EFFECTS OF HIGH HYDROSTATIC PRESSURE (HHP) ON QUALITY PARAMETERS AND SHELF-LIFE OF LAGER BEER

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ABSTRACT

EFFECTS OF HIGH HYDROSTATIC PRESSURE (HHP) ON QUALITY PARAMETERS AND SHELF-LIFE OF LAGER BEER

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Filtered bright and unfiltered hazy lager beer samples were either treated with high hydrostatic pressure (200, 250, 300, 350 MPa for 3, 5 and 10 min at 10°C and 20°C) or conventional heat pasteurization (60°C for 15 min).

Treatments did not affect ethanol, extract, fermentation degree, density and pH in comparison with untreated beers. Both treatments produced microbiologically stable products. Bitterness, color, protein sensitivity and chill haze parameters were affected by both the HHP and heat treatment.

A storage period of 56 days showed that HHP and heat pasteurization had similar results in terms of pH and color. However, HHP treated samples had lower bitterness and protein sensitivity and higher chill haze values than the heat pasteurized samples which indicates HHP treatment had a positive effect on bitterness and protein sensitivity at the end of the storage period. The microbiological stability of HHP treated beers was comparable with heat-treated beers, and the development of both lactic and acetic acid bacteria was inhibited for 56 days of storage.

Unfiltered beer samples had 7. 48, 7.15 and 2.64 $\log_{10}$cfu/ml of total yeasts, total aerobic and lactic acid bacteria counts, respectively. No colony formation of lactic acid bacteria was observed when the samples were pressurized at pressures equal to or higher than 300 MPa at 10°C and 20°C for 5 and 10 min. Total aerobic and total yeasts counts demonstrated more than 6 and 7 log-cycle reduction when pressurized at 350 MPa at 10°C and 20°C for 10 min, respectively. Heat treatment gave similar results in terms of log reductions as HHP.

Key words: Lager beer, High hydrostatic pressure, Heat pasteurization

ÖZ

YÜKSEK HİDROSTATİK BASINCIN (YHB) LAGER BİRANIN KALİTE PARAMETRELERİ VE RAF ÖMRÜ ÜZERİNDEKİ ETKİSİ

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Filitre edilmiş berrak ve filitre edilmemiş bulanık lager bira örnekleri hem yüksek hidrostatik basınca (200, 250, 300, 350 MPa 10°C ve 20°C'de 3, 5 ve 10 dakika) hem de geleneksel ısıl pastörizasyona (60°C'de 15 dakika) tabi tutulmuşlardır.

Hiç işlem görmemiş biralarla karşılaştırıldıklarında, bu iki işlemde biranın etanol, ekstrakt, fermentasyon derecesi, yoğunluk ve pH'sını etkilememişlerdir. Her iki işlemde mikrobiyolojik olarak kararlı ürünler ortaya çıkarmıştır. Acılık,

renk, protein hassasiyeti ve soğuk bulanıklık parametreleri hem YHB hem de ısıl pastörizasyon işleminden etkilenmişlerdir.

56 günlük bir saklama süreci YHB'nin ve ısıl pastörizasyonun pH ve renk üzerinde benzer sonuçlar verdiğini göstermiştir. Ancak, YHB uygulanmış örneklerin ısıyla pastörize edilmiş örneklerden daha düşük acılık, protein hassasiyeti ve daha yüksek soğuk bulanıklık değerlerine sahip oldukları gözlemlenmiştir. Bu da saklama süresinin sonunda, YHB işleminin acılık ve protein hassasiyetine olumlu bir etkisi olduğunu göstermektedir. YHB uygulanmış biraların mikrobiyolojik kararlılığı ısıl işlem görmüş biralarınkiyle karşılaştırılaştırılmış ve hem laktik hem de asetik asit bakterilerinin üremesinin 56 günlük bir süreçte engellendiği gözlemlenmiştir.

