogenization pressures on the stability and rheological properties of pineapple pulp. The pineapple pulp showed shear thinning behavior with rising flow index. Also, the pulps which include smaller particles showed less serum cloudiness and the sedimentation tests showed the highest stability for pulp homogenized between 200 and 300 bar (Silva et al., 2010).

High pressure homogenization provides to sustain the process without exogenous chemicals, few flavor compounds and nutritional components loss (Liu et al., 2009). This treatment affects the fruit juice quality minimally. Because of this reason, high

pressure homogenization has gained popularity as a fruit juice processing technique. Thereby, many studies related to the effect of HPH on fruit juice was contributed to the literature.

1.6 Nuclear Magnetic Resonance (NMR)

Applying different techniques and adding different chemicals or enzymes to the fruit mash can change the water distribution of it. Normally, common techniques or analyzes are used in order to determine the physical and chemical changes of the fruit juice exposed to different techniques and chemicals or enzymes. Total phenolic content determination, antioxidant capacity determination and total soluble solid content measurement can be considered as common technique. However, Nuclear Magnetic Resonance (NMR) is a different technique which offers the opportunity of studying foods in their wholeness, in a non-invasive and non-destructive way (Otero & Préstamo, 2009). This technique permits the quantification of relaxation time processes. The spin-lattice relaxation time, T_1, represents the constant for the spin recovery in the direction of the external magnetic field and the spin-spin relaxation time, T_2 , represents the rate of the relaxation in the direction perpendicular to the external magnetic field and the relaxation process includes energy loss to its neighboring spins and the environments (Zhang & McCarthy, 2013). With the help of Nuclear Magnetic Resonance (NMR) technique, gathering the qualitative and quantitative data on physical and chemical properties of a wide range of samples is possible (Kirtil & Oztop, 2015).

1.7 Aim of the Study

There are several studies related to the Ottoman strawberry. One of these studies is the determination of aroma compounds in fresh and frozen Ottoman strawberry fruits. Kafkas et al., (2004) evaluated that aliphatic esters and furanones were found as parent compounds in Ottoman strawberry fruits. Moreover, Kafkas et al., (2004) examined the alteration of sugar and organic acid content during Ottoman strawberry

fruits ripening period. Furthermore, an ethnobotanical survey which includes Ottoman strawberry was conducted by Sağıroğlu et al., (2012). However, there is not any study related to the Ottoman strawberry juice exposed to any thermal or non-thermal process in the literature. So, this study can provide a technical support for other applications of high-pressure homogenization technique in Ottoman strawberry juice.

The main objective of this study was to evaluate the effect of high-pressure homogenization (microfluidization) on the physical and chemical properties of Ottoman strawberry juice. Moreover, the enzyme and microfluidization effects on the ordinary strawberry juice and commercial strawberry juice were investigated. Furthermore, Ottoman strawberry, ordinary strawberry and commercial strawberry juices were compared with each other in this study.

CHAPTER 2

MATERIALS AND METHODS

2.1 Ottoman Strawberry Juice Preparation

Ottoman Strawberries, which were harvested from "Karadeniz Eregli", were used in the study. After fruits were brought to the laboratory, they were frozen at -18 ˚C. For each experiment, frozen strawberries were thawed and used. The whole strawberry fruits were grinded (20-30 sec) into the mash by a food processor (K 1190 Arçelik-Robolio, Turkey). Then the mash was drained with a cloth to eliminate all seeds and pulp. Afterwards, it was centrifuged at 10000xg for 5 min, and the supernatant was collected as Ottoman Strawberry juice (pH 3.79, °Brix 9.8).

2.2 Ordinary Strawberry Juice Preparation

Ordinary strawberries, which were harvested from a local variety (Ankara), were used in the study. After fruits were brought to the laboratory, they were frozen at -18 ˚C. For each experiment, frozen strawberries were thawed and used. The whole strawberry fruits were grinded (20-30 sec) into the mash by a food processor (K 1190 Arçelik-Robolio, Turkey). The mash was centrifuged at 10000xg for 5 min.

A commercial strawberry juice prepared from strawberries harvested in Silifke, Mersin was used as the commercial standard. The commercial juice was not a pure juice and included glucose syrup and black carrot juice. The aim to sue the juice is to compare the findings of the study with a commercial product. These juices were stored at the refrigerator during the study. The juice was centrifuged at 10000xg for 5 min before the experiments as it was a pulpy juice. Total phenolic content, antioxidant capacity experiments and NMR analyses were conducted, $CIE_{L^*a^*b^*}$ values were measured.

2.3 Homogenization of the Juice

2.3.1 High Shear Homogenization (Silent Crusher) and Enzyme Treatment

To evaluate the effect of homogenization on the clarity of the strawberry juice before applying high pressure homogenization, trials were conducted using a high shear homogenizer (Silent Crusher – Heidolph Instruments GmbH & Co. KG, Germany).

Concentrated purified pectolytic enzyme preparation (7.600 PGNU/g Polygalacturonase - SIHAZYM® Extro) was used for the enzyme treatment.

Crushed ordinary strawberry samples were centrifuged at 10000xg for 10 min at 25 °C. Then, samples were divided into three groups. The first one was heated to 55-60 °C approximately and pectinase was added to sample (0.1g/100 ml). The second one was passed through the silent crusher at 75000 rpm for 1 min and heated to 55-60 °C and also pectinase was added to sample (0.1g/100 ml). The third one was used as control sample and no treatment was applied. All these three samples were kept for 1 day and passed through a filter paper before the measurement. All these steps were indicated in Fig. 2.1.

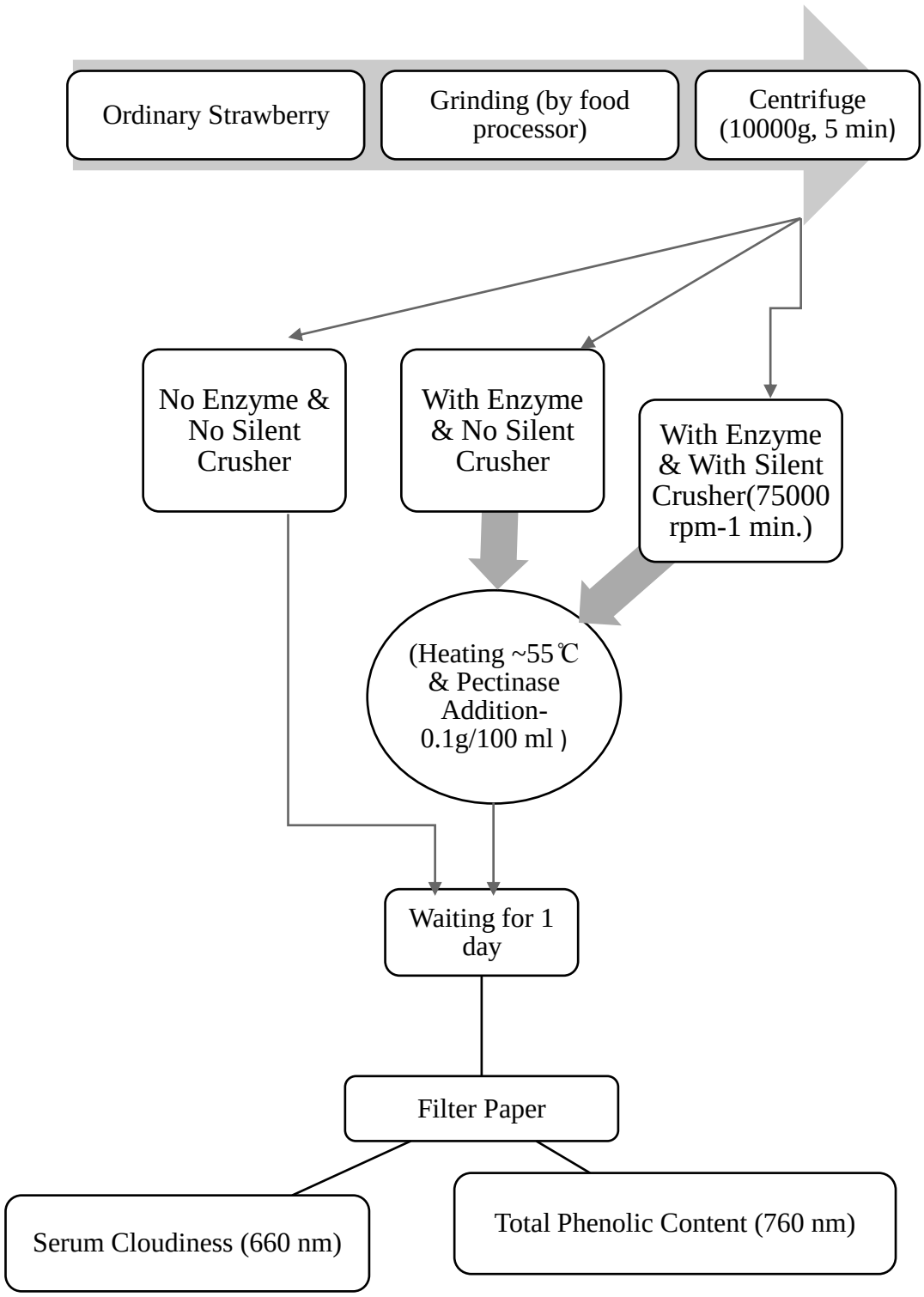

Figure 2.1 Juice preparation for silent crusher and enzyme effect experiment

2.3.2 Microfluidization

The juice was placed in a reservoir with 0.3 L and subjected to high pressure microfluidization (Nano Disperser - NLM 100, South Korea). The sample was treated at two different pressures (600 bar & 1000 bar) with two different passes (2 & 5). While the temperature of sample was measured as 37.3 ˚C for 2 passes at 600 bar, the temperature of sample was measured as 46 ˚C for 5 passes at 600 bar. At 1000 bar pressure, the temperature of sample was measured as 43.3 ˚C and 56.6 ˚C for 2 passes and 5 passes, respectively. After microfluidization treatment, the sample was taken and physical and chemical analyses were conducted. Untreated sample was used as a control. Three replicates were used for all experiments.

