# PREVENTION OF TURBIDITY IN POMEGRANATE JUICE CONCENTRATE DURING STORAGE TIME

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# ABSTRACT

## PREVENTION OF TURBIDITY IN POMEGRANATE JUICE CONCENTRATE DURING STORAGE TIME

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This study aimed at finding a solution to turbidity problem in pomegranate juice concentrate during storage without reprocessing. In this study Hicaz type pomegranates (*Punica Granatum L.* ) (Hicaznar) were processed into clear fruit juice. Based on the literature studies and the interviews with the sector representatives (Novozymes and Erbslöh), the experiments were carried out on the effect of gelatin in preventing turbidity. In addition, the effect of filtration process of oxalic acid crystals, which is one of the turbidity factors, were also determined. For this purpose, four different trial plans were developed considering all possibilities. In the first experimental design, fresh fruits firstly are sorted, washed and removed from shells. Then crushing, pressing, pasteurization, enzymation, clarification, filtration and concentration stages have been followed. In the second experimental design, after ultrafiltration process pomegranate juice is brought to 30-35 brix, stored at cold temperatures (at three different temperatures) and then passed through paper filter. In the third experimental design, in the clarification stage gelatin is added and followed as in first plan. In the last experimental design, gelatin, cold storage and filter stages are applied. Experimental design 1 and 2; experimental design 3 and 4 is conducted at the same time interval. In this study; sorting, washing and milling steps were conducted in production factories DÖHLER A.Ş. and production of pomegranate juice

concentrate were simulated with the studies that started in the pilot plant and continued in the laboratory and the solution to turbidity was sought.

It was determined that stability problems were not observed in plans using gelatin. In cases where gelatin is not used, it is shown that the waiting period in production should be selected at least 24 hours for three different temperature values to provide stability of products.

Keywords: Pomegranate Juice Concentrate, Turbidity, Gelatin

# ÖZ

## NAR SUYU KONSANTRESİNDE DEPOLAMA SÜRESİ BOYUNCA OLUŞAN BULANIKLIĞIN ENGELLENMESİ

Sert, Dilara
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Bu çalışmanın amacı, depolama süresince nar suyu konsantresindeki bulanıklık problemine bir çözüm bulmaktır. Bu çalışmada Hicaz tipi narlar (Punica granatum L. Hicaznar) berrak meyve suyu olarak işlenmiştir. Literatür çalışmaları ve sektör temsilcileri (Novozymes ve Erbslöh) ile yapılan görüşmelere dayanarak jelatinin bulanıklığı önlemedeki etkisine ilişkin deneyler yapılmıştır. Ek olarak, filtrasyon işleminin bulanıklık faktörlerinden biri olan oksalik asit kristalleri üzerindeki etkisi de gözlenmiştir. Bu amaçla, tüm olasılıklar göz önünde bulundurularak dört farklı deneme planı geliştirilmiştir. Birinci deneme planında, nar hammadde ayıklanmış, yıkanmış ve parçalanmıştır. Daha sonra pilot tesiste presleme, pastörizasyon, enzimasyon, klarifikasyon, ultrafiltrasyon ve konsantrasyon aşamaları takip edilmiştir. İkinci deneme planında, ultrafiltrasyon işleminden sonra nar suyu 30-35 brikse getirilmiş, üç farklı sıcaklık değerinde bekletilmiştir. Üçüncü deneme planında ise klarifikasyon aşamasında jelatin eklenmiş ve diğer aşamalar ilk deneme planındaki gibi takip edilmiştir. Son deneme planında ise jelatin eklenmiş, üç farklı sıcaklık derecesinde bekletilmiş ve filtrasyon uygulanmıştır. Deneme planı 1 ve 2; 3 ve 4 aynı zaman aralığında gerçekleşmiştir.

Jelatin kullanılan planlarda stabilite sorunun gözlemlenmediği belirlenmiştir. Jelatinin kullanılmadığı planlarda, ürünlerin stabilitesini sağlamak için üretim prosesinde bekleme süresinin en az 24 saat seçilmesi gerektiği sonucu ortaya çıkmıştır.

Anahtar Kelimeler: Nar suyu konsantresi, Bulanıklık, Jelatin

I dedicate to this work to my family.

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ABBREVIATIONS

UF: Ultrafiltration

J.C. : Juice Concentrate

# CHAPTER 1

# INTRODUCTION

## 1.1. Pomegranate

Pomegranate is one of the oldest known fruit species. It has been written in the sources that human beings know, eat and heal for 6500 years. First written sources in BC 1550, it is estimated to have been written and is the Ebers Medical Papyrus in Egypt. The word pomegranate is used in Turkish was translated from Persian. The Latin name is Punica granatum. Iran, India and Pakistan are reported to be pomegranate's homeland. Today, especially in Iran, China and India are grown. Turkey, ranks 4th in terms of pomegranate production. Pakistan, Azerbaijan and Spain are other important producer countries. High adaptability of pomegranates, giving fruits 3-4 years after planting tree, rediscovery of the durability and benefits of the fruit is becoming more common day by day. Pomegranate is still produced in all Mediterranean countries, Middle Eastern countries, Asian countries starting from Crimea to Azerbaijan, China, and in USA and South American countries (Karaca, 2011).

Pomegranate is a fruit that can be grown smoothly in many climatic conditions. It is appropriate to have long and warm summers and warm and rainy winters. The need for pomegranate coldening is almost non-existent. In order to ripen the fruits, a high temperature sum is required during the vegetation period. Pomegranate, our country's climate in the cooler regions (such as Central Anatolia) in May, while a little warmer in the Mediterranean (such as Mediterranean regions) starts to bloom in April. Flowering continues until June.

An average annual rainfall of 500 mm is sufficient for aquaculture. The precipitation in summer disrupts the quality of the fruit and causes fruit cracking especially in periods close to maturity. Dry weather conditions during fruit growth period are

important for the formation of quality fruits. Pomegranate should not be grown in areas with severe wind. If it is to be cultivated, windbreakers should be built on the garden side. Otherwise, the fruits will be damaged by the thorns of the plant and branch friction and the fruit quality will decrease and fruit losses will be lost (Gölükcü, 2008).

The pomegranate can be grown in tropical and subtropical regions and can be grown in temperate climates. Pomegranate cultivation is suitable for warm and dry summers in winter and hot and dry winters where temperatures do not fall below -10 ° C. Under this temperature, if the fresh shoots are below -18 ° C, the main body is damaged by cold. Hard winds are not suitable pomegranate cultivation areas in winter because they cause lower temperatures in winter. In order to, make pomegranate cultivation in these areas, windbreakers should be installed in advance. Although the pomegranates are the plants that can withstand pistachio and heat and drought the most, they want regular water during the summer. In the light of this information, pomegranate regions from Çanakkale to Siirt can be grown and cultivated in the southeastern Anatolia region with western and southern passage areas (*nar @ www.meyed.org.tr*, n.d.).

Pomegranate can adapt to a larger variety of soils than most fruit trees. Deep, alluvial soils are the most suitable soil for pomegranate cultivation. But it is also grown in sandy, clayey and calcareous soils. It is moderately resistant to salinity. The pomegranate, which has a wide range of tolerance from soil to heavy gravel, can be grown in partially salty and calcareous areas. However, the best growth medium permeability for pomegranate is good humid and cool soils with good water content and high water retention capacity. For this reason, it is necessary to understand whether the pomegranate is suitable for planting and also for the fertilization of the plant and other years must be soil analysis (Gölükcü, 2008).

Some cultivated pomegranate varieties 07 N 08 Hicaznar, 33 N 16 Silifke Vaccine, 33 N 26 Seedless (VI), 01 N 03 Fellahyemez II, 26/3 Seedless, 33 N 24 Beynarı, Suruç, Ernar and Erdemli-Aşınar (33 N is 11). Pomegranate varieties produced in Çukurova

region, which are widely produced and especially sought in the market, are Hicaznar and Silifke Vaccines.

Hicaznar (07 N 08) obtained by selection along with many local varieties in our country, 1479-20, 1483-2, 1472-20. 1465-20, 1461-30, 1481-20, 1487-15 A, 1478-15A, 1445-20, 1466-10, 1458-30A, 1265-25, Beads, 1469-15 A, 1480-15A, Tarbey, Bird pomegranate, 1261-35A, 1986-10 and 1267-15A, Suruc. 07 N 03 Fellahyemez province 2/3 Japanese pomegranate, 33 N 26 Seedless VI, 26/3 Seedless, Bey pomegranate, 07 N 14 (Mayhosh IV, Alanya), 33 N l6 (Silifke vaccine, Silifke), 33 N 24 and Nizip varieties such as pomegranate are available. These varieties are different in terms of taste, color and earliness. Since the aim of pomegranate cultivation is to make money, the varieties with the highest market demand should be preferred. Among these varieties Hicaznar is a variety that should be preferred for those who think about making this market considering that it has been preferred more in recent years in European markets. Although the pomegranate is consumed more freshly in our country, it is widely used in fruit juice and pomegranate juice. Pomegranate and pomegranate products are used in food, chemical, pharmaceutical and cosmetic industries (*nar @ www.meyed.org.tr*, n.d.).

### 1.1.1. The Reasons of Turbidity in Pomegranate Juice Concentrate

The reasons for the turbidity that occurs during the post-bottling or storage in the clear juices / concentrates are related to the complex reactions in which many elements originating from phenolic substances, proteins, starch, copper and iron are found naturally in the structure of the fruit. Cause of turbidity these elements can be found naturally in the structure of the pomegranate juice or can be exposed to the processes applied to the juice during processing. The processes used in the production of clear pomegranate juice are maceration, depectinization, clarification, ultrafiltration and pasteurization, respectively. Different levels of pectinases and cartanases are used in the maceration and depuration stages. The enzymes used in this step themselves may cause turbidity. Therefore, it is very important to determine the optimum dosages of

the enzymes to be used in the production of clear juice. Also the type of fruit processed, storage conditions are also among the causes of turbidity (Yemi, 2016).

Many studies have been conducted on pomegranate juice clarification. Mirsaeedghazi et al. (2010), studied microfiltration process with two different pore sizes polyvinylidene fluoride membranes to clarify pomegranate juice. They concluded that microfiltration provides clarification of pomegranate juices without any change in terms of chemical composition and color.

Recently, Benucci et al. (2016), found that combination of pectolytic and proteolytic enzymes reduces the turbidity of pomegranate juice and did not chance the total amount of pectins, proteins and phenols. Also, it was observed that they have no effect on color and anthocyanin amount.

Tastan et al. (2015), revealed that chitosan precipitates with suspended particles in juices and provides seperation of them from beverages. In this study, optimum dosage, temperature and process time were investigated and using chitosan resulted in clarified pomegranate juice.