Filitre edilmemiş bira örnekleri sırasıyla 7.48, 7.15 ve 2.64 logaritmik değer toplam maya, toplam aerobik ve laktik asit bakteri sayısına sahiplerdir. Örneklere 10°C ve 20°C'de, 5 veya 10 dakika, 300 MPa ve daha yüksek basınç uygulandığında laktik asit bakterilerinin koloni oluşturmalarının engellendiği gözlemlenmiştir. 10°C ve 20°C'de, 10 dakika 350 MPa basınç, toplam aerobik ve toplam maya sayılarının sırasıyla 6 ve 7 logaritmik değerden daha fazla azalmasına neden olmuştur. Isıl işlem de logaritmik azalma bakımından YHB'ye benzer sonuçlar vermiştir.

Anahtar kelimeler: Lager bira, Yüksek hidrostatik basınç, Isıl pastörizasyon

To my mother and father,

and also to my brother Anıl and Esra...

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INTRODUCTION

1.1 Recent Developments in Food Processing and High Hydrostatic Pressure (HHP)

Consumer trends and therefore the food markets are changing and will change more in the future. Foods with high quality and more fresh-like attributes are in demand, consequently less extreme treatments and/or fewer additives are required [1]. This growing demand for safe “fresh products” and if possible with longer shelf-life, is influencing the development of industries that process food [2].

Many of the food preservation methods that are used at present have several drawbacks. High heat treatment used for canned foods destroys the heat labile vitamins and adversely effects the natural color, texture and flavor of foods. Low heat-treated pasteurized foods need to be stored at refrigerated temperature and have a limited shelf-life. Drying and freezing with time, also reduce nutritional and acceptance qualities. Many of the chemical preservatives used are non-food origin and have limited efficiency[3].

Consumers have a concern about the possible adverse effects that non-food preservatives may have on their health and health of future generations. This

concern has shifted the desire of the consumers for nutritious and “natural” foods that are not processed by “harsh techniques” and preserved by “harsh chemicals” [3].

To satisfy these needs, several non-traditional food processing and preservation techniques are being studied. The efficiency of some non-thermal processing methods are magnetic or electric fields, ionization, light pulses, high hydrostatic pressures, ultra sounds and the application of chemical or biological agents such as carbon dioxide, policationic polymers, bacteriocins and lytic enzymes. Some of these systems already have regulatory approval and are commonly used in the industry, while others continue to be developed and evaluated for potential commercial application [2, 3].

Among these non-thermal techniques high hydrostatic pressure (HHP) is gaining in popularity with food processors not only because of its food preservation capability but also because of its potential to achieve interesting functional effects. HHP technology has generated international research and development activity and within a short time produced several commercial products in the market [3, 4].

1.1.1 Brief History of the General Use of HHP on Food Products

HHP has had application for years in other industries which process or use ceramics, carbon graphite, diamond, steel/alloy, and plastics [4]. High pressure treatment to kill bacteria was first described in 1895 by Roger [5]. However, the most important work involving microbial inactivation in food science and technology was published in June of 1899 by Bert Hite [6]. Hite reported the effects of HHP on “food” microorganisms by subjecting the milk to pressures of 650 Mega Pascal (MPa) and obtaining a reduction in the viable numbers of microbes. Hite and his coworkers [7] of the University of West Virginia examined

a wide range of foods and beverages for the potential use of high pressure processing.

Although the concept of using HHP as a method of food processing is not new, the technical impossibility of working at industrial level reduced interest in such a treatment. However, for the last 15 years, the use of HHP has been explored extensively in food industry and related research institutions due to the increased demand by consumers for improved nutritional and sensory characteristics of food without loss of "fresh" taste [4]. In April 23, 1990, the first high pressure products, three kinds of jam (strawberries, kiwifruit and apple) made using high pressure treatment without application of heat, were introduced to the Japanese retail market [8]. In 1991, yogurts, fruit jellies, salad dressings and fruit sauces were also introduced and two Japanese fruit juice processors installed semi-continuous high pressure equipment for citrus juice bulk processing [1, 8].

