EFFECT OF HIGH HYDROSTATIC PRESSURE ON MICROBIAL LOAD AND QUALITY PARAMETERS OF GRAPE JUICE

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EFFECT OF HIGH HYDROSTATIC PRESSURE ON MICROBIAL LOAD AND QUALITY PARAMETERS OF GRAPE JUICE

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

EFFECT OF HIGH HYDROSTATIC PRESSURE ON MICROBIAL LOAD AND QUALITY PARAMETERS OF GRAPE JUICE

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Effect of high hydrostatic pressure (150-200-250 MPa) on the microbial load and quality parameters (pH, color, 5-hydroxymethylfurfural-HMF) of white (Sultaniye) and red (Alicante Bouschet) grape juices with combination of temperature (20-30-40°C) and holding time (5-10-15 min) was studied. Increased pressure and temperature showed significant effect on microbial reduction in white and red grape juices ($p<0.05$). The effect of pressure and time on pH drop was found to be insignificant ($p>0.05$). HHP resulted in $\Delta E<1$ for white grape and $\Delta E<7$ for red grape juice samples. Shelf life analysis for HHP treated white grape juice (200 MPa-40°C-10min) and red grape juice (250 MPa-40°C-10min) revealed no microbial growth up to 90 days when stored at 25°C. Although HMF formation was observed in industrially manufactured, pasteurized samples (65°C for 30 min), no HMF was detected in HHP treated white and red grape juices. HHP at the suggested conditions can be recommended as a better production alternative to heat treatment for white and red grape juice with respect to microbial load and studied quality parameters even at temperatures lower than required for pasteurization.

Key Words: High Pressure, Grape Juice, Microbiological Analysis, Color, HMF

ÖZ

YÜKSEK HİDROSTATİK BASINCIN ÜZÜM SUYU MİKROBİYAL YÜK VE KALİTE PARAMETRELERİ ÜZERİNE ETKİSİ

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Yüksek hidrostatik basıncın (YHB) (150-200-250 MPa) beyaz (Sultaniye) ve kırmızı (Alicante Bouschet) üzüm sularının mikrobiyal yükü ve kalite parametreleri (pH, renk, 5-hidroksimetilfurfural-HMF) üzerine etkileri, sıcaklık (20-30-40°C) ve zaman (5-10-15 dakika) kombinasyonlarında araştırılmıştır. Artan sıcaklık ve basınç beyaz ve kırmızı üzüm suyunun mikrobiyal yükünün azalmasına önemli katkı sağlamıştır ($p<0.05$). Uygulanan basınç ve süre üzüm sularının pH değerlerinde önemli bir değişim yaratmamıştır ($p>0.05$). YHB işleminin toplam renk indeksi (ΔE) üzerine doğrudan etkisi, beyaz üzüm suyu için $\Delta E<1$ ve kırmızı üzüm suyu için $\Delta E<7$'dir. YHB uygulaması ile işlem görmüş beyaz (200 MPa-40°C-10 dakika) ve kırmızı (250 MPa-40°C-10 dakika) üzüm sularında 25°C'de 90 günlük raf ömrü çalışması sonunda microbiyal üreme gözlemlenmemiştir. Endüstriyel olarak pastörize edilen (65°C' de 30 dakika) üzüm sularında HMF oluşumu belirlenirken; YHB işlemi uygulanmış örneklerde HMF tesbit edilmemiştir. YHB'ın beyaz ve kırmızı üzüm suyu için çalışılan kalite parametrelerinde bağlı olarak ısıl işleme alternatif olabileceği ve pastörizasyon sıcaklığından daha düşük sıcaklılarda yüksek kaliteli ürün elde edilebileceği görülmüştür.

Anahtar Kelimeler: YHB, Üzüm Suyu, Mikrobiyolojik Analiz, Renk, HMF

To My Parents

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INTRODUCTION

1.1 Novel Technologies and High Hydrostatic Pressure (HHP) in Food

Preservation methods are different depending on the type and the physical condition of the foods. These methods are basically applied so as to eliminate or to slow down the spoilage of foods due to micro-organisms. The preservation methods such as pasteurization, freezing, addition of preservative agents (i.e., sodium benzoate), drying, and smoking are traditionally used for specific types of foods. Although these applications ensure longer shelf life and add characteristic sensory attributes to the processed food, they have some undesirable effects on the nutritive value, chemical composition, flavor, odor and appearance of the food compared with the unprocessed one (Ohlsson T, 1994).

As the technological developments emerge and some side effects of traditional preservation methods on health are discovered, as well as increased awareness of nutritive issues, triggered the trend among the consumers turn to consume foods fresher and more natural (Thakur and Nelson, 1998). Consequently, the novel technologies have been investigated as an alternative to traditional preservation methods. In order to alter the traditional methods, novel technology studies are focused on to "minimal processing" of foods.

Minimal processing has been defined as the minimal possible treatment to preserve the quality characteristics of a food while, at the same time, enabling longer and suitable shelf-life to the food during storage and distribution (Huis in't Veld, 1996). As novel technologies, alternative to traditional preservation techniques, especially for heat treatment, irradiation, ultraviolet radiation, ultrasound, pulsed electric field (PEF), modified atmosphere packaging (MAP), pulsed white light and high hydrostatic pressure (HHP) are subjected to new researches (Hoover, 1997). Despite being non-thermal treatment methods, some of these novel techniques have unsolved problems. The concerns arise mainly on public resistance for unknown long term effects on human health that may be caused due to by-products, inadequate analyses for nutritional value, pathogenic bacterial resistance and operator safety (Webb and Lang 1987 and 1990). Nevertheless the novel technologies such as HHP, PEF and ultrasound gain some special interest from industrial research and investigated as an alternative to heat treatment being as nonthermal treatment which causes no significant undesirable changes in foods. Therefore, this enhances the final product's quality as also being safe to consume (Knorr D, 1995). However free radical formation probability due to the interactions between foods and electrode materials is an emerging concern for each type of food during PEF treatment (Knorr et. al., 2002) and showing limited effect on pasteurizing or sterilizing foods by using the ultrasound without combining with other treatments like pH modification, chlorination and heating (Lillard, 1994) are the main problems for these treatments in food safety.

HHP treatment is applied in foods so as to inactivate micro-organisms at lower temperatures, compared to thermal processing. Owing to this reason

HHP offers nearly the same nutritional and sensory qualities for the treated foods as the untreated ones (Chen and Hoover, 2003). The demand of consumers for minimally processed foods referring safe and no long time effect on health makes a unique opportunity for food industry to use HHP rather than using conventional processing methods (Knorr et. al., 2002).

1.1.1 Development of High Hydrostatic Pressure (HHP) in Food Applications

The use of High Pressure was subjected from mid 1800s especially in chemistry and physics in the experiments of compressibility of gases and liquids. In 1895, H. Roger reported the effect of HHP on killing bacteria. Although this was the first report on inactivation of micro-organisms by HHP, in 1899 B. Hite researched the effect of HHP treatment on inactivation of bacteria in food, for the first time in the history. The experiment showed that the shelf-life of raw milk could be extended for about 4 days with a pressure treatment at 600MPa for 1 hour at room temperature. Despite the fact that Hite's introduction of inactivation experiments by using HHP treatment, the food industry did not pay attention to HHP technique until mid 1980s.

The use of HHP in other industrial applications such as in ceramics, super-alloys, diamonds, simulators and sheet metal forming have been a familiar issue in 20th century. As the applications in ceramics and metallurgical industry, which is used as forming technique at pressure ranges between 50-500MPa in a medium of water or oil, progress during 1970s and 1980s, HHP gained significant interests from the food industry (Deplace and Mertens,

1992). In 1991 first commercial HHP product was introduced in the market in Japan (Yaldagard et. al., 2008). HHP treatment has currently been used to preserve juices, salad dressings, fruit jams, salsas, soups, oysters, guacamole, hams and yogurt. (Hendrickx et. al., 2002; Rahman, 2007).

1.1.2 Principle of HHP Mechanism

The basic principle of HHP treatment is the instantaneous and uniform pressure distribution (isostatic) among the food regardless of size, shape and the composition. In addition to this, in package treatments, the size, shape and composition of the package does not affect the processing factors. (Farkas and Hoover, 2000). Whilst the pressure is applied to food in a specific volume, there is an increase of temperature due to the compressive work against intermolecular forces, which is defined as adiabatic or compression heating (Denys et. al., 2000). Due to this fact, depending on the compression rate, initial temperature and composition of the food, the adiabatic heating increase during the compression is about 2-3 °C per 100 MPa. The counter phenomenon, decrease in temperature, is also valid during the decompression of the foods unless no heat is lost or gained through the pressurization chamber (Buzrul et. al, 2008). In Figure 1.1, pressure and temperature profiles of water, ethylene glycol and ethanol is shown as a function of initial temperature (Buzrul et. al, 2008).

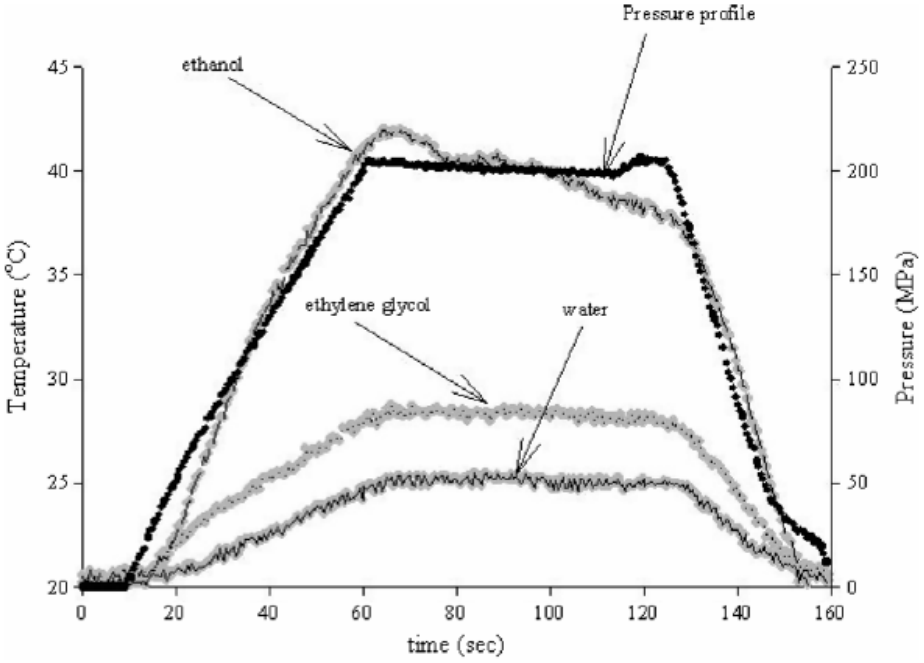

Figure 1.1 Pressure and temperature profiles of water, ethylene glycol and ethanol. Initial temperature of the substances was 20 °C and compression rate was set to 200 MPa/min (Buzrul et. al, 2008).

The chemical and biological effects due to pressurization are investigated according to the La Chatelier principle. Reactions, structural or phase changes, that cause a volume reduction occur primarily under pressure, while those accompanied by a volume increase are inhibited (Thakur and Nelson, 1998).

1.1.3 Effect of HHP on Food Constituents

Since the food constituents, carbohydrates, proteins, vitamins and minerals, determine the nutritional value of the food, effect of HHP on food constituents has been investigated, as the HHP started as an alternative method of pasteurization. It is shown that pressures used up to 300-400 MPa

in food have little or no effect on covalent bonds under room temperature, on the other hand ionic bonds, hydrogen and hydrophobic bonds are more sensible than covalent bonds (Alpas et. al., 2003a, Tauscher 1998, 1999).

The effect of HHP on monosaccharide and oligosaccharides is insignificant due to the covalent bonds. However, the polysaccharides, such as starch, can be affected from HHP because of the weak bond chains. Depending on the type of starch, degree of pressurization, time and temperature, the changes in starch varies, but alteration in the structure is almost like the one after heat treatment: gelatinization (Kawai et. al. 2007).

Proteins form of linear polymers of amino acids that are constituted in three or four structural levels. Primary level is the complete covalent forming unit of amino acid sequence. The secondary structure can be defined as the hydrogen bonded polypeptide chains. The tertiary level is the configuration of a three-dimensional folding due to non-covalent bonds between amino acid chains, and the quaternary structure is the non-covalent bonded polypeptide subunits, which forms spatial arrangement (Coultate 2002 and Yaldagard et. al, 2008). Due to the non-covalent bonds secondary, tertiary and quaternary levels of protein structure are sensible to HHP treatment (Masson, 1992)

The effect of HHP on lipids is basically on the phospholipids. Pressure treatments above 200 MPa causes pressure induced phase transitions in phospholipids (Kato and Hayashi, 1999). Though some experiments show oxidation of fats to be decreased, there are some inconsistent statements about the issue, since the reason of oxidation is associated with the storage

conditions (Angsupanich and Ledward, 1998; Cheah and Ledward, 1995)

Vitamins are the collection of complex nutrients that are bound covalently. As expected, the effect of HHP treatment on both, water soluble and fat soluble vitamins, is insignificant or not (Fernandez et. al. 2001; Sanchez-Moreno et. al. 2003; Tauscher 1998).

1.1.4 Effect of HHP on Microorganisms

The effect of HHP on microorganisms has been the greatest concern from the initial HHP treatments of H.Roger (1895) and B. Hite (1899) to the most recent studies and experiments. Since the microbial load decrease in milk was shown by B. Hite, HHP has been used in industrial food manufacturing as an alternative to pasteurization. There are many studies that explain the effect of HHP on microorganisms.

The parameters that are affecting sensitivity of microorganism by pressurizing are magnitude of pressure, pressurization time and temperature, type of microorganism, antimicrobial substances, such as bacteriocins and lysozyme, pH, cell growth phase and the characteristics of the suspending media. (Alpas and Bozoglu, 2000a).

According to results of these studies, the pressure magnitude, pressurization time and temperature have synergistic effect in different types of foods for both pressure sensitive and pressure resistant bacteria. (Gervilla et. al., 1997a; Gervilla et. al.1997b; Patterson and Kilpatrick, 1998; Ponce et. al., 1998; Alpas et al. 1999, Alpas et al., 2000b). Gram negative bacteria and the cells in the

exponential growth phase are determined to be the less sensitive than Gram positive bacteria and the cells in stationary phase (Cheftel 1995; Mackey et. al. 1995). Pressure resistance of the bacteria also vary between the strains of a specific species at moderate temperatures, however as the temperature rises up to 50°C, the resistance factor becomes ineffective (Alpas et. al. 1999).

Bacterial cells subjected to pressure between 20-35°C are more sensitive than the cells pressurized above 35°C. This is explained by the phase transition of the membrane lipids(Ludwig et. al., 1992; Kalchayanand et. al., 1998a, 1998b). It is reported that cells that are sublethally injured due to pressure are more susceptible to be inactivated by other means of treatments or combinations (Metrick et. al., 1989). Pressure with combination of low pH causes significant bacterial cell reduction. (Satomi et. al., 1995; Steward et. al., 1997). Pressure- induced injury on the recovery of injured bacteria is also related with the suspended media (Fuji et. al., 1995). In some studies, the effect of pH on injured cells is shown directly in a complex food. In order to obtain high destruction levels in low acid foods, the combination of pressure and antimicrobials, such as bacteriocins, can be used (Garcia-Graells et. al., 1998; Alpas and Bozoglu, 2002).

The inactivation mechanism of HHP on bacterial cells has been explained by some hypotheses and experimental results. It is reported that during the pressurization the cell membrane and cell wall lose their function due to some physiological changes (Kalchayanand et. al., 2002). In addition, the theory of dissociation of ribosomes, thermotropic phase changes in membrane lipids, and protein denaturation are proposed as cellular inactivation mechanism of HHP, the primary pressure damage at pressures

of 400 MPa or higher is observed, however the damage of ribosomal units was at pressures lower than 400 MPa (Alpas et. al., 2003b)

Inactivation of spores is more difficult and harder than the inactivation of vegetative cells. Although they are more pressure resistant, the resistance to pressure varies between the spore of strains and the condition of sporulation. As the vegetative cells are terminated with pressure levels between 300-400 MPa at moderate temperatures, so as to eliminate spores, pressure combinations (two stage pressurizations and oscillating pressurizations) and combinations with temperature above 70°C, combination with additives and other nonthermal techniques showed positive results (Butz et. al., 1990; Seyderhelm and Knorr, 1992; Ludwing et. al., 1992; Hayakawa et. al., 1994; Okazai et. al., 1996; Wuytack et. al., 1998)

The vegetative cells of yeasts are also pressure sensitive and can be inactivated at 300 MPa- 25°C treatment (Butz et. al., 1996). Although there has been not enough studies made about the inactivation of viruses, it is reported that the pressure resistance of viruses varies. Viruses can be inactivated by pressurization but their immunogenic properties are preserved, because usually it does not markedly change the viral structure (Mor-Mur and Yuste, 2005)

1.1.5 HHP Equipment Design and Operations

The equipment for high hydrostatic pressure application consists of five main parts: pressure vessel, high pressure pump, closures, seals and the process control instruments. According to the operation, whether it could be

batch or semi batch, there may be some additional parts for the design of equipments, such as feed inlet pump or the conveyor system for the package transportation in to the pressure vessel.

In batch operations, as in laboratory scale one, the product is treated mainly in a flexible, pressure resistant package that is in a medium which is generally water, for industrial applications, or oil. Product, in the package, is put in to the pressure vessel, then the closure is tightened and the pressurization is applied by the help of high pressure pump. In industrial applications, the packages are carried in to the vessel on a conveyor and pressurized, and then the packages are again send to palletizing for shipment or warehouse on the conveyor. (Figure 1.2)

In semi-batch operations the feed, which is a fluid, is pumped directly in to the vessel by an external low pressure pump. The pressurization is applied to the feed and then the product is filled into the pre-sterilized packages aseptically. However, there are some negative aspects of these operations, one of them is the cleaning in place (CIP) problem of the vessel and the feed line, the other one is the aseptic line and aseptic filling of the product into the packages. In order to overwhelm these problems, some additional investments should be done to the equipment design.

Figure 1.2 Industrial Scale HHP Equipment Which Runs for Batch Operations

(Adopted from http://www.avure.com/food/products/350l-600-system.asp)

1.2 Fruit Juice Processing

Fruit juices are regarded as: healthy, thirst quenching, fresh, nutritional and natural amongst the soft drinks and beverages. Throughout the history, human has developed many types of processing methods for each kind of fruit or vegetable juice. The processing methods in fruit juice industry basically developed on squeezing, clarifying and the inactivation of microorganisms and enzymes. By this way, specific processes cause some undesirable effects on the final product.

The microbial problems occurring in fruit juice industry is divided into two main groups. One is the, microbial spoilage which generally occurs during the storage of the juice after the treatment and causes detoriation. The other problem is, food borne poisoning due to the pathogenic bacteria presence in the package. Besides these, the enzyme pectin naturally found in fruit juice should be eliminated so as to observe no gelation in the final product (Ashurst, 2005).