Ozıycı et al. (2012), studied that pomegranate juices are produced by two extraction methods (pressing with husks and without husks), hot and cold clarification and two different filtration (kieselguhr and active charcoal). In cold clarification bentonite and gelatin added at 5°C and they held for 8h. In hot clarification kieselsol and gelatin added at 50°C and waited 3h. It was concluded that hot clarification technique has a negative effect on color and turbidity stability during shelf life.

Vardin et al. (2003), concluded that application of gelatin and PVPP to juice decreased turbidity level and preserved anthocyanin and color value. Also, according to organoleptic evaluations the samples which added gelatin were found succesfull.

#### 1.1.1.1. Pectins

Fruit juices generally consist of polysaccharides such as pectin, cellulose, hemicellulose, protein and tannins. Pectin is a high molecular weight carbohydrate

polymer. Pectin contains a series of polymers and these polymers shows differences according to molecular weight, chemical configuration, and content of neutral sugars. The pectin consists of a galacturonic acid unit chain linked by α-1,4 glycosidic bonds (Flutto, 2003) (Figure 1.1).

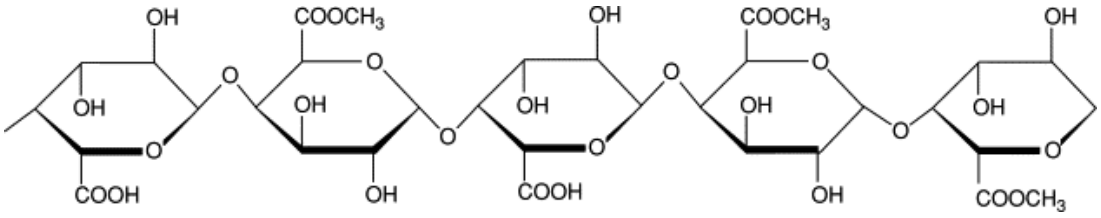


**Figure 1.1. Pectin consists of long sequences of anhydro galacturonic acid completely or partly esterified with methanol (Flutto, 2003).**

There are some factors that affect the dissolution of pectin in water. These factors are the degree of polymerization, the number and distribution of methyl ester groups. At the same time, pH and temperature are among the factors affecting dissolution. Pectin (-) charged in fruit juices at low pH levels and other colloidal materials are (-) electrically charged. These substances do not collapse due to repulsion of each other, they also surround other (+) charged particles in the dispersed state to prevent them from collapsing.

Pectin is able to form gel under certain conditions and has been used for a long time in the jam and canning industry. A temperature of 77–82 °C is preferable and enough for gel-making procedure. In the fruit juice industry, during the evaporation process, non-degraded large molecule pectins can form gels. Therefore, pectin acts as a protective colloid in fruit juice (Smith, 2003).

One of the biggest problems encountered by consumers in the marketing of fruit juices is the turbidity caused by the presence of pectins. A fresh pomegranate juice contained 14 mg of pectin per liter. The breakdown of pectin into small molecules by enzymatic depectinization is a solution in preventing turbidity. Pectins can be hydrolysed using

enzymes that act as pectinases. Thus, the juice contains less pectin and less viscosity. This facilitates the subsequent filtration. Also, with the disintegration of pectin, it is possible to concentrate the fruit juice without making gel (Hmid, 2016).

#### 1.1.1.2. Polyphenols

Phenolic acids and flavonoids, which are phenolic compounds, are found in the structure of vegetables, fruit, tea, wine and fruit juices. The distribution of phenolics shows both quantitative and qualitative differences as a function of species, sowing, ripening, cultivation and storage. Furthermore, phenolics contribute to the sensory quality of fruit and juice due to their effects on color, bitterness, sharpness and flavor. Phenolic compounds are found in almost all fruits and vegetables and play an important role in their color and taste. Furthermore, due to their antimicrobial properties, phenolic compounds and their oxidation products are known to have a significant effect on the defense mechanism of plants. During pressing pomegranates and obtaining pomegranate juice, some polyphenols especially high molecular weight phenolic compounds pass the juice and can cause turbidity problem. High molecular weight phenolic compounds; condensable phenolics (proanthocyanidins) and hydrolyzable phenolics (ellagitannins and gallotanenes) are divided into two groups. Pomegranate shells are extremely rich in hydrolysable phenolics and contain less amounts of punicalin (4,6-galla-gylglucose), gallic acid, ellagic acid and ellagic acid glycosides (hexoside, pentoside, rhmnoside, etc.), mainly ellajitanen and isomers. The most important group among the ellagitanens is punicalagens. Punicalagine is hydrolyzed to form punicalin and ellagic acid (Martinez, 2017).

Pomegranate contains significant phenolic substances. A significant proportion of these phenolics, particularly high molecular weight phenolics, are also present in the shell. The extract obtained from pomegranate peels (249.4 mg / L) contained about 10 times more total phenolic substances than the extract obtained from pulp (24.4 mg / L). Compared with other phenolic rich products; pomegranate juices (2566 mg / L) and red wine (2036 mg / L) contain approximately the same amount of phenolic

substances, whereas pomegranate juices are reported to contain about 2 times more phenolic substances than green tea (1029 mg / L).Pomegranate juice also includes highly hydrolyzable tannins (especially ellagitannins: gallic acid and ellagic acid).In addition, anthocyanins (cyanidine, delfinidine, pelargonidine) and phenolic acids (ellagic acid, caffeic acid and chlorogenic acid) are present in the structure of phenolic compounds. Regular daily consumption of pomegranate juice is beneficial for humans in terms of CAS (carotid artery stenosis) (Karaca, 2011).

Polyphenols have an important place in foods. Polyphenols are present in foods as colorants and antioxidants. Research has focused on their antioxidant properties because of their positive effects on chronic diseases, cardiovascular diseases and cancer. By entering the oxidation reactions themselves, they can increase the shelf life of some foods by taking the role of antioxidant in food industry. For example, oxidized foods do not cause problems only because of their bad odor and taste. They also have negative effects on human health due to the free radicals they contain. Polyphenols can be classified according to the number of carbon atoms. There are simple soluble forms found in vacuoles, polymerized forms such as tannins and no insoluble forms such as lignins (Cooper and Nicola, 2014).

Chemical properties are given by phenolic rings and the simplest structural states are devoid of nitrogen-based functional group. Polyphenols can be classified according to the phenol rings they contain and the structural elements connecting these rings. They can be grouped into two main headings, flavonoids and nonflavonoids (Piccolella and Pacifico, 2015). The classification of polyphenols is as below in Figure 1.2.

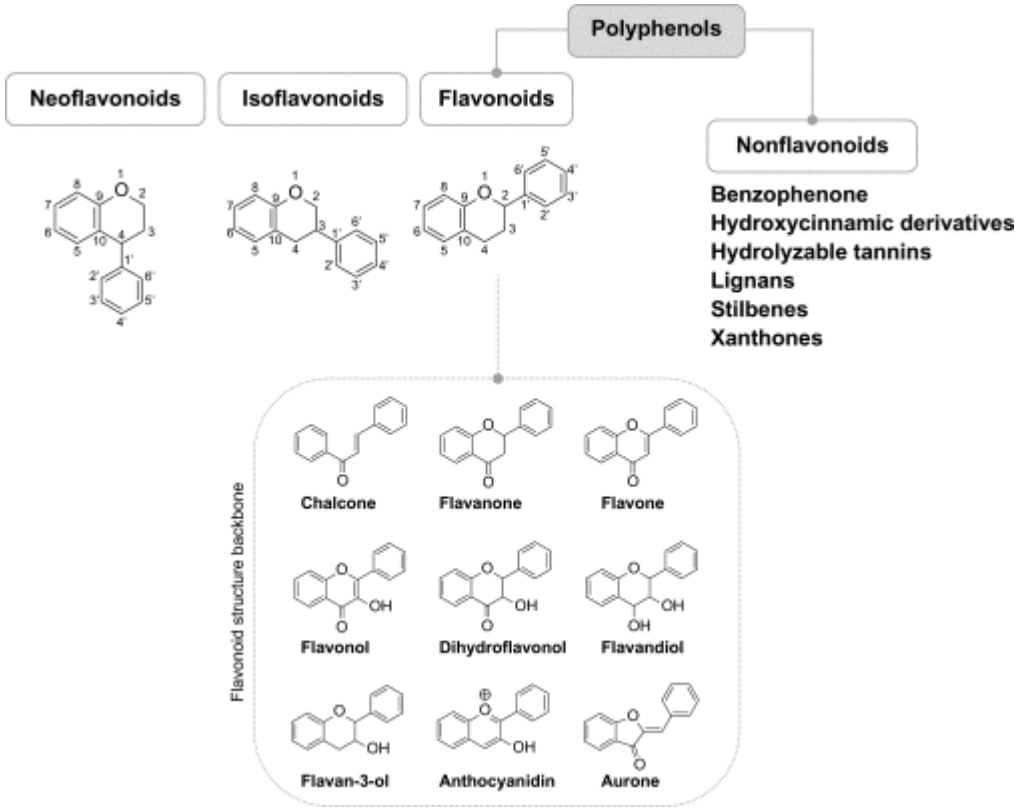


**Figure 1.2. Chemical classification of polyphenols (Piccolella and Pacifico, 2015)**

### 1.1.1.2.1 Nonflavonoids

Nonflavonoid phenols are phenolic acids which are divided into two groups as benzoic and cinnamic acids. Phenolic acids are generally not free in viable plant tissues, but during hydrolysis of plants. Carboxyl groups can react with carbohydrates, glycosides, amino acids or proteins. The hydroxyl groups of phenolic acids which are attached to the phenol ring are also very active and combine with sugars to form glycosides. The amounts of phenolic acids in fruits vary according to their maturity (*nar @ www.meyed.org.tr*, n.d.).

Hydroxycinamic acids are commonly found in plant foods and have different properties depending on the number and location of the hydroxyl group bound to the phenylpropane. Among these, ferulic acid, caffeic acid, o-coumaric acid and pkumaric acid are important. Hydroxycinamic acids are only present in very small amounts in

free form, often in the form of acid derivatives. The esters of hydroxycinamic acid are also very common in foods. Hydroxycinamic acid glycosides and amides are also present in many plants. Hydroxybenzoic acids are usually present in trace amounts (10 ppm) or not at all in the structure of plant foods. These include salicylic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, gallic acid and vanylic acid (Cvejić et al., 2017).

#### 1.1.1.2.2. Flavonoids

The flavonoids are C6-C3-C6 diphenylpropane and the triple carbon bridge between the phenyl groups forms the ring with oxygen (flavan ring). Differences between different flavonoids; from the number of hydroxyl groups bound, the degree of unsaturation and the oxidation level of the triple carbon segment arises. Flavonoids are the most important group of phenolic compounds, flavan (2-phenol-benzo-dihydro-pyran) derivatives (Martinez, 2017).