1.1.2 General Principle and Mechanism of HHP

Two principles underlie the effect of HHP. Firstly, the principle of le Chatelier, according to which any phenomenon (phase transition, chemical reaction, change in molecular configuration) accompanied by a decrease in volume can be enhanced by pressure. Secondly, pressure is instantaneously and uniformly transmitted independent of size and geometry of the food i.e., the food will be compressed by a uniform pressure from every direction and then return to its original shape when the pressure has been released. This is known as isostatic pressure [1-4, 9-11]. The principle of isostatic processing is presented in Figure 1.1.

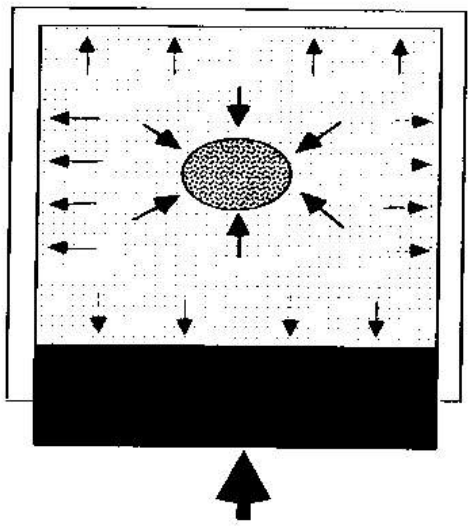

Figure 1.1. The principle of isostatic processing. (Adapted from Ref. 1.)

HHP affects non-covalent bonds (hydrogen, ionic and hydrophobic bonds) substantially as some non-covalent bonds are very sensitive to pressure, which means that low molecular weight food components (responsible for nutritional and sensory characteristics) are not affected, whereas high molecular weight components (whose tertiary structure is important for functionality-determination) are sensitive [3, 4, 8, 12].

1.1.3 High Pressure Equipment and Operation

A typical high pressure system consists of a high pressure vessel and its closure, a pressure generation system, a temperature control device and a material handling system [1, 3, 13]. Once loaded and closed, the vessel is filled with a pressure transmitting medium. Air is removed from the vessel by means of a low pressure fast fill and drain pump, in combination with an automatic de-aeration valve, and high hydrostatic pressure is then generated. High pressures can be generated by direct or indirect compression or by heating the pressure medium [13].

Heating of the pressure medium utilizes expansion of the pressure medium with increasing temperature to generate high pressure. Heating of the pressure medium is therefore used when high pressure is applied in combination with high temperature and requires very accurate temperature control within the entire internal volume of the pressure vessel [13].

The basis for applying high pressure to food is to compress the pressure-transferring medium (most often water) surrounding the food. A decrease in volume of water with increasing pressure is very minimal compared to gases [1, 4]. The volume decrease for water is approximately 4 % at 100 MPa, 7 % at 200 MPa, 11.5 % at 400 MPa, 15 % at 600 MPa at 22°C [12, 14].

A sterile container filled with food is vacuum sealed and placed in the pressure chamber for pressurizing. Ethylene-vinyl alcohol copolymer (EVOH) and polyvinyl alcohol (PVOH) films are recommended for packaging food for high pressure treatment. Also the existing multi-layer plastic and some aluminum packages may be used for high pressure processing. No deformation of the package occurs because the pressure is uniform. The shape of the package needs to be designed to fill the vessel volume as far as possible to increase the economical feasibility of the process [1].

Another interesting rule concerns the small energy needed to compress a solid or liquid to 500 MPa as compared to heating 100°C, because compresibility is small. The processing cost as a function of time and pressure is presented in Figure 1.2. Processing at 800 MPa with a holding time of 10 min is twice as expensive as processing at 400 MPa with a holding time of 10 min [15]. The combination of pressure, time and temperature at which the product is processed must therefore be evaluated carefully. A low maximum operating pressure can cause drastic reductions in the fabrication costs. High pressure processing may be combined with moderately high temperatures, so the operating pressures required are not extremely high [1].

Figure 1.2. Process cost as a function of exposure time at different pressures. (Adapted from Ref. 15)

1.1.4 Uses in Food Science

1.1.4.1 Effect of HHP on Microorganisms

Food preservation is based primarily on the inactivation, growth delay or prevention of spoilage and pathogenic microorganisms [1]. A major function of high pressure processing of food is destruction of microorganisms [3]. The effectiveness of HHP on microbial inactivation has to be studied in great detail to ensure the safety of food treated in this manner [4].