The fruit juices are served into the market either pasteurized or unpasteurized. Since the fruit juice market has a great potential of consumers, the juice industry offers different types of processes for the treatment of fruit juices. The unpasteurized ones are the either "fresh" squeezed and served in a short time or treated with non-thermal processing methods and filled "cold" in aseptic bags or bottles. On the other hand, pasteurized fruit juices are "hot filled" or "cold filled". In hot filling, juice is pasteurized and filled in to the package, which is usually bottle, at 75-85 °C, therefore enabling the bottle to be pasteurized. In cold filling, the juice is pasteurized between 85-95°C, then cooled to 20-25°C and either filled into the bottle or stored in "aseptic tank" or in "clean tank", which are buffer tanks before the filling machine and protects the juice from re-treatment. Another method of pasteurization is the "tunnel pasteurization" in which the juice is filled freshly into the bottles and then both the bottle and the fruit juice is pasteurized under hot water bath, for about 20-30 minutes at 65-75°C.

1.2.1 Microbiological Concerns in Fruit Juice Processing

Microbiological problems in fruit juice are either microbial spoilage or food borne diseases.

The microbial spoilage of the final products is mainly due to yeast and moulds which deteriorate the juice by producing by-products such as CO_2, acid and tainting compounds (Hocking and Jensen, 2001). The source of contamination or the spoilage is mainly from the fruit itself, in addition to this, contamination can be from water and other chemical added to fruit juice (Davenport, 1996). The strains of *Saccharomyces cerevisiae* show moderate to high resistance to preservatives. The byproducts or undesirable effects that are formed are alcohol, pressure rise and haze. The other yeast that is a spoilage problem in fruit juices is the sub species of *Zygosaccharomyces*, however the species of this kind basically contaminates the juice via sugar addition or growth during the concentration and this may be a serious problem in soft drink industry (Lund, 2000).

Alicyclobacillus acidoterrestris, a thermo acidophilic, nonpathogenic and spore forming bacteria, has been detected in the last decade in several spoiled commercial pasteurized fruit juices and concentrates. *Alicyclobacillus acidoterrestris* spores are not damaged by pasteurization and growing vegetative cells cause to spoilage of fruit juice and concentrates and production of patulin (Yamazaki et. al., 1997). D values of the bacteria are reported as at 85°C, 60.8-94.5 minutes, at 90°C, 10.0-20.6 minutes and at 95°C between 2.5-8.7 minutes. Therefore, in the orange juice stored about 30°C after Ultra High Temperature (UHT) treatment at 95°C, bacteria have the

capability of growth, which causes possible spoilage of the fruit juice (Eiora et. al., 1999)

The low pH of the fruit juices generally plays an important role against the growth of most pathogens, however bacteria like *Escherichia coli* O157:H7 are acid tolerant and can survive at pH ranges 3-3.5 (Buchanan et. al., 1999). It is reported that the strains of *E. coli* and *Salmonella* in fruit juices that were not pasteurized caused significant outbreaks that became lethal (Formanek, 2001).

1.2.2 Other Mechanisms that Detoriate Fruit Juice

The fruit juice processing involves physical and chemical changes that negatively modify the quality. Browning, off-flavor formation, discoloration with some other chemical additives manipulates the original flavor, color and the nutritional value of the fruit juices.

Color is an important factor in the selection of fruit and fruit derivatives. Color of fruits is formed by naturally occurring pigments such as chlorophyll, anthocyanins and carotenoids. During the processing of fruits, sometimes, the color changes caused by enzymatic reactions or non-enzymatic reactions may occur. These color changes form browning in fruit and it is mostly undesirable for the consumers and the producers (Coultate, 2002).

The enzymatic reactions that cause browning in fruit juice processing is due to the oxidation of the phenolic compounds to quinones during the grinding

or the extraction of fruits. The mechanism is induced by the naturally occurring enzyme, polyphenoloxidase (PPO).

The non-enzymatic reactions in fruit juice processing are listed in three main categories. First one is the pyrolysis which is the burning due to the loss of water from sugar molecule and what makes the final product inedible. The second one is the caramelization in which heat-induced transformation of reducing sugars alone in a concentrated solution occurs especially in the pH ranges of 2-7. The third one is the Maillard reactions that caused by the interactions of amino acids and carbohydrates at even moderate temperatures and increase rate by 2–3 times for each 10°C rise in temperature, that also induces some by-product formations (Lozano, 2006).

1.2.3 HHP Applications on Fruit Juice

As an alternative to pasteurization, applications of HHP on fruit and vegetable juices has been vastly investigated since the effect of HHP on pathogenic and spoilage microorganisms were shown.

Orange juice is one of the major experimental materials that are to be studied, since not only being excessively consumed fruit juice but also having a low pH and inhibitory effect on most vegetative cells. Therefore, the effect of pH with combination of HHP has been extensively investigated. Linton et al. (1999) reported the effect of HHP on the survival of a pressure-resistant strain of *Escherichia coli* O157:H7 (NCTC 12079), which was pre-inoculated in orange juice, and a 6 log-cycle reduction after pressurizing at 550 MPa for 5 min at 20 C from pH 3.4 to 4.5 was observed and Alpas and

Bozoglu (2000) found 8 log reduction of *E. coli,* in orange juice with a pH 3.76, treated at 345 MPa for 5 min at 50°C. Ogawa et al (1990) showed the effect of HHP on *Aspergillus awamori* and *Saccharomyces cerevisiae* in mandarin juice, at 250 MPa for 10 minutes, 4 log reduction was observed for both of them. Sterilization of peach juice, contaminated with *E. coli* at 600MPa and 25°C took 12 minutes (Erkmen and Dogan 2003). The combination of pressure with moderate heat treatment can affirmatively affect the inactivation of bacteria in fruit juices that are especially acidic such as citrus juices.

The enzymes, which are natural or from microbial source, play important role in fruit juice processing due to reactions that cause by-product formation, texture, color change or undesirable odor formation. The enzyme inactivation in fruit juice processing is generally achieved by heat treatment or addition of other enzymes (Ashurst, 2005). Enzymes are denaturated or inactivated only when very high-pressure treatments are applied, however at lower pressures the activation effects could be attributed to reversible configuration and conformation changes on the enzyme or substrate molecules (Anese et. al., 1995; Balny and Mason, 1993). Anese et. al. (1995) reported complete activity of peroxidase, from a carrot cell-free extract, was lost only when treated under pressure 900 MPa for 1 min. In the range of 300–500 MPa enzyme activation was observed. In addition to this, when apple cell-free extract was treated at 900 MPa for 1 min, at pH 7.0, 5.4, and 4.5, polyphenoloxidase activity showed a significant reduction in enzyme activity. For both enzymes, a pH dependence on residual activity was observed after the pressure treatment. Pectinesterase in Satsuma mandarin juice is inactivated with a pressure treatment of 300–400 MPa, purified

pectinesterase is also inactivated at pressures of 300 MPa or higher (Ogawa et. al., 1990). Peroxidase, polyphenoloxidase, and pectin methyl esterase activities in strawberry and orange juice with a combination of HHP and temperature were studied by Cano et. al. (1997). At 230 MPa and 43°C, inactivation of peroxidase in strawberry puree was achieved; also combination of HHP with temperature at 35°C effectively reduced peroxidase in orange juice. The effects of pressure and temperature on pectin methyl esterase activity in orange juice were observed similar to those for peroxidase. In the case of pectin methyl esterase in juices, when pressurization at 600-1000 MPa for 10 min at temperatures 57 and 20°C is applied; a stable product without microbial deterioration can be archived but against pectin methyl esterase activity, mild blanching, refrigerated storage and inhibitory enzymes should be acquired (Cheftel, 1992). The effect of HHP on enzyme inactivation has some beneficial aspects on fruit juice quality parameters and can be used as an alternative method for heat treatment; however for inactivation of specific enzymes higher pressure rates with combinations of heat and pH are needed.

1.2.4 Grape Juice and Grape Juice Processing

Grape is one of the most harvested fruit in the world due to being non-climatic, perennial and deciduous plant. According to the Food and Agriculture Organization (FAO) statistics in 2007, 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit. However, only a small portion of grape production is used for producing grape juice, which is offered to consumers as canned or "100% fruit juice".

Turkey is also one of the significant grape producing countries in the world, which contributes with 6% share to the whole grape production. Especially with the increasing conscious and demand for the more health prompting fruit juices that are regarded as 100% fruit juices, grape, pomegranate and tomato juice promoted about 23% increase in the processing of fruit juices in Turkey (Anon, 2008a).

Grape juice has almost the same composition as the whole grape has, except oil and crude fiber that constituted in the seed. The composition of the grape juice may change according to the type of grape, but as in general, the sugars found in grape juice are glucose, fructose and sucrose. In addition to these, acids, methyl anthranilate, volatile esters, alcohols, and aldehydes are major flavor constituents. The nonvolatile acids of grape juice are tartaric and malic acid. Also the mineral elements of sodium, potassium, calcium, phosphorus, iron, copper, and manganese and organic compounds of biotin, niacin, inositol, pantothenic acid, pyridoxine hydrochloride, thiamine, folic acid, ascorbic acid, choline, and trace amounts of riboflavin and vitamin B_{12} contributes to the grape juice composition (Barrett et. al., 2004).

The processing of grape juice is almost the same as the other fruit juices. The processing techniques varies whether the final product be from fruit concentrate or be single strenght juice. After harvesting grape, first the stem is removed, then the grapes are crushed. It is either cold pressed or hot pressed. During cold pressing operation pectolytic enzymes are added to the must in order to degrade the naturally occurring pectin. The filtration, rotary vacuum filter, kieselguhr or ultrafilter, is applied to the must so as to remove the suspended solids. The clear juice is then either pasteurized in a heat

exchanger at 85-95°C and filled to the bottles or filled to the bottles and then pasteurized in bottles at about 65°C for 30 min. (tunnel pasteurization). (Appendix B)

1.2.4.1 Factors that Affects Grape Juice Quality

It is difficult to define quality factors for grape juice because of many parameters associated to the whole grape characteristics. The quality parameters of grape juices start from the cultivar type, climate and soil type, vineyard management, processing type and storage conditions. However there are some general quality parameters that ensure grape juice manipulation such as color, anthocyanidin amount, cloudiness, sugar content or acid pattern(Hui, 2006).

Color is one of the most important aspect of grape juice quality parameters. Heating during extraction or heating during pasteurization influences the color of grape juice (Ponting et. al., 1960; Sastry and Tischer, 1952; Sistrunk and Cash, 1974; Skalski and Sistrunk, 1973). White and red grapes do not have the browning effect of enzymes due to the lack of polyphenoloxidase and of a low pH (Sims et. al. 1991) . The Maillard reactions, that cause non-enzymatic browning, during heat processing and storage of fruit juices triggers the formation of 5- hydroxymethyl furfural (HMF) which is also proposed to complement the color data of fruit juices (Askar 1984; Toribo and Lozano 1987).

The formation of HMF, which is not naturally occurring product in fruits, is associated with the duration and temperature during processing and storage

(Babsky et. al., 1986; Askar 1984). The HMF indication in fruit juices is regarded as the temperature abuse in the final product in which off-flavor and cooked taste formation can develop (Meydav and Berk, 1978; Lozano et al., 1984). When the treatments are optimally applied to fresh juice, the desirable effects occur, however the long and high temperature treatments yield the formation of HMF and cause manipulation in the composition and degrade the nutritional value (Rada-Mendoza et. al., 2002).

1.3 Objectives of the Study

The objectives of the study are:

- Determine best high pressure, temperature and time parameters for inactivation of the microorganisms in red (Alicante Bouschet) and white (Sultaniye, *Vitis vinifera* L. cv. Sultan) grape juices.
- Analysis of quality parameters, pH, color and HMF amount in red (Alicante Bouschet) and white (Sultaniye, *Vitis vinifera* L. cv. Sultan) grape juice under HHP treatment.
- Shelf-life determination.
- Comparison between HHP treated grape juices and industrially produced grape juices that are pasteurized with heat treatment.

The initial objective is to determine the best conditions for the inactivation of vegetative cells in both red (Alicante Bouschet) and white (Sultaniye, *Vitis vinifera* L. cv. Sultan) grape juices under pre-determined pressure (150-200-250 MPa), temperature (20-30-40°C) and time (5-10-15min) combinations. The parameters were selected according to the previous studies of literature and the economical aspects of the operation.

The quality parameters were also selected according to the literature surveys and the industrial problems that are to be occurring by incorporation with Kavaklıdere Winery. Color, HMF amount and pH are determined as the quality parameters for both red (Alicante Bouschet) and white (Sultaniye) grape juices and the treated samples under 150-200-250 MPa, at 20-30-40°C for 5-10 and 15 minutes are analyzed.

Shelf life analyses were performed so as to determine the microbiological stability of the HHP treated red and white grape juices. Treatments were performed according to the selected pressure, temperature, time combination from the inactivation of microorganisms and the samples were stored at 25°C for 3 months at which the sampling is performed at 2^{nd}, 7^{th}, 15^{th}, 30^{th}, 60^{th} and 90^{th} day.

Finally, industrially produced (Kavaklıdere Winery) grape juices, which are pasteurized at 65°C for 30 min, and the HHP treated grape juice samples were compared so as to compare and determine HHP as an alternative processing technique to heat treatment.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples

Both red (Alicante Bouschet) and white (Sultaniye,) grape juices which are single strength, 100% fruit juice, are taken from Kavaklıdere Winery. The samples are firstly pressed for the juice extraction and then filtered, so the clear juice is obtained, and then filled cold into the bottles. Fresh samples are taken just before the pasteurization and the samples for the comparison are taken after "tunnel pasteurization", in which the juices are heat treated at 65°C for 30 minutes. The fresh samples that were unpasteurized were carried in ice bathes to the refrigerators in Food Engineering Department (FDE) in Middle East Technical University (METU). The samples were held under 4°C so as to keep its freshness and natural composition.

2.2 Sample Preparation and Processing

The samples, which are stored in refrigerator at 4°C, were just taken out before the experiment and kept in ice water bath till the sampling.

The samples that are packaged and sealed in the glass bottles were first opened and observed for any carbonation formation. This procedure is applied for each bottle of unpasteurized grape juice since the yeast activity may cause fermentation and CO_2 formation. If any carbonation or the

bubbling is seen, then the sample becomes negative for the sample taking and the procedure starts again from the initial step. If no carbonation is observed, then the sample is taken from the bottle and treated with HHP, however, at the same time, the total microbial count determination is made in order to compare the total microbial count of the new sample and the one of the initial sample that is just made after the samples were taken from the Kavaklıdere Winery, which is < 10 cfu/mL.

The shelf life analyses were performed in a closed cabinet in which the temperature is held constant at ± 25°C. The samples were stored as wrapped in opaque paper so as to avoid any reaction caused by sun light.

2.3 Treatments

The samples were treated with HHP according to the pre-determined combinations of pressure, temperature and time in order to find the optimal conditions for the selection of the suitable combination after the both microbial and chemical analyses of grape juices. The heat pasteurized ones were directly taken after the "tunnel pasteurization" in Kavaklıdere Winery and the shelf life analyses were performed at room temperature according to the best suitable treatment conditions that ensures the microbial safety.

2.3.1 HHP Applications

High hydrostatic pressure treatments were performed by HHP equipment in Food Engineering Department (FDE) in METU (Fig. 2.1). The equipment operates up to 350 MPa and by the help of an external heating and cooling

device (Hoefer Scientific Instruments RCB300, San Francisco) temperature of the medium can be adjusted between the ranges of -5°C to 80°C. The capacity of the pressurization chamber is about 5 ml and as a pressurizing medium mineral oil (Shell 20W) is used.

The equipment is designed for the laboratory scale and consists of four main parts: pressurization chamber, high pressure hydraulic pump, mechanical top and bottom closures with ethylene propylene diene Monomer (M-class) rubber (EPDM) seals and a thermocouple.

Pressurization chamber is a cylindrical metal which is made up of hot galvanized carbon steel. It is manufactured specific for the high pressure treatments, there are two end closures that are tightened with special bolts, in front of which are sealed with EPDM seals. The pressurization chamber is between these closures. After the sample is put in to the chamber, the mineral oil is fed into the vessel as a pressurizing medium. The air, which is uncompressible, is eliminated from the medium by lifting up and down the hydraulic piston. The pressure application is performed, after the closures are tightened, by the piston of high pressure pump. The piston is moved up or down, for the compression and decompression, inside the pressurization vessel by the help of control panel. The temperature is measured by a thermocouple inside the vessel, and the temperature of the system is held constant by circulating water around the jacket of the vessel via using an external heat exchanger (Hoefer Scientific Instruments RCB300, San Francisco).

Holding times are adjusted in this way: the chronometer is started just after

reaching the desired pressure and stopped before the release of the pressure. The pressure increase and release times are not included in this study.

Figure 2.1 HHP Unit in FDE in METU

2.3.2 Experimental Design

The experimental design in this study is based on the HHP treatment, heat treatment and the shelf life analysis. The parameters determined for the HHP treatment were based upon the previous studies and the economical aspects with respect to heat treatment and operational costs. The heat treated samples, which were used for the comparison, were the same ones that are commercially sold and the shelf life study is determined according to the optimum microbial inhibition parameters that were obtained after HHP

treatments.

2.3.2.1 HHP Treatment and Combinations

HHP treatments of 150, 200 and 250 MPa, at 20 °C, 30°C and 40°C for a holding time of 5, 10 and 15 minutes combinations were used in this study for each experiment of both white (Sultaniye) and red (Alicante Bouschet) grape juices (Table 2.1). The samples were filled in to ~4 mL sterile cryovials (Simport Plastic, Canada), avoiding as much air as possible inside the tubes and then placed inside the pressurization chamber for the HHP application. Before pressure treatment, it was waited for 1-2 minutes so as to ensure the temperature equilibrium of the sample in the cryovial and the medium. Experiments and measurements were duplicated on separate days, in order to justify the data obtained.

Table 2.1 HHP Treatments with Combinations of Temperature and Time

	Pressure (MPa) / Time (min.)	150	200	250
20 ºC	5	+	+	+
	10	+	+	+
	15	+	+	+
30 ºC	5	+	+	+
	10	+	+	+
	15	+	+	+
40 ºC	5	+	+	+
	10	+	+	+
	15	+	+	+

2.3.2.2 Shelf Life Analysis

Shelf life analysis of both red (Alicante Bouschet) and white (Sultaniye) grape juices were performed according to the data obtained after microbiological analysis. The samples in duplicate were pressure treated and kept at room temperature (25°C) up to 3 months (Table 2.2). Each time new cryovial was used for the determination of microbial growth and data was obtained from the analysis of two separate samples.