#### 1.1.1.2.3. Anthocyanidins

The anthocyanidins are flavonoids. Anthocyanidins are present in plants in the form of glycosides or anthocyanins, which are usually formed by sugars, not in free form. Red, blue, purple and violet colors of fruits and vegetables originate from anthocyanins. They are classified according to the binding of the R groups to H, OH and OCH3. As the number of -OH groups in the phenolic parts of the anthocyanins increases the blue, the redness increases as the number of OCH3 groups increases. Major anthocyanidins; pelargonidine, cyanidine, delfinidine, peonidine and malvidine. Although there are 23 anthocyanidins in nature; the number of hydroxyl groups in the molecule, the degree of methylation of hydroxyl groups, and the number of sugar bound to the molecule and depending on the binding position of the sugar , and also the structure and number of aliphatic and aromatic acids bound to sugar in the molecule more than 500 anthocyanins are formed (Martinez, 2017).

#### 1.1.1.2.4. Flavanones

Flavanones are usually found in nature in glycoside form. Flavanone glycosides are commonly found in citrus fruits. For example; naringin, hesperidin, naringenin. Naringin gives citrus juice a bitter taste (Martinez, 2017).

#### 1.1.1.2.5. Catechins (Flavan-3-ol)

Catechins are colorless compounds. Catechins present in almost every fruit are intermediate products in flavonoid biosynthesis. They form the most common flavonoid group in foods. The catechins are systematically called flavan-3-ol because they contain an OH group at the C3 atom. Since the catechins have two asymmetric carbon atoms in their structures, they have four isomers (Martinez, 2017).

#### 1.1.1.2.6. Proanthocyanidins

Proanthocyanidins are referred as condensed tannins. Condensed tannins are dimmers or polymers with C-C bonds between flavan-3-ol subunits (Benaiges and Guillen, 2007). The most important proanthocyanidins are catechins and their condensed forms catechin, epicatechin, gallocatechin, epigallocatechin, epicathecin gallate and epigallocatechin gallate. The binding of catechin units in the formation of proanthocyanidins is generally in the form of C4-C8 condensation. So mostly linear (linear) consists of proanthocyanidins (Basalekou et al., 2019).

The concentration of tannins, the size and content of the polymers have a great effect on taste. For example, tannins with a long polymer chain form a bitter taste in the mouth (Vidal et al., 2003).

Although short chain length molecules are colorless, their colors change from yellow to brown as their degree of polymerization increases. However, when heated in an acid environment, they turn into anthocyanidins and take a typical red purple color. Therefore, it is called proanthocyanidin (Basalekou et al., 2019).

#### 1.1.1.2.7. Isoflavanoids

Isoflavanoids are compounds found in some fruits and vegetables, in particular legumes, particularly soybeans. Clinical studies in recent years have shown that isoflavonoids are bioactive compounds and play an important role in reducing blood cholesterol levels with soy proteins (Cemeroğlu, 2007).

#### 1.1.1.3. Polyphenol-Protein Interaction

One of the most important turbidity factors in fruit juices is the interaction of protein and polyphenols. This is also related to their ability to form haze. Many studies have been conducted on haze active substances in fruit juice. After centrifugation process, haze or sediment material can be collected and analysed in terms of chemical and amino acid content (Benucci et al., 2016).

Benucci et al. (2016), shown that the proline-rich protein class, the alcohol-soluble part of barley prolamines, is associated with malt polyphenols. In one study, it was observed that in a catechin buffer model system, the mole percent of proline in a polypeptide was linearly related to the amount of haze formed and proline-free amino acid chains did not form haze. The enzymes used in the juice process have protease activities as well as carbohydrate hydrolyzing activities, which in turn affect the formation of haze.

Haze active polyphenols in beer are proanthocyanidins and are also present in pomegranate juice. These include catechin, epicatechin and gallocatechin polymers, dimers, trimers and higher polymers. Of these, the concentrations of the two dimeric proanthocyanidins, prosyanidin B3 and prodelphinidin B3 in beer are closely related to haze formation. The polymerization ability of proanthocyanide in haze formation is more effective than the number of OH groups on the ring (Benucci et al., 2016)

Between the protein-polyphenol bonds formed by hydrophobic bonds. Benucci et al. (2016), also revealed that the interactions between polyphenols and proline-containing proteins are complexes formed by the overlap of the rings of the two compounds.

In this study, haze formation in beer is explained for a variety of reasons. Beer contains high haze-active proteins but low haze-active polyphenols. In beer, it is desirable to remove the haze active material while maintaining the foam-forming protein. In one method, the beer is placed in the tank and stored at low temperatures, allowing the haze to form sediment, but this requires a long wait. Another method is to use certain amounts of enzyme to attack the haze active protein and delay the onset of haze formation. Although effective, it has been observed to impair foam formation. The most effective methods used today are fining agents or adsorbents. In beer, bentonite removes both haze active and foam active proteins. Silica gels are more effective in removing haze active proteins. In beverages rich in polyphenols, silica gels do not work as effectively as beer because the proline portions of proteins are bound by polyphenols. Bentonite effectively removes protein and is used in fruit juice and wine stabilization. Ultrafiltration stabilizes fruit juices, holding all proteins larger than their membrane pore size.

#### 1.1.1.4. Oxalic Acid

Oxalic acid crystals readily release proton ions to form oxalate ions and oxalate salts. They combine with metal ions to form salt. Oxalate ions, oxalate salts and calcium oxalate crystals are found in many foods and plants. Some legumes, fruits, cereals and green leaves have a high concentration of oxalate (Norton, 2018).

Oxalic acid crystallizes in colorless, transparent form. Crystals are odorless and have a strong acidic taste. They are soluble in water at 15.5 degrees. Furthermore, calcium oxalate is insoluble and can be used as a test for the presence of oxalic acid calcium. The calcium sulfate solution forms a bluish precipitate with oxalic acid (Hussain, 2012).

To our knowledge, this is the first study on pomegranate juice, which using together of pectolytic and proteolytic enzymes as well as gelatin and other clarifying agents (silicasol and aktivit). Pectin forms a layer around proteins and carries a negative charge in acidic environments. This causes them to push each other. The pectinases

break down the pectin and thereby produce positively charged proteins. This reduces the electrostatic repulsion so that they are collected together. The enzymes are involved in this stage, they will break down the large molecules and provide convenience for the filter stage. Treatment of fruit juices with gelatin and bentonite is a common practice. These thinning agents adhere to the particles, making them large and heavy, and so the particles settle to the bottom. A clear juice can be obtained by removing these particles by means of filters. The success and purpose of gelatin in this study is to adhere to large polyphenols. The Klar-Sol used in the process also causes complexation of proteins and rapidly precipitate (Benitez and Lozano, 2007). At the same time, considering the presence of oxalic acid crystals in pomegranate, cold storage and filtration studies were carried out. The effect of crystals on turbidity was tested at three different temperatures.

The aim of this study is finding a solution to turbidity problem in pomegranate juice concentrate during storage without the reproses. In this study Hicaz type pomegranates (*Punica granatum* L. Hicaznar) are processed into clear fruit juice. Based on the literature studies and the interviews with the sector representatives (Novozymes and Erbslöh), experiments have been carried out on the effect of gelatin in preventing turbidity. In addition, the effect of filtration process on oxalic acid crystals, which is one of the turbidity factors, has also been observed. For this purpose, four trial plans were developed considering all the factors that cause turbidity. In all plans, enzymes were used to break down the pectin and Aktivit and Klarsol were used in the clarification stage. In only two trial plans, the product was waited at three different temperatures and the filtration process was applied. The use of gelatin was again applied in two different plans. Some special enzymes were used for this purpose. Enerzym HT provides disintegration of starch, dextrin and oligosaccharides, hydrolyzes 1,4 - α - D - glycosidic bonds in starch, dextrins and oligosaccharides. In this process, D - glucose units are separated. The optimum pH level is 3.4-6.0 and temperature is 55°C. Exact dosage recommendations can be determined by laboratory studies. Fructozym Color and Fructozym P6L keeps color stable during process and

provides complete pectin degradation. At the same time, they reduce the viscosity by allowing the pectin to divide rapidly. They provide clarification and prevent the formation of sediment. Aktivit is a calcium-sodium bentonite used in stabilization of fruit juices and wine. In the case of combination with silicasol and proteins supports advanced flocculation. Klar-Sol is used for clarification of fruit juices and wine, it forms complex with proteins (like gelatin) and precipitates. It contains high charge load and can form precipitate easily.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1. Materials

In this study, Hicaz pomegranate variety was used. Pomegranates used in the production of pomegranate juice concentrate were obtained from Antalya-Hatay-Mersin region. Approximately 300 kg pomegranate was sorted, crushed and milled in production site of factory, then mash is brought to pilot plant and continued with pressing process. Enerzym HT, Fructozym Color, and Fructozym P6L the enzymes and clarification agents; Aktivit, Klar-Sol were taken from Erbsloeh Co. Inc (Geisenheim, Germany). Gelatin (bovine gelatin 80-100 bloom) was taken from SelJel Co. Inc (Istanbul, Turkey). Enerzym HT provides disintegration of starch, dextrin and oligosaccharides, hydrolyzes 1,4 - α - D - glycosidic bonds in starch, dextrins and oligosaccharides. In this process, D - glucose units are separated. The optimum pH level is 3.4-6.0 and temperature is 55°C. Fructozym Color and Fructozym P6L keeps color stable during process and provides complete pectin degradation. At the same time, they reduce the viscosity by allowing pectin to divide rapidly. They provide clarification and prevent the formation of sediment. Aktivit is a calcium-sodium bentonite used in stabilization of fruit juices and wine. In the case of combination with silicasol and proteins supports advanced flocculation. Klar-Sol is used for clarification of fruit juices and wine, it forms complex with proteins (like gelatin) and precipitates. It contains high charge load and can form precipitate easily.

## 2.2. Methods

### 2.2.1. Applied Methods During Process

For production of pomegranate juice concentrate, pomegranates were undergone washing, sorting, shredding, press, enzymation, pasteurization, clarification and

ultrafiltration starting from raw material respectively in production factories Döhler A.Ş.. In this study, the steps in the production of pomegranate juice concentrate were simulated with the studies that had been started in the pilot plant and continued in the laboratory and the solution to turbidity was sought. In the production of pomegranate juice concentrate, four processes were implemented at the pilot production facility of Döhler Gıda A.Ş. In the first experimental design, fresh fruits firstly were sorted, washed and removed from shells. Then crushing, pressing, pasteurization, enzymation, clarification, filtration and concentration stages were followed.

In the second experimental design, after ultrafiltration process pomegranate juice was brought to 30-35 brix, stored at cold temperatures (at three different temperatures) and then passed through J16 paper filters (Erbslöh-J series/ 3.0-1.5 μm).