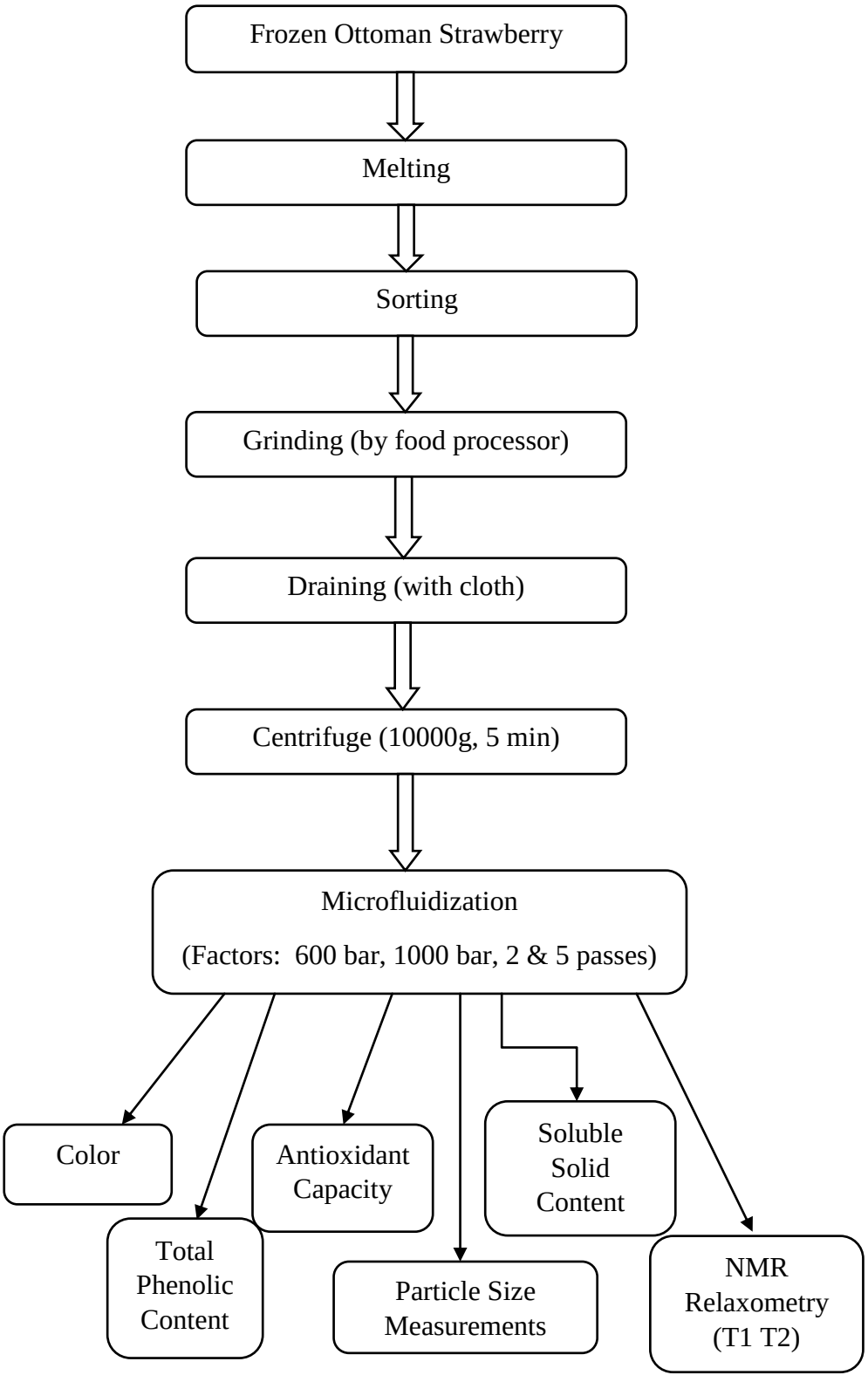

Figure 2.2 Juice preparation for microfluidization experiments

2.4 Total Phenolic Content (TPC) Determination

TPC was measured by using Folin-Ciocalteau reagent (Krawitzky et al., 2014) with some alterations. Three ml concentrate was dissolved in three ml ethanol:water: acetic acid mixture (50:42:8 v/v). These chemicals were mixed with the help of vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min. Then, the mixture was passed through micro-filter (0.45 µm Chromafil CA-45/25 S, Düren). 2.5 ml 0.2 N Folin-Ciocalteau (2N, Sigma-Aldrich F9252) reagent was added into 500 µl diluted sample and mixed with vortex. This mixture was kept in dark for 5 min. And then, 2 ml of 75 g/L sodium carbonate (Sigma-Aldrich S7795) solution was added into the mixture and stirred with vortex. Samples which were hold in dark place at 25℃ (room temperature) for 1 h, absorption values of these samples were measured at 760 nanometer with the help of UV/VIS spectrophotometer T 70, (PG Instruments LTD, UK). TPC of Ottoman Strawberry juice was stated as gallic acid equivalents (GAE) in milligrams per liter fresh juice.

2.5 Antioxidant Activity with DPPH Radical Scavenging Method

The antioxidant capacity was measured by using DPPH (2,2 - Diphenyl-1-picrylhydrazyl) method as described by Çilek (2012) with some slight alterations. Three ml juice was dissolved in three ml ethanol:water:acetic acid mixture (50:42:8 v/v). These chemicals were mixed with the help of vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min. Then, the mixture was passed through micro-filter (0.45 µm Chromafil CA-45/25 S, Düren). Samples were diluted at a ratio of 1:20. 100 µl of diluted samples were mixed with 3.9 ml DPPH radical solution and these samples were kept in dark for 1 h. This waiting period was sufficient in order to complete the reaction of DPPH solutions. Absorptions of samples were measured (A_2) at 517 nm with the help of UV/VIS spectrophotometer T 70, (PG Instruments LTD, UK). 3.9 ml of 25 ppm (2.5 mg DPPH with 100 ml MetOH) DPPH solution and 100 µl of methanol were agitated. Then, its absorption value was determined (A_1) as blank. There was no waiting period for the blank measurement. With A_1 and A_2 values, concentrations (C_1 and C_2) were calculated using the calibration curve

prepared at different concentrations of DPPH in methanol. The results of antioxidant capacity experiment were determined by using below equation:

$$\text{AA (ml DPPH / ml juice)} = \frac{C_1 - C_2}{V_{sample}} * V_{total} * d$$

where C_1 represents the concentration of DPPH immediately after DPPH solution and sample was mixed, and C_2 represents the concentration of DPPH which was measured after 1h waiting period, d represents dilution rate, V_{total} represents the volume of the mixture of strawberry juice and ethanol:water:acetic acid mixture in ml and V_{sample} represents the strawberry juice volume in ml.

2.6 Particle Size Measurements

Particle size of the homogenized juice was measured with the help of Malvern Mastersizer 3000 system (Malvern Instruments Limited, Worcestershire, U.K.) The red light source was only used for the measurements. The values of refractive index and absorption index for Ottoman Strawberry juice were 1.0 and 0.8, respectively. Absorption index was determined at 530 nm. Sample particles were assumed to be non-spherical. The particle size was described by the volume-based mean diameter D [4, 3].

2.7 Color

Color measurements were performed by using a CM-5 Spectrophotometer (Konica Minolta, Inc., Japan) with illuminant D65 and angle of 10°. White calibration was used for the instrument standardization. Pure water (L^*_{ref} =100.0, a^*_{ref} =0.0, b^*_{ref} =0.0) was used as the reference material. The samples were placed in glass cells and measurement was done at 740 nm. L*, a*, b* color space was used for the measurement. Total color difference (ΔE*) was determined by using below formula:

$$\Delta E^* = \sqrt{(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2}$$

2.8 NMR (Nuclear Magnetic Resonance) Experiments

NMR data were acquired on a 0.5T, 13.52 MHz low resolution system (Spin Track, Russia) with a 16 mm inside diameter coil. T_1 and T_2 relaxation times of the samples were measured. T_1 and T_2 experiments were performed by using Saturation Recovery and Carr-Purcell-Meiboom-Gill (CPMG) sequences respectively. CPMG sequence was performed with a relaxation period of 300 ms, an echo time (TE) of 1000 us, and 64 scans. Saturation Recovery was applied with delay times changing between 0.01 s and 8 s, 32 scans, and a repetition delay of 8 s.

2.9 Total Soluble Solid Content

Total soluble solid of the juice was determined at room temperature with a manual refractometer (Kyowa, Japan). Results were indicated as °Brix.

2.10 Serum Cloudiness (Turbidity)

The turbidity was measured after homogenizing the samples with the silent crusher and enzyme addition. Crushed ordinary strawberry samples were centrifuged at 10000xg for 10 min at 25 °C. Then, samples were divided into three groups. The first one was heated to 55-60 °C approximately and pectinase was added to sample (0.1g/100 ml). The second one was passed through the silent crusher at 75000 rpm for 1 min and heated to 55-60 °C and also pectinase was added to sample (0.1g/100 ml). The third one was used as control sample and no treatment was applied. All these three samples were kept for 1 day and passed through a filter paper before the measurement. All these steps were indicated in Fig. 2.1. For turbidity, absorbance of the samples (i.e., the juice serum cloudiness) was determined at 660 nm with the help of UV/VIS spectrophotometer T 70, (PG Instruments LTD, UK).

2.11 Data Analysis

The reported results were the averages of three measurements. Data were indicated as mean value. Analysis of variance (ANOVA) was performed and Tukey test was applied as the multiple comparison test by using Minitab (ver.16.2.0.0, Minitab Inc., United Kingdom).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Effect of High Shear Homogenization (Silent Crusher) and Enzyme Addition on the Serum Cloudiness (Turbidity) of Ordinary Strawberry Juice

Strawberry juice is one of the products that require the clarification process. For the strawberry juice, clarification is an important step. In order to obtain a clear juice, the use of enzyme is necessary.

For serum cloudiness experiments, the suspended particles in the sample are responsible for the absorption of radiation, so the absorbance directly represents the sample cloudiness/turbidity (Kubo et al., 2013). In this study, enzyme addition and homogenization with silent crusher significantly changed the turbidity of the ordinary strawberry juice. Results are given in Fig. 3.1. When control (without enzyme and silent crusher) and depectinized samples (sample with enzyme) were compared, it was seen in the figure that depectinized sample had low absorbance values. With enzyme addition, pectin molecules are broken down and pectin-protein flocculation occurs resulting in a clear juice (Saxena et al., 2014). Due to precipitation, particles that absorbed the beam decreased, so low absorbance values were obtained from the depectinized samples. That is, turbidity decreased. Homogenizing with the silent crusher and enzyme addition at the same time decreased the turbidity when compared with control sample. However, use of silent crusher resulted in a different behavior. The turbidity of depectinized sample passed through the silent crusher was higher than the turbidity of depectinized sample without any process. Homogenization with silent crusher aims to decrease the particle size. Kubo et al. (2013) stated that the smaller particles tend to remain in suspension, which results in an increase in the turbidity and absorbance values. So,

using silent crusher might have increased the turbidity of the depectinized sample due to the same effect. According to ANOVA results (Table A.1), control sample, depectinized sample and depectinized sample passed through silent crusher were found to be significantly different from each other in terms of turbidity, ($p \leq 0.05$).

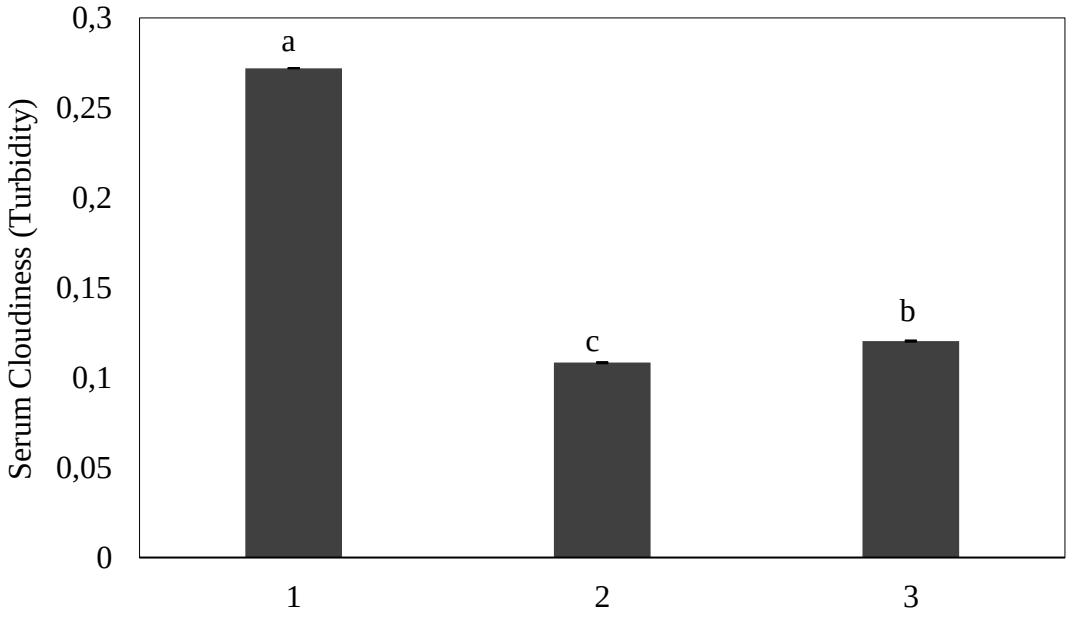

Figure 3.1 Serum cloudiness (turbidity) values of the ordinary strawberry juice (1: Control sample, 2: Depectinized sample, 3: Depectinized sample with silent crusher). Different letters represent significant difference ($p \leq 0.05$)

3.2 Silent Crusher and Enzyme Effect on the Total Phenolic Content of Ordinary Strawberry Juice

Strawberry phenolics are very instable and undergo destruction during the juice production process (Oszmiański & Wojdyło, 2008). Because of the instability of phenolics, each individual processing step can affect the total phenolic content of the strawberry. In this part of the study, effect of homogenization with silent crusher and enzyme addition on phenolic content was examined. Enzyme addition did not affect

the total phenolic content of the strawberry juice when control sample and depectinized sample were compared (Fig. 3.2). However, results showed that depectinized sample passed through silent crusher had higher total phenolic content than others. According to ANOVA results (Table A.2), applying silent crusher to the juice increased the total phenolic content, significantly ($p \leq 0.05$). This process may have released more phenolic compounds. In the study conducted by Patras et al., (2009), it was stated that total phenolic content of grape by-products increased following high pressure processing, ultrasonics and pulsed electric field. This increase in total phenolic content may be related to an increased extractability of some of the antioxidant components (Ankit Patras et al., 2009). Similarly, total phenolic content of the strawberry juice may have been increased by the homogenizing through the silent crusher. With the help of this process, the extractability of some of the antioxidant components, which contributes to the total phenolic content, may have increased.