It can be expected that the mode of action of pressure on whole organisms is not necessary the same, but dependent on the pressure level. Elevated hydrostatic pressures between 30 and 50 MPa can influence gene expression and protein

synthesis. At pressures of about 100 MPa the nuclear membrane of yeasts was affected, and at more than 400-600 MPa further alteration occurred in the mitochondria and the cytoplasm. In particular, metal ions are released at pressures over 300 MPa. Pressure-inactivation is also accompained by an increase of extracellular ATP showing leakage of the membrane [9].

The result is an increase in the permeability of cell membranes and possible inhibition of enzymes vital for survival and reproduction of the bacterial cells [3, 4]. Exponentially growing cells are more sensitive to pressure than cells in the stationary phase. Stress might be induced during the stationary phase (e. g. through starvation or acidification). Gram-negative bacteria are inactivated at a lower pressure than Gram-positive bacteria [3, 4, 9, 16-19]. The lower resistance of Gram-negative bacteria compared with Gram-positives has attributed to their lack of teichoic acid, which is responsible for the rigidity of the cell wall of Gram-positive bacteria [3, 19].

Bacterial cells are relatively less sensitive to hydrostatic pressure at 20 to 35°C but become more sensitive to pressurization above 35°C, due to phase transition of membrane lipids [3, 16-18]. Microbial cells surviving pressurization also become sublethally injured and developed sensitivity to physical and chemical environments to which normal cells are resistant [3].

In general, bacterial spores are more resistant to HHP than vegetative cells [3, 9, 19, 20]. This has been attributed to the protection afforded by dipicolinic acid (DPA) of the spore proteins against solvation and excessive ionization that are responsible for cell death [3]. Bacterial spores are killed directly by pressures higher than 1000 MPa. However, spores are sensitive to pressures between 50 and 300 MPa. Hydrostatic pressure can cause spore germination. Release of DPA, which is not present in vegetative bacteria, is one of the first events in germination. *Bacillus subtilis* in physiological saline loses 80 % of its DPA after 60 MPa at 30°C. At pressures over 1000 MPa, spores are killed more rapidly at low pH values [9].

1.1.4.2 Effect of HHP on Proteins and Enzymes

High pressure can denature protein molecules. The four levels of protein structure are characterized as: primary (amino acids in a polypeptide chain joined by covalent bonding), secondary (coiling of peptide chains joined with hydrogen bonding), tertiary (arrangement of chains into globular shape by non-covalent bonding) and quaternary (various compact structures or sub-units joined by non-covalent bonding) [4]. Pressure denaturation of proteins is a complex phenomenon depending on the protein structure, pressure range, temperature, pH and solvent composition [1]. The secondary, tertiary and quaternary structures can be significantly affected by HHP (because high pressure affects non-covalent bonds); therefore HHP may result in novel functional properties because tertiary structure is important in determining protein functionality [4]. The main targets of pressure are the electrostatic and hydrophobic bonds in protein molecules. Protein denaturation becomes irreversible beyond a given pressure threshold which depends on the protein [1].

HHP affects enzymes in a variety of ways depending on other parameters of processing and also the type of the enzyme [4]. Exposure to high pressure may activate or inactivate enzymes. Pressure inactivation of enzymes is influenced by the pH, substrate concentration, subunit structure of enzyme and temperature during pressurization. Pressure effects on enzyme activity are expected to occur at the substrate-enzyme interaction. If the substrate is a macromolecule, then the effects may occur on the conformation of the macromolecule, which can make the enzyme action easier or more difficult. Pressure enzyme inactivation can also be attributed to alteration of intermolecular structures or conformational changes at the active side. Inactivation of some enzymes pressurized to 100-300 MPa is reversible. Reactivation after decompression depends on the degree of distortion of the molecule. The changes of reactivation decrease with an increase in pressure beyond 300 MPa [1].