Table 2.2 Shelf Life Analysis based on the Total Microbial Growth after HHP treatment at the Selected Combination for Both White (Sultaniye) and Red (Alicante Bouschet) Grape Juice

2 Days	7 Days	15 Days	30 Days	60 Days	90 Days
+	+	+	+	+	+

2.4 Microbiological Analysis

Total microbial count method that is performed by spread plate technique is used for the determination of microbial analysis. The cultivations are performed on tryptic soy agar (TSA) (Merck, Germany), where duplicate agar plates incubated at 37°C ± 1°C for 48 hours are used for each sample. Plates containing 25-250 cfu/mL were selected for counting.

The samples are first diluted in 0.1% peptone (LabM, U.K.) water by taking 1 mL of the sample and mixing it in 9 mL of peptone water solution. Serial dilutions are performed by this protocol and then cultivated in to TSA plates.

TSA plates are prepared by dissolving 1g of yeast extract (Merck, Germany) and 40g of tryptic soy agar (Merck, Germany) in 1 liter of pure water. The solution is then sterilized at 120°C for 30 min. After it is cooled to 50-45°C, the liquid agar is poured in to the pre sterilized agar plates. Each experiment was repeated twice so as to justify the data obtained.

2.5 5-Hydroxymethylfurfural (HMF) Determination

5- hydroxymethylfurfural (HMF) determination of both white and red grape juice samples were performed by using high performance liquid chromatography (HPLC) system Agilent 1100 (Waldbronn, Germany) consisting of a quaternary pump, an auto sampler, a diode array detector and a temperature-controlled column oven.

The HMF determination experiments were performed in Food Engineering Department of Hacettepe University in Ankara. Both white and red grape juice samples were directly injected to HPLC instrument. The analysis was performed according to the method given by Li et. al. (1988).

Stock solution of HMF was prepared at a concentration of 1.0 mg/ml in distilled water. Standards were prepared by diluting the stock solution to concentrations of 0.05, 0.10, 0.25, 0.50 and 1.0 g/ml with distilled water. The HMF content of the samples was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions. Duplicate measurements were performed so as to justify the data.

2.6 pH Determination

The determination of pH is performed by using a pH meter (MP200, Mettler Toledo, Switzerland). The pH of high pressure treated samples; control samples and heat treated samples are measured at 25°C. Duplicate measurements were performed so as to justify the data.

2.7 Color Measurement

The color measurements are performed by using spectrophotometer (AVANTES, AvaSpec-2048) and the data is obtained by using the software of AVASOFT. The Hunter, L, a, b scale is used in the determination of color changes. First the color of control sample, which is fresh, untreated grape juice, is measured and data stored as standard. The color of the samples, HHP treated and heat treated, are measured also in L, a, b scale and evaluated as ΔL, Δa, Δb and ΔE. The measurements of calculations were performed according to the procedure of Hunter Lab (Anon, 2008b) Where:

- ΔL: $L_{sample} - L_{standard}$: Positive result indicates lighter than the standard, negative result indicates darker than the standard.
- Δa: $a_{Sample} - a_{Standard}$: Positive result indicates redder than the standard, negative result indicates greener than the standard.
- Δb: $b_{Sample} - b_{Standard}$: Positive result indicates yellowier than the standard, negative result indicates bluer than the standard.
- $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$: The result indicates the total color change

The samples of HHP treated grape juice were stored for 1 day at 4°C and the measurements were performed after this process. Duplicate measurements were performed so as to justify the data.

2.8 Statistical Analysis

The results of the experiments were analyzed statistically so as to determine the differences and the changes between data. Analysis of Variance (ANOVA) is used for the analysis of microbiological effect of HHP, color measurement and pH changes after treatments with a probability limit of $p<0.05$. The values $p>0.05$ are reported as insignificant. Throughout the statistical analysis, Minitab Release 14 and Microsoft Excel 2000 were used.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Microbiological Analysis

The microbiological analyses are based on the total microbial count of aerobic cells. The microbial load of the fresh red (Alicante Bouschet) and white (Sultaniye) grape juices are measured for the control sample. However, since the fresh juices contain less than 10 cfu/ml grape juice, in order to demonstrate the effect of HHP on inactivation of microorganisms, fresh grape juices were kept at 25°C for 2 days so as to increase the microbial load of the samples. The juices were then kept at 4°C in the refrigerator. After keeping for 2 days at room temperature, the initial loads of the grape juices were determined as a control sample. The period for the HHP treatment and cultivation of samples kept very short and every day the microbial load of the control sample was measured for any significant increase.

The results of the effect of high pressure with combination of temperature on red (Alicante Bouschet) and white (Sultaniye) grape juice samples are analyzed in sections 3.1.1, 3.1.2 and 3.1.3. The results of the statistical analysis and the detailed data are given in the Appendix A.

3.1.1 Microbiological Analysis of White (Sultaniye) Grape Juice

The microbiological analysis of the white (Sultaniye) grape juices were performed for the pressure (150- 200-250MPa), temperature (20-30-40°C)

and time (5-10-15 min) combinations. With respect to initial load, the total aerobic microorganisms were counted by spread plate method. The microbial load of the samples were measured and monitored in $\log_{10}$ logarithmic-scale. Initial load of the white (Sultaniye) grape juice was determined, after storing at room temperature for 2 days, as 7.3 log cfu/ml grape juice.

The results of the HHP treatment on inactivation of total aerobic bacteria at 20°C are represented in figure 3.1. The bars indicate the log reduction values at the specific treatment.

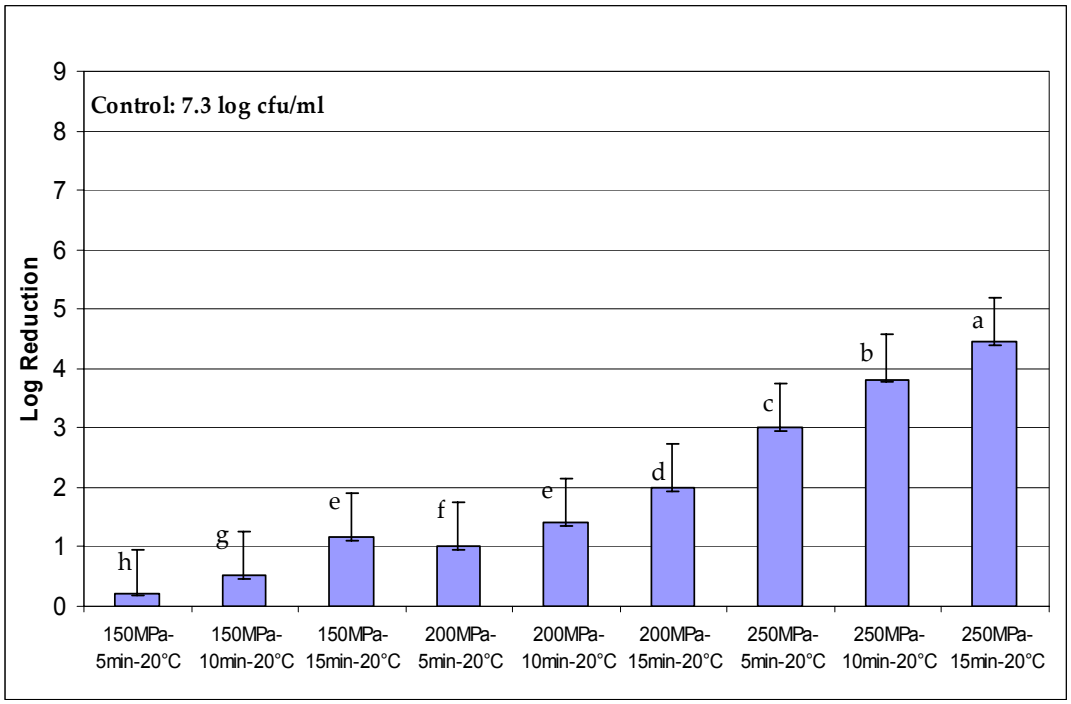

Figure 3.1 Total Microbial Reduction in White (Sultaniye) Grape Juice Treated at 20°C at 150-200-250 MPa for 5-10-15 min with an initial load of 7.3 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

According to the figure 3.1 the results indicate that the lowest microbial

reduction is observed at 150 MPa- 5 min which is about 0.2 log cycle. It is seen that as the degree of pressurization and the duration increase the reduction in the microbial load also increases. Only the treatments at 250 MPa exceeded 3 log cycle reduction. In figure 3.1, treatment for 250 MPa for 15 minutes represents the highest microbial log reduction, with about 4.5 log cycle decrease.

The results indicate that the effect of pressure at 20°C is significant ($p<0.05$). However the effect of time is found to be insignificant according to ANOVA ($p >0.05$). It is also seen from figure 3.1 that the log reductions are considerably changing with increasing pressure magnitudes.

Total microbial log reduction at 30°C by HHP treatments in white (Sultaniye) grape juice is represented in figure 3.2.

With respect to figure 3.2 the lowest log reduction rate is about 0.5 log cycle, is observed at 150 MPa- 5min treatment and the highest log reduction, 4.8 log cycle, is attained at 250 MPa-15min treatment. On the other hand, at 200 MPa destruction values exceed 3 log cycle as the holding time duration increases. Treatments at 250 MPa are resulted a log reduction above 4 cfu/ml.

According to the ANOVA calculations the effect of pressure on total microbial log reduction is stated as significant ($p<0.05$) and effect of time is found to be insignificant on total microbial log reduction at 30°C ($p>0.05$).

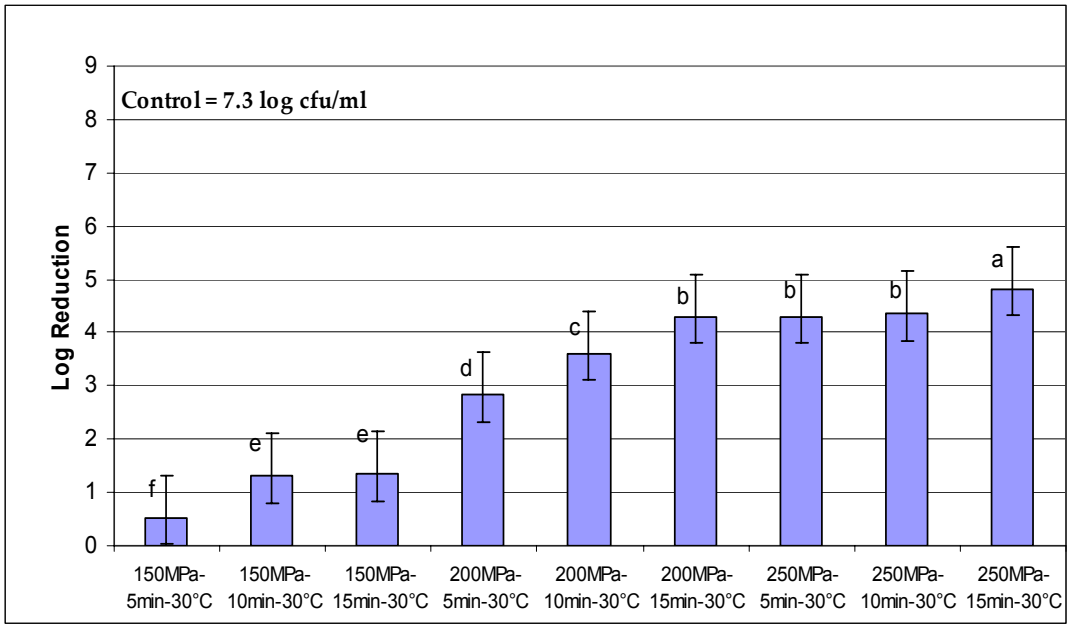

Figure 3.2 Total Microbial Reduction in White (Sultaniye) Grape Juice Treated at 30°C at 150-200-250 MPa for 5-10-15 min with an initial load of 7.3 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of the HHP treatment on inactivation of total aerobic bacteria at 40°C are represented in figure 3.3.

The results represent that the lowest log reduction, 2 log cycle, is attained at 150 MPa-5min treatment and highest log reduction is obtained at 200 and 250 MPa treatments. In 200 and 250 MPa treatments at 40°C, total inactivation of microorganisms is achieved. However at 150 MPa treatments considerable differences are observed between 5 min treatment and 10 and 15 min treatments.

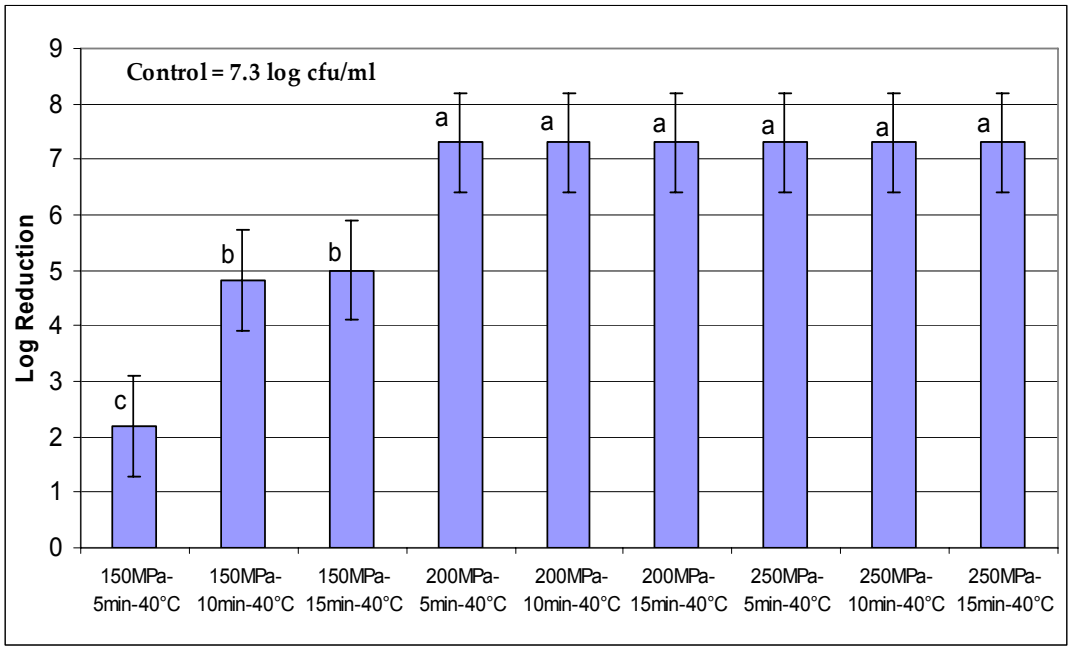

Figure 3.3 Total Microbial Reduction in White (Sultaniye) Grape Juice Treated at 40°C at 150-200-250 MPa for 5-10-15 min with an initial load of 7.3 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The statistical analysis of the data given in Figure 3.3 states that the effect of pressure on total microbial log reduction at 40°C is significant ($p<0.05$) and the effect of time on log reduction at 40°C is insignificant ($p>0.05$).

3.1.2 Microbiological Analysis of Red (Alicante Bouschet) Grape Juice

The microbiological analysis of the red (Alicante Bouschet) grape juices were performed at the pressure (150-200-250 MPa), temperature (20-30-40°C) and time (5-10-15 min) combinations. With respect to initial load and the treatments, the total aerobic microorganisms were counted by using spread plate method. The microbial load of the samples were measured and monitored in $\log_{10}$ logarithmic-scale. Initial load of the red (Alicante Bouschet) grape juice was measured, after storing at room temperature for 2 days, as 5 log cfu/ml grape juice.

The results of the HHP treatment on inactivation of total aerobic bacteria at 20°C are represented in figure 3.4. The bars indicate the log reduction values at the specific treatment.

In all 150-200 and 250 MPa treatments as the duration of time increases, the total microbial load reduction also increases.

From the data it is clear that the effect of pressure on the log reduction of total microbial at 20°C is significant referring to ANOVA ($p<0.05$) and the effect of time on microbial log reduction is insignificant ($p>0.05$).

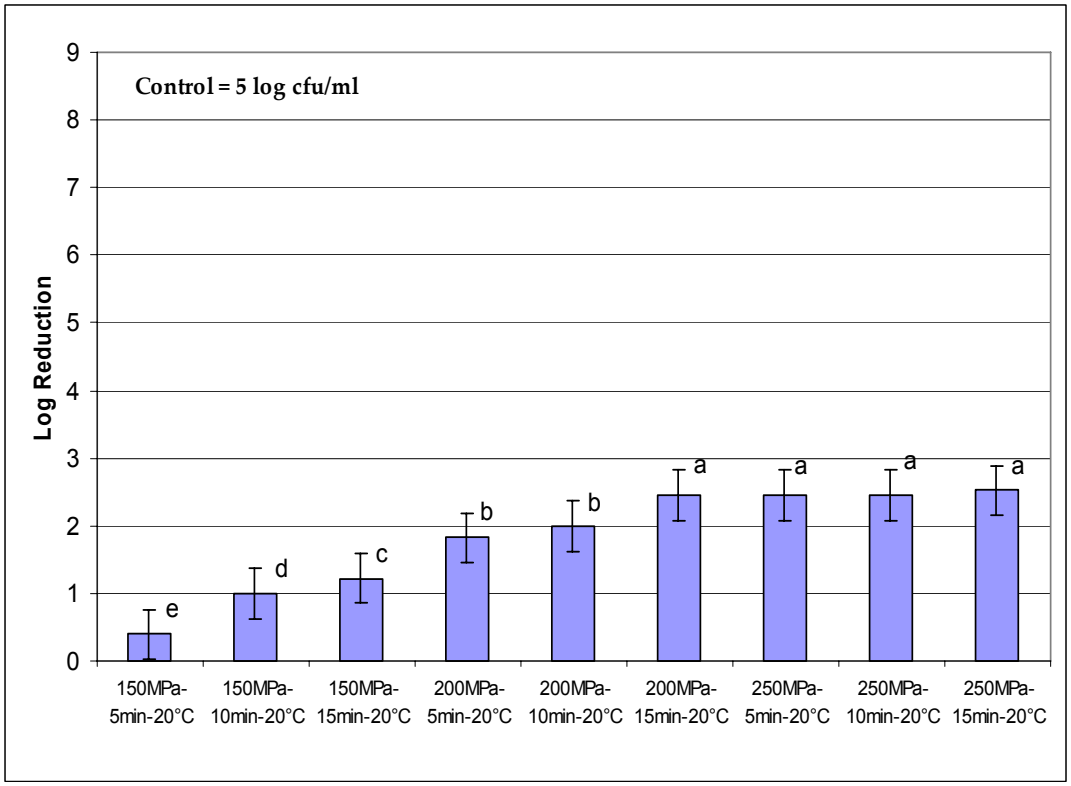

Figure 3.4 Total Microbial Reduction in Red (Alicante Bouschet) Grape Juice Treated at 20°C at 150-200-250 MPa for 5-10-15 min with an initial load of 5 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of the HHP treatment on inactivation of total aerobic bacteria at 30°C are represented in figure 3.5. The bars indicate the log reduction values at the specific treatment.

Concerning with Figure 3.5, 1.2 log reduction at 150 MPa-5 min. treatment is the lowest log reduction attained at 30°C. In addition to this, at 250 MPa-15 min treatment, 3.2 log is the highest reduction obtained at 30°C. Except 150 MPa-5 min, 150 MPa-10min and 250 MPa-5 min treatments, all other treatments exceeded 2 log reduction. The holding time is also

effective in log reduction rate. As the duration increases, the microbial reduction also increases.