In the third experimental design, in the clarification stage gelatin was added and followed as in first plan.

In the last experimental design, gelatin, cold storage and filter stages were all applied. Experimental design 1 and 2; experimental design 3 and 4 were conducted at the same time interval. After consultation with the sector representatives, a test method called hot-cold stability test was practised. According to this test, the beginning of the product brought to 65 brix NTU was measured and called N. After the juice concentrate was boiled, it was placed at -18 °C and stored there for one day. After melting, the NTU value was measured and and called N1. A third NTU value was measured at the end of the second day and was called N2. All NTU values of pomegranate juice concentrate was measured at single strength brix values (15°brix). If the NTU <5 and N2/N1<2, pomegranate juice concentrate was stable in terms of experiments (Maier, 2016). This result gives an idea whether the product will remain stable after one month of storage.

### 2.2.2. Pomegranate Juice Concentrate Production Stages

#### 2.2.2.1. Raw Material Input, Washing and Sorting

Foreign substances such as stones, soil and leaves on the pomegranates were removed by extraction and washing processes. Then rotten, raw and unfavorable fruit was removed.

#### 2.2.2.2. Mash

Washed pomegranates were sent to the drum screen with conveyor belts. The hammer shafts connected to the screw inside the sieve shredded the pomegranates and pomegranate seeds were dropped from the sieve holes and sent to the conveyor pump through the conveyor belt.

#### 2.2.2.3. Pressing

Pressing was performed to obtain the fruit juice from the mash. The applied pressure was approximately 1.5 bar. In the Figure 2.1. NIKO VP11e type press was used and pomegranate juice was obtained.

**Figure 2.1. Press machine**

### 2.2.2.4. Pre-Concentration

After press, pomegranate juices were pre-concentrated to 15°Bx with Yuanan evaporation machine at 70°C. With the preconcentration process, both heat treatment is applied to fruit juice and it is brought to brix value where enzymation and clarification process is applied.

### 2.2.2.5. Tubular Heat Exchangers

The raw fruit juice obtained after the press carries a microbial load. In particular, raw fruit juices are rapidly subjected to heat treatment in large capacity productions. For this purpose, heat treatment is applied with NIKO, 2HE3000 type tubular heat exchangers, (Figure 2.2.) to the juice. By using a tubular heat exchanger, the outlet temperature of the liquid flowing in is brought to the desired value. The outlet temperature of the liquid in the pipe is generally controlled by changing the temperature of outside liquid stream. After the product enters the heat exchanger, the product temperature is increased to 95 °C with the help of the liquid flowing out. The exit temperature of the product from the heat exchanger is set to 50 °C, which is the appropriate temperature for the enzymatic process.

**Figure 2.2. Tubular heat exchanger**

### 2.2.2.6. Enzymation

Enzymation procedure was applied to the fruit juice in tanks by adding enzyme. Enzymes were prepared by diluting with water. Enerzyme HT (5g /ton), Fructozym Color (50g / ton), Fructozym P6L (100g / ton) enzymes were used at 50°C. Enzyme time was one hour and alcohol test was applied to determine whether pectin is degraded or not. Enzyme duration was followed by alcohol test and when the alcohol test gave negative results, the enzymation process was terminated.

#### 2.2.2.7. Clarification

For clarification process, aktivit (500g /ton), gelatin (500g /ton) and klarsol (2500g/ton) is applied to the juice and waited for one hour. Prior to process aktivit should be added into 8-10 fold water amount and stirred for 8-12 hours. Enzyme and clarification processes were performed in the tanks shown in Figure 2.3.


**Figure 2.3. Tanks**

#### 2.2.2.8. Ultrafiltration

Enzyme process completed fruit juice the ultrafiltration spiral membranes (100kDa) from Alfa Laval (Figure 2.4.) are based on a polypropylene or polyester support material.

**Figure 2.4. Ultrafiltration**

### 2.2.2.9. Evaporation

The type of evaporator used is the forced circulation evaporator. The liquid is drived by force through the evaporator tubes and with high efficiency circulation, designed for large volumes. Filtered pomegranate juice was sent to a evaporator with three effects (Yuanan evaporation machine) in the Figure 2.5. and concentrated to 65°Bx. The evaporation temperature is approximately 70 °C and the product is at these temperatures after the evaporation process. Yuanan evaporator is suitable for heat sensitive, low and high concentration foods. The capacity is up to 300 kg/h.

**Figure 2.5. Evaporation machine**

### 2.2.2.10. Storage of Pomegranate Juice Concentrates

Pomegranate juice concentrates were stored at -18°C for 6 months. These samples were analyzed once every 2 months for turbidity analysis. In the production of pomegranate juice concentrate, four processes were implemented.

Four experimental designs flowcharts are below in the figures 2.6, 2.7, 2.8 and 2.9 respectively. Experimental design 1 is the same flowchart used in production. After the raw material was washed, sorted and milled in production, brought to the pilot plant. Pressing process was performed to obtain the fruit juice from the mash. After press, pomegranate juices were pre-concentrated to 15°Bx where enzymation and clarification process is applied. Heat treatment process is applied to the raw fruit juice obtained after the press by tubular heat exchanger. After the product enters the heat exchanger, the product temperature is increased to 95 °C with the help of the liquid flowing out. The exit temperature of the product from the heat exchanger is set to 50 °C, which is the appropriate temperature for the enzymatic process. Enzymation and clarification processes are applied to fruit juice during 1hour process time. After enzyme and clarification process, juice was filtered by using ultrafiltration. Filtered pomegranate juice was sent to a evaporator to obtain juice concentrate.

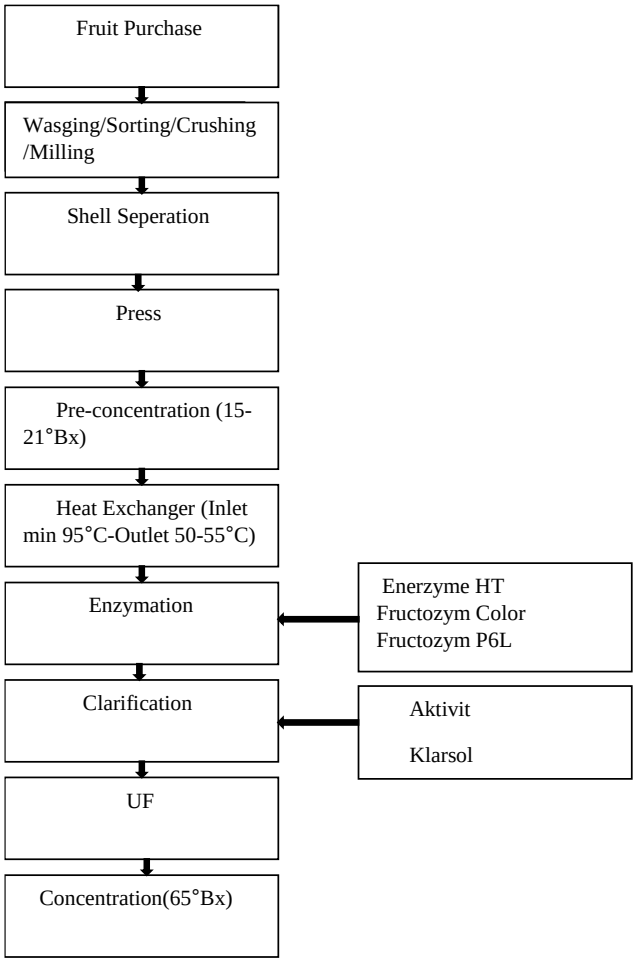


**Figure 2.6. Flowchart of experimental design 1**

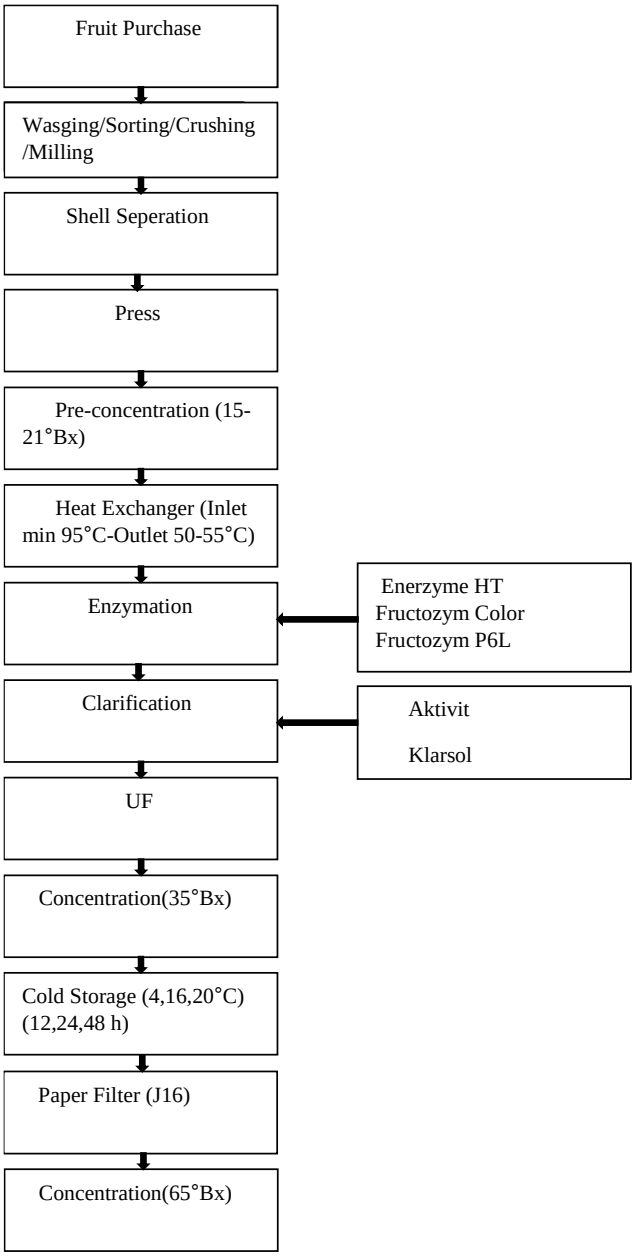


**Figure 2.7. Flowchart of experimental design 2**

In experimental design 2 (Figure 2.7), after ultrafiltration process pomegranate juice was brought to 30-35 brix. After concentration process, nine samples were taken in a 500 ml beaker and kept at three different temperature values (4, 16, 20°C) and three different time values (12, 24, 48 h) provided under laboratory conditions. After 12 hours, three samples were stored at specified temperatures, filtered using J16 paper

filters in the laboratory and the products were concentrated. These procedures were repeated after 24 hours and 48 hours. In experimental design 3(Figure 2.8), the same process steps as in experimental design 1 were applied, only gelatin was added during clarification process. In experimental design 4, both the effect of gelatin on clarification stage and the effect of holding at three different temperatures were observed.