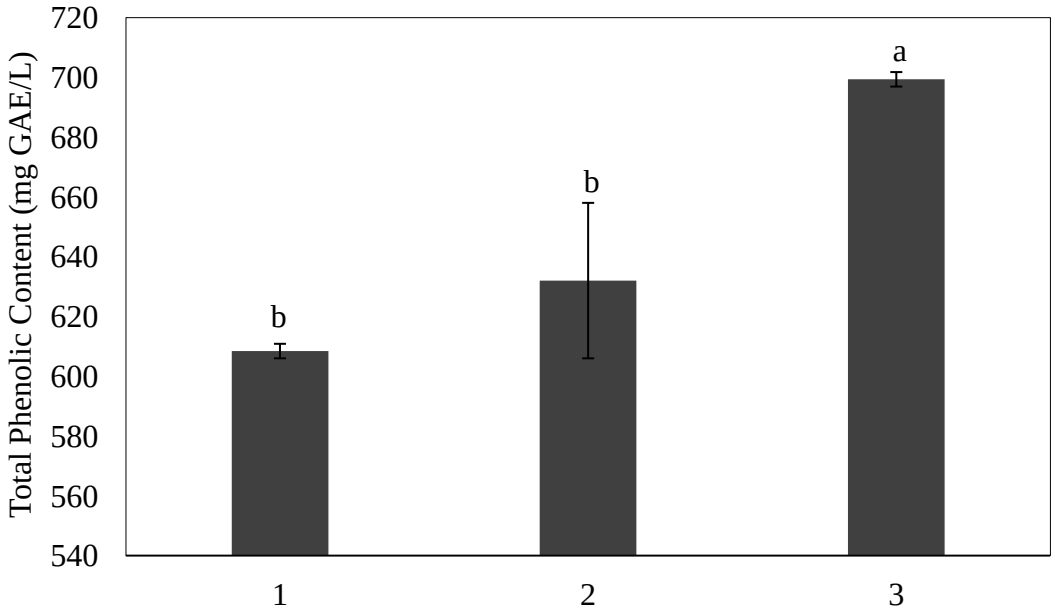

Figure 3.2 Total phenolic content of ordinary strawberry juice (1: Control sample, 2: Depectinized sample, 3: Depectinized sample with silent crusher). Different letters represent significant difference ($p \leq 0.05$)

3.3 Effect of Microfluidization and Enzyme Addition on the Total Phenolic Content of Ordinary Strawberry Juice

Both microfluidization and enzyme effect on total phenolic content of ordinary strawberry juice was also investigated. To understand the effects of these treatments, only microfluidized sample, only depectinized sample (sample with enzyme) and both microfluidized and depectinized samples were used. Microfluidization was performed at 600 bar with two pass number. Total phenolic content of depectinized sample was determined as 510.77±6.172 mg GAE/L and total phenolic content of microfluidized sample was determined as 541.75±1.166 mg GAE/L. The highest total phenolic content, which belonged to both microfluidized and depectinized sample, was found as 658.92±8.165 mg GAE/L. Results are given in Fig. 3.3. ANOVA results (Table A.3) showed that these three samples were significantly different from each other ($p \leq 0.05$). Patras et al. (2009) stated that the content of phenols in strawberry puree increased by high hydrostatic pressure (HHP) treatment. Moreover, it was stated that the highest pressure conditions enhanced the phenolic compound extraction of tomato puree due to cell disruption in another study (Pérez-Conesa et al., 2009). Similar results were also obtained in our experiments. Microfluidization process reduced the particle size of the juice so the reduction in particle size may have eased the release of phenolic compounds (Fig. 3.3). Furthermore, using enzyme and microfluidization increased the total phenolic content of the ordinary fruit juice. It was mentioned earlier that microfluidization provided to release the phenolic compounds. When enzyme effect was added to the microfluidization effect, the phenolic content showed an increasing behavior. If results are examined from Fig. 3.3, it can be seen that total phenolic content of the ordinary strawberry juice that was exposed to both microfluidization process and enzymatic treatment was significantly higher compared to other cases. The results were consistent with the experiments conducted by the silent crusher.

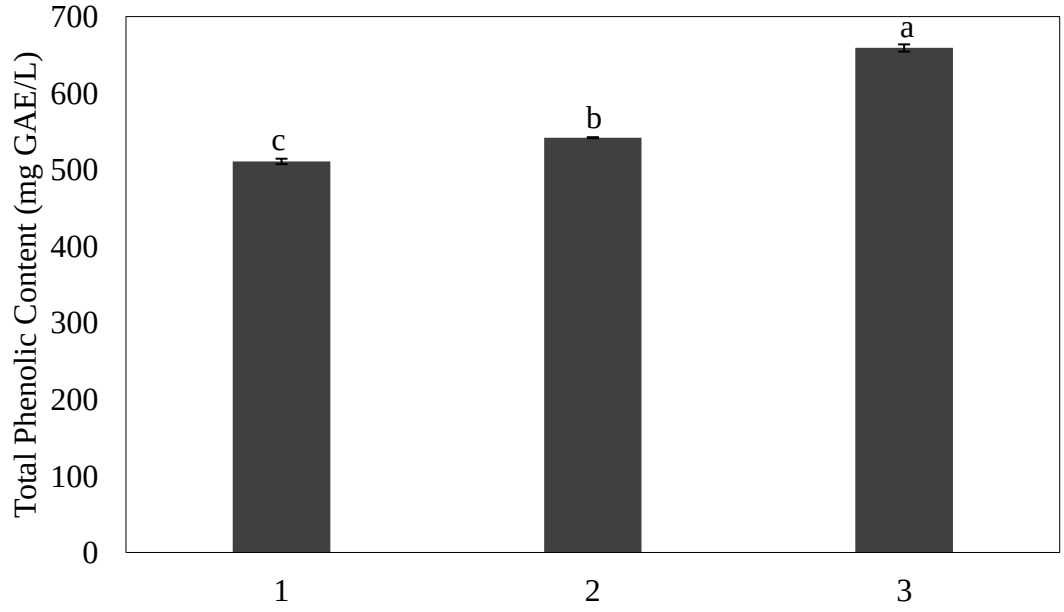

Figure 3.3 Total phenolic content of ordinary strawberry juice (1: Depectinized sample, 2: Microfluidized sample, 3: Both microfluidized and depectinized sample). Different letters represent significant difference ($p \leq 0.05$)

3.4 Comparison among the Total Phenolic Contents of Ordinary Strawberry, Ottoman Strawberry (*F. ananassa*) and Commercial Strawberry Juices

Total phenolic contents of strawberry juices, which were centrifuged after passing through food processor, were investigated. Besides ordinary strawberry and Ottoman strawberry juices, a commercial strawberry juice was included in the investigation of total phenolic content. Fig. 3.4 showed that total phenolic contents of three different strawberry juices were significantly different from each other ($p \leq 0.05$). Moreover, enzyme addition changed significantly the total phenolic contents of these juices. When samples without enzyme treatment were compared, it was seen that total phenolic content of commercial strawberry juice was lower than others. Commercial strawberry juice was exposed to different processes such as pasteurization during the production. These processes could have deteriorated the phenolics in the strawberry juice since strawberry phenolics are very instable and undergo destruction during the juice production process (Oszmiański & Wojdyło, 2008).

In order to understand how the enzyme affects the phenolic content of strawberry juices, the total phenolic contents of depectinized strawberry juice samples were determined (Fig. 3.4). ANOVA results (Table A.4) showed that there was significant difference among the three types of strawberry juices ($p \leq 0.05$).

The other aspect was that enzyme addition caused a decrease in total phenolic contents of ordinary strawberry and Ottoman strawberry juices but it gave rise to an increase in total phenolic content of commercial strawberry juice. With enzyme addition, some of the phenolics may have agglomerated with the pectin particles. As commercial juice has a different composition there could be a couple of reasons on the increase in phenolic content with enzyme addition. Commercial juice has additional sugar and black carrot concentrate. These ingredients may have affected inversely the phenolic content of depectinized commercial juice.

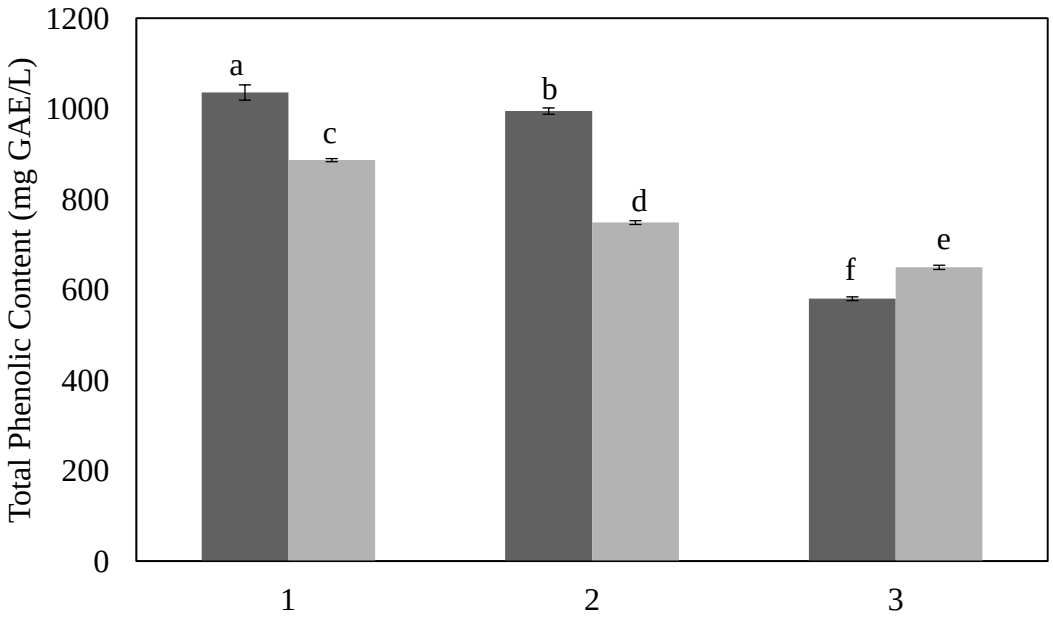

Figure 3.4 Total phenolic contents of strawberry juices without enzyme: (■) and depectinized strawberry juices: (■). (1: Ordinary strawberry juice, 2: Ottoman strawberry juice, 3: Commercial strawberry juice). Different letters represent significant difference ($p \leq 0.05$)

3.5 Comparison among the Antioxidant Activities of Ordinary Strawberry, Ottoman Strawberry (*F. ananassa*) and Commercial Strawberry Juices

Antioxidant activities of strawberry juices, which were centrifuged after passing through food processor, were also investigated. ANOVA results (Table A.5) showed that there was no significant difference between the ordinary strawberry juice and ottoman strawberry juice but commercial strawberry juice was significantly different from these juices ($p \leq 0.05$) and it had the lowest antioxidant capacity (Fig. 3.5). The reason of this results was that the commercial strawberry juice was exposed to different processes during the production.