According to ANOVA, effect of pressure on the log reduction of total microbial at 30°C is significant referring to ANOVA ($p<0.05$) and the effect of time on microbial log reduction is insignificant ($p>0.05$).

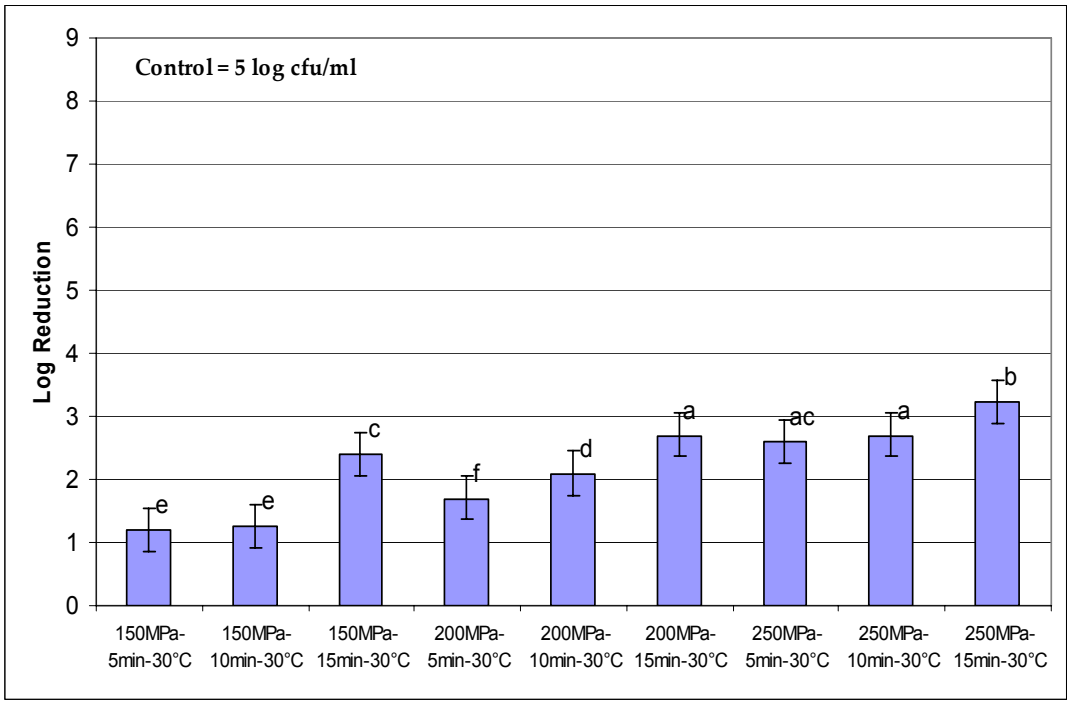

Figure 3.5 Total Microbial Log Reduction in Red (Alicante Bouschet) Grape Juice Treated at 30°C at 150-200-250 MPa for 5-10-15 min with an initial load of 5 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of the HHP treatment on inactivation of total aerobic bacteria at 40°C are represented in Figure 3.6. The bars indicate the log reduction values at the specific treatment.

Referring to Figure 3.6, only at 250 MPa-10min and 250 MPa-15min treatments total inhibition microorganisms is achieved. Except the treatments at 250 MPa, the whole other treatments remain between 2 and 3 log cycle. It is also observed that, as the duration of time increased, total log reduction was also increased. The lowest rate attained is at the treatment of 150 MPa-5min.

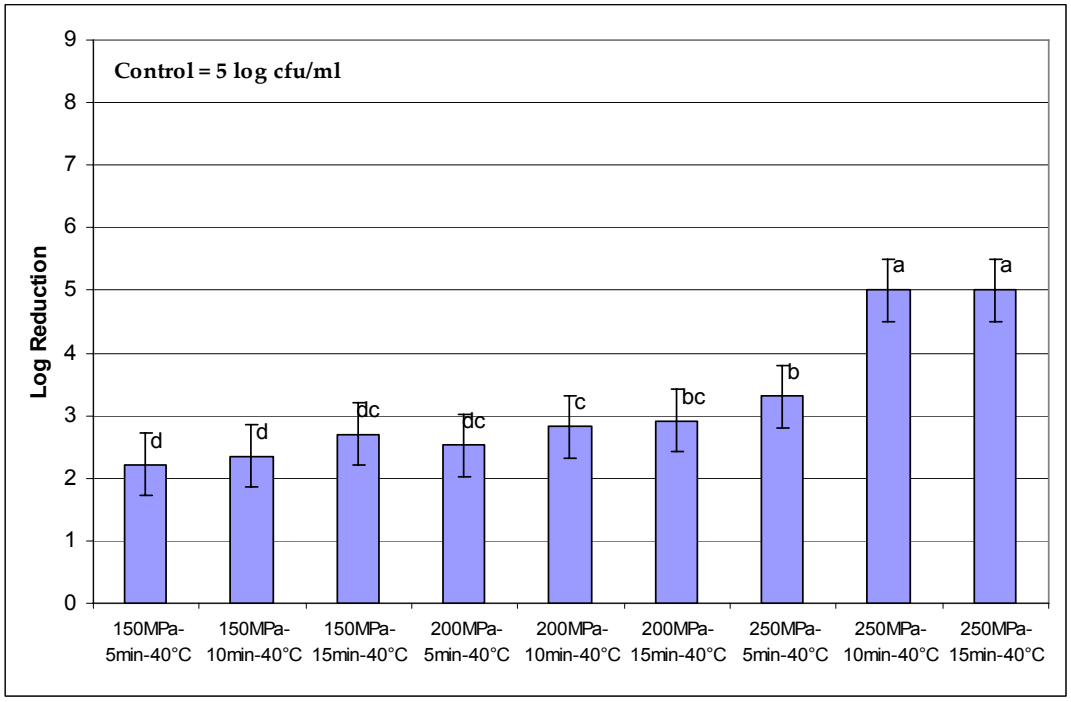

Figure 3.6 Total Microbial Log Reduction in Red (Alicante Bouschet) Grape Juice Treated at 40°C at 150-200-250 MPa for 5-10-15 min with an initial load of 5 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The ANOVA test statistically refers that the effect of pressure on the inactivation of microorganisms at 40°C is significant ($p<0.05$) and effect of

time is insignificant ($p>0.05$)

3.1.3 Summary and Discussion of Microbiological Analysis of White (Sultaniye) and Red (Alicante Bouschet) Grape Juice

The general and the comprehensive summaries of the microbiological analysis of white (Sultaniye) and red (Alicante Bouschet) grape juice is given in figure 3.7 and 3.8, respectively.

According to the figure 3.7, it is seen that the effect of HHP on the total microbial reduction is effective as the magnitude of pressurization, temperature of the medium and the holding time increase. At 40°C the total inhibition of microorganisms was achieved in pressure ranges of 200 and 250 MPa for the whole holding time ranges. In addition to this, at 150 MPa-15 min-40°C application also results 5 log reduction. At 20°C treatments, only at 250 MPa-15 min application above a reduction of 4.5 log cycle is observed. However, for treatments of 250 MPa and 200 MPa-15 min at 30°C were subjected log reductions above 4 log cycle. In conclusion, there is almost a steady increase in log reduction in white (Sultaniye) grape juice as the pressurization and time value increase at 20, 30 and 40°C treatments.

The statistical results of ANOVA indicate the effect of pressure on microbial load reduction is significant ($p<0.05$) for the whole experimental data attained. Statistical analysis also states that the effect of temperature on microbial log reduction is significant ($p<0.05$). However, effect of holding time on log reduction of microorganisms is found to be insignificant ($p>0.05$).

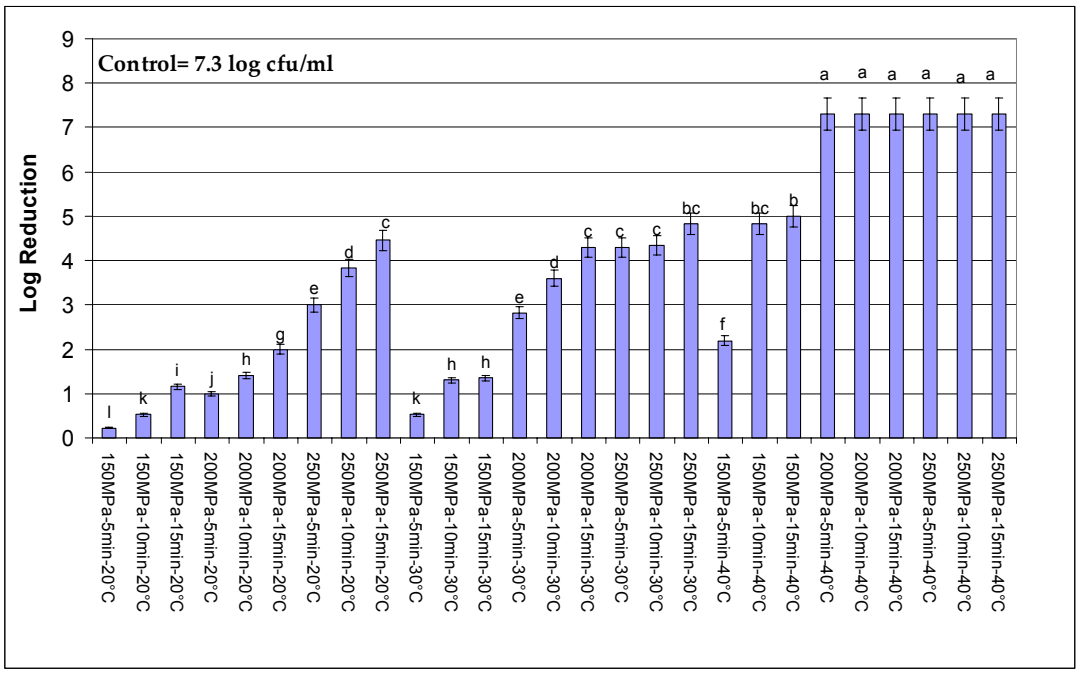

Figure 3.7 Comprehensive Data Analysis of Total Aerobic Bacterial Reduction After HHP treatment (150-200-250MPa) of White (Sultaniye) Grape Juice with an Initial Microbial Load of 7.3 log cfu/ml at 20-30-40°C for Specific Holding Time (5-10-15 min). The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

Total inhibition of aerobic microorganisms in red (Alicante Bouschet) grape juice was achieved at 250 MPa-10 min-40°C and 250 MPa-10 min-40°C treatments (Figure 3.8). At applications of 250 MPa-5 min-40°C and 200 MPa-15 min-30°C inactivation exceeds 3 log cycle whereas treatments of 250 MPa at 20 and 30°C results an inactivation between 2 and 3 log cycle reduction, depending on the holding time. Although 150MPa applications at 40°C showed increased log reduction, between 2.2 and 2.7 log cycle, when compared with 200 and 250 MPa applications, 150 MPa treatments stayed

more ineffective. As the temperature of the media is increased, the effect of pressurization is also increased.

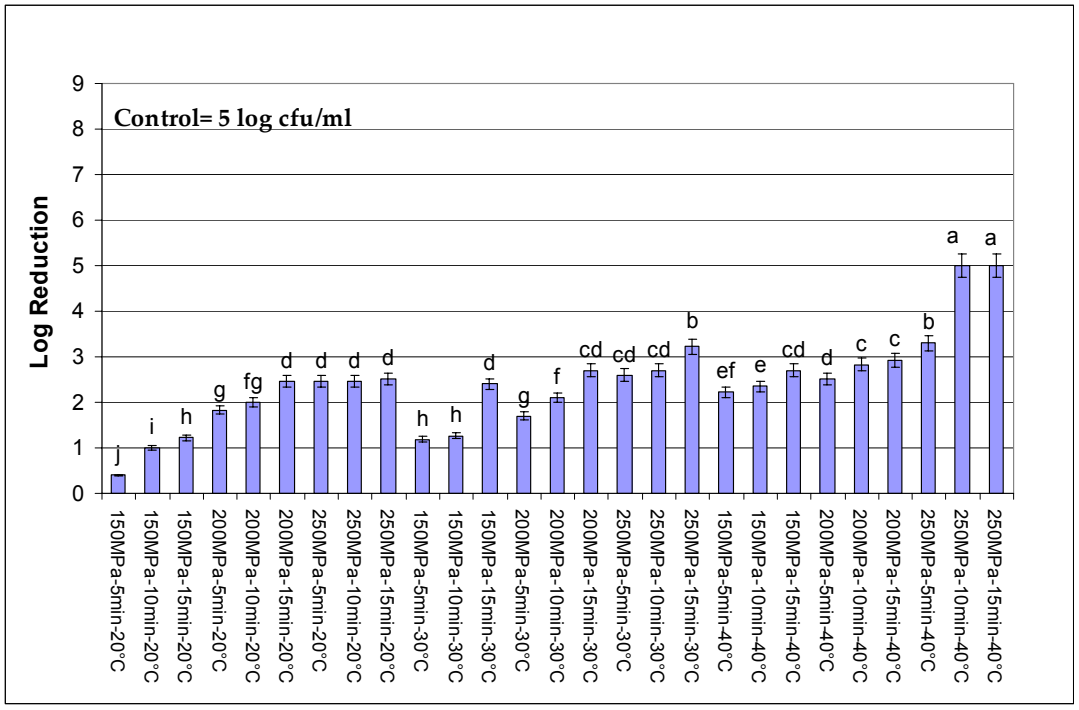

Figure 3.8 Comprehensive Data Analysis of Total Aerobic Bacterial Reduction After HHP treatment (150-200-250MPa) of Red (Alicante Bouschet) Grape Juice with an Initial Microbial Load of 5 log cfu/ml at 20-30-40°C for Specific Holding Time (5-10-15 min). The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of ANOVA state the effect of pressure on microbial load reduction is significant ($p<0.05$) for the whole experimental data attained. The effect of temperature on microbial log reduction is significant ($p<0.05$). However, effect of holding time on log reduction of microorganisms is found to be insignificant ($p>0.05$).

Total aerobic bacteria log reduction was used as an indicator of effect of HHP on microbial spoilage. The difference between the initial microbial counts and the total reduction rates could be related with the increased resveratrol content of red grape juice (Creasy M and Creasy L, 1998). Since resveratrol acts as an antimicrobial agent, the reason for low initial load attained in red grape juice, compared to white grape juice, could be due to this fact. Fonberg-Broczek M., (2005), reported as the magnitude of pressure increases, at constant temperature and holding time values, effect on microbial inactivation also increases when compared 200, 300, 400 and 500 MPa applications at 5°C for 5 min and reported up to 3 log cycle reduction difference in carrot juice between the treatments of 500 and 200 MPa applications . In this study, the effect of pressure on microbial log reduction was found to be significant ($p<0.05$) and as the magnitude of pressure increases the microbial reduction increases too. Alpas and Bozoglu (2000a) reported up to 8 log cycle reduction of pressure resistant pathogens in orange juice at 345 MPa-50°C-5min application while Linton et. al. (1999) reported a 6 log cycle reduction for *Escherichia coli* O157:H7 in orange juice for the treatment of 550 MPa for 5 min 20°C, which indicates the effect of temperature on microbial iactivation. The results of this study state that the effect of temperature on microbial reduction is also significant ($p<0.05$). The rate of microbial reduction increases with increasing temperature. Although significant log reductions were observed at 20 and 30°C treatments for 200 and 250 MPa treatments, especially for 10 and 15 min treatments, the effective treatments were attained at 40°C. The reason for this may be the alterations in the phase transition of membrane lipids (Ludwig et al. 1992; Kalchayanand et al. 1998a, 1998b). In this study the combined effect of pressure, temperature and time on total microbial log reduction in white

and red grape juice is shown.

Linton et. al, 1999, reported a reduction of *E. coli* O157:H7 about 6 log-cycle in orange juice after pressurizing at 550 MPa for 5 min at 20° C from pH 3.4 to 4.5. The effect of low pH is important factor in inhibition of bacterial strains, since low pH affects most microbes that become more susceptible to HPP inactivation, and causes sublethally injured cells not to repair (Linton et. al., 1999). In this study, the effect of pH is effective on the microbial load reduction of both white and red grape juice samples that have the control pH of 3.35 and 3.47, respectively. For white grape juice, application of 200 MPa for 10 min at 40°C resulted in 7.3 log cycle reduction and for red grape juice, application of 250 MPa for 10 min at 40°C resulted in 5 log cycle reduction.

Although the effect of time has been reported as insignificant by statistical calculations, at specific pressure magnitude and temperature parameters, effect of holding time on microbial inactivation is obvious. Treatments of 5 min applications always resulted with lower microbial log reduction rates.

Before the shelf-life analysis of grape juices, best conditions of HHP treatment were selected as an alternative to heat treatment based on the inhibition of microorganisms, economical aspects of operation and the results of statistical calculations performed.

3.2 pH Analysis

pH analysis of both white (Sultaniye) and red (Alicante) grape juices were performed at 25°C after HHP processing. The detailed results and the statistical calculations of ANOVA are given in Appendix A.

Differences between pH of HHP treated grape juices and the pH of the control sample is represented in Figure 3.9 and 3.10.

3.2.1 pH Analysis of White (Sultaniye) Grape Juice

The pH analysis of the white (Sultaniye) grape juices were performed according to the pressure (150-200-250 MPa), temperature (20-30-40°C) and time (5-10-15 min) combinations. The measured pH of each treatment and the pH of control sample are compared and the difference is shown in figure 3.9. The difference occurred is due to decrease of pH after treatment.

Referring to figure 3.9, it is seen that the pH of HHP treated white grape juices were slightly decreased comparing with the untreated one. The maximum difference attained, compared to untreated grape juice sample, was about 0.06 unit pH decrease at 40°C treatments of 150 MPa-5min and 10min, 200 MPa-10min and 15 min and 250 MPa-10 min applications. The pH change after HHP application with time and temperature combination in white (Sultaniye) grape juice is between 0.06 and 0.03 units pH. It should be noted that as the temperature increases the pH drop recorded also increases.