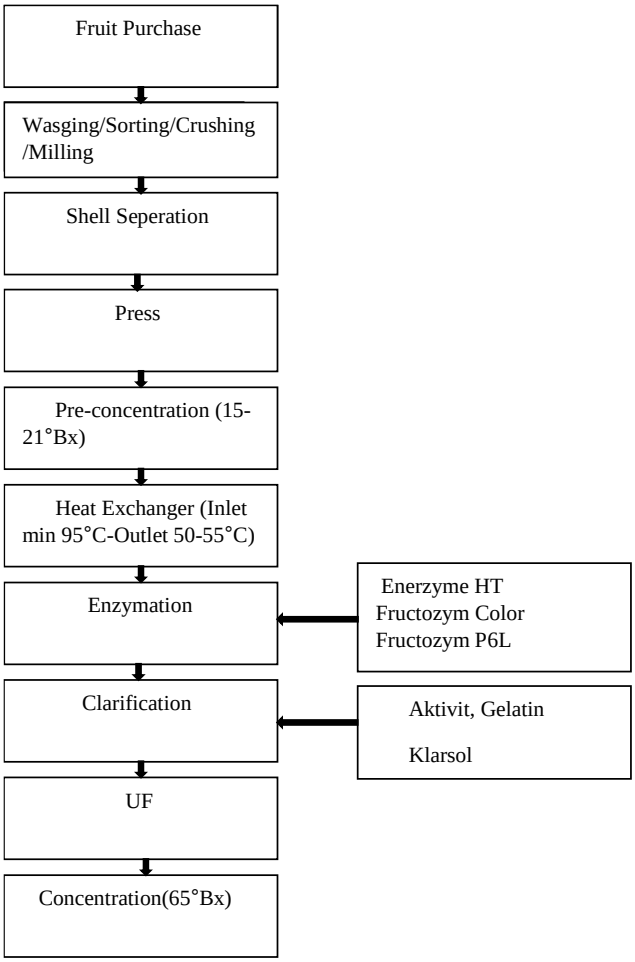


**Figure 2.8. Flowchart of experimental design 3**

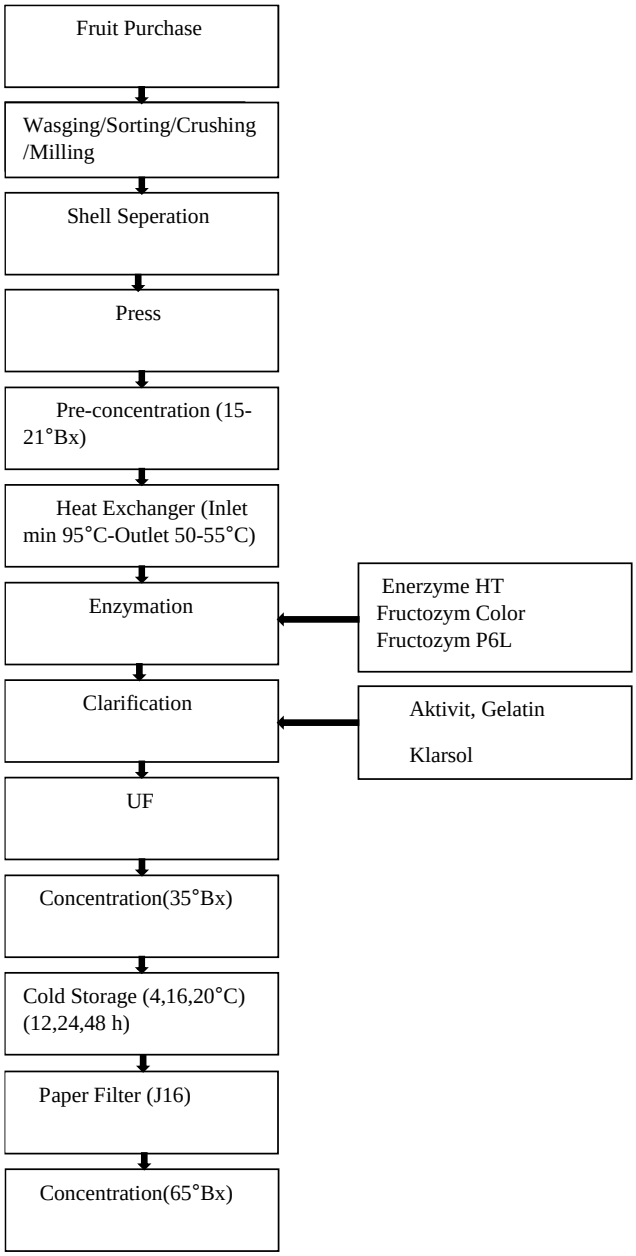


**Figure 2.9. Flowchart of experimental design 4**

### 2.2.3. Determination of pH

The pH of the samples was determined using the SevenCompact S220 model pH meter (MetlerToledo, China).

### 2.2.4. Water Soluble Dry Matter Determination

In solutions containing dissolved substances, the light is broken when passing from one environment to another. The breakage of light is characteristic of the substance dissolved in water and is a measure of its concentration. Based on the principle of refraction of light, the RX-5000CX (ATAGO, JAPAN) brand refractometer and the brix values of the samples were read.

### 2.2.5. Determination of Turbidity

The turbidity level of the pomegranate juice samples was determined with the aid of turbidimeter (HACH 2100 turbidimeter Ratio / XR USA 2008). The level of turbidity is expressed by the value "NTU (Nephelometric Turbidity Unit)". The level of turbidity was determined directly in pomegranate juice and in concentrates after diluting the natural pomegranate juice to brix (15 ° Bx). Zero setting of turbidimeter is made with distilled water used for dilution of concentrates

### 2.2.6. Determination of Color

Pomegranate juice and concentrate samples are prepared in pH 3 buffer solution. To prepare the pH 3 buffer solution, 10 g of trisodium citrate are dissolved in 1000 ml of purified water. The pH of the tricitrate solution is adjusted to the pH 3 by using 50% citric acid solution. The pomegranate juice concentrate color value is measured by 5 g of concentrate diluted with 500 g pH=3 buffer solution. The sample, in special tube made of hard plastic or quartz, is placed to the spectrometer (SHIMADZU/ UV-1800) and the absorbance value is measured at wavelengths 420, 520, 580 and 620 nm (Maier, 2016).

### 2.2.7. Laboratory Filter Process

After cold holding process, samples were filtered by vacuum monifold filter system (KNF LABOPORT) with J16 paper filters (Erbslöh-J series/ 3.0-1.5 µm).

### 2.2.8. Pectin Test (Alcohol Test)

This test is used to detect pectins in fruit juices and to see if the enzymation is effective. The pectins need to be broken down until a negative test is obtained. For this purpose, two volumes (10 ml) of acidified ethanol (add 1 ml 37% hydrochloric acid to 100 ml 96% ethanol) are added to one volume of fruit juice (5 ml) and filtered through a paper filter. The purpose of ethanol acidification to ensure that only pectin will cause a precipitation. The test tube is slowly turned upside down 2 or 3 times, and waited 15 minutes to make a decision. If the pectin is degraded, gel or flocculation is not observed as in Figure 2.10.

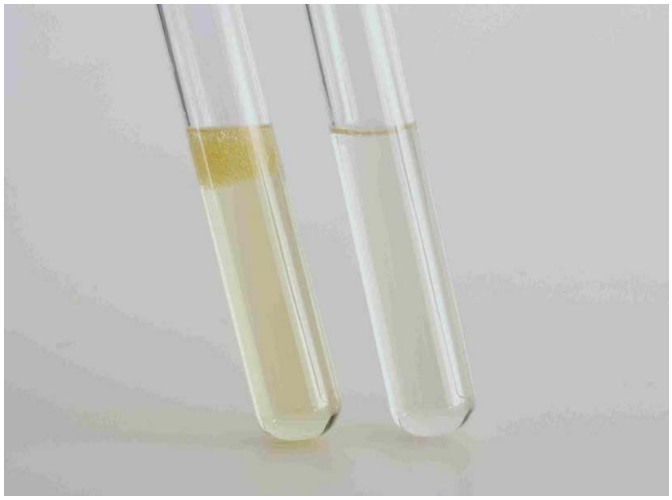

**Figure 2.10. Pectin test ( Alcohol test) (Maier, 2016)**

Left: positive (gelification)

Right: negative (pectin degraded)

Experiments were conducted under laboratory conditions to test whether the amount of enzyme were sufficient or not. After the pomegranate juice passes through the heat

exchanger, its temperature is suitable for enzyme application. The enzymation treatments used 500 ml of juice samples, stirred with different concentrations (5, 10 and 15 ppm) of Enerzyme HT and (50, 100, 150 ppm) of Fructozym P6L respectively. Then, the treated pomegranate juices were incubated in a water bath at 50°C for 1 h, then alcohol test is applied. If the alcohol test is negative, this indicates that the enzyme treatment time and enzyme dosage are sufficient.

### 2.2.9. Statistical Analysis

ANOVA was used by GraphPad statistical program to analyze experiment results and Tukey Single Range Test was used to compare if significant difference was obtained, means $p \leq 0.05$

# CHAPTER 3

# RESULTS AND DISCUSSION

## 3.1. Change in Process Parameters During Pomegranate Juice Concentrate Production

The pH values of the pomegranate juices taken from pomegranate raw material to the end of the process ranged from 3.3 ± 0.1 to 3.5 ± 0.2. It is thought that this change in pH values is caused by the applied processes and the different amount of buffer material contained in the pomegranate juices (Cemeroğlu,2007). Cemeroğlu et al. (2004), determined that pH values of pomegranate juice obtained by using 120 kinds of pomegranate samples were minimum 2.40 and maximum 3.53.

While the average brix value of the raw material was 16 ± 0.3, the average brix value of the samples at the paper filter outlet was 14 ± 0.2. Cemeroğlu et al. (1992), reported that the water-soluble dry matter in pomegranate juice varied between 12-18% and Velioğlu et al. (1997), reported that this value varied between 13.2-18.7%. Gölükcü and Tokgöz (2008), reported that brix values of pomegranate juices obtained from sweet pomegranate varieties grown in Turkey ranged from 13.00 to 17.18.

Enzyme dosage was determined by alcohol test in laboratory studies. The appropriate dosage for Enerzyme HT is selected as 5 ppm, for Fructozym Color is 50 ppm.