For the fruit juice production, the importance of enzyme addition was stated. The depectinization process has the possibility of altering the antioxidant capacity. To determine whether a change in antioxidant capacity occurred, the antioxidant capacities of depectinized strawberry juices was investigated. Enzyme treatment caused decrease in the antioxidant capacities of ordinary strawberry and Ottoman strawberry juices. This was similar to total phenolic content results, since phenolic content and antioxidant activity is known to be correlated. However, enzyme addition gave rise to a different result on the commercial strawberry juice. The enzyme treatment caused an increase in total phenolic content of commercial strawberry juice (Fig. 3.4) but it did not change the antioxidant capacity of the commercial strawberry juice (Fig. 3.5). This phenomena could be explained that some phenolics in commercial strawberry juice released due to the depectinization process. However, these phenolics might have not antioxidant properties so the increase in total phenolic content did not contribute to the increase in antioxidant activity of the commercial strawberry juice.

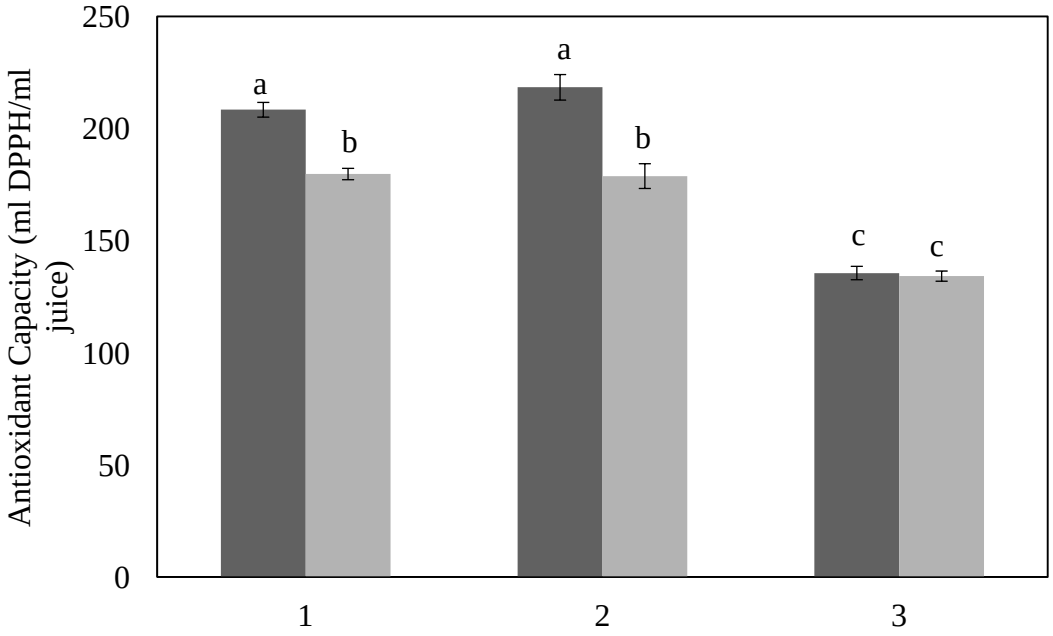

Figure 3.5 Antioxidant activities of strawberry juices without enzyme treatment: (■) and depectinized strawberry juices: (■). (1: Ordinary strawberry juice, 2: Ottoman strawberry juice, 3: Commercial strawberry juice). Different letters represent significant difference ($p \leq 0.05$)

3.6 Comparison of $CIE_{L^*a^*b^*}$ values of Ordinary Strawberry, Ottoman Strawberry (*F. ananassa*) and Commercial Strawberry Juices

In particular, significant color differences were determined among the strawberry juice samples. Ottoman strawberry and ordinary strawberry juices without centrifuging were compared with the commercial strawberry juice. Color values were indicated in Table 3.1. Lightness (L^*) values of the strawberry juices were significantly different from each other (Table A.6) ($p \leq 0.05$). Commercial strawberry juice had the highest lightness value and ottoman strawberry juice had the lowest lightness value. For the strawberry juice, redness is the most important quality parameter. In the study, significant difference was observed among the redness (a^*) values of the strawberry juice samples (Table A.7) ($p \leq 0.05$). While

commercial strawberry juice had the highest redness value, the lowest redness value was observed in Ottoman strawberry juice. Actually, this case is related to the nature of the ottoman strawberry fruit because original color of the Ottoman strawberry is light pink. This results in lower redness values. Furthermore, yellowness (b^*) and color difference (ΔE^*) values of the strawberry juice samples were significantly different (Tables A.8 & A.9) ($p \leq 0.05$). Commercial strawberry juice had the highest yellowness value and Ottoman strawberry juice had the lowest yellowness value. On the other hand, the highest color difference belonged to Ottoman strawberry juice and the lowest one belonged to commercial strawberry juice.

Table 3.1 Lightness (L^*), redness (a^*), yellowness (b^*) and difference (ΔE^*) values of three different types of strawberry juices

Type of Strawberry Juice	**L^***	**a^***	**b^***	**ΔE^***
Ordinary SJ	6.57 ± 0.251^b	30.48 ± 0.469^b	11.32 ± 0.425^b	98.92 ± 0.056^b
Ottoman SJ	0.69 ± 0.070^c	3.37 ± 0.304^c	1.09 ± 0.095^c	99.37 ± 0.059^a
Commercial SJ	15.96 ± 0.180^a	35.92 ± 0.079^a	27.16 ± 0.256^a	95.35 ± 0.058^c

*Different letters represent significant difference ($p \leq 0.05$). SJ: Strawberry juice

3.7 Enzyme Effect on the $CIE_{L^*a^*b^*}$ values of the Ordinary Strawberry Juice and Ottoman Strawberry (*F. ananassa*) Juice

Enzyme treatment decreases the juice turbidity because of the hydrolysis of pectin substances and causing pectin protein complex to flocculate (Saxena et al., 2014). This can also change the color of the fruit juice since the flocculation of pectin particles, which cause the decrease in turbidity, affect the lightness directly. Furthermore, it causes clear appearance due to the flocculation so redness is affected significantly. $CIE_{L^*a^*b^*}$ values of the ordinary strawberry juice were given in Table 3.2. According to ANOVA results, color values of depectinized juice were found to be higher than the color values of the juice without enzyme treatment (Tables A.10, A.11, A.12 & A.13). An attractive red color is important for the strawberry juice. With the help of enzyme, desirable red color was achieved. Significant difference was observed between the redness values of the depectinized juice and the juice without enzyme ($p \leq 0.05$). Besides redness values, enzyme treatment affected the lightness and yellowness.

Table 3.2 Lightness (L^*), redness (a^*), yellowness (b^*) and difference (ΔE^*) values of the ordinary strawberry juice (without enzyme and with enzyme)

Ordinary Strawberry Juice	L^*	a^*	b^*	ΔE^*
Juice without enzyme	50.03±0.155[b]	65.29±0.100[b]	81.93±0.198[b]	116.07±0.128[b]
Juice with enzyme (Depectinized juice)	50.32±0.01[a]	66.09±0.010[a]	83.89±0.012[a]	117.79±0.005[a]

*Different letters represent significant difference ($p \leq 0.05$).

For Ottoman strawberry juice, $CIE_{L^*a^*b^*}$ color values were indicated in Table 3.3. As can be seen in Table 3.3, there was significant difference between depectinized juice and the juice without enzyme for lightness, redness, yellowness and color difference values (Tables A.14, A.15, A.16 & A.17) ($p \leq 0.05$). In ordinary strawberry juice, enzyme caused to increase all color values. On the other hand, lightness and redness values of Ottoman strawberry juice showed increasing behavior but yellowness and ΔE^* values decreased with the addition of enzyme. These results revealed that color values of ordinary strawberry and Ottoman strawberry juices were affected differently from the enzyme treatment. Nevertheless, the redness value is the most important quality parameter for the strawberry juice. As expected, redness value of Ottoman strawberry juice increased with depectinization.

Table 3.3 Lightness (L^*), redness (a^*), yellowness (b^*) and difference (ΔE^*) values of the Ottoman strawberry (*F.ananassa*) juice (without enzyme and with enzyme)

Ottoman Strawberry Juice	**L^***	**a^***	**b^***	**ΔE^***
Juice without enzyme	73.82 ± 0.162^b	23.24 ± 0.075^b	24.28 ± 0.055^a	42.61 ± 0.168^a
Juice with enzyme (Depectinized juice)	81.74 ± 0.163^a	26.96 ± 0.352^a	18.81 ± 0.241^b	37.60 ± 0.450^b

*Different letters represent significant difference ($p \leq 0.05$).

3.8 Nuclear Magnetic Resonance (NMR) Measurement for Ordinary Strawberry Juice and Commercial Strawberry Juice

3.8.1 NMR T_1 values of Ordinary Strawberry Juice and Commercial Strawberry Juice

One of the parameters of Nuclear Magnetic Resonance is T_1 that can provide information about how water is structurally bound in the tissues (Otero & Préstamo, 2009). To understand clearly the water content change in depectinized ordinary strawberry and commercial strawberry juices, NMR T_1 values were determined. These values were given in Fig.3.6. Enzyme treatment affected significantly the T_1 value of the ordinary strawberry juice according to ANOVA results (Table A.18) ($p \leq 0.05$). It caused disruption of the pectin substances and juice became less viscous (Hubert et al., 2007). And then, T_1 value of the juice increased because of the rise in water content. Although there was approximately 14% increase in the T_1 values of ordinary strawberry juice due to enzyme treatment, the increase in the T_1 values of the commercial strawberry juice was approximately 8%. This phenomena can be explained that commercial strawberry juice exposed to some processes such as filtration and pasteurization. These processes could have affected the pectin content or total soluble solid content during the production. So, the enzyme treatment became less effective in the commercial strawberry juice. Significant difference was not observed between the commercial juice without enzyme treatment and depectinized commercial juice.