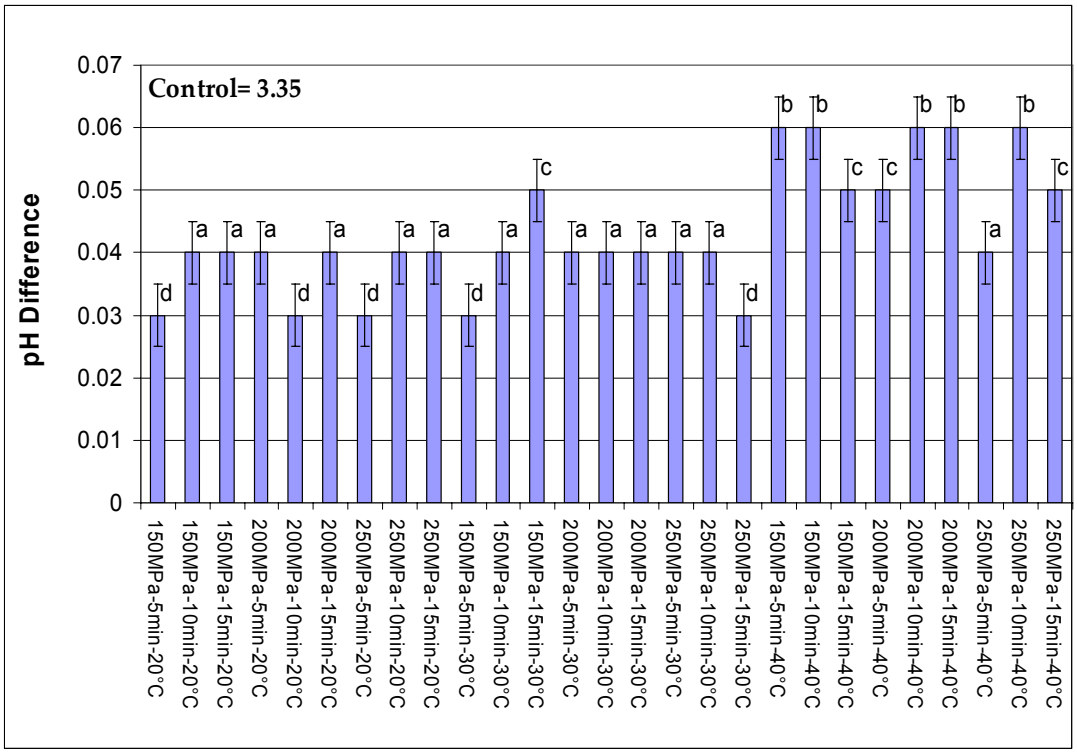

Figure 3.9 pH Difference Between HHP Treated White (Sultaniye) Grape Juice Samples and the Untreated Fresh Grape Juice with a pH of 3.35. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

According to the statistical calculations, it is stated that the effect of pressure and time on pH decrease of white grape juice is insignificant ($p>0.05$), however effect of temperature on pH decrease is found to be significant ($p<0.05$).

3.2.2 pH Analysis of Red (Alicante Bouschet) Grape Juice

The pH analysis of the red (Alicante) grape juices were performed after the treatments of HHP (150-200-250MPa) with temperature (20-30-40°C) and time (5-10-15 min) combinations. The measured pH of each treatment and

the pH of control sample are compared and the difference, which is due to the decrease of pH after treatment, is shown in Figure 3.10.

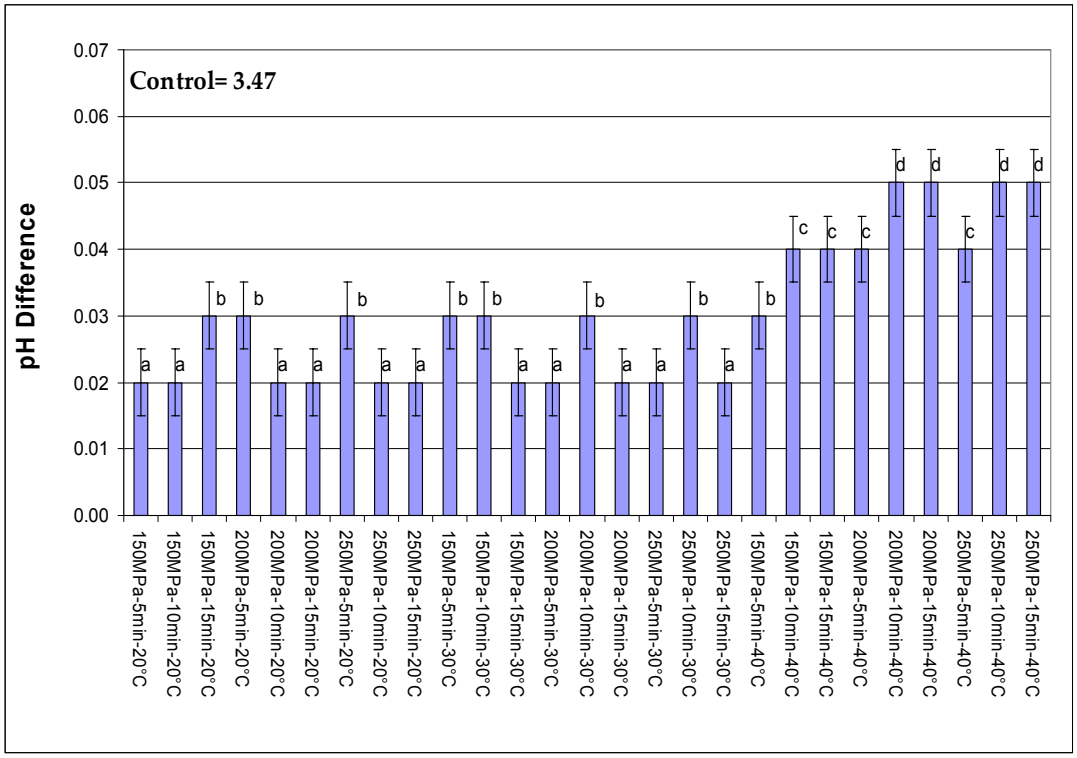

Figure 3.10 pH Difference Between HHP Treated Red (Alicante Bouschet) Grape Juice Samples and the Untreated Fresh Grape Juice with a pH of 3.47. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

After HHP treatment the pH of red grape juice samples were slightly decreased. From figure 3.10 it is seen that at 20 and 30°C resulted with similar pH drops, however at 40°C a slight increase of pH drop was observed. Treatments of 200 and 250 MPa- 10 and 15min at 40°C resulted in 0.05 unit pH drop compared to the control sample. Then the other treatments performed at 40°C influenced 0.04 unit pH drop and the other treatments almost showed a similar decrease of 0.02 and 0.03 unit pH. The pH

drop after HHP application with time and temperature combination in white grape juice is between 0.05 and 0.02 units of pH when compared to untreated grape juice sample.

The results obtained from ANOVA test represents that the effect of time and pressure on pH drop of red grape juice is insignificant ($p>0.05$). However, the effect of temperature on pH drop of red grape juice after HHP treatment is found to be significant ($p<0.05$).

3.2.3 Summary and Discussion of pH Analysis of White (Sultaniye) and Red (Alicante Bouschet) Grape Juices

After pressurizing the white and red grape juices, between 0.06 and 0.02 units of pH decrease is observed for both white and red grape juice samples. The pH drop of white grape juice at 20 and 30°C is monitored between 0.03 and 0.04 unit pH and at 40°C the pH drop exceeded up to 0.06 unit. In red grape juice, the pH drop at 20 and 30°C is observed between the values of 0.02 and 0.03 unit and, as in white grape juice treatment, the HHP applications of red grape juice resulted in significant pH drop, 0.05 pH unit, when compared with 20 and 30°C applications. Consequently, temperature affected the pH drop rates concerning with figures 3.9 and 3.10. According to statistical analysis, it can be stated that the effect of temperature on pH change of HHP treated grape juice samples is significant ($p<0.05$). Although pH drop is observed after HHP treatment, the slight differences in pH drop of samples can be attributed to measuring sensitivity of pH meter.

Hermans (1995), reported 0.2 units decrease in pH of apple juice for every 100 MPa increase in pressure. In this study, pH drop after HHP treatment is

also observed. However, the increase in pH drop is correlated with the rise in temperature rather than increase in magnitude of pressure according to the ANOVA results. The reason for the pH drop is correlated with the increased ionization of water and acid molecules under high pressure resulting in an increase of proton concentration and a pH reduction in the medium (Earnshaw et. al, 1995).

3.3 5- Hydroxymethylfurfural (HMF) Analysis of HHP Treated White (Sultaniye) and Red (Alicante Bouschet) Grape Juices.

The HMF analysis of the white and red grape juice samples were performed after the HHP treatment (150-200-250 MPa) with temperature (20-30-40°C) and time (15 min) combinations. The samples were held at 4°C for 3 days before the analysis. The objective of this analysis is to determine any HMF formation by HHP treatment at 20, 30 and 40°C for 15 min.

In this study, any formation of HMF by HHP treatment at 20, 30 and 40°C is not detected. It is reported by Hayashi (1995) that high pressure processing does not trigger the chemical reactions occurring in food systems such as Maillard reaction and formation of cooked flavors that generally occur during heat treatment.

HMF is an intermediate product of Maillard reaction that can start at lower temperatures and at higher dilutions, as in clarified fruit juices (Toribio and Lozano, 1984). Maillard reaction rate is increased 4 fold by the increment of every 10°C increase (Eskin, 1990). In addition to this, conditions and duration of storage have also similar effects of temperature in HMF formation (Toribo and Lozano, 1984). Although this study does not cover the storage

conditions of grape juices, treatments of 40°C were suspicious for HMF formation.

However, no formation of HMF is attained at 20, 30 and 40°C pressurizations for 15 min holding time. The reason for this is presumed to be short holding time and moderate temperatures applied.

3.4 Color Measurement of HHP Treated White (Sultaniye) and Red (Alicante Bouschet) Grape Juices.

The color measurements of HHP treated white (Sultaniye) and Red (Alicante Boushcet) grape juices were performed by using the Hunter L-a-b scale. The measurements of HHP treated samples were performed after 1 day storage at 4°C. The changes in color (ΔE) and luminosity (L) were taken as basis for the determination of alterations in color. The detailed results of data obtained and the calculation of statistical analysis is given in Appendix A.

3.4.1 Analysis of Color Measurements of HHP Treated White (Sultaniye) Grape Juice

Color changes and L value alterations of HHP treated white grape juice samples are represented in figure 3.11 and 3.12. The bars in each figure indicate the total color change (ΔE) and L value after treatment, respectively.

The color change values (ΔE) are between 0.2 and 0.7 units for the treatments of white grape juice. The distribution of color change is clustered specifically for the temperature, and the values of 20 and 40°C show a bit higher ΔE

values. The ΔL value obtained for the all treatments were positive, which indicates a lighter color tendency after HHP processing.

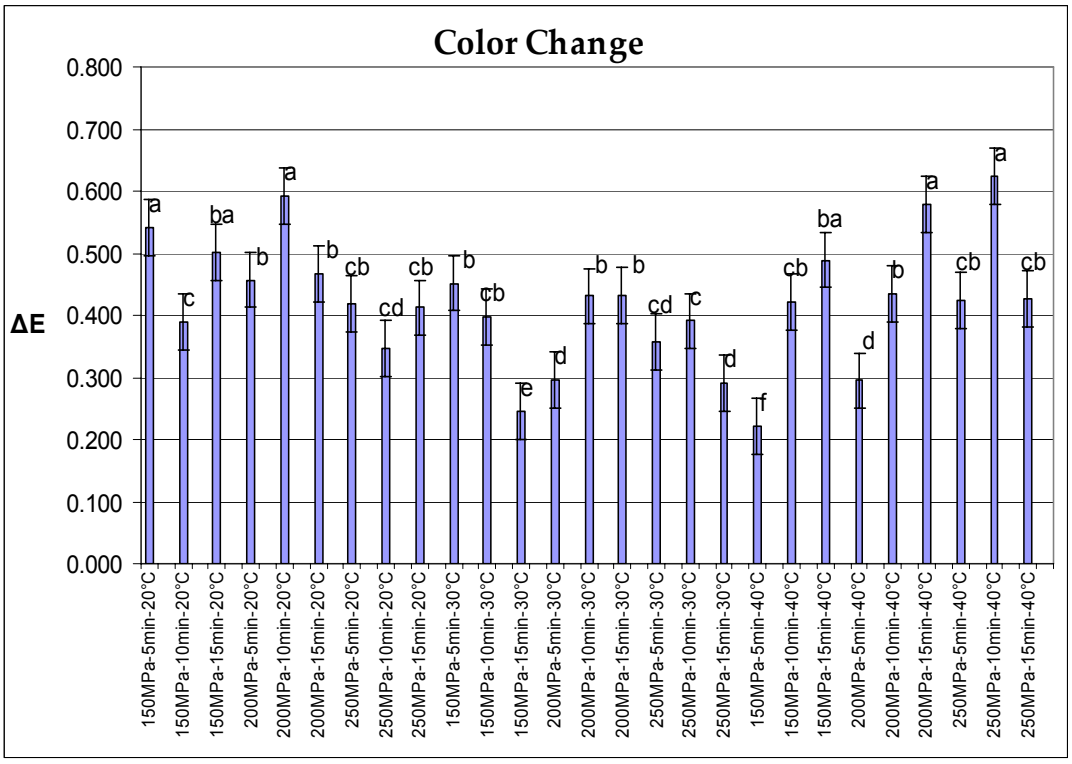

Figure 3.11 Total Color Change (ΔE) of HHP Treated White (Sultaniye) Grape Juice Samples. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The changes in ΔE and L values are statistically analyzed. The effect of pressure and time are found to be insignificant on the total color change value (ΔE) of white grape juice ($p>0.05$). However the effect of temperature on ΔE is stated as significant according to ANOVA ($p<0.05$). The L value, which is used as an indication of whiteness and blackness, does not change by the effect of pressure and time significantly ($p>0.05$), but the effect of temperature on L value is found to be significant ($p<0.05$).

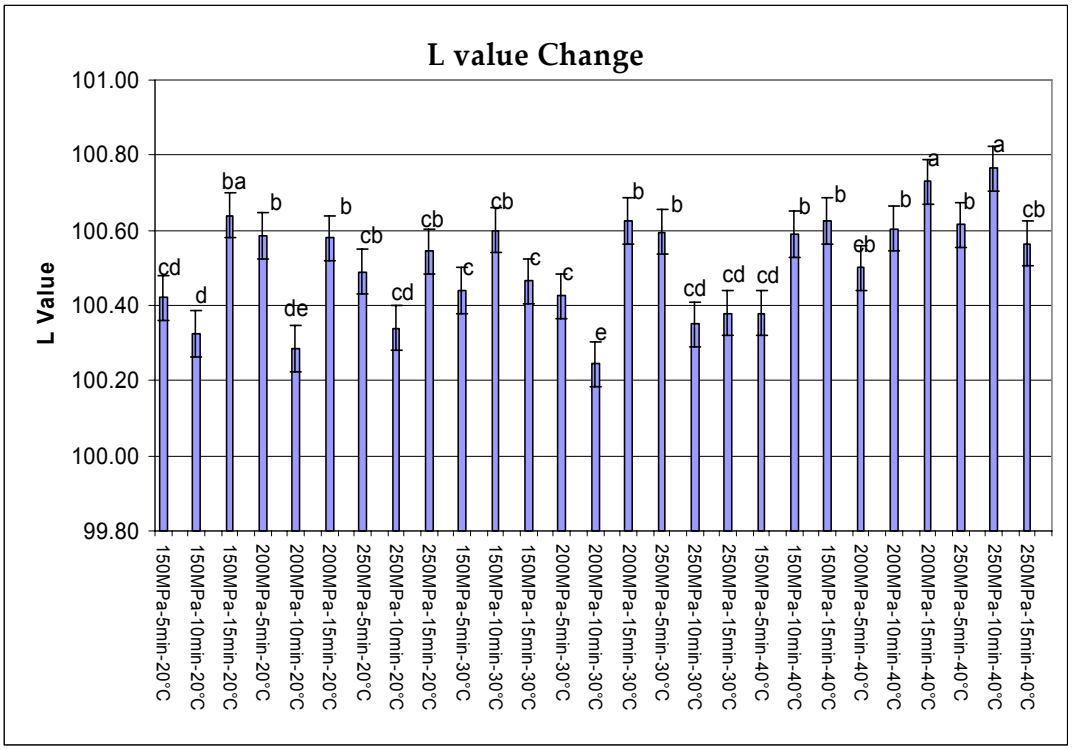

Figure 3.12 L value change of HHP Treated White (Sultaniye) Grape Juice Samples where the L value of the untreated sample is 100.24. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

3.4.2 Analysis of Color Measurements of HHP Treated Red (Alicante Bouschet) Grape Juice

Color changes (ΔE) and L value alterations of HHP treated red grape juice samples are represented in figure 3.13 and 3.14. The bars in each figure indicate the total color change (ΔE) and L value after treatment, respectively.

The color change values (ΔE) are between 1 and 7 units for the treatments of red grape juice. The ΔL value obtained for the all treatments were negative, which indicates a darker color tendency after HHP processing. For red

grape juice samples more color change with a darker color formation is attained compared to the control sample.

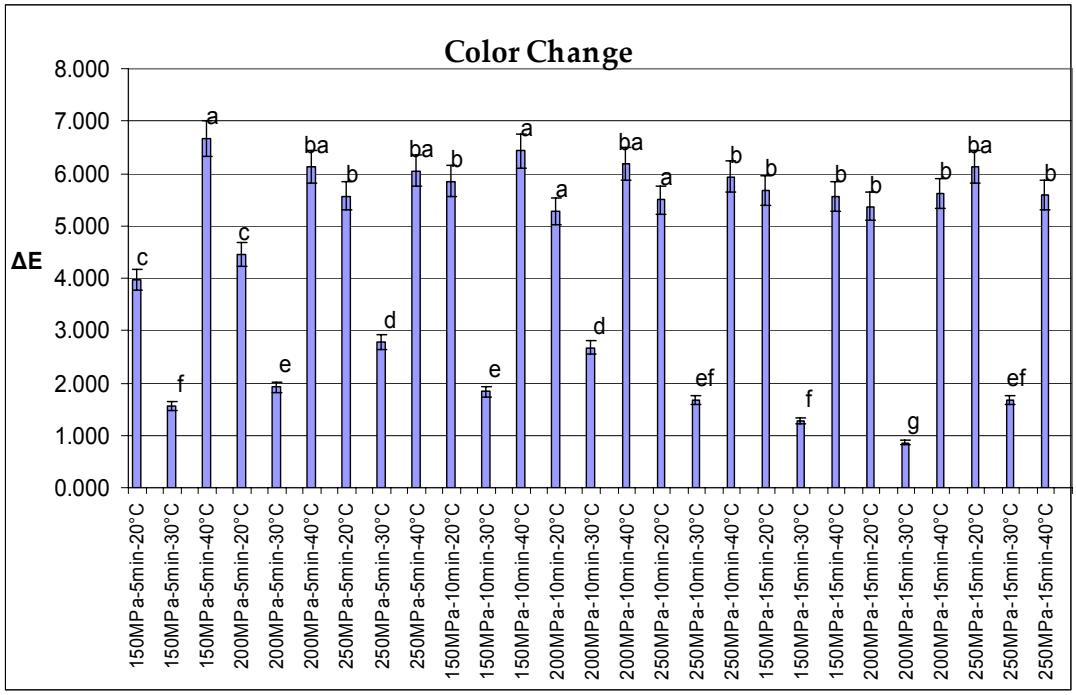

Figure 3.13 Total Color Change (ΔE) of HHP Treated Red (Alicante Bouschet) Grape Juice Samples. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

According to the statistical analysis of ANOVA the effect of pressure and time on the total color change (ΔE) of red grape juice is found to be insignificant ($p>0.05$). However, the effect of temperature on ΔE of red grape juice is stated as significant ($p<0.05$). The similar results were also obtained in the L value change of red grape juice. The statistical analysis indicate that the effect of pressure and time is insignificant ($p>0.05$) on L value change, but the effect of temperature is significant ($p<0.05$).