1.1.4.3 Effect of HHP on Sensory and Nutritional Characteristics

The principal advantage of high pressure technology is its relatively small effect on food composition and hence, on sensory and nutritional attributes. For instance; grapefruit juice manufactured by high pressure technology does not possess the bitter taste of limonene present in conventional thermal processed grape fruit juice [1]. The jams obtained by high pressure processing retain the taste and color of fresh fruit, unlike conventional jams produced by heat [1, 21].

Protein, nucleic acid and starches, whose tertiary structures are composed of non-covalent bonds, change their structures at high pressure and lead to denaturation, coagulation or gelatinization. Pressure effects are, thus, similar to heat effects on biological materials and foods. In other words, high pressure is as useful as high temperature (Table 1.1).

Table 1.1 Possible uses of high pressure in processing and preservation of foods as compared to high temperature processing. (Adapted from Ref. 8)

Phenomenon	Temperature	Pressure
Sterilization of microorganisms	√	√
Killing of insects and parasites	√	√
Denaturation of protein	√	√
Coagulation of protein	√	√
Gelatinization of starch	√	√
Chemical changes*	√	X
Enzyme inactivation	√	√

√ : possible, X : not possible, *e. g. Maillard reactions, off-flavor and vitamin destruction

The unique advantage of the high pressure treatment is that the covalent bonds are kept intact as the liquid water was compressed. A wide variety of effects and changes in food flavor, texture, physical appearance and structure could result after the application of pressure and these changes depend on the type of food and its composition and structure. However, effects such as Maillard reaction and formation of cooked flavors do not occur during the pressure treatment. Thus, it is possible to retain natural flavor and taste by application of high pressure treatment to foods [8].

Commercial high-pressure food processing, however poses specific requirements in relation to sanitation and cleaning, material handling, package design and operational cost. The most difficult challange lies in the uncertain field of marketing, i.e., identifying those niche applications for which the higher cost for high-pressure processing is justified by superior or unique product properties [3].

1.2. Beer and Brief History of Brewing

Beer refers to a beverage obtained by alcoholic fermentation of malted cereal, usually barley malt, with or without other starchy materials and to which usually hops have been added [22, 23].

The manufacture of alcoholic liquor is at least 6000 years old. Some of the oldest records of brewing are from Egypt, China, Greece and Rome [23]. The oldest mention of beer is in Mesopotamian cuneiform writing, from the year 2800 BC, which describes the distribution of the daily ration of beer and bread to the work force [24].

Egyptian legend has it that Osiris, god of earth and vegetation, was the inventor of beer. Whilst tracing such origins is of course a matter of faith, there is evidence that beer in those far off days not only comprised a valuable constituent of

the diet, but also served a diversity of medicinal functions, including mouthwash, enema and applicant to wounds [25].

In Europe the change to a brewing "industry" occured in the breweries of Christian religious foundations where beer was produced not only for their own consumption but was also supplied to others for a payment. The use of hops as the sole flavoring ingredient occurred in the 14th century. In the 15th century the commercial position of the brewer was established, but it was restricted by a large number of regulations [24].

The invention and installation of steam engine and refrigeration machine in 18th and 19th century respectively and the development of transport by the building of the railway network led to the building and expansion of large breweries in all developed countries. As a result of these developments, in the middle of the last century a large number of now industrially operated breweries were founded in Europe and America, or existing older breweries were industrially modernised [24].

1.2.1 Modern Brewing

Beer was produced without any knowledge of microorganisms or enzymes thousands years ago. Because of this extremely long history brewing is often mentioned as a typical example of traditional or old biotechnology. It is true, of course, that brewing has old traditions, but it is also true that brewing has been and still is in the forefront of biotechnological development [26].

Today beer is produced and drunk in almost every country in the world, although in widely differing amounts. Nowadays, the largest groups of breweries are to be found spread out over all four corners of the world; but some of them have 100 or more breweries. The sizes of brewing companies are greatly dependent on the brewing history of the country [24].