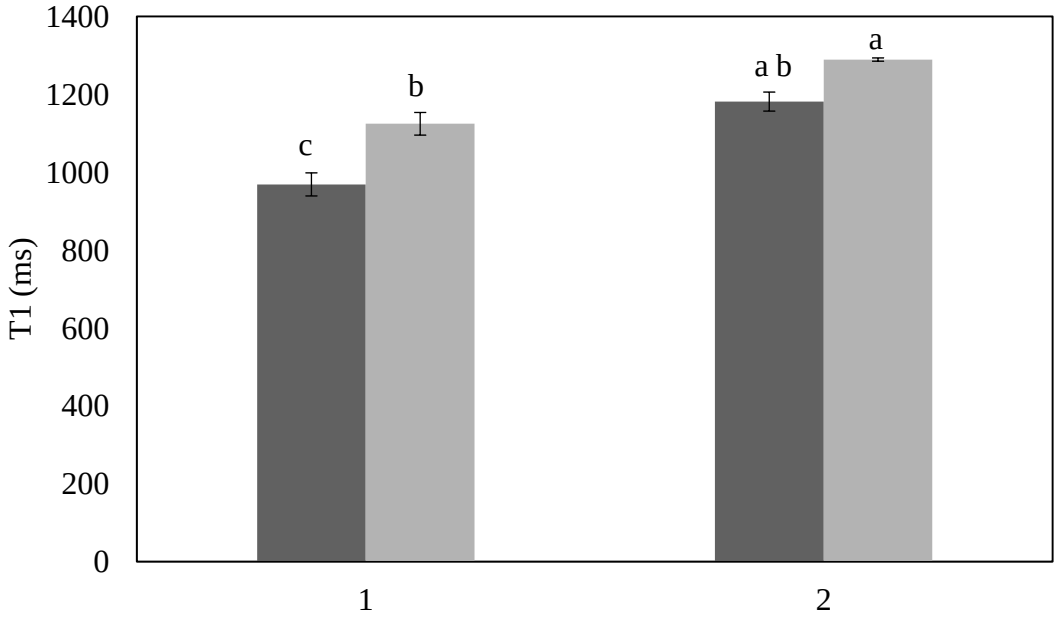

Figure 3.6 T_1 values of strawberry juices without enzyme treatment: (■) and depectinized strawberry juices: (■). (1: Ordinary strawberry juice, 2: Commercial strawberry juice). Different letters represent significant difference ($p \leq 0.05$)

3.8.2 NMR T_2 values of Ordinary Strawberry Juice and Commercial Strawberry Juice

NMR T_2 values of ordinary strawberry juice and commercial strawberry juice were given in Fig. 3.7. The enzyme treatment did not affect T_2 values of the samples (Table A.19) ($p > 0.05$). When comparison was done between the ordinary strawberry juice and commercial strawberry juice, it was seen that enzyme treatment did not change the T_1 and T_2 values of the commercial strawberry juice, significantly (Fig. 3.6 and Fig.3.7). On the other hand, enzyme treatment gave rise to an increase in T_1 values of the ordinary strawberry juice but it did not change the T_2 values of the ordinary strawberry juice, significantly. In this case, T_1 value can be used as a parameter to determine the quality of depectinized ordinary strawberry juice in the experiments.

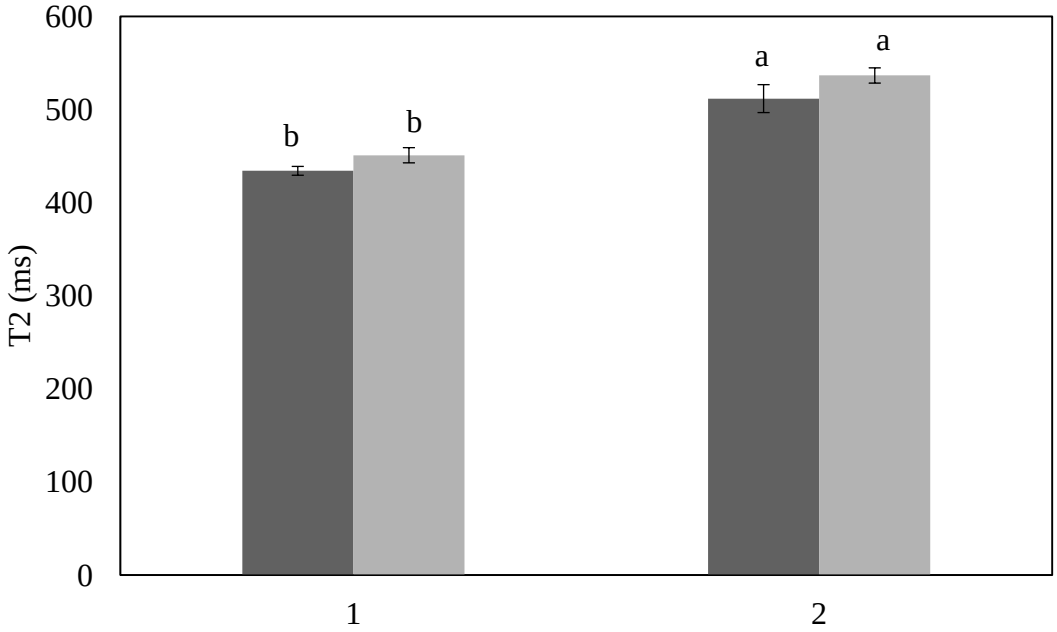

Figure 3.7 T_2 values of strawberry juices without enzyme treatment: (■) and depectinized strawberry juices: (■). (1: Ordinary strawberry juice, 2: Commercial strawberry juice). Different letters represent significant difference ($p \leq 0.05$)

3.9 Effect of Microfluidization on the Ottoman Strawberry (*F.ananassa*) Juice

3.9.1 Total Phenolic Content (TPC)

TPC of Ottoman strawberry juice samples was evaluated using Folin-Ciocalteau method. The total phenolic content of control sample was determined as 594.3 mg GAE/L (Fig. 3.8). The total phenolic contents of 2 and 5 passes treated samples under 600 bar pressure were determined as 573.7±1.428 and 601.01±0.000 mg GAE/L, respectively (Fig. 3.8). Similar to antioxidant activity results, there was no significant difference between control and treated samples under the pressure of 600 bar (Table A.20) ($p>0.05$). For 1000 bar treatment, there was significant difference between control and 2 passes and also 5 passes treated samples (Table A.21) ($p\leq0.05$). The total phenolic contents of 2 and 5 passes treated samples were

determined as 662.9±3.086 and 656.2±6.172 mg GAE/L, respectively when 1000 bar pressure was applied. Applying the pressure treatment to the juice at 1000 bar resulted an increase on the total phenolic content of Ottoman strawberry juice. From these results, it can be concluded that the effect of HPH on the total phenolic content of Ottoman strawberry juice showed similarity with antioxidant activity results. That is, applying high pressure improved both total phenolic content and antioxidant capacity of the juice. The reason of this phenomena is that phenolic compounds exhibit strong antioxidant activity. And also, there has been strong correlations between total phenolics and antioxidant capacity (Gündüz & Özdemir, 2014). Moreover, Pearson correlation between total phenolic content and antioxidant activity was found as 0.993 for Ottoman strawberry juice ($p \leq 0.05$) which was consistent with the published literature. Normally, the decrease of phenols in processed strawberries occur by polyphenol oxidase (PPO) and peroxidase (POD) enzymes (Cao et al., 2012). However, these two enzymes were considered to be inactivated in homogenization process in this study and enzymatic degradation of total phenols was not expected because the temperature of sample was measured as 43.3 ˚C and 56.6 ˚C for 2 passes and 5 passes, respectively, at 1000 bar pressure. Terefe et al. (2009) evaluated that high pressure combined with mild temperature gave rise to substantial inactivation of peroxidase in strawberries at 60 ˚C. Furthermore, Cano et al. (1997) found that pressurization/depressurization treatments gave rise to a significant loss of strawberry polyphenol oxidase up to 250 MPa and peroxidase activity up to 230 MPa. Thus, the total phenolic content of Ottoman strawberry juice increased, that is, homogenization process released more phenolic compounds and these compounds might have not been adversely affected due to the inactivation of these two enzymes. Patras et al. (2009) also indicated that the content of phenols in strawberry puree increased by high hydrostatic pressure (HHP) treatment.

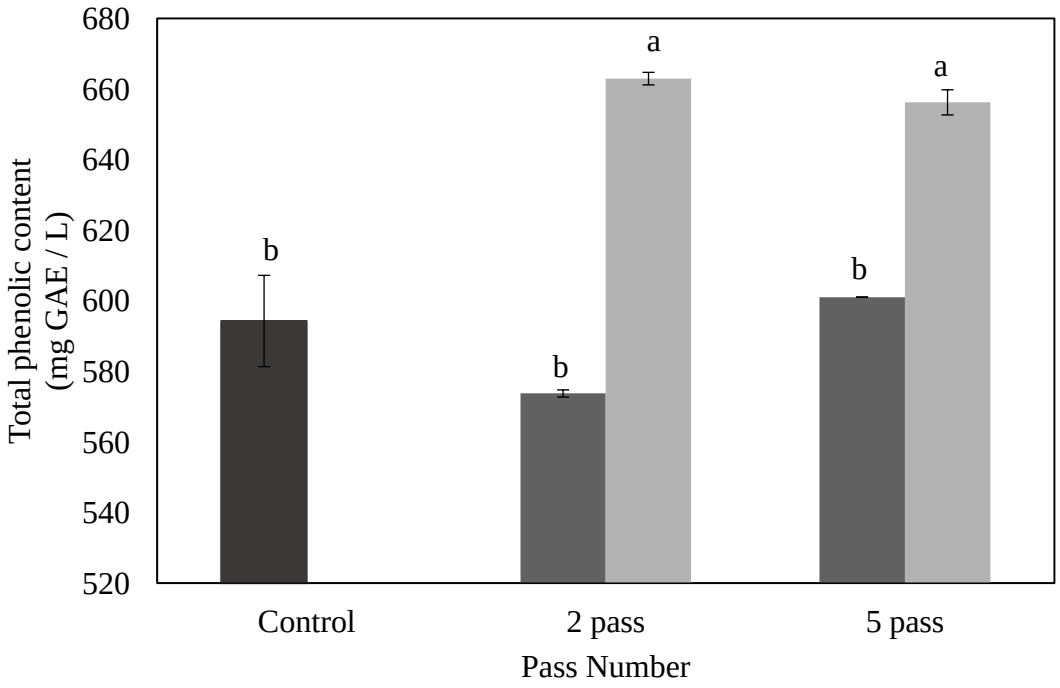

Figure 3.8 Total phenolic content of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number (ml GAE/ L) and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

3.9.2 Antioxidant Activity

Antioxidant activities of ottoman strawberry juice homogenized under pressures of 600 bar and 1000 bar for 2 and 5 passes, and also control sample (no homogenization) are shown in Fig. 3.9. The antioxidant activity of control sample was determined as 112.5±8.168 ml DPPH/ml juice while for 600 bar treatment, the antioxidant activities of 2 and 5 passes treated samples were determined as 117.4±3.861 ml DPPH/ml juice and 125.6±0.000 ml DPPH/ml juice, respectively. ANOVA results showed that, there was no significant difference between control and treated samples under the pressure of 600 bar (Table A.22) ($p>0.05$). As a result, it was concluded that microfluidization at 600 bar did not alter the antioxidant

activity of the juice, significantly. Velázquez-Estrada et al. (2013) evaluated the ultra-high pressure homogenization (UHPH) treatments on the antioxidant activity of orange juice. Fresh squeezed orange juice was pre-warmed at two inlet temperatures (10 and 20 °C) and processed at 100, 200 and 300 MPa and then, no significant differences were observed on the antioxidant capacity values between the fresh orange juice and orange juice samples treated with ultra-high pressure homogenization. For the pressure of 1000 bar, there was significant difference between control sample and 2 passes treated samples (Table A.23) ($p \leq 0.05$). The antioxidant activity of 2 passes treated samples was determined as 133.8±0.000 ml DPPH/ml juice. The antioxidant activity of the juice homogenized under pressure of 1000 bar for 2 passes increased approximately by 16% as compared to that of control sample. Thus, it was shown that microfluidization at higher pressures could improve the antioxidant capacity. Similarly in another study conducted by Wang et al. (2013), it was demonstrated that the microfluidization could improve physicochemical properties of wheat bran when pass number was increased and found that the antioxidant capacity increased with increase in the extent of the treatment. Although the decrease in the antioxidant activity was observed when pass number was increased from 2 to 5, this decrease was not found to be significant ($p > 0.05$). At the end of 5 passes, the temperature of the juice was 56°C and at this temperature, anthocyanins in the juice may have degraded. This phenomena probably caused the decrease in antioxidant capacity of the juice at the end of 5 passes because there was strong correlations between anthocyanins and antioxidant capacity (Gündüz & Özdemir, 2014).