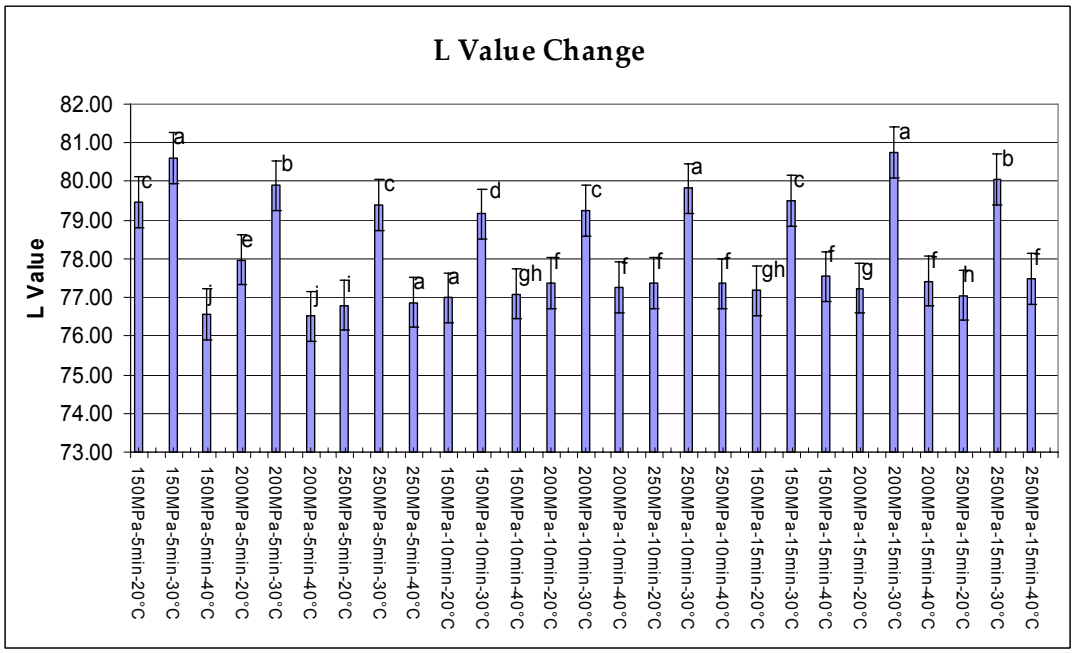

Figure 3.14 L value change of HHP Treated Red (Alicante Bouschet) Grape Juice Samples where the L value of the untreated sample is 80.71. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

3.4.3 Summary and Discussion of Color Measurements of HHP Treated White (Sultaniye) and Red (Alicante Bouschet) Grape Juice

The color measurements of both HHP treated white and red grape juice samples were performed by using L-a-b Hunter color scale. L value, which indicates the lightness and the darkness and the total color change (ΔE) values were analyzed. In this study the combined effect of pressure, temperature and time on the color change of grape juices is analyzed. The effect of storage time and temperature was not conducted.

Anthocyanins are the colorant substances of fruits, vegetables and flowers which form red, pink, mauve, violet or the blue colors. Anthocyanins are regarded as stable when low pH of medium in juice is maintained during the processing of fruits. The grape contains five of the six anthocyanins (malvidin, cyaniding, petunidin, peonidin and delphinidin) (Coultate 2002). There have been no studies performed about the effect of high pressure on the structure of antocyanins of grape. However, high pressure treatment of pelargonidin forms (pelargonidin-3-glucuside and pelargonidin-3-rutinoside) , which is not found in grapes, at moderate temperatures in red raspberry and strawberry (800MPa,18-20°C,15 min) showed stable attitude and did not change formation (Garcia-Palazon et. al.,2004). Therefore, no significant effect of HHP on anthocyanins could enhance the quality of HHP processed juices by preserving its natural color.

In this study, total color change and luminosity change of HHP treated white and red grape juice samples with combination of temperature (20-30-40°C) for 5-10 and 15 min were analyzed. The measurements were performed one day after the treatment and the samples were kept at 4°C before the analysis. In white grape juice samples, the total color change measured was between 0.2 and 0.6 units. Daudi et al (2002) showed the similar results of this study for the white grape juice at 400 and 500MPa, at 2 and 40°C for 10 min treatment. The L value change attained after 1 day storage is positive, which refers to the alteration in luminosity tends to become lighter. The HHP treated samples also showed positive value change in this study. However the statistical analysis states that the temperature has significant effect on ΔE and L value change of white grape juices that immediate color measurement were performed after the HHP treatment.

The color change values and L value change compared to untreated samples of HHP treated red grape juices showed higher rates than the white grape juice samples. The ΔE value change attained after HHP treatment was between the values of 1 to 7 units. The L value change compared to control sample was in negative value indicating a darker juice color after HHP treatment with combination of temperature and time. The ANOVA results of color analysis of red grape juice samples indicate the effect of temperature on both total color change value and L value alteration is significant ($p<0.05$). Pressure and holding time were found to be insignificant on ΔE and L value change. The difference between the color change of red grape juice and white grape juice may be due to the pH of the control samples. Since white grape juice has lower pH, that may be protective effect on anthocyanins not to alter formation. According to the results obtained, difference of "a" value, of which positive value indicates redder and negative value indicates greener than sample, found to be positive for treatments of 20 and 40°C but negative for 30°C treatments. The data attained showed similarities for the L and ΔE value changes. Treatments of both white and red grape juices at 30°C showed minimal luminosity and total color change. The reason for this may be explained by the reaction rate kinetics of anthocyanins specific to temperature changes. Mori et al (2007) showed the effect of high temperature (25 and 35°C) have considerable effect on the decrease of anthocyanins in the grape skin due to chemical or enzymatic degradation. Since the anthocyanins present in grape juice, the reason for obtaining different results at 30°C treatments could be due to this phenomenon.

3.5 Shelf Life Analysis of HHP Treated White (Sultaniye) and Red (Alicante Bouschet) Grape Juices.

The best parameters of HHP treatment of grape juice samples were selected with respect to microbial inactivation rates and the economical aspects of the study, which will be presented as an alternative to heat treatment operation. First of all, the microbial inactivation rates, which were obtained after HHP treatment of grape juice samples with combination of temperature and holding time, were evaluated according to figures 3.7 and 3.8 and the parameters that achieve full inactivation of microorganisms were taken into consideration. After that, economical aspects keeping the temperature and the holding time as possible as at low values, was considered as the next priority. Finally for the white and red grape juice 200 MPa-40°C-10 min and 250 MPa-40°C-10 min parameters were selected; respectively for the shelf life study. The shelf life study was kept up to three months at 25 °C at dark. The results obtained are represented in Tables 3.1 and 3.2.

Table 3.1 Microbial results of shelf life analysis of white grape juice treated at 200 MPa-40°C-10 min. Control $4x10^2$ cfu/ml.

2 Days	7 Days	15 Days	30 Days	60 Days	90 Days
*N/D**	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*

* Not detected

Table 3.2 Microbial results of shelf life analysis of red grape juice treated at 250 MPa-40°C-10 min. Control $3x10^1$ cfu/ml.

2 Days	7 Days	15 Days	30 Days	60 Days	90 Days
*N/D**	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*

* Not detected

Before HHP treatment, microbial load of both white and red grape juice samples were measured. For the white grape juice $4x10^2$ cfu/ml total aerobic bacteria and for the red grape juice $3x10^1$ cfu/ml total aerobic bacteria were detected. As it is seen from the tables above no growth of microbial was detected up to 90 days storage at 25°C. The results correlate also with the previous studies performed with low pH foods. Alpas and Bozoglu (2000a) reported the combined effect of pressure, temperature and low pH on inactivation of pressure resistant strains of pathogenic bacteria. Acid media affects most microbes that become more susceptible to HPP inactivation, and causes sublethally injured cells not to repair (Linton et. al, 1999). Therefore, longer shelf life durations of grape juices (pH 3.35 and 3.47 for white and red; respectively) was achieved.

3.6 Comparison of Pasteurized and HHP Treated White and Red Grape Juices

HHP was introduced as a minimal food processing technique and an alternative method to heat treatment (Knorr et. al., 2002). Therefore, one of the main aims of this study was the evaluation of HHP treatment against pasteurization and the possible use of HHP for shelf-life extension of

unpasteurized white and red grape juices.

The experiments of microbiological analysis were performed to determine the effectiveness of HHP treatment at studied temperature range (20-40°C). In HHP treatment of white and red grape juice samples at 40°C every HHP treatment showed high rates of microbial inactivation. However, for complete inactivation of microorganisms, grape juice is heated up to 65°C and hold for 30 min in Kavaklıdere Winery. When the results of this study are evaluated, combination of 200 MPa at 40°C for holding times of 5 minutes in white grape juice and treatments of 250 MPa at 40°C for holding times of 10 minutes for red grape juice resulted with complete inactivation of microorganisms. Consequently, moderate temperatures and shorter holding times of HHP application achieved the same level of microbial inactivation of grape juices compared with the heat treated ones at 65°C for 30 min in the industry. In the light of data obtained from microbiological analysis of HHP treatments, shelf life studies were also performed in order to evaluate the microbiological stability of grape juice samples and with respect to the quality parameters. The samples treated with the best combination of pressure, temperature and time showed no microbial growth up to 90 days of storage at 25°C.

As quality parameters, pH, HMF amount and color change were monitored. pH change of HHP treated grape juice samples were insignificant ($p>0.05$) as compared to untreated grape juice samples.

One of the objectives of this study was to measure the HMF formation and color change immediately after HHP treatment. HMF, which is regarded as

an indicator of the Maillard reaction and potential of browning formation, is often presented as a quality parameter in processed foods (Lee and Nagy, 1988). Bozkurt et al (1999) showed that increasing the temperature from 55 to 75°C affected the rates of HMF formation significantly in grape juice. In this study no formation of HMF was observed after HHP treatment of grape juice samples at 20-30-40°C for 15 minutes and stored for 3 days at 4°C. However with the heat treated samples at 65°C for 30 min and then stored for 15 days at 4°C, had the accumulation of HMF. The HMF amounts obtained for heat treated white and red grape juices were 0.22 and 0.25 mg/kg, respectively. Although the formation of HMF is not only dependent on heat abuse but also on the storage time and temperature (Lozano, 2006), the heat treated samples of Kavaklıdere Winery which are stored at 4°C also showed formation of HMF.

The other quality parameter evaluated is the color change of both white and red grape juice samples which were HHP treated and stored at 4°C for one day. The color change, likewise in HMF formation, is dependent on both temperature increase during processing and storage temperature and duration. Morris et al (1986) showed the effect of temperature on increase of browning index of red grape juice as the treatment temperature rises from 60°C to 99°C. However in this study the effect of HHP on white grape juice color change value and luminosity (L value) was limited but for red grape juice L value change indicated darker color formation as in the case of heat treatment. When HMF and color measurements are evaluated together, the color change attained in red grape juice is not due to the formation of HMF, which is accepted as an indicator for non-enzymatic browning due to Maillard reaction, but due to the alterations in anthocyanin structure and

formation. However, the fact is not valid for the heat treated white and red grape juice samples since the HMF amount is detected. It is also shown by Saguy et al (1978) that the accumulation of HMF increased as the duration of storage time increased with a dependency of storage temperature.

Table 3.3 Comparison Between HHP Treated and Heat Treated White and Red Grape Juice

	Microbial Reduction	**pH Change**	**HMF Accumulation**	**Shelf Life**
HHP Treated White Grape Juice (200 MPa-40°C-10min)	Yes	-0.06	N/D*	Up to90 days
Heat Treated White Grape Juice (Kavaklıdere-65°C - 30 min)	Yes	-0.03	0.22 mg/kg	2 years
HHP Treated Red Grape Juice (250 MPa-40°C-10min)	Yes	-0.05	N/D*	Up to90 days
Heat Treated Red Grape Juice (Kavaklıdere-65°C - 30 min)	Yes	-0.12	0.25 mg/kg	2 years

* Not detected

CHAPTER 4

CONCLUSIONS AND RECCOMENDATIONS

HHP was introduced as an alternative processing method for heat treatment and in many countries different types of products has been commercially processed under high pressure. Therefore, the main objective of this study is to represent HHP as an alternative method for heat treated, 65°C for 30 min, white and red grape juice samples. With respect to the main objective, effect of high pressure (150-200-250 MPa) with combination of temperature (20-30-40°C) and holding times (5-10-15min) were used as set parameters so as to determine the best conditions for microbial inactivation in red and white grape juices, observe the changes in quality parameters of grape juice and monitor microbial stability during shelf-life analysis.

The microbiological analysis, inactivation of total aerobic bacteria by HHP was conducted so as to determine the best conditions for microbial inactivation. The results were evaluated by using ANOVA and the effect of pressure, temperature and time on microbial reduction was statistically analyzed. According the results obtained from the microbiological analyses and evaluating the economical aspects of model HHP system, parameters were determined in order to monitor shelf-life of white and red grape juices for 90 days stored at 25°C.

Increased pressure and temperature showed significant effect on microbial reduction in white and red grape juices ($p<0.05$). Shelf life analysis of

microbial stability for HHP treated white grape juice (200 MPa-40°C-10min) and red grape juice (250 MPa-40°C-10min) showed no growth up to 90 days.

The quality parameters were selected as the pH, color change and HMF amount obtained after HHP treatment. The effect of pressure and time on pH drop found to be insignificant ($p>0.05$) but the effect of temperature is significant ($p<0.05$). The statistical results indicate that the effect of pressure on both color change and L value change is insignificant ($p>0.05$), however the effect of temperature on ΔE and L value change is significant ($p<0.05$). Immediate effects of HHP on color resulted in $\Delta E<1$ for white grape juice and $\Delta E<7$ for red grape juice samples. After HHP treatment, L value changes in white grape juice tend to result in lighter color compared to control sample and in red grape juice tend to form darker color.

No HMF was detected in HHP treated white and red grape juice samples after storage at 4°C for 3 days. However, commercially manufactured, heat treated white and red grape juice samples had HMF. When the storage conditions are considered it is probable to increase the accumulation of HMF as it was proven by the previous experiments.

White grape juice treated at 200 MPa for 10 min at 40°C and red grape juice treated at 250 MPa for 10 min at 40°C showed microbial stability up to 90 days. Consequently, the HHP treatment could be an alternative to heat treatment- pasteurization for white and red grape juices.

Finally, as a recommendation, the analysis of color, turbidity and HMF amount of HHP treated white and red grape juices, with combination of temperature and time, could be performed with regard to storage conditions such as the storage temperature and the duration of storage. After the determination of best conditions of storage analysis, sensory evaluations could be conducted so as to introduce a model system for commercial manufacturing.

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APPENDIX A

RESULTS AND CALCULATIONS

Table A.1 Effect of HHP on Aerobic Microorganisms in White Grape Juice, Total Microbial Counts and Log Reductions After Treatment.

	Meas. 1	Meas. 2	Meas. 3	Meas. 4	Mean	Log Mean	Log Reduction	
Control	2E+07	2E+07	2E+07	2E+07	2E+07	7.301		
150MPa-5min-20°C	1E+07	1E+07	1E+07	1E+07	1E+07	7.079	0.2218487	
150MPa-10min-20°C	6E+06	8E+06	6E+06	6E+06	6E+06	6.778	0.5228787	
150MPa-15min-20°C	1E+06	1E+06	2E+06	2E+06	1E+06	6.146	1.154902	
200MPa-5min-20°C	2E+06	2E+06	2E+06	3E+06	2E+06	6.301	1	**20°C**
200MPa-10min-20°C	8E+05	7E+05	8E+05	9E+05	8E+05	5.903	1.39794	
200MPa-15min-20°C	2E+05	2E+05	2E+05	2E+05	2E+05	5.301	2	
250MPa-5min-20°C	20000	25000	20000	15000	20000	4.301	3	
250MPa-10min-20°C	2500	3000	3000	3500	3000	3.477	3.8239087	
250MPa-15min-20°C	750	600	600	800	700	2.845	4.455932	
150MPa-5min-30°C	6E+06	6E+06	7E+06	5E+06	6E+06	6.778	0.5228787	
150MPa-10min-30°C	1E+06	1E+06	1E+06	9E+05	1E+06	6	1.30103	
150MPa-15min-30°C	9E+05	9E+05	9E+05	9E+06	9E+05	5.954	1.3467875	**30°C**
200MPa-5min-30°C	30000	30000	30000	25000	30000	4.477	2.8239087	
200MPa-10min-30°C	5000	5500	5000	4000	5000	3.699	3.60206	
200MPa-15min-30°C	900	1000	1100	950	1000	3	4.30103	

Table A.1 (continued)

250MPa-5min-30°C	1000	1100	1000	900	1000	3	4.30103	**30°C**
250MPa-10min-30°C	950	900	800	900	900	2.954	4.3467875	
250MPa-15min-30°C	250	250	400	200	300	2.477	4.8239087	
150MPa-5min-40°C	1E+05	1E+05	1E+05	1E+05	1E+05	5.114	2.1870866	**40°C**
150MPa-10min-40°C	300	250	300	300	300	2.477	4.8239087	
150MPa-15min-40°C	200	250	250	150	200	2.301	5	
200MPa-5min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	
200MPa-10min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	
200MPa-15min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	
250MPa-5min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	
250MPa-10min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	
250MPa-15min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	7.30103	

Table A.2 Effect of HHP on Aerobic Microorganisms in Red Grape Juice, Total Microbial Counts and Log Reductions After Treatment.