### 3.1.1. Color Determination

Pomegranate juice, in its original form, has a blurred appearance. Pomegranate fruit contains significant amounts of phenolic compounds, enzymes, proteins, pectins and insoluble complexes. Phenolic compounds give pomegranate juice color, astringency and bitterness. These compounds, including colloids and proteins, are also responsible for the formation of the blurred appearance of fruit juices during concentration and

storage (Valero, 2014). The red color of the pomegranate juice is determined using a spectrophotometer (SHIMADZU / UV-1800) using a pH 3 buffer solution. The color measurements are shown all figures (Figure 3.1, 3.2, 3.3, 3.4).

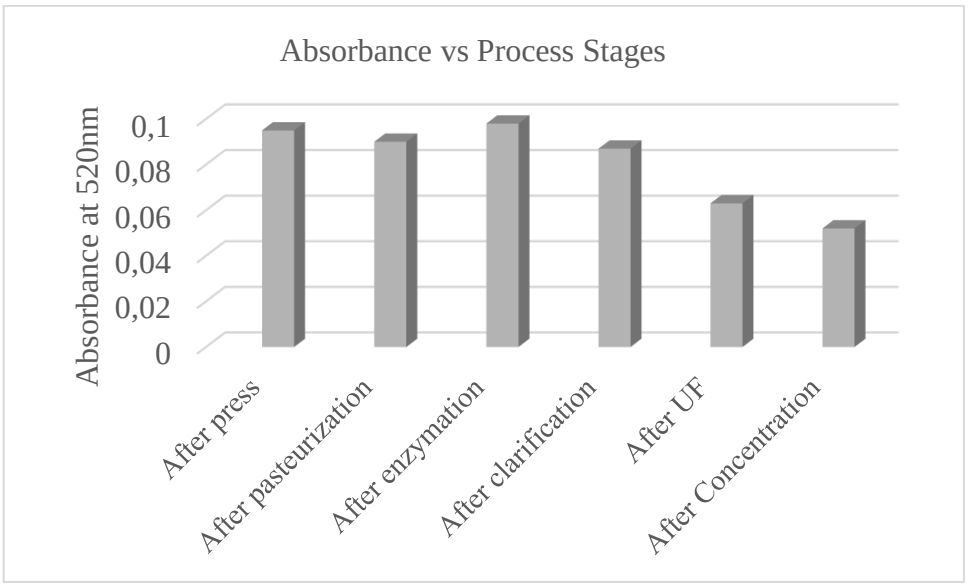


**Figure 3.1. Change of color values of experimental design 1 during process**

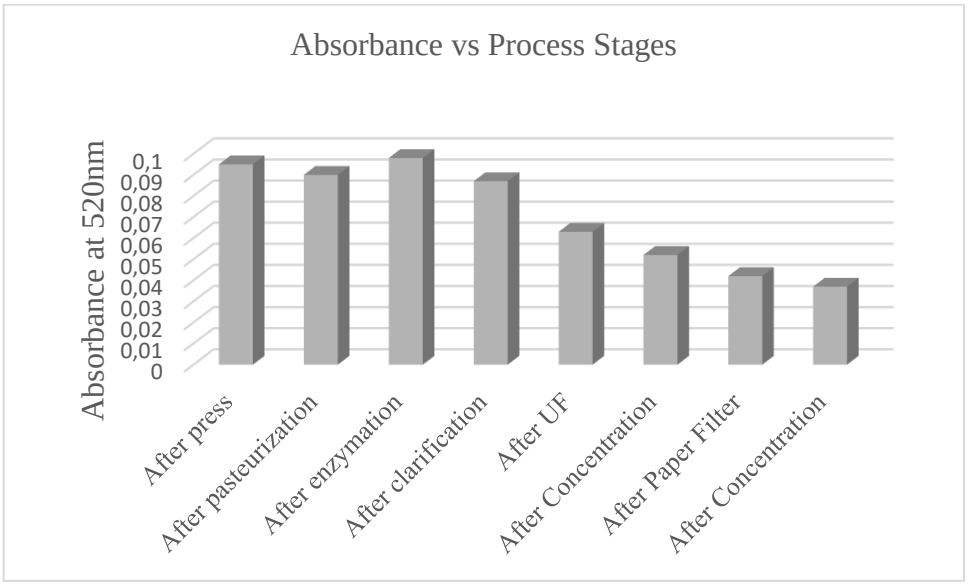


**Figure 3.2. Change of color values of experimental design 2 during process**

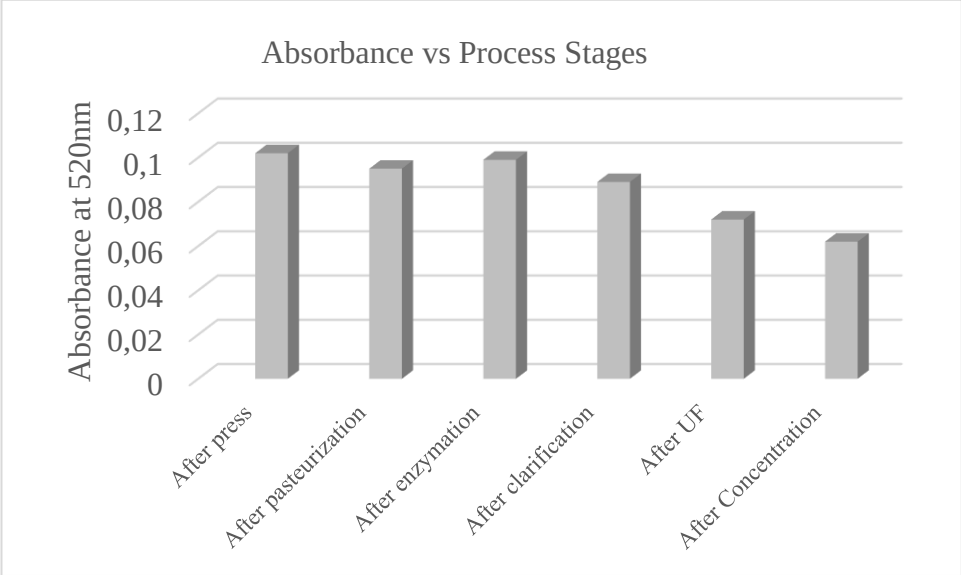


**Figure 3.3. Change of color values of experimental design 3 during process**

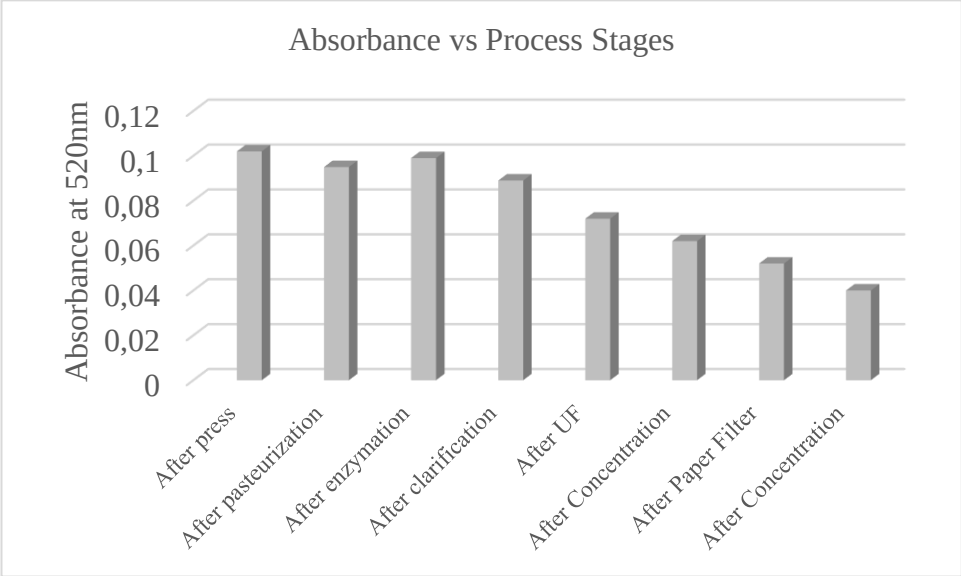


**Figure 3.4. Change of color values of experimental design 4 during process**

Pomegranate juice color intensity can be determined by measuring its absorbance values at 520 nm ($A_{520}$), the wavelength of maximum absorbance of the present monomeric anthocyanins, which give the pomegranate juice the characteristic red color (Vegara, 2013).

According to Valero et al. (2014), the operating costs of using membrane processes are considerably lower than those of more conventional processes. In this study, the effect of membrane clarity on color, turbidity, total soluble solids (TSS), total phenolic content (TPC) and biologically active compounds (anthocyanins, ellagitanins and ellagid acid) of pomegranate juice was evaluated using uf membrane. In many of these parameters, changes were also examined and compared using bentonite or albumin. The procedure helps remove active haze precursors and thus reduces the potential for haze formation during storage. Albumin and bentonite are two clarifying agents used in the fruit juices industry. The strong negative charge of the bentonite surface has a significant effect on the positively charged proteins of the fruit juice which causes its agglomeration (Valero, 2014). According to the results, the use of bentonite or albumin was observed to decrease in the absorbance value at 520 nm as shown all figures (Figure 3.1, 3.2, 3.3, 3.4) but no significant change was observed in the filter process.

Alper et al. (2005), concluded that Pomegranate juices were produced by different treatment techniques (PVPP, ultrafiltration). According to the results, it is found that conventional thinning together with PVPP process is the most effective method of removing phenolic compounds (Alper, 2005). As can be seen in all the figures, after clarification and filtration processes, not significant but noticeable decrease in 520 nm was observed.

In recent years, due to the healthy properties of natural pigments found in these fruits, such as anthocyanins, there has been an increase in consumption of red drinks such as red oranges, grapes, fruit and pomegranate juice. Unfortunately, anthocyanins are unstable and susceptible to degradation and are damaged during heat treatment. Thermal treatment is the most common method to prolong the shelf life of fruit juices by neutralizing microorganisms and enzymes. In this study, the formation of red color loss in pasteurized pomegranate juice samples during storage has been revealed. Measurement of A520 immediately after heat treatment showed red color loss

(Vegara, 2013). The decrease of 520 nm, after pasteurization process could be explained by heat treatment.

Fructozym Color is an enzyme specially developed for fruit processing. Fructozym Color protects the color of the products by keeping the color stable and is a property which is responsible for the dark red color of fruit juice or fruit concentrate containing the coloring agent and polyphenols. Fructozyme Color eliminates a selected mode of action that prevents the release of unwanted fractions of fruit tissue. The rapid disintegration of pectin substances leads to a severe reduction of the mash viscosity, effectively breaks the fruit tissue and thus provides a precondition for its permeability. The increase in color value after enzyme application can be explained in this way.