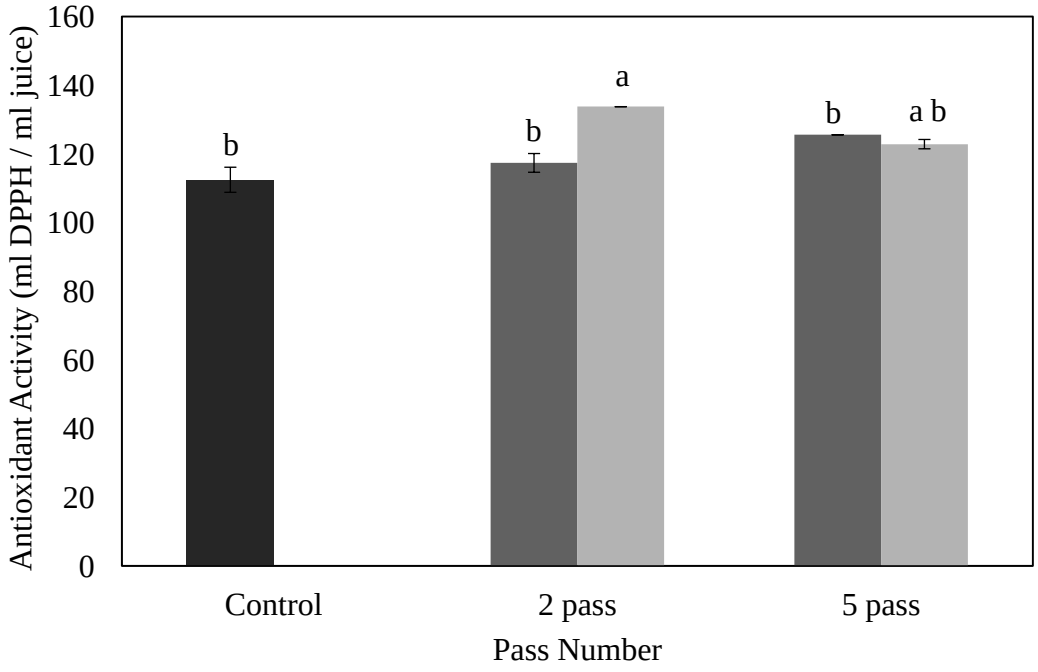

Figure 3.9 Antioxidant activity of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number (ml DPPH/ml juice) and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

3.9.3 Particle Size

The particle size distribution of Ottoman strawberry juice homogenized under pressures of 600 and 1000 bar according to pass number is shown in Fig. 3.10. Mean particle sizes of the juice decreased as the homogenization pass number increased for two different pressure treatments. Pressure did not affect the particle size. There was significant difference between samples treated with different pass number (Tables A.24 & A.25) ($p \leq 0.05$). As expected, the high pressure homogenization process decreased the mean particle diameter, as previously observed for tomato juice

(Augusto, Ibarz, & Cristianini, 2012), passion fruit juice (Okoth, Kaahwa, & Imungi, 2000), citrus juices (Betoret, Betoret, Carbonell, & Fito, 2009; Sentandreu, Gurrea, Betoret, & Navarro, 2011; Lacroix et al., 2005), and apple juice (Donsi, Esposito, Lenza, Senatore, & Ferrari, 2009).

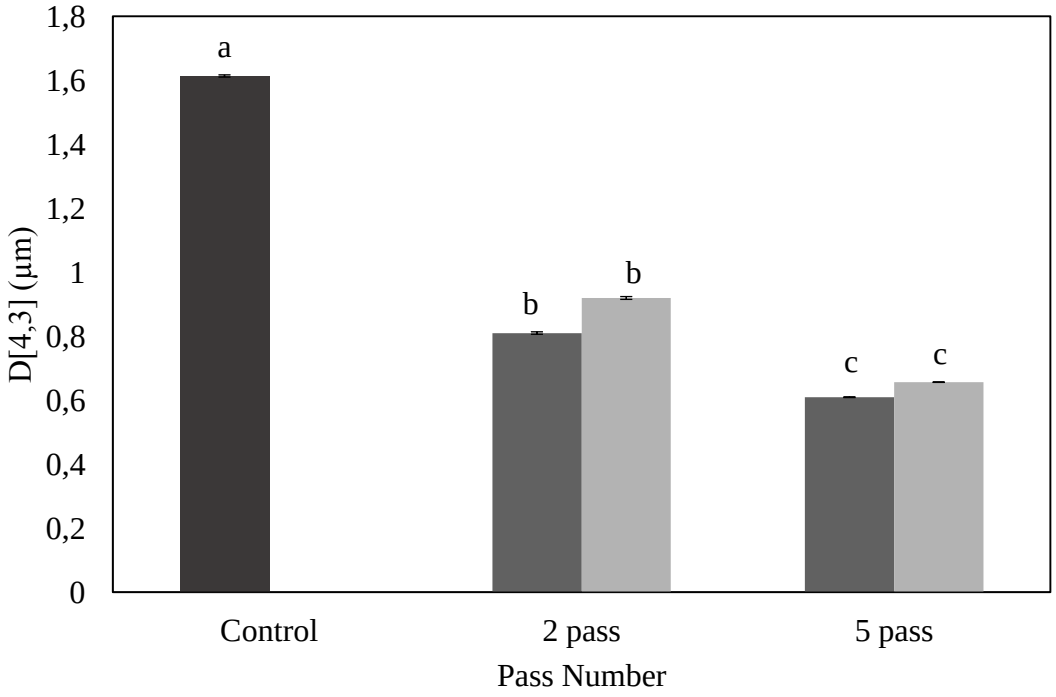

Figure 3.10 Particle size distribution of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number (µm) and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

3.9.4 Nuclear Magnetic Resonance (NMR)

3.9.4.1 Spin-Lattice Relaxation Time (T_1)

The spin-lattice relaxation time, T_1, represents the spin recovery constant in the direction of the external magnetic field (Zhang & McCarthy, 2013). This parameter, like T_2, can provide information about how water is structurally bound in the tissues (Otero & Préstamo, 2009). NMR T_1 values of Ottoman strawberry juice treated at the pressures of 600 bar and 1000 bar are shown in Fig. 3.11. For 600 bar treatment, no significant difference was observed between control and treated samples (Table A.26) ($p>0.05$). However, T_1 values of the samples treated at 1000 bar showed significant difference (Table A.27) ($p \leq 0.05$). Decrease in T_1 value was observed when sample was homogenized with 2 passes as compared to control sample. From control sample to 2 passes treated sample, T_1 value of the juice decreased (Fig. 3.11). Decrease in T_1 value was explained due to the substantial increase in viscosity (Kerr & Wicker, 2000).

Fig. 3.12 shows the brix values of the homogenized juice. Brix reflects the total soluble solid content and affects the water content of the fruit, directly. A direct relation was sought between brix and T_1 values of the juice but a meaningful interpretation could not be made. For 600 bar treatment, significant difference was observed between control and 5 passes treated samples (Table A.28) ($p \leq 0.05$), that is, the high pressure homogenization increased the total soluble solid content of the juice at 600 bar. For 1000 bar treatment, there was no significant difference between untreated and treated samples (Table A.29) ($p>0.05$).

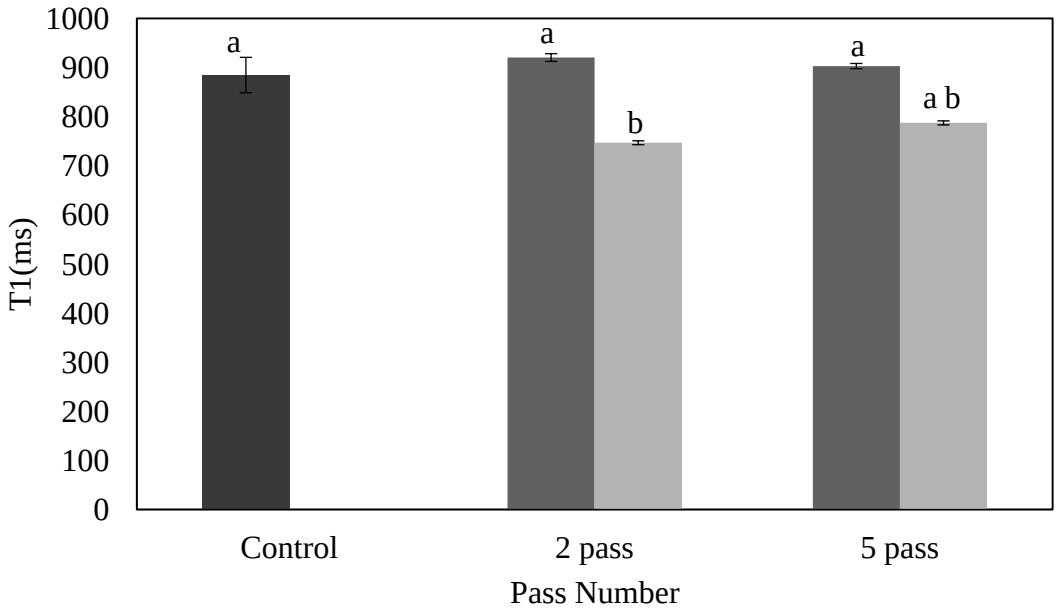

Figure 3.11 T_1 values of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number (ms) and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

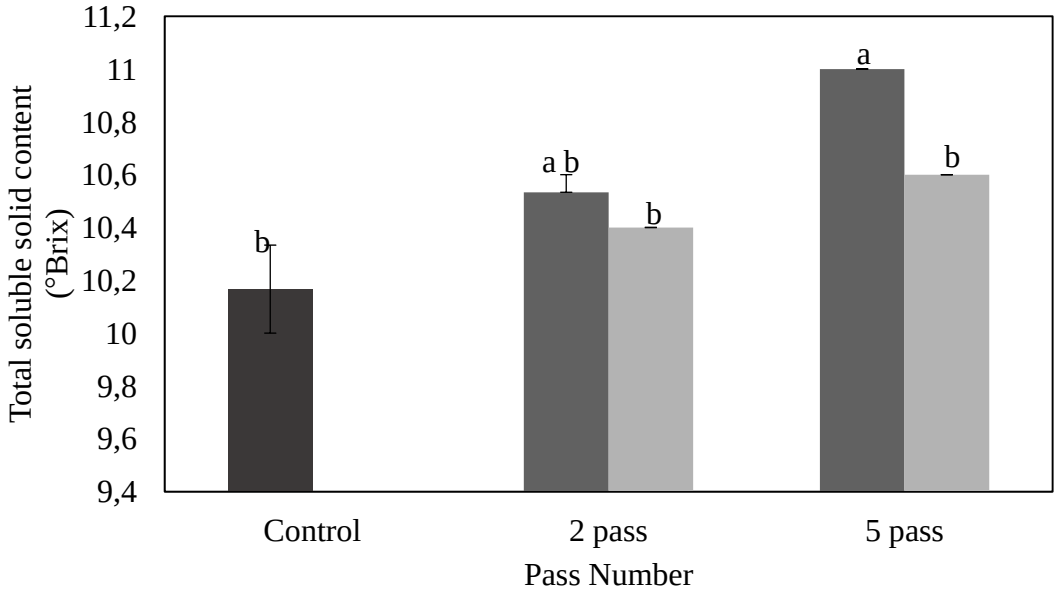

Figure 3.12 Total soluble solid content of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

3.9.4.2 Spin-Spin Relaxation Time (T_2)

The spin-spin relaxation time, T_2, represents the rate of the relaxation in the direction perpendicular to the external magnetic field and the relaxation process includes energy loss to its neighboring spins and the environments (Zhang & McCarthy, 2013). NMR T_2 relaxation measurement gives information on the water distribution of plant tissue (Zhang & McCarthy, 2013). In this study, T_2 relaxation measurement was performed in order to understand the physiological changes accompanied by microfluidization. Nuclear magnetic resonance T_2 (spin-spin relaxation) times of Ottoman strawberry juice treated at the pressures of 600 bar and 1000 bar are shown in Fig. 3.13. For 1000 bar treatment, no significant difference was found between T_2 values of 2 passes and 5 passes treated samples and control. However, there was significant difference between the T_2 times of the juice treated at 600 bar and untreated samples (Table A.30) ($p \leq 0.05$). It could be concluded that applying low pressure was sufficient to change the T_2 value of the juice, significantly.