	Meas. 1	Meas. 2	Meas. 3	Meas. 4	Mean	Log Mean	Log Red.	
Control	1E+05	100000	1E+05	1E+05	100000	5		
150MPa-5min-20°C	40000	35000	50000	40000	40000	4.602	0.398	
150MPa-10min-20°C	9000	11000	12000	10000	10000	4	1	
150MPa-15min-20°C	6000	6000	6000	5500	6000	3.778	1.222	
200MPa-5min-20°C	1200	1600	1400	1700	1500	3.176	1.824	**20°C**
200MPa-10min-20°C	1000	900	1000	1000	1000	3	2	
200MPa-15min-20°C	350	400	300	350	350	2.544	2.456	
250MPa-5min-20°C	350	350	350	300	350	2.544	2.456	
250MPa-10min-20°C	1500	2000	400	2000	350	2.544	2.456	
250MPa-15min-20°C	200	300	350	300	300	2.477	2.523	
150MPa-5min-30°C	6000	7000	6000	7000	6500	3.813	1.187	
150MPa-10min-30°C	5500	5500	5000	5500	5500	3.74	1.26	
150MPa-15min-30°C	400	300	400	400	400	2.602	2.398	
200MPa-5min-30°C	2000	2500	1500	2000	2000	3.301	1.699	
200MPa-10min-30°C	750	800	900	750	800	2.903	2.097	**30°C**
200MPa-15min-30°C	200	150	200	200	200	2.301	2.699	
250MPa-5min-30°C	300	250	200	250	250	2.398	2.602	
250MPa-10min-30°C	200	150	200	250	200	2.301	2.699	
250MPa-15min-30°C	65	65	55	50	60	1.778	3.222	
150MPa-5min-40°C	550	600	600	650	600	2.778	2.222	
150MPa-10min-40°C	450	450	500	400	450	2.653	2.347	
150MPa-15min-40°C	200	200	250	150	200	2.301	2.699	**40°C**
200MPa-5min-40°C	250	350	350	250	300	2.477	2.523	
200MPa-10min-40°C	200	100	150	150	150	2.176	2.824	

Table A.2 (continued)

200MPa-15min-40°C	120	115	130	110	120	2.079	2.921	**40°C**
250MPa-5min-40°C	45	55	50	40	50	1.699	3.301	
250MPa-10min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	5	
250MPa-15min-40°C	No Growth	No Growth	No Growth	No Growth	No Growth	0	5	

Table A.3 ANOVA table for the effect of pressure on the total microbial reduction in white grape juice at 20°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	15.340	7.67	24.45	0.001
Error	6	1.883	0.314		
Total	8	17.223			
S=	0.5601		R-Sq=	89.07%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
150    3  0.6332  0.4762   (-----*------)
200    3  1.4660  0.5035          (-----*------)
250    3  3.7236  0.6790                              (------*------)
                           -+---------+---------+---------+--------
                          0.0       1.2       2.4       3.6

Pooled StDev = 0.5601
```

Table A.4 ANOVA table for the effect of time on the total microbial reduction in white grape juice at 20°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	1.8	0.9	0.35	0.719
Error	6	15.43	2.57		
Total	8	17.22			
S=	1.603		R-Sq=	10.43%	

```
                    Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  ------+---------+---------+---------+---
 5     3  1.407  1.433  (--------------*--------------)
10     3  1.915  1.710      (--------------*--------------)
15     3  2.501  1.654          (--------------*--------------)
                        ------+---------+---------+---------+---

 Pooled StDev = 1.603         0.0       1.5       3.0       4.5
```

Table A.5 ANOVA table for the effect of pressure on the total microbial reduction in white grape juice at 30°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	18.971	9.486	33.71	0.001
Error	6	1.689	0.281		
Total	8	20.66			
S=	0.5305		R-Sq=	91.83%	

```
Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
150    3  1.0569  0.4630  (----*----)
200    3  3.5757  0.7389                (----*----)
250    3  4.4906  0.2896                      (----*----)
                        --------+---------+---------+---------+-
                              1.5       3.0       4.5       6.0
Pooled StDev = 0.5305
```

Table A.6 ANOVA table for the effect of time on the total microbial reduction in white grape juice at 30°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	1.34	0.67	0.21	0.818
Error	6	19.32	3.22		
Total	8	20.66			
S=	1.795		R-Sq=	6.47%	

```
        Individual 95% CIs For Mean Based on
                       Pooled StDev
Level  N   Mean  StDev    +---------+---------+---------+---------
 5     3  2.549  1.904    (---------------*---------------)
10     3  3.083  1.588       (---------------*---------------)
15     3  3.491  1.875          (---------------*---------------)
                           +---------+---------+---------+---------
                         0.0       1.6       3.2       4.8

Pooled StDev = 1.795
```

Table A.7 ANOVA table for the effect of pressure on the total microbial reduction in white grape juice at 40°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	21.745	10.873	13.41	0.006
Error	6	4.965	0.828		
Total	8	26.711			
S=	0.9097		R-Sq=	81.41%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --+---------+---------+---------+-------
150    3  4.0037  1.5757  (--------*-------)
200    3  7.3010  0.0000                    (--------*-------)
250    3  7.3010  0.0000                    (--------*-------)
                          --+---------+---------+---------+-------
                          3.0       4.5       6.0       7.5

Pooled StDev = 0.9097
```

Table A.8 ANOVA table for the effect of time on the total microbial reduction in white grape juice at 40°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	1.66	0.83	0.20	0.825
Error	6	25.06	4.18		
Total	8	26.71			
S=	2.044		R-Sq=	6.20%	

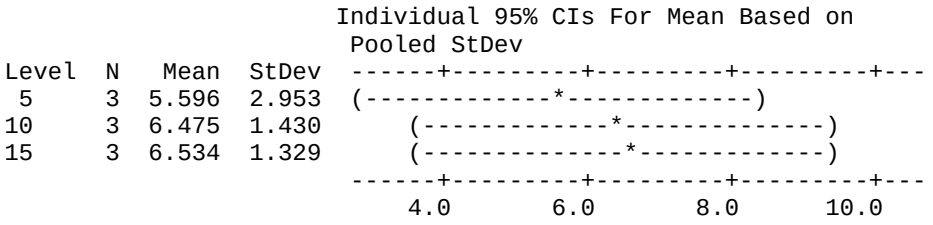


Table A.9 ANOVA table for the effect of pressure on the total microbial reduction in red grape juice at 20°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	4.2126	2.1063	21.82	0.002
Error	6	0.5793	0.0965		
Total	8	4.792			
S=	0.3107		R-Sq=	83.88%	

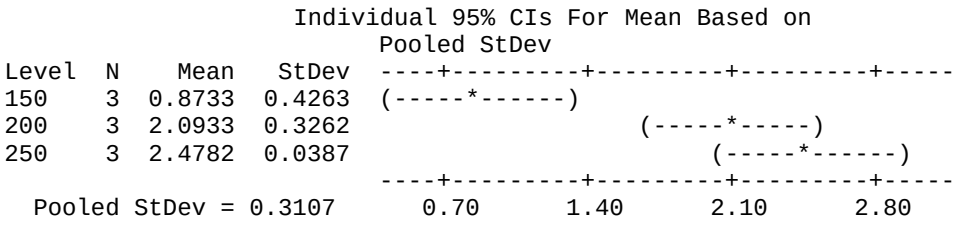


Table A.10 ANOVA table for the effect of time on the total microbial reduction in red grape juice at 20°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.387	0.193	0.26	0.777
Error	6	4.405	0.734		
Total	8	4.792			
S=	0.8569		R-Sq=	8.07%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
 5     3  1.5593  1.0542  (--------------*--------------)
10     3  1.8186  0.7447      (--------------*--------------)
15     3  2.0669  0.7326         (--------------*--------------)
                          ------+---------+---------+---------+---
                              0.80      1.60      2.40      3.20

Pooled StDev = 0.8569
```

Table A.11 ANOVA table for the effect of pressure on the total microbial reduction in red grape juice at 30°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	2.263	1.131	4.11	0.075
Error	6	1.652	0.275		
Total	8	3.914			
S=	0.5247		R-Sq=	57.81%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
150    3  1.6149  0.6791  (----------*----------)
200    3  2.1650  0.5035          (----------*----------)
250    3  2.8410  0.3334                    (----------*---------)
                          --------+---------+---------+---------+-
                                1.40      2.10      2.80      3.50

Pooled StDev = 0.5247
```

Table A.12 ANOVA table for the effect of time on the total microbial reduction in red grape juice at 30°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	1.496	0.748	1.85	0.236
Error	6	2.419	0.403		
Total	8	3.914			
S=	0.6350		R-Sq=	38.2%	

```
                          Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
 5     3  1.8294  0.7164  (------------*------------)
10     3  2.0185  0.7229     (------------*------------)
15     3  2.7729  0.4169                 (------------*-----------)
                          -------+---------+---------+---------+--
                               1.40      2.10      2.80      3.50

Pooled StDev = 0.6350
```

Table A.13 ANOVA table for the effect of pressure on the total microbial reduction in red grape juice at 40°C.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	6.971	3.485	9.8	0.013
Error	6	2.133	0.355		
Total	8	9.104			
S=	0.5962		R-Sq=	68.76%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ----+---------+---------+---------+-----
150    3  2.4225  0.2474  (-------*--------)
200    3  2.7559  0.2075     (--------*-------)
250    3  4.4337  0.9809                      (-------*--------)
                          ----+---------+---------+---------+-----
                            2.0       3.0       4.0       5.0

Pooled StDev = 0.5962
```

Table A.14 ANOVA table for the effect of time on the total microbial reduction in red grape juice at 40°C.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	1.26	0.63	0.48	0.640
Error	6	7.84	1.31		
Total	8	9.1			
S=	1.143		R-Sq=	13.84%	

```
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N   Mean  StDev   -+---------+---------+---------+--------
 5     3  2.682  0.557   (------------*-------------)
10     3  3.390  1.414         (------------*-------------)
15     3  3.540  1.269          (------------*-------------)
                         -+---------+---------+---------+--------
                        1.2       2.4       3.6       4.8

Pooled StDev = 1.143
```

Table A.15 ANOVA table for the effect of pressure on the total microbial reduction in white grape juice.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	50.24	25.12	5.89	0.008
Error	24	102.42	4.27		
Total	26	152.67			
S=	2.066		R-Sq=	27.32%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  -------+---------+---------+---------+--
150    9  1.898  1.805  (--------*--------)
200    9  4.114  2.597               (--------*--------)
250    9  5.172  1.672                     (--------*--------)
                          -------+---------+---------+---------+--
                               1.6       3.2       4.8       6.4

Pooled StDev = 2.066
```

Table A.16 ANOVA table for the effect of temperature on the total microbial reduction in white grape juice.

One-way ANOVA: Log Reduction vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Temperature	2	88.07	44.04	16.36	0.000
Error	24	64.59	2.69		
Total	26	152.67			
S=	1.641		R-Sq=	57.69%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  ------+---------+---------+---------+---
20     9  1.941  1.467  (-----*----)
30     9  3.041  1.607        (----*-----)
40     9  6.202  1.827                    (-----*-----)
                          ------+---------+---------+---------+---
                              2.0       4.0       6.0       8.0

Pooled StDev = 1.641
```

Table A.17 ANOVA table for the effect of time on the total microbial reduction in white grape juice.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	4.54	2.27	0.37	0.696
Error	24	148.12	6.17		
Total	26	152.67			
S=	2.484		R-Sq=	2.98%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  --------+---------+---------+---------+-
 5     9  3.184  2.668  (--------------*-------------)
10     9  3.825  2.466        (-------------*------------)
15     9  4.175  2.306           (-------------*------------)
                          --------+---------+---------+---------+-
                                2.4       3.6       4.8       6.0

Pooled StDev = 2.484
```

Table A.18 ANOVA table for the effect of pressure on the total microbial reduction in red grape juice.

One-way ANOVA: Log Reduction vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	11.791	5.895	9.28	0.001
Error	24	15.252	0.635		
Total	26	27.042			
S=	0.7972		R-Sq=	43.6%	

```
                          Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N    Mean   StDev  ----+---------+---------+---------+-----
150    9  1.6369  0.7914  (------*-------)
200    9  2.3380  0.4471            (------*-------)
250    9  3.2510  1.0393                      (------*-------)
                          ----+---------+---------+---------+-----
                            1.40      2.10      2.80      3.50

Pooled StDev = 0.7972
```

Table A.19 ANOVA table for the effect of temperature on the total microbial reduction in red grape juice.

One-way ANOVA: Log Reduction vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Temperature	2	9.232	4.616	6.22	0.007
Error	24	17.81	0.742		
Total	26	27.042			
S=	0.8614		R-Sq=	34.14%	

```
Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
20     9  1.8149  0.7739  (--------*-------)
30     9  2.2069  0.6995        (--------*-------)
40     9  3.2040  1.0668                  (--------*-------)
                          ---+---------+---------+---------+------
                           1.40      2.10      2.80      3.50

Pooled StDev = 0.8614
```

Table A.20 ANOVA table for the effect of time on the total microbial reduction in red grape juice.

One-way ANOVA: Log Reduction vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	2.67	1.33	1.31	0.288
Error	24	24.38	1.02		
Total	26	27.04			
S=	1.008		R-Sq=	9.86%	

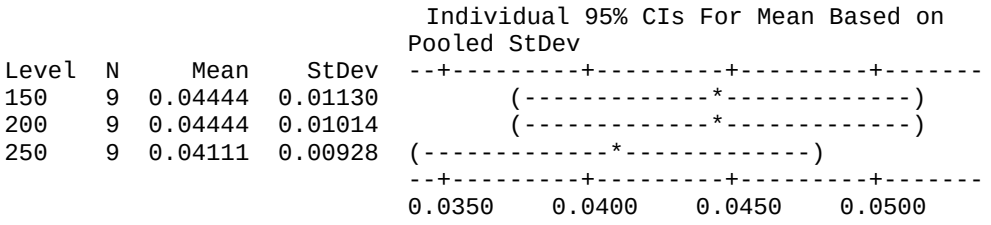
```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  --------+---------+---------+---------+-
 5     9  2.024  0.861  (-----------*----------)
10     9  2.409  1.148        (----------*-----------)
15     9  2.793  0.994              (-----------*----------)
                          --------+---------+---------+---------+-
                                1.80      2.40      3.00      3.60

Pooled StDev = 1.008
```

Table A.21 pH Measurements of HHP Treated White Grape Juices.

	Meas. 1	Meas. 2	Mean	Difference
Control	3.35	3.35	3.35	
150MPa-5min-20°C	3.32	3.32	3.32	0.03
150MPa-10min-20°C	3.31	3.30	3.31	0.04
150MPa-15min-20°C	3.31	3.30	3.31	0.04
200MPa-5min-20°C	3.30	3.31	3.31	0.04
200MPa-10min-20°C	3.31	3.32	3.32	0.03
200MPa-15min-20°C	3.31	3.31	3.31	0.04
250MPa-5min-20°C	3.32	3.32	3.32	0.03
250MPa-10min-20°C	3.31	3.31	3.31	0.04
250MPa-15min-20°C	3.30	3.31	3.31	0.04
150MPa-5min-30°C	3.32	3.32	3.32	0.03
150MPa-10min-30°C	3.30	3.31	3.31	0.04
150MPa-15min-30°C	3.40	3.20	3.3	0.05
200MPa-5min-30°C	3.10	3.10	3.31	0.04
200MPa-10min-30°C	3.10	3.00	3.31	0.04
200MPa-15min-30°C	3.31	3.31	3.31	0.04
250MPa-5min-30°C	3.31	3.31	3.31	0.04
250MPa-10min-30°C	3.30	3.31	3.31	0.04
250MPa-15min-30°C	3.32	3.31	3.32	0.03
150MPa-5min-40°C	3.29	3.29	3.29	0.06
150MPa-10min-40°C	3.29	3.28	3.29	0.06
150MPa-15min-40°C	3.40	3.30	3.3	0.05
200MPa-5min-40°C	3.40	3.30	3.3	0.05
200MPa-10min-40°C	3.28	3.29	3.29	0.06
200MPa-15min-40°C	3.29	3.29	3.29	0.06
250MPa-5min-40°C	3.30	3.31	3.31	0.04
250MPa-10min-40°C	3.28	3.29	3.29	0.06
250MPa-15min-40°C	3.20	3.30	3.3	0.05

Table A.22 ANOVA table for the effect of pressure on the pH change of white grape juice after HHP treatment.

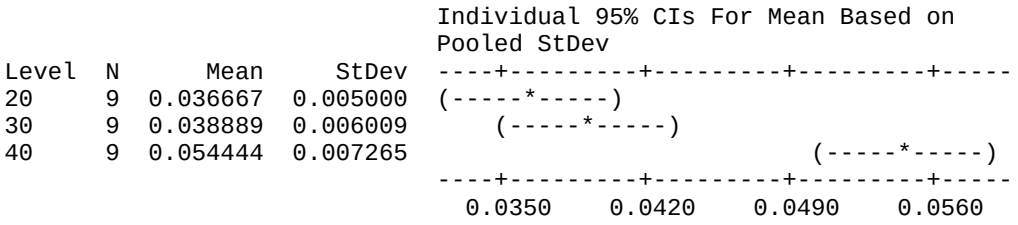
One-way ANOVA: pH Difference vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	0.000067	0.000033	0.32	0.732
Error	24	0.002533	0.000106		
Total	26	0.0026			
S=	0.01027		R-Sq=	2.56%	



Pooled StDev = 0.01027

Table A.23 ANOVA table for the effect of temperature on the pH change of white grape juice after HHP treatment.

One-way ANOVA: pH Difference vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Temperature	2	0.001689	0.000844	22.24	0.000
Error	24	0.000911	0.000038		
Total	26	0.0026			
S=	0.006161		R-Sq=	64.96%	



Pooled StDev = 0.006161

Table A.24 ANOVA table for the effect of time on the pH change of white grape juice after HHP treatment.