### 3.1.2. NTU Determination (Hot-Cold Stability Test)

In clear fruit juices and concentrates, it is desirable for the clarity to remain stable until the product is consumed. As with all clear fruit juices, the stability of clarity is one of the most important quality criteria in pomegranate juices. The pomegranate raw juice naturally contains suspended fruit tissue particles, active enzymes, phenolic substances in many different amounts and types. These turbidity elements are removed by the process called clarification (depectinization + clarification) and clear pomegranate juice is obtained. The biggest problem in pomegranate juice production technology is that the clear fruit juice becomes extremely cloudy and loses its clarity over time and these turbidity elements turn into an insoluble sediment and settle at the bottom of the packaging. Although the resulting turbidity and sediment do not pose any health risk, the consumer does not know the cause of this turbidity and does not perceive it as a visual defect and reduces its interest in pomegranate juice (Yemi, 2016).

Meyer et al. (2001), revealed that various alternative strategies have been examined by conducting laboratory scale experiments for the treatment of cherry juice. Centrifugation of freshly squeezed juice from 1000 g to 35000 g resulted in low turbidity. Effect on turbidity and haze formation pectinase, acid protease, bromelain,

gallic acid and gelatin-silica sol. The gelatin-silica sol had the best effect on continuous juice clarity. Centrifugation of cherry juice prior to purification (10000 g for 15 minutes) significantly increased the clarity of the juice and reduced haze formation during cold storage of the juice. The NTU measurements are shown all figures (Table 3.1, 3.2, 3.3, 3.4, 3.5, 3.6).

**Table 3.1. NTU values of experimental design 1**

| **Initial** | After first day | After second day |
|---|---|---|
| **20.21±0.2** | 33.3±0.3 | 38.8±0.4 |

**Table 3.2. NTU values of experimental design 3**

| **Initial** | 1.day | 2.day |
|---|---|---|
| **1.03±0.2** | 1.13±0.3 | 1.48±0.4 |

Benitez et al. (2007), investigated that purification of apple juice by precipitation with bentonite and gelatin is explained by determining turbidity and zeta potential. Apple juice was treated with Poly-vinylpolypyrrolidone (PVPP) to remove total polyphenol. The gelatin particle complex was evaluated as an increase in juice turbidity after addition of tannic acid. The slope change during zeta potential determination indicated that electrostatic forces predominate at low gelatin content and that hydrophobic and hydrophilic interactions occur at a higher gelatin content. The results also showed that the tannic acid test is useful for determining the optimal gelatin concentration for clarification. Also, they revealed that the traditional clarification process has been replaced by the use of ultrafiltration membranes. However, clarification of juice by ultrafiltration alone does not remove active haze precursors and allows haze to form during storage. Bentonite, polyvinylpolypyrrolidone (PVPP) and activated charcoal are used to eliminate natural polyphenols found in fruit juices. It was also claimed that

unlike PVPP, which eliminates all fruit juice polyphenols, gelatin will only remove haze-forming polyphenols after bottling. Therefore, gelatin is a commonly used cleaning agent currently used by the fruit processing industries. This result shows that consumption of gelatin is primarily a function of the colloidal particle content, not from the chemical composition of the solution (soluble solids, acidity, degraded pectin, etc.). Finally, the results showed that the risk of free gelatin in free juice requires at least 10 times more gelatin than the optimum dosage for purification. As can be seen from the results, the turbidity value of the experimental design 1 (Table 3.1) was considerably higher than the experimental design 3 (Table 3.2) and turbidity values was not stable. According to previous definitions, for hot-cold stability test the beginning of the product brought to 65 brix NTU is measured and called N. After the juice concentrate is boiled, it is placed at -18 °C and stored there for one day. After melting, the NTU value is measured and and called N1. A third NTU value is measured at the end of the second day and is called N2. All NTU values of pomegranate juice concentrate is measured at single strength brix value(15°brix). If the NTU <5 and N2/N1<2, pomegranate juice concentrate is stable in terms of experiments (Maier, 2016). Hot-cold test was not applied for trial design 1 because the turbidity values were quite high. On the contrary experimental design 1, NTU values of experimental design 3 under 5 and N2/N1<2. In experimental design 3, the success of gelatin use on clarification and stability can be observed.

**Table 3.3. Changes in NTU values of pomegranate j.c. end of 12 h**

| | N2/N1(NTU) | | |
|---|---|---|---|
| | 4°C | 16°C | 20°C |
| **Experimental Design 2** | $18.29\pm0.20^{aA}$ | $3.62\pm0.28^{bA}$ | $5.1\pm0.26^{cA}$ |
| **Experimental Design 4** | $1.89\pm0.36^{aB}$ | $1.33\pm0.65^{aB}$ | $1.04\pm0.25^{aB}$ |

**Table 3.4. Changes in NTU values of pomegranate j.c. end of 24 h**

| | N2/N1(NTU) | | |
|---|---|---|---|
| | 4°C | 16°C | 20°C |
| **Experimental Design 2** | $1.24\pm0.25^{aA}$ | $1.04\pm0.25^{aB}$ | $0,86\pm0.35^{aC}$ |
| **Experimental Design 4** | $1.15\pm0.25^{aA}$ | $1.06\pm0.01^{aB}$ | $1.26\pm1.5^{aC}$ |

**Table 3.5. Changes in NTU values of pomegranate j.c. end of 48 h**

| | N2/N1(NTU) | | |
|---|---|---|---|
| | 4°C | 16°C | 20°C |
| **Experimental Design 2** | $1.12\pm0.12^{aA}$ | $1.43\pm0.21^{aB}$ | $1.34\pm0.10^{aC}$ |
| **Experimental Design 4** | $0.95\pm0.25^{aA}$ | $1.12\pm0.13^{aB}$ | $1.28\pm0.10^{aC}$ |

Superscript lower letters in each row indicate statistically significant difference ($p<0.05$) holding at different temperatures. Superscript upper letters indicate statistically significant difference ($p\leq0.05$) between two different plans.

**Table 3.6. NTU Values of experimental design 3 storage time**

| **Initial** | 5.day | 10.day | 20.day | 30.day |
|---|---|---|---|---|
| **1.03±0.2** | 1.50±0.3 | 1.60±0.5 | 2.10±0.4 | 2.20±0.3 |

Yemi et al. (2016), investigated that gelatin-clarified and non-clarified samples were observed for turbidity development during the storage process. Similar increases were observed within 30 days for both samples. It was observed that turbidity formation at 4 and 10 ° C continued in the clarified samples for up to 90 days, while it continued until 120th day in the unclarified samples. While there was a statistically significant difference between the turbidity values reached at 4, 10 and 20 ° C at the end of storage in the clarified samples, no such difference was found in the unclarified samples. It was found that turbidity was higher in the clarified samples at 4°C. Turbidity occurring at low temperatures was not observed in non-clarified samples and turbidity formation at 20 °C was higher than that at 4 and 10°C. Turbidity formation for both clarified and non-clarified samples was found to be substantially complete after 90 days. No significant changes in turbidity levels were observed during the subsequent storage process.

Mirsaeedghazi et al. (2010), concluded that the most important reason for membrane processing in pomegranate juice concentrate was to reduce turbidity. According to the results of this study, both MF and UF did not show any difference in terms of clarification success under the applied conditions. However, permeate flux and volume in MF were higher than in UF process. MF alone can result in a desirably clarified juice and further processing of the juice by UF treatment does not improve the juice properties (in terms of clarity). According to statistical results, it was observed that experimental design 2 in Table 3.3 has a difference between all three temperature values after 12 hours and N2/N1>2, stability was not observed. It was found that there is a difference between experimental design 2 and 4 at the end of the 12 hour, effect and success of gelatin in clarification and stabilization process can be

observed in this way. At the end of 24 and 48 hours, no such difference between the N2/N1 results of experimental design 2 and 4. Also, for both experimental design there was no difference between three different temperature values (Table 3.4 and 3.5). It can be concluded that, oxalic acid in pomegranate juice precipitates after these two waiting times and is held by a filter.

Lee et al. (2007), concluded that haze formation during storage could be reduced by fining treatment in banana juice. The results gained from these treatments showed that bentonite provided least turbid juice during 24 weeks storage and all treated juice samples were found to be a stable during storage. The turbidity values of experimental design 3 (Table 3.6) were measured during the storage period. The NTU value at the end of one-month storage was $2.20 \pm 0.3<5$. According to these results, the accuracy of the hot cold test was demonstrated.

The reason of choosing these temperatures and waiting time values was that they are applicable in production side. These results indicated that there is no stability problem in both cases when gelatin is used, stability and clarification can be achieved for filtered and unfiltered processes. In cases where gelatin is not used, it is shown that the waiting period in production should be selected at least 24 hours for three different temperature values.

# CHAPTER 4

# CONCLUSION AND RECOMMENDATION

## 4.1. Conclusion

In this study it was aimed to find a solution to turbidity problem in pomegranate juice concentrate during storage without the reprocess.

In this study, the steps in the production of pomegranate juice concentrate were simulated with the studies that started in the pilot plant and continued in the laboratory and the solution to turbidity was sought. In the production of pomegranate juice concentrate, four processes were implemented at the pilot production facility of Döhler Gıda A.Ş. In the first experimental design, after juice pasteurization, enzymation, clarification, filtration and concentration stages were followed. In the second experimental design, after ultrafiltration process pomegranate juice is brought to 30-35 brix, stored at cold temperatures (at three different temperatures) and then passed through paper filter. In the third experimental design, in the clarification stage gelatin is added and followed as in first plan. In the last experimental design, gelatin, cold storage and filter stages were all applied.

Experimental design 1 and 2; experimental design 3 and 4 were conducted at the same time interval.

Analysis parameters were obtained as pH, brix, turbidity, color and statistical analysis. In this study, it was focused on preventing color lost during pomegranate juice production and the ways of keeping the turbidity levels stable. Although the turbidity is not harmful to health, the consumers do not prefer the appearance of sediment and do not select products. Pomegranate juice color intensity can be determined by measuring its absorbance values at 520 nm ($A_{520}$). According to the results, with the use of bentonite and filtration processes was observed to decrease in the absorbance value at 520 nm. Also, the decrease of 520 nm, after pasteurization process could be

explained by heat treatment. The increase in color value after enzyme application can be explained by use of enzyme in process. The loss of color is noticeable, but not too much, and to prevent excessive color loss, enzyme was used to keep the color stable. Since turbidity is the most important problem, bentonite and filter papers are required for clarification and this loss is not considered very important.

The stability of clarity is one of the most important quality criteria in pomegranate juices. The results showed that the use of gelatin in the process as described in experimental design 3 and 4, provides a stable product in both cases, whether or not in holding at three different temperatures and filtering process. Also, it was found that in cases where gelatin is not used as described experimental design 2, after 12 hours the stability can not be achieved in the process only by holding at different temperatures and filter processing. It is shown that the waiting period in production should be selected at least 24 hours for three different temperature values.