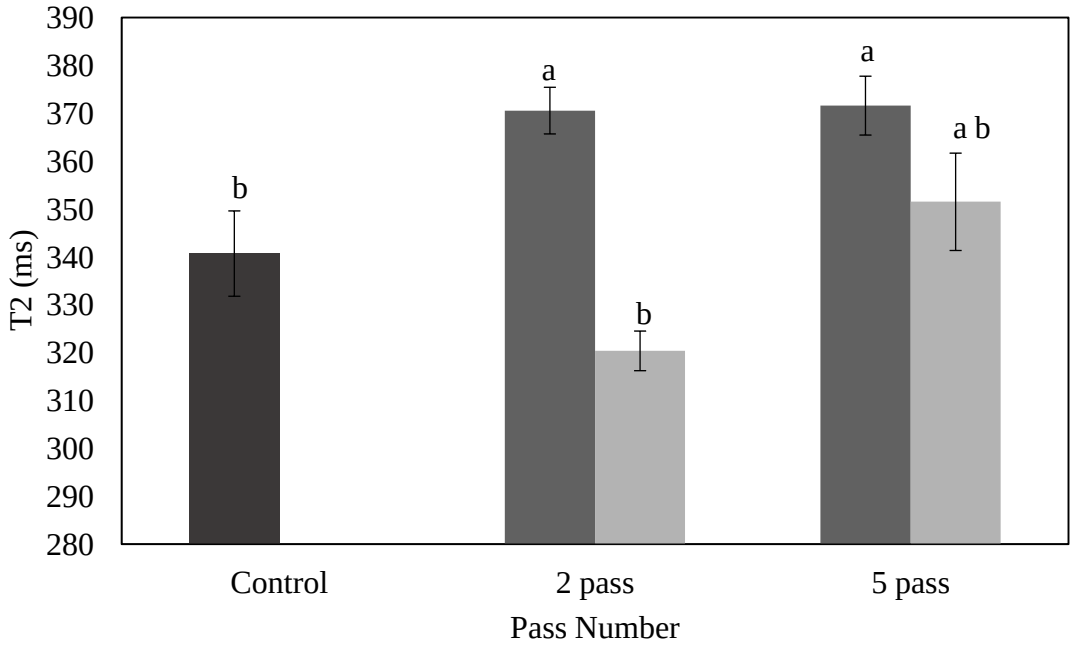

Figure 3.13 T_2 values of Ottoman strawberry juice homogenized at the pressures of 600 bar: (■) and 1000 bar: (■) according to the pass number (ms) and control sample: (■). Different letters represent significant difference ($p \leq 0.05$)

3.9.5 Color

High pressure homogenization gave rise to color modifications of the sample. In particular, significant color differences were determined between control and treated samples. Color values were indicated in Table 3.4. For the pressure of 600 bar, there was significant difference between control and homogenized samples ($p \leq 0.05$) for L* (lightness) values of the juice (Table A.31). As the pass number increased, the lightness of the juice decreased. On the other hand, a* (redness) values of the juice increased, as the pass number increased. There was significant difference between control sample, 2 passes treated sample and 5 passes treated sample ($p \leq 0.05$) for a* values of the juice (Table A.32). This could be explained by the anthocyanin content

of the juice because anthocyanin was responsible for the red color of the strawberry. With the effect of high pressure homogenization, anthocyanin content could increase during the process, so the redness could increase as the anthocyanins content of the organic strawberry cultivation was consistent with $CIE_{L^*a^*b^*}$ color determinations (Crecente-Campo et al., 2012). The color parameters, except b^* value, were closely correlated ($p \leq 0.05$) to anthocyanin contents in strawberry juices (Cao et al., 2012). Moreover, b* (yellowness) and ΔE* (color difference) values of the juice increased, as the pass number increased. Significant differences were found between all samples for b* and ΔE* values of the juice (Tables A.33 & A.34) ($p \leq 0.05$).

For the pressure of 1000 bar, as the number of passes increased, L* (lightness) value of the juice decreased similar to 600 bar. Furthermore, b* and ΔE* values of the juice increased with increasing pass number. Significant difference was found between all samples for L*, b* and ΔE* values of the juice ($p \leq 0.05$). On the other hand, a* value of the juice homogenized at 1000 bar showed a different behavior than ones treated at 600 bar. From control to 2 passes treated one, redness increased similar to the increase in the antioxidant activity and total phenolic content for 1000 bar since there was strong correlations between total phenolics, anthocyanins and antioxidant capacity (Gündüz & Özdemir, 2014). However, redness value of the juice showed a decreasing behavior from 2 passes treated sample to 5 passes treated sample. The degradation of anthocyanins could be influenced by different parameters such as temperature (Zabetakis et al., 2000). Temperature was high (56.6 °C) for 1000 bar treatment at the end of 5 passes. Anthocyanins of the juice could have degraded at this temperature and redness decreased as the pass number increased. The degradation of anthocyanin at higher temperature may also be related to the Maillard reaction (non-enzymatic browning) that occurred in the presence of sugars and proteins during food processing at high temperatures (Tonon, Brabet, & Hubinger, 2010). According to Von Elbe & Schwartz (1996), the presence of sugars or products resulting from their degradation could cause an acceleration on the anthocyanin degradation because this reaction rate provided the conversion of sugar to furfural and furfural was a product resulting from the Maillard reaction that condensed together with the anthocyanins, leading to the compound formation which caused the brown color. This reaction was highly dependent on temperature,

occurring more frequently in fruit juices (Tonon et al., 2010). According to ANOVA results, significant difference between untreated and treated samples homogenized at 1000 bar was observed for $CIE_{L^*a^*b^*}$ color values (Tables A.35, A.36, A.37 & A.38) ($p \leq 0.05$).

Table 3.4 Lightness (L^*), redness (a^*), yellowness (b^*) and difference (ΔE^*) values of microfluidized Ottoman strawberry (*F.ananassa*) juice

Pressure (Bar)	**Pass Number**	**L^***	**a^***	**b^***	**ΔE^***
600	0	66.79 ± 0.015^{b}	28.36 ± 0.00^{f}	28.94 ± 0.011^{e}	52.38 ± 0.011^{f}
	2	58.85 ± 0.026^{c}	29.49 ± 0.011^{e}	31.59 ± 0.015^{c}	59.67 ± 0.024^{d}
	5	56.33 ± 0.021^{e}	30.65 ± 0.017^{d}	33.13 ± 0.015^{a}	62.80 ± 0.030^{b}
1000	0	67.09 ± 0.02^{a}	31.00 ± 0.017^{b}	29.31 ± 0.005^{d}	53.88 ± 0.015^{e}
	2	58.06 ± 0.01^{d}	31.22 ± 0.00^{a}	32.12 ± 0.010^{b}	61.36 ± 0.010^{c}
	5	52.93 ± 0.01^{f}	30.83 ± 0.02^{c}	33.09 ± 0.005^{a}	65.28 ± 0.016^{a}

*Different letters represent significant difference ($p \leq 0.05$).

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Enzyme addition for depectinization causes changes in the physical and chemical properties of fruit juice during the production.

In this study, it was observed that enzyme addition did not affect the total phenolic content of ordinary strawberry juice but it changed the turbidity of strawberry juice significantly.

Without enzyme treatment, total phenolic content and antioxidant activity of commercial juice were found to be lower than the other types of strawberry juices and there was no difference between Ottoman strawberry juice and ordinary strawberry juice in terms of antioxidant capacity. However, these juices had different total phenolic contents. Enzyme addition caused changes in the total phenolic contents of Ottoman strawberry, ordinary strawberry and commercial strawberry juices. These juices became significantly different in terms of total phenolic content. Also, their antioxidant activities were affected from enzyme treatment except for commercial strawberry juice. Color analysis was conducted without enzyme in order to make a comparison among three different types of strawberry juices. Significant differences were determined among these strawberry juices.

Enzyme effect on color values of ordinary strawberry juice and Ottoman strawberry juice was investigated, separately. $CIE_{L^*a^*b^*}$ color values of depectinized ordinary strawberry juice were found to be higher than the color values of the juice without enzyme. For Ottoman strawberry juice, lightness and redness values increased but yellowness and color difference values decreased with the enzyme treatment. Also, NMR measurements were conducted to understand the water content change in depectinized ordinary strawberry and commercial strawberry juices.

In addition to the enzyme treatment, particle size change can affect the physical and chemical properties of fruit juice. Silent crusher changed the turbidity and total phenolic content of ordinary strawberry juice. It caused an increase in the turbidity and total phenolic content. Moreover, microfluidization treatment increased the total phenolic content of ordinary strawberry juice.

Microfluidization treatment caused changes in the physical and chemical properties of Ottoman strawberry juice.

For future study, effect of different processing conditions on the aroma compounds of Ottoman strawberry could be investigated. Optimum process conditions could be determined to prevent the loss of strawberry aroma during the juice production.

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APPENDIX A

STATISTICAL ANALYSES

Table A.1 One way ANOVA for silent crusher and enzyme effect on the serum cloudiness (turbidity) of ordinary strawberry juice

Source	DF	SS	MS	F	P
Enz.&Sil.Cr.Effect	2	0.0499336	0.0249668	112350.50	0.000
Error	6	0.0000013	0.0000002		
Total	8	0.0499349			

Table A.2 One way ANOVA for silent crusher and enzyme effect on the total phenolic content of ordinary strawberry juice

Source	DF	SS	MS	F	P
Enz.&Sil.Cr.Effect	2	13355	6677	9.70	0.013
Error	6	4130	688		
Total	8	17485			

Table A.3 One way ANOVA for microfluidization and enzyme effect on the total phenolic content of ordinary strawberry juice

Source	DF	SS	MS	F	P
Microf.&Enz.	2	36636.6	18318.3	517.90	0.000
Error	6	212.2	35.4		
Total	8	36848.8			

Table A.4 Two way ANOVA for total phenolic contents of strawberry juices without enzyme and depectinized strawberry juices (Ordinary strawberry juice, Ottoman strawberry juice, Commercial strawberry juice)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Type of juice	2	388116	388116	194058	970.39	0.000
Enzyme Type	1	53334	53334	53334	266.70	0.000
Juice.T.*Enz.T.	2	78523	78523	39262	196.33	0.000
Error	12	2400	2400	200		
Total	17	52237				

Table A.5 Two way ANOVA for antioxidant activities of strawberry juices without enzyme and depectinized strawberry juices (Ordinary strawberry juice, Ottoman strawberry juice, Commercial strawberry juice)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Type of juice	2	15158.4	15158.4	7579.2	161.23	0.000
Enzyme Type	1	2423.8	2423.8	2423.8	51.56	0.000
Juice T.*Enz.T.	2	1163.0	1163.0	581.5	12.37	0.001
Error	12	564.1	564.1	47.0		
Total	17	19309.3				

Table A.6 One way ANOVA for lightness (L^*) values of three different types of strawberry juices

Source	DF	SS	MS	F	P
Juice Type	2	355.7550	177.8775	5323.90	0.000
Error	6	0.2005	0.0334		
Total	8	355.9555			

Table A.7 One way ANOVA for redness (a^*) values of three different types of strawberry juices

Source	DF	SS	MS	F	P
Juice Type	2	1824.446	912.223	8596.87	0.000
Error	6	0.637	0.106		
Total	8	1825.083			

Table A.8 One way ANOVA for yellowness (b^*) values of three different types of strawberry juices

Source	DF	SS	MS	F	P
Juice Type	2	1035.166	517.583	6074.13	0.000
Error	6	0.511	0.085		
Total	8	1035.67			