One-way ANOVA: pH Difference vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.000156	0.000078	0.76	0.477
Error	24	0.002444	0.000102		
Total	26	0.0026			
S=	0.01009		R-Sq=	5.98%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
 5     9  0.04000  0.01000  (-------------*-------------)
10     9  0.04556  0.01130            (-------------*-------------)
15     9  0.04444  0.00882          (-------------*-------------)
                          ----+---------+---------+---------+-----
                            0.0350    0.0400    0.0450    0.0500

Pooled StDev = 0.01009
```

Table A.25 pH Measurements of HHP Treated Red Grape Juices.

	Meas. 1	**Meas. 2**	**Mean**	**Difference**
Control	3.47	3.47	3.47	
150MPa-5min-20°C	3.44	3.45	3.45	0.02
150MPa-10min-20°C	3.45	3.44	3.45	0.02
150MPa-15min-20°C	3.44	3.44	3.44	0.03
200MPa-5min-20°C	3.44	3.44	3.44	0.03
200MPa-10min-20°C	3.45	3.44	3.45	0.02
200MPa-15min-20°C	3.45	3.45	3.45	0.02
250MPa-5min-20°C	3.44	3.44	3.44	0.03
250MPa-10min-20°C	3.45	3.45	3.45	0.02
250MPa-15min-20°C	3.45	3.44	3.45	0.02
150MPa-5min-30°C	3.44	3.44	3.44	0.03
150MPa-10min-30°C	3.44	3.44	3.44	0.03
150MPa-15min-30°C	3.45	3.44	3.45	0.02

Table A.25 (continued)

200MPa-5min-30°C	3.44	3.45	3.45	0.02
200MPa-10min-30°C	3.45	3.44	3.44	0.03
200MPa-15min-30°C	3.45	3.44	3.45	0.02
250MPa-5min-30°C	3.45	3.44	3.45	0.02
250MPa-10min-30°C	3.44	3.44	3.44	0.03
250MPa-15min-30°C	3.45	3.45	3.45	0.02
150MPa-5min-40°C	3.44	3.43	3.44	0.03
150MPa-10min-40°C	3.43	3.43	3.43	0.04
150MPa-15min-40°C	3.43	3.42	3.43	0.04
200MPa-5min-40°C	3.42	3.44	3.43	0.04
200MPa-10min-40°C	3.41	3.43	3.42	0.05
200MPa-15min-40°C	3.41	3.42	3.42	0.05
250MPa-5min-40°C	3.43	3.43	3.43	0.04
250MPa-10min-40°C	3.42	3.42	3.42	0.05
250MPa-15min-40°C	3.42	3.42	3.42	0.05

Table A.26 ANOVA table for the effect of pressure on the pH change of red grape juice after HHP treatment.

One-way ANOVA: pH Difference vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Pressure	2	0.000007	0.000004	0.02	0.975
Error	24	0.003578	0.000149		
Total	26	0.003585			
S=	0.01221		R-Sq=	0.21%	

```
          Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N     Mean    StDev  -------+---------+---------+---------+--
150    9  0.03111  0.01054    (----------------*----------------)
200    9  0.03000  0.01323  (----------------*----------------)
250    9  0.03111  0.01269    (----------------*----------------)
                              -------+---------+---------+---------+--
                                   0.0250    0.0300    0.0350    0.0400

Pooled StDev = 0.01221
```

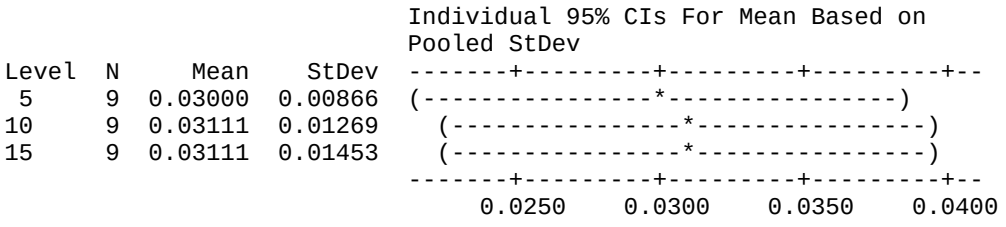
Table A.27 ANOVA table for the effect of temperature on the pH change of red grape juice after HHP treatment.

One-way ANOVA: pH Difference vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Temperature	2	0.002963	0.0014815	57.14	0.000
Error	24	0.000622	0.0000259		
Total	26	0.0035852			
S=	0.005092		R-Sq=	82.64%	

```
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  -----+---------+---------+---------+----
20     9  0.023333  0.005000  (---*----)
30     9  0.023333  0.005000  (---*----)
40     9  0.045556  0.005270                          (---*---)
                              -----+---------+---------+---------+----
                                 0.0240    0.0320    0.0400    0.0480

Pooled StDev = 0.005092
```

Table A.28 ANOVA table for the effect of time on the pH change of red grape juice after HHP treatment.

One-way ANOVA: pH Difference vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.000007	0.000004	0.02	0.975
Error	24	0.003578	0.000149		
Total	26	0.003585			
S=	0.01221		R-Sq=	0.21%	



Pooled StDev = 0.01221

Table A.29 Color Measurement of HHP Treated White Grape Juice- L-a-b Hunter.

	L	L (Mean)	a	a (Mean)	b	b (Mean)	ΔE
Control 1		100.24		-0.31		0.65	
150MPa-5min-20°C	*100.68*	100.42	-0.08	-0.05	*1.14*		
	100.16		*-0.02*		*1.04*	1.09	0.542
150MPa-10min-20°C	*100.13*	100.33	*-0.16*	-0.18	*0.96*		
	100.52		*-0.19*		*1.05*	1.01	0.389
150MPa-15min-20°C	*100.73*	100.64	*-0.05*	-0.09	*0.9*		
	100.55		*-0.12*		*0.8*	0.85	0.501
200MPa-5min-20°C	*100.44*	100.59	-0.25	-0.19	*0.86*		
	100.73		-0.13		*0.99*	0.93	0.457
200MPa-10min-20°C	*100.3*	100.29	*-0.16*	-0.18	*1.28*		
	100.27		*-0.19*		*1.17*	1.23	0.592
200MPa-15min-20°C	*100.72*	100.58	*-0.1*	-0.14	*1.01*		
	100.44		*-0.18*		*0.83*	0.92	0.466
250MPa-5min-20°C	*100.26*	100.49	*-0.16*	-0.14	*0.99*		
	100.72		*-0.12*		*0.89*	0.94	0.419
250MPa-10min-20°C	*100.15*	100.34	*-0.22*	-0.22	*0.88*		
	100.53		*-0.22*		*1.06*	0.97	0.347
250MPa-15min-20°C	*100.66*	100.55	*-0.09*	-0.16	*0.92*		
	100.43		*-0.22*		*0.84*	0.88	0.412
150MPa-5min-30°C	*100.32*	100.44	*-0.35*	-0.32	*1.01*		
	100.56		*-0.29*		*1.1*	1.06	0.452
150MPa-10min-30°C	*100.65*	100.60	*-0.23*	-0.25	*0.9*		
	100.55		*-0.26*		*0.71*	0.81	0.397
150MPa-15min-30°C	*100.63*	100.47	*-0.28*	-0.25	*0.63*		
	100.3		*-0.22*		*0.83*	0.73	0.246
200MPa-5min-30°C	*100.29*	100.43	*-0.2*	-0.23	*0.91*		
	100.56		*-0.25*		*0.82*	0.87	0.296
200MPa-10min-30°C	*100.22*	100.25	*-0.2*	-0.20	*0.96*		
	100.27		*-0.19*		*1.17*	1.07	0.431

Table A.29 (Continued)

200MPa-15min-30°C	*100.79*	100.63	*-0.24*	-0.24	*0.94*	0.84	0.433
	100.46		*-0.24*		*0.73*		
250MPa-5min-30°C	*99.99*	100.60	*-0.34*	-0.34	*0.66*	0.67	0.357
	101.2		*-0.34*		*0.67*		
250MPa-10min-30°C	*100.28*	100.35	*-0.38*	-0.32	*0.97*	1.03	0.391
	100.42		*-0.26*		*1.08*		
250MPa-15min-30°C	*100.01*	100.38	*-0.31*	-0.30	*0.43*	0.40	0.291
	100.75		*-0.29*		*0.36*		
150MPa-5min-40°C	*100.12*	100.38	*-0.22*	-0.22	*0.66*	0.80	0.221
	100.64		*-0.22*		*0.93*		
150MPa-10min-40°C	*100.62*	100.59	*-0.23*	-0.24	*0.86*	0.88	0.422
	100.56		*-0.25*		*0.89*		
150MPa-15min-40°C	*100.56*	100.63	*-0.19*	-0.18	*0.88*	0.92	0.489
	100.69		*-0.16*		*0.96*		
200MPa-5min-40°C	*100.35*	100.50	*-0.26*	-0.26	*0.72*	0.78	0.295
	100.65		*-0.26*		*0.84*		
200MPa-10min-40°C	*100.69*	100.61	*-0.21*	-0.24	*0.89*	0.88	0.435
	100.52		*-0.26*		*0.86*		
200MPa-15min-40°C	*100.64*	100.73	*-0.18*	-0.16	*0.88*	0.92	0.578
	100.82		*-0.13*		*0.95*		
250MPa-5min-40°C	*100.55*	100.62	*-0.24*	-0.18	*0.6*	0.80	0.424
	100.68		*-0.12*		*1*		
250MPa-10min-40°C	*100.62*	100.77	*-0.12*	-0.14	*1.02*	0.94	0.623
	100.91		*-0.16*		*0.86*		
250MPa-15min-40°C	*100.34*	100.57	*-0.31*	-0.22	*0.82*	0.91	0.426
	100.79		*-0.13*		*1*		

Table A.30 ANOVA table for the effect of temperature on the color change of white grape juice after HHP treatment.

One-way ANOVA: ΔE vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.0806	0.0403	3.55	0.045
Error	24	0.2725	0.0114		
Total	26				
S=	0.1066		R-Sq=	22.82%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
20     9  0.4572  0.0772                    (---------*----------)
30     9  0.3315  0.1118  (---------*----------)
40     9  0.4340  0.1250                (---------*---------)
                          ---+---------+---------+---------+------
                           0.280     0.350     0.420     0.490

Pooled StDev = 0.1066
```

Table A.31 ANOVA table for the effect of pressure on the color change of white grape juice after HHP treatment.

One-way ANOVA: ΔE vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.0194	0.0097	0.7	0.508
Error	24	0.3338	0.0139		
Total	26	0.3531			
S=	0.1179		R-Sq=	5.48%	

```
Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
150    9  0.4055  0.1099        (-------------*------------)
200    9  0.4414  0.1030              (-------------*------------)
250    9  0.3759  0.1379   (-------------*------------
                           -+---------+---------+---------+--------
                          0.300     0.360     0.420     0.480

Pooled StDev = 0.1179
```

Table A.32 ANOVA table for the effect of time on the color change of white grape juice after HHP treatment.

One-way ANOVA: ΔE vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.0338	0.0169	1.27	0.299
Error	24	0.3193	0.0133		
Total	26	0.3531			
S=	0.1153		R-Sq=	9.58%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
 5     9  0.3605  0.1277    (----------*----------)
10     9  0.4458  0.0952              (----------*----------)
15     9  0.4164  0.1206            (----------*----------)
                            +---------+---------+---------+---------
                           0.280     0.350     0.420     0.490

Pooled StDev = 0.1153
```

Table A.33 ANOVA table for the effect of temperature on the L value change of white grape juice after HHP treatment.

One-way ANOVA: L vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.235	0.1175	5.12	0.014
Error	24	0.5510	0.023		
Total	26	0.786			
S=	0.1515		R-Sq=	29.9%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean  StDev  ----+---------+---------+---------+-----
20     9  100.470  0.129          (--------*-------)
30     9  100.372  0.197  (-------*--------)
40     9  100.600  0.115                    (-------*--------)
                          ----+---------+---------+---------+-----
                          100.32    100.44    100.56    100.68

Pooled StDev = 0.152
```

Table A.34 ANOVA table for the effect of pressure on L change of white grape juice after HHP treatment.

One-way ANOVA: L vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.0354	0.0177	0.57	0.575
Error	24	0.7506	0.0313		
Total	26	0.786			
S=	0.1768		R-Sq=	4.51%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean  StDev  ---------+---------+---------+---------+
150    9  100.500  0.117         (-----------*-----------)
200    9  100.512  0.161          (-----------*-----------)
250    9  100.430  0.233  (-----------*-----------)
                          ---------+---------+---------+---------+
                               100.40    100.50    100.60    100.70

Pooled StDev = 0.177
```

Table A.35 ANOVA table for the effect of time on L change of white grape juice after HHP treatment.

One-way ANOVA: L vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.0525	0.0262	0.86	0.437
Error	24	0.7335	0.0306		
Total	26	0.786			
S=	0.1748		R-Sq=	6.67%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean  StDev  --------+---------+---------+---------+-
 5     9  100.441  0.150  (-----------*-----------)
10     9  100.459  0.184    (-----------*-----------)
15     9  100.542  0.188             (-----------*-----------)
                          --------+---------+---------+---------+-
                              100.40    100.50    100.60    100.70

Pooled StDev = 0.175
```

Table A.36 Color Measurement of HHP Treated Red Grape Juice- L-a-b Hunter.

	L	L (Mean)	a	a (Mean)	b	b (Mean)	ΔE
Control 1		80.71		19.16		12.86	
150MPa-5min-20°C	*79.05*	79.47	*23.25*	22.67	*14.71*		
	79.88		*22.09*		*13.84*	14.28	3.984
150MPa-5min-30°C	*80.25*	80.61	*16.99*	17.61	*12.94*		
	80.96		*18.22*		*12.82*	12.88	1.559
150MPa-5min-40°C	*76.52*	76.56	*23.8*	23.69	*15.54*		
	76.6		*23.57*		*15.4*	15.47	6.672
200MPa-5min-20°C	*77.87*	77.98	*22.86*	22.61	*13.56*		
	78.08		*22.36*		*13.53*	13.55	4.456
200MPa-5min-30°C	*80.66*	79.90	*19.66*	18.91	*11.25*		
	79.13		*18.16*		*11.02*	11.14	1.924
200MPa-5min-40°C	*77.73*	76.51	*23*	23.04	*14.85*		
	75.29		*23.07*		*15.27*	15.06	6.123
250MPa-5min-20°C	*76.36*	76.80	*23.67*	22.95	*14.41*		
	77.23		*22.23*		*13.63*	14.02	5.571
250MPa-5min-30°C	*79.34*	79.40	*16.89*	17.57	*10.96*		
	79.45		*18.25*		*11.06*	11.01	2.771
250MPa-5min-40°C	*77.54*	76.88	*23.44*	23.22	*15.28*		
	76.21		*23*		*15.1*	15.19	6.051
150MPa-10min-20°C	*77.64*	76.99	*23.27*	23.41	*14.38*		
	76.34		*23.55*		*14.42*	14.40	5.854
150MPa-10min-30°C	*78.96*	79.16	*20.55*	19.56	*12.36*		
	79.35		*18.56*		*11.58*	11.97	1.835
150MPa-10min-40°C	*77.11*	77.09	*23.98*	23.72	*15.66*		
	77.06		*23.46*		*15.52*	15.59	6.433
200MPa-10min-20°C	*77.38*	77.37	*23.15*	23.17	*14.15*		
	77.35		*23.19*		*13.03*	13.59	5.273

Table A.36 (continued)

200MPa-10min-30°C	*79.03*	79.25	*17.56*	18.02	*11.33*	10.93	2.678
	79.46		*18.48*		*10.53*		
200MPa-10min-40°C	*77.28*	77.26	*23.75*	23.60	*15.56*	15.42	6.175
	77.24		*23.44*		*15.28*		
250MPa-10min-20°C	*77.58*	77.38	*23.29*	23.32	*14.16*	14.17	5.490
	77.17		*23.35*		*14.18*		
250MPa-10min-30°C	*79.44*	79.83	*17.69*	18.42	*11.27*	11.66	1.670
	80.21		*19.14*		*12.04*		
250MPa-10min-40°C	*77.31*	77.36	*23.56*	23.53	*15.02*	15.07	5.933
	77.41		*23.5*		*15.12*		
150MPa-15min-20°C	*77.24*	77.18	*23.08*	23.47	*13.73*	13.90	5.670
	77.11		*23.86*		*14.06*		
150MPa-15min-30°C	*79.33*	79.50	*18.55*	18.79	*13.02*	12.68	1.278
	79.67		*19.03*		*12.34*		
150MPa-15min-40°C	*77.89*	77.54	*23.36*	23.23	*14.81*	14.94	5.561
	77.19		*23.1*		*15.06*		
200MPa-15min-20°C	*77.95*	77.24	*22.19*	23.05	*13.43*	14.18	5.372
	76.53		*23.9*		*14.92*		
200MPa-15min-30°C	*80.99*	80.76	*20.11*	19.61	*11.36*	12.13	0.859
	80.52		*19.11*		*12.9*		
200MPa-15min-40°C	*77.6*	77.42	*23.12*	23.17	*14.89*	15.00	5.609
	77.24		*23.22*		*15.1*		
250MPa-15min-20°C	*77.7*	77.06	*23.18*	23.73	*14.04*	14.69	6.126
	76.41		*24.27*		*15.33*		
250MPa-15min-30°C	*80.12*	80.05	*16.95*	17.87	*12.03*	12.01	1.680
	79.98		*18.79*		*11.99*		
250MPa-15min-40°C	*77.68*	77.49	*23.14*	23.28	*14.68*	14.82	5.585
	77.29		*23.42*		*14.95*		

Table A.37 ANOVA table for the effect of pressure on the color change of red grape juice after HHP treatment.

One-way ANOVA: ΔE vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.27	0.14	0.03	0.966
Error	24	96.15	4.01		
Total	26	96.43			
S=	2.002		R-Sq=	0.28%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  ---+---------+---------+---------+------
150    9  4.337  2.179  (----------------*----------------)
200    9  4.320  1.899  (----------------*----------------)
250    9  4.542  1.915     (----------------*----------------)
                         ---+---------+---------+---------+------
                          3.20      4.00      4.80      5.60

Pooled StDev = 2.002
```

Table A.38 ANOVA table for the effect of temperature on the color change of red grape juice after HHP treatment.

One-way ANOVA: ΔE vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	88.442	44.221	132.92	0.000
Error	24	7.984	0.333		
Total	26	96.427			
S=	0.5768		R-Sq=	91.72%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
20     9  5.3107  0.6787                        (-*--)
30     9  1.8728  0.6217    (-*--)
40     9  6.0158  0.3885                             (--*--)
                           +---------+---------+---------+---------
                          1.5       3.0       4.5       6.0

Pooled StDev = 0.5768
```

Table A.39 ANOVA table for the effect of time on the color change of red grape juice after HHP treatment.

One-way ANOVA: ΔE vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.78	0.39	0.10	0.907
Error	24	95.65	3.99		
Total	26	96.43			
S=	1.996		R-Sq=	0.81%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N   Mean  StDev  -----+---------+---------+---------+----
 5     9  4.385  1.858     (-----------------*----------------)
10     9  4.614  1.915        (----------------*----------------)
15     9  4.200  2.199  (----------------*-----------------)
                          -----+---------+---------+---------+----
                             3.20      4.00      4.80      5.60

Pooled StDev = 1.996
```

Table A.40 ANOVA table for the effect of pressure on L value change of red grape juice after HHP treatment.

One-way ANOVA: L vs Pressure					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.21	0.10	0.05	0.949
Error	24	47.57	1.98		
Total	26	47.78			
S=	1.408		R-Sq=	0.44%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
150    9  78.233  1.453      (---------------*---------------)
200    9  78.188  1.438     (---------------*---------------)
250    9  78.028  1.329  (---------------*----------------)
                          ------+---------+---------+---------+---
                             77.40     78.00     78.60     79.20

Pooled StDev = 1.408
```

Table A.41 ANOVA table for the effect of temperature on L value change of red grape juice after HHP treatment.

One-way ANOVA: L vs Temperature					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	38.696	19.348	51.10	0.00
Error	24	9.086	0.379		
Total	26	47.78			
S=	0.6153		R-Sq=	80.49%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
20     9  77.497  0.811      (---*---)
30     9  79.829  0.571                           (---*----)
40     9  77.123  0.391  (---*---)
                          ---+---------+---------+---------+------
                          77.0      78.0      79.0      80.0

Pooled StDev = 0.615
```

Table A.42 ANOVA table for the effect of time on L value change of red grape juice after HHP treatment.

One-way ANOVA: L vs Time					
Source	***DF***	***SS***	***MS***	***F***	***P***
Time	2	0.46	0.23	0.12	0.891
Error	24	47.32	1.97		
Total	26	47.78			
S=	1.404		R-Sq=	0.96%	

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 5     9  78.234  1.622       (---------------*---------------)
10     9  77.966  1.109  (---------------*---------------)
15     9  78.249  1.434       (---------------*---------------)
                          -------+---------+---------+---------+--
                              77.40     78.00     78.60     79.20

Pooled StDev = 1.404
```

APPENDIX B

PROCESS DIAGRAMS

Figure B.1 Block diagram of heat treated grape juice processing.