It can be concluded that when keep the product and interrupt the process flow while processing fruit juices at certain hours in factories, it was not feasible. As a result; with use of gelatin, a clear and stable product can be provided without these steps.

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# APPENDICES

## A. Statistical Analysis

### Table A.1. Statistical Analysis of Experimental Design 2 After 12h

| | | | | | |
|---|---|---|---|---|---|
| **Table Analyzed** | **Data 1** | | | | |
| **Data sets analyzed** | A-C | | | | |
| | | | | | |
| **ANOVA summary** | | | | | |
| **F** | 3242 | | | | |
| **P value** | <0,0001 | | | | |
| **P value summary** | **** | | | | |
| **Significant diff. among means (P < 0.05)?** | Yes | | | | |
| **R square** | 0,9991 | | | | |
| | | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 0,08454 (2, 6) | | | | |
| **P value** | 0,9200 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| | | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 392,0 | 2 | 196,0 | F (2, 6) = 3242 | P<0,0001 |
| **Residual (within columns)** | 0,3627 | 6 | 0,06044 | | |
| **Total** | 392,3 | 8 | | | |
| | | | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.2. Statistical Analysis of Experimental Design 2 After 24 h

| **Table Analyzed** | **Data 1** | | | | |
|---|---|---|---|---|---|
| **Data sets analyzed** | A-C | | | | |
| | | | | | |
| **ANOVA summary** | | | | | |
| **F** | 1,369 | | | | |
| **P value** | 0,3237 | | | | |
| **P value summary** | ns | | | | |
| **Significant diff. among means (P < 0.05)?** | No | | | | |
| **R square** | 0,3134 | | | | |
| | | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 0,1653 (2, 6) | | | | |
| **P value** | 0,8514 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| | | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 0,2246 | 2 | 0,1123 | F (2, 6) = 1,369 | P=0,3237 |
| **Residual (within columns)** | 0,4922 | 6 | 0,08203 | | |
| **Total** | 0,7168 | 8 | | | |
| | | | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.3. Statistical Analysis of Experimental Design 2 After 48 h

| **Table Analyzed** | **Data 1** | | | | |
|---|---|---|---|---|---|
| **Data sets analyzed** | A-C | | | | |
| | | | | | |
| **ANOVA summary** | | | | | |
| **F** | 4,542 | | | | |
| **P value** | 0,0629 | | | | |
| **P value summary** | ns | | | | |
| **Significant diff. among means (P < 0.05)?** | No | | | | |
| **R square** | 0,6022 | | | | |
| | | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 1,062 (2, 6) | | | | |
| **P value** | 0,4030 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| | | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 0,1544 | 2 | 0,07721 | F (2, 6) = 4,542 | P=0,0629 |
| **Residual (within columns)** | 0,1020 | 6 | 0,01700 | | |
| **Total** | 0,2564 | 8 | | | |
| | | | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.4. Statistical Analysis of Experimental Design 4 After 12 h

| **Table Analyzed** | **Data 1** | | | | |
|---|---|---|---|---|---|
| **Data sets analyzed** | A-C | | | | |
| **ANOVA summary** | | | | | |
| **F** | 2,991 | | | | |
| **P value** | 0,1256 | | | | |
| **P value summary** | ns | | | | |
| **Significant diff. among means (P < 0.05)?** | No | | | | |
| **R square** | 0,4992 | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 1,174 (2, 6) | | | | |
| **P value** | 0,3712 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 0,1650 | 2 | 0,08248 | F (2, 6) = 2,991 | P=0,1256 |
| **Residual (within columns)** | 0,1655 | 6 | 0,02758 | | |
| **Total** | 0,3304 | 8 | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.5. Statistical Analysis of Experimental Design 4 After 24 h

| | | | | | |
|---|---|---|---|---|---|
| **Table Analyzed** | **Data 1** | | | | |
| **Data sets analyzed** | A-C | | | | |
| **ANOVA summary** | | | | | |
| **F** | 1,040 | | | | |
| **P value** | 0,4093 | | | | |
| **P value summary** | ns | | | | |
| **Significant diff. among means (P < 0.05)?** | No | | | | |
| **R square** | 0,2575 | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 1,545 (2, 6) | | | | |
| **P value** | 0,2876 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| | | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 0,06009 | 2 | 0,03004 | F (2, 6) = 1,040 | P=0,4093 |
| **Residual (within columns)** | 0,1733 | 6 | 0,02888 | | |
| **Total** | 0,2334 | 8 | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.6. Statistical Analysis of Experimental Design 4 After 48 h

| **Table Analyzed** | **Data 1** | | | | |
|---|---|---|---|---|---|
| **Data sets analyzed** | A-C | | | | |
| **ANOVA summary** | | | | | |
| **F** | 2,991 | | | | |
| **P value** | 0,1256 | | | | |
| **P value summary** | ns | | | | |
| **Significant diff. among means (P < 0.05)?** | No | | | | |
| **R square** | 0,4992 | | | | |
| | | | | | |
| **Brown-Forsythe test** | | | | | |
| **F (DFn, DFd)** | 1,174<br>(2, 6) | | | | |
| **P value** | 0,3712 | | | | |
| **P value summary** | ns | | | | |
| **Are SDs significantly different (P < 0.05)?** | No | | | | |
| | | | | | |
| **Bartlett's test** | | | | | |
| **Bartlett's statistic (corrected)** | | | | | |
| **P value** | | | | | |
| **P value summary** | | | | | |
| **Are SDs significantly different (P < 0.05)?** | | | | | |
| **ANOVA table** | SS | DF | MS | F (DFn, DFd) | P value |
| **Treatment (between columns)** | 0,1650 | 2 | 0,08248 | F (2, 6) = 2,991 | P=0,1256 |
| **Residual (within columns)** | 0,1655 | 6 | 0,02758 | | |
| **Total** | 0,3304 | 8 | | | |
| **Data summary** | | | | | |
| **Number of treatments (columns)** | 3 | | | | |
| **Number of values (total)** | 9 | | | | |

## Table A.7. Statistical Analysis of Experimental Design 2 and 4 After 12 h at 4°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | <0,0001 |
| **P value summary** | **** |
| **Significantly different (P < 0.05)?** | Yes |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=69,56, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 18,29 |
| **Mean of column B** | 1,887 |
| **Difference between means (B - A) ± SEM** | -16,40 ± 0,2358 |
| **95% confidence interval** | -17,06 to -15,75 |
| **R squared (eta squared)** | 0,9992 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 3,140, 2, 2 |
| **P value** | 0,4831 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.8. Statistical Analysis of Experimental Design 2 and 4 After 12 h at 16°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,0050 |
| **P value summary** | ** |
| **Significantly different (P < 0.05)?** | Yes |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=5,598, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 3,617 |
| **Mean of column B** | 1,333 |
| **Difference between means (B - A) ± SEM** | -2,283 ± 0,4079 |
| **95% confidence interval** | -3,416 to -1,151 |
| **R squared (eta squared)** | 0,8868 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 5,582, 2, 2 |
| **P value** | 0,3038 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.9. Statistical Analysis of Experimental Design 2 and 4 After 12 h at 20°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | <0,0001 |
| **P value summary** | **** |
| **Significantly different (P < 0.05)?** | Yes |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=19,56, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 5,080 |
| **Mean of column B** | 1,040 |
| **Difference between means (B - A) ± SEM** | -4,040 ± 0,2066 |
| **95% confidence interval** | -4,614 to -3,466 |
| **R squared (eta squared)** | 0,9897 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 1,038, 2, 2 |
| **P value** | 0,9813 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.10. Statistical Analysis of Experimental Design 2 and 4 After 24 h at 4°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,6796 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=0,4445, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 1,243 |
| **Mean of column B** | 1,153 |
| **Difference between means (B - A) ± SEM** | -0,09000 ± 0,2025 |
| **95% confidence interval** | -0,6521 to 0,4721 |
| **R squared (eta squared)** | 0,04708 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 1,035, 2, 2 |
| **P value** | 0,9829 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.11. Statistical Analysis of Experimental Design 2 and 4 After 24 h at 16°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,8811 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=0,1593, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 1,037 |
| **Mean of column B** | 1,060 |
| **Difference between means (B - A) ± SEM** | 0,02333 ± 0,1464 |
| **95% confidence interval** | -0,3832 to 0,4299 |
| **R squared (eta squared)** | 0,006307 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 48,49, 2, 2 |
| **P value** | 0,0404 |
| **P value summary** | * |
| **Significantly different (P < 0.05)?** | Yes |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.12. Statistical Analysis of Experimental Design 2 and 4 After 24 h at 20°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,1409 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=1,832, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 0,8567 |
| **Mean of column B** | 1,260 |
| **Difference between means (B - A) ± SEM** | 0,4033 ± 0,2202 |
| **95% confidence interval** | -0,2080 to 1,015 |
| **R squared (eta squared)** | 0,4562 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 5,379, 2, 2 |
| **P value** | 0,3135 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.13. Statistical Analysis of Experimental Design 2 and 4 After 48 h at 4°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,3314 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=1,104, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 1,117 |
| **Mean of column B** | 0,9500 |
| **Difference between means (B - A) ± SEM** | -0,1667 ± 0,1509 |
| **95% confidence interval** | -0,5857 to 0,2524 |
| **R squared (eta squared)** | 0,2336 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 10,71, 2, 2 |
| **P value** | 0,1707 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.14. Statistical Analysis of Experimental Design 2 and 4 After 48 h at 16°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,0687 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=2,474, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 1,427 |
| **Mean of column B** | 1,087 |
| **Difference between means (B - A) ± SEM** | -0,3400 ± 0,1374 |
| **95% confidence interval** | -0,7216 to 0,04159 |
| **R squared (eta squared)** | 0,6047 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 2,899, 2, 2 |
| **P value** | 0,5129 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |

## Table A.15. Statistical Analysis of Experimental Design 2 and 4 After 48 h at 20°C

| Table Analyzed | Data 1 |
|---|---|
| **Column B** | plan 4 |
| **vs.** | vs, |
| **Column A** | plan 2 |
| **Unpaired t test** | |
| **P value** | 0,3056 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **One- or two-tailed P value?** | Two-tailed |
| **t, df** | t=1,174, df=4 |
| **How big is the difference?** | |
| **Mean of column A** | 1,343 |
| **Mean of column B** | 1,280 |
| **Difference between means (B - A) ± SEM** | -0,06333 ± 0,05395 |
| **95% confidence interval** | -0,2131 to 0,08647 |
| **R squared (eta squared)** | 0,2562 |
| **F test to compare variances** | |
| **F, DFn, Dfd** | 1,879, 2, 2 |
| **P value** | 0,6947 |
| **P value summary** | ns |
| **Significantly different (P < 0.05)?** | No |
| **Data analyzed** | |
| **Sample size, column A** | 3 |
| **Sample size, column B** | 3 |