Table A.9 One way ANOVA for color difference (ΔE^*) values of three different types of strawberry juices

Source	DF	SS	MS	F	P
Juice Type	2	29.21584	14.60792	4366.81	0.000
Error	6	0.02007	0.00335		
Total	8	29.23591			

Table A.10 One way ANOVA for lightness (L^*) values of the ordinary strawberry juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ord.Str.	1	0.1261	0.1261	10.43	0.032
Error	4	0.0484	0.0121		
Total	5	0.1745			

Table A.11 One way ANOVA for redness (a^*) values of the ordinary strawberry juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ord.Str.	1	0.96000	0.96000	190.10	0.000
Error	4	0.02020	0.00505		
Total	5	0.98020			

Table A.12 One way ANOVA for yellowness (b^*) values of the ordinary strawberry juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ord.Str.	1	5.7820	5.7820	295.25	0.000
Error	4	0.0783	0.0196		
Total	5	5.8604			

Table A.13 One way ANOVA for color difference (ΔE^*) values of the ordinary strawberry juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ord.Str.	1	4.42663	4.42663	535.48	0.000
Error	4	0.03307	0.00827		
Total	5	4.45969			

Table A.14 One way ANOVA for lightness (L^*) values of the Ottoman strawberry (*F.ananassa*) juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ott.Str.	1	94.2481	94.2481	3585.85	0.000
Error	4	0.1051	0.0263		
Total	5	94.3532			

Table A.15 One way ANOVA for redness (a^*) values of the Ottoman strawberry (*F.ananassa*) juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ott.Str.	1	20.7576	20.7576	321.08	0.000
Error	4	0.2586	0.0647		
Total	5	21.0162			

Table A.16 One way ANOVA for yellowness (b^*) values of the Ottoman strawberry (*F.ananassa*) juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ott.Str.	1	44.9908	44.9908	1468.69	0.000
Error	4	0.1225	0.0306		
Total	5	45.1134			

Table A.17 One way ANOVA for color difference (ΔE^*) values of the Ottoman strawberry (*F.ananassa*) juice (without enzyme and with enzyme)

Source	DF	SS	MS	F	P
Ord.Str.	1	37.587	37.587	325.23	0.000
Error	4	0.462	0.116		
Total	5	38.050			

Table A.18 Two way ANOVA for T_1 values of ordinary strawberry juice and commercial strawberry juice (without enzyme and with enzyme)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Type of juice	1	107081	107081	107081	61.05	0.000
Type of enzyme	1	52122	52122	52122	29.71	0.001
Juice T.*Enz.T.	1	1727	1727	1727	0.98	0.350
Error	8	14033	14033	1754		
Total	11	174963				

Table A.19 Two way ANOVA for T_2 values of ordinary strawberry juice and commercial strawberry juice (without enzyme and with enzyme)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Type of juice	1	20004.0	20004.0	20004.0	69.93	0.000
Type of enzyme	1	1297.0	1297.0	1297.0	4.53	0.066
Juice T.*Enz.T.	1	49.6	49.6	49.6	0.17	0.688
Error	8	2288.5	2288.5	286.1		
Total	11	23639.1				

Table A.20 One way ANOVA for total phenolic content of Ottoman strawberry juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	940	470	0.75	0.503
Error	8	5019	627		
Total	10	5959			

Table A.21 One way ANOVA for total phenolic content of Ottoman strawberry juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	14333	7167	15.75	0.002
Error	8	3641	455		
Total	10	17974			

Table A.22 One way ANOVA for antioxidant activity of Ottoman strawberry juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	247.5	123.8	2.63	0.151
Error	6	281.8	47.0		
Total	8	529.3			

Table A.23 One way ANOVA for antioxidant activity of Ottoman strawberry juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	676.6	338.3	7.50	0.023
Error	6	270.6	45.1		
Total	8	947.2			

Table A.24 One way ANOVA for particle size distribution of Ottoman strawberry juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	1.694833	0.847416	32316.72	0.000
Error	6	0.000157	0.000026		
Total	8	1.694990			

Table A.25 One way ANOVA for particle size distribution of Ottoman strawberry juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	1.467468	0.733734	23753.97	0.000
Error	6	0.000185	0.000031		
Total	8	1.467653			

Table A.26 One way ANOVA for T_1 values of Ottoman strawberry juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	2201	1101	0.38	0.695
Error	7	20062	2866		
Total	9	22263			

Table A.27 One way ANOVA for T_1 values of Ottoman strawberry juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	35664	17832	6.33	0.027
Error	7	19714	2816		
Total	9	55378			

Table A.28 One way ANOVA for total soluble solid content of Ottoman strawberry juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	1.4067	0.7033	7.36	0.013
Error	9	0.8600	0.0956		
Total	11	2.2667			

Table A.29 One way ANOVA for total soluble solid content of Ottoman strawberry juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	0.3933	0.1967	2.12	0.176
Error	9	0.8333	0.0926		
Total	11	1.2267			

Table A.30 Two way ANOVA for T_2 values of Ottoman strawberry juice homogenized at the pressures of 600 bar and 1000 bar according to the pass number

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	1404.7	1073.4	1073.4	10.01	0.016
Pass	2	1655.9	1591.2	795.6	7.42	0.019
Press.*Pass	2	2331.1	2331.1	1165.5	10.87	0.007
Error	7	750.5	750.5	107.2		
Total	12	6142.0				

Table A.31 One way ANOVA for lightness (L^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	179.1376	89.5688	196614.46	0.000
Error	6	0.0027	0.0005		
Total	8	179.1404			

Table A.32 One way ANOVA for redness (a^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	7.866822	3.933411	27231.31	0.000
Error	6	0.000867	0.000144		
Total	8	7.867689			

Table A.33 One way ANOVA for yellowness (b^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	26.96509	13.48254	67412.72	0.000
Error	6	0.00120	0.00020		
Total	8	26.96629			

Table A.34 One way ANOVA for color difference (ΔE^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 600 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	171.4740	85.7370	160245.24	0.000
Error	6	0.0032	0.0005		
Total	8	171.4773			

Table A.35 One way ANOVA for lightness (L^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	308.5180	154.2590	730700.58	0.000
Error	6	0.0013	0.0002		
Total	8	308.5193			

Table A.36 One way ANOVA for redness (a^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	0.229400	0.114700	491.57	0.000
Error	6	0.001400	0.000233		
Total	8	0.230800			

Table A.37 One way ANOVA for yellowness (b^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	23.23282	11.61641	209095.40	0.000
Error	6	0.00033	0.00006		
Total	8	23.23316			

Table A.38 One way ANOVA for color difference (ΔE^*) values of Ottoman strawberry (*F.ananassa*) juice homogenized at the pressure of 1000 bar according to the pass number

Source	DF	SS	MS	F	P
Homogenization	2	201.4043	100.7021	480911.76	0.000
Error	6	0.0013	0.0002		
Total	8	201.4055			

APPENDIX B

CALIBRATION CURVES

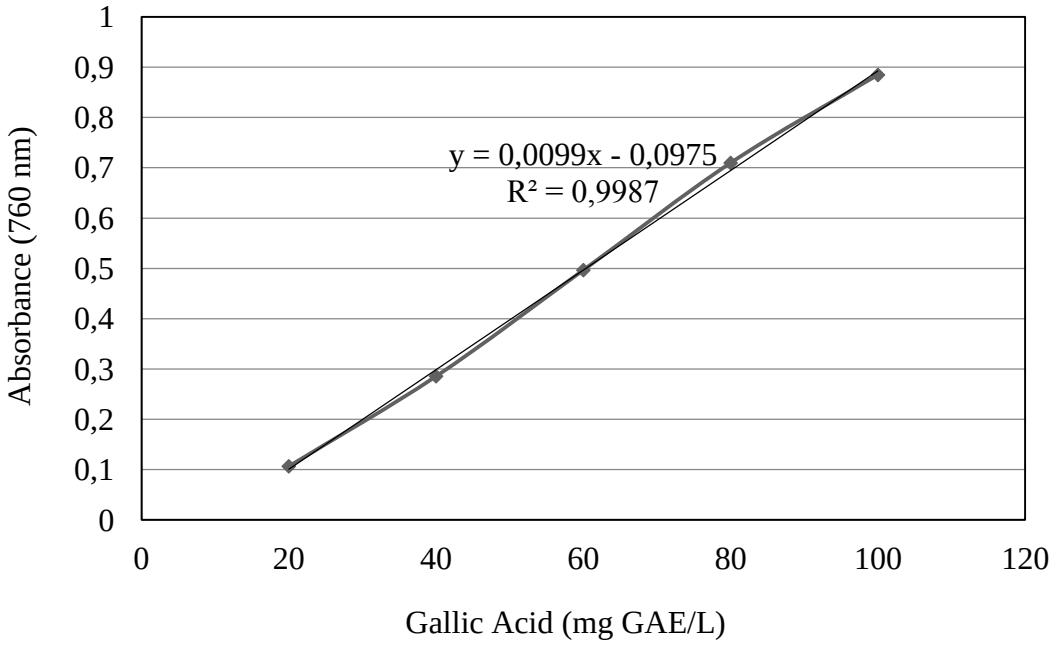

Figure B.1 Calibration curve prepared by gallic acid in ethanol:acetic acid:water mixture (50:8:42 v/v) for determination of total phenolic contents

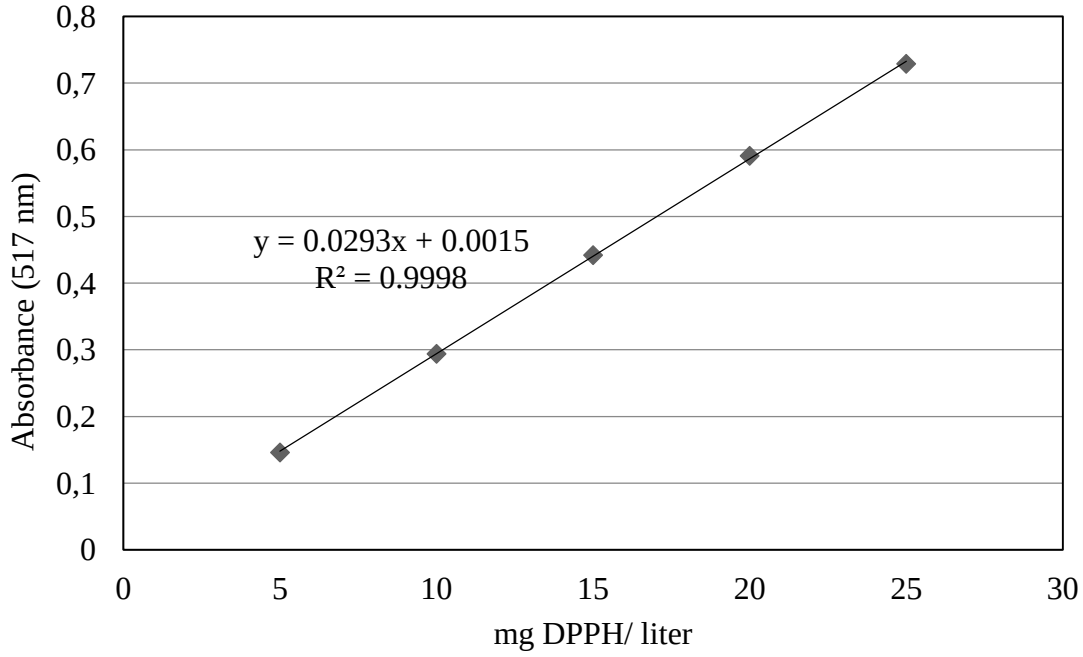

Figure B.2 Calibration curve prepared by DPPH radical in methanol for determination of antioxidant activity (Çilek, 2012)