1.2.2 The Brewing Process

The conventional brewing process is outlined in Figure D.1. The whole beer production procedure consists of four stages: (1) malting (based on germination of barley); (2) wort production (mashing, i.e., extraction and hydrolysis of the components of malt and possibly other cereals, followed by separation of non-soluble components and boiling with hops or hop extracts); (3) fermentation (in most cases divided into primary or main fermentation and secondary fermentation); (4) down-stream processing (filtration, stabilization, bottling, etc.) [26].

Beer is complex mixture; over 400 differents compounds have been characterized in beer which, in addition, contains macromolecules such as proteins, nucleic acids, carbohydrates and lipids. Some of the constituents of beer are derived from the raw materials. Others are the result of chemical and biochemical transformation of the raw materials during malting, mashing, boiling, fermentation and conditioning [22]. Together all these constituents make up the character of beer but, in general, different beers and lagers contain different propotions of the same compounds rather than novel constituents resulting with different quality standards. Therefore in this study the quality standards of Anadolu Efes Biracılık ve Malt Sanayi A.Ş was used (Table E.1).

1.2.3 Microbiology and Microbial Contaminants of Beer

Brewers are said to employ top yeasts (top fermentation) or bottom yeasts (bottom fermentation). The distinction arises from the practice of removing the yeast from the top of the fermentor by a skimming process or from the bottom of the vessel. It is current practice to classify all brewer's yeast as *S. cerevisiae*, although in brewing practice ale yeasts are referred to as *S. cerevisiae* whereas lager strains are called *S. carlsbergensis* (or *S. uvarum*). The requirements of the brewer are threefold: (1) to maintain and store yeast; (2) to monitor yeast quantity

and vitality; (3) to monitor the equipment, process and product for the presence of unwanted and undesirable microbes [27, 28].

Unlike most other food products and beverages, beer is generally resistant to microbiological spoilage. This is principally due to the bacteriostatic properties of hop compounds present in beer. Hop components contribute to the desirable bitter flavor and characteristic aroma while acting as a natural preservative against most Gram-positive bacteria. There are, however, some microorganisms which can grow in beer [29]. The bacteria generally regarded as most hazardous for modern breweries are the Gram-positive bacteria belonging to the genera *Lactobacillus* and *Pediococus*. Among the lactobacilli the most important spoilage organisms according to the brewing literature are *Lactobacillus brevis*, *L. lindneri*, *L. curvatus*, *L. casei*, *L.buchneri*, *L.coryneformis* and *L. plantarum*. In addition, the following potential beer spoilage species have been reported: *L. brevisimilis*, *L. malefermentans* and *L. parabuchneri*. *L. delbrueckii*, *L. fermentum* and *L. fructivorans* have been reported to occur in beer but their spoilage potential is low [28, 30]. Among the pediococci only *Pediococcus damnosus*, *P. inopinatus* and to some extent *P. dextrinicus* are of importance for spoilage of beer. However, growth of *P. inopinatus* and *P. dextrinicus* is only possible above pH 4.2 and at low ethanol and hop bitter concentrations. It also appears that only some strains of the above species are capable of growth in beer. *P. acidilactici* and *P. pentosaceus* are found on malt and can grow during the early stages of wort production as long as the temperature is below 50°C and hops have not been added, but they have never been reported to cause any defect in beer produced. The Gram-negative beer spoilage organisms include a number of species belonging to various genera. Among these the strictly anaerobic bacteria *Pectinatus cerevisiiphilus, P. frisingensis* and *Selenomonas lacticifex* have been reported as obligate beer spoilage organisms. Within the genus *Zymophilus* which is phylogenetically close to the genus *Pectinatus*, two species have been isolated from brewery samples*: Z. raffinosivorans* and *Z. paucivorans* although only the first mentioned has been reported as a beer spoilage organism. Another strictly anaerobic Gram-negative beer spoilage organism is the coccus *Megasphaera cerevisiae* [30].

Of the importance for primed (added sugars) beer is the anaerobic but oxygen tolerant Gram-negative bacterium *Zymomonas mobilis* which is resistant to hop bitters and able to grow at pH above 3.4 and ethanol concentrations lower than 10 % (w/v). *Zymomonas* spp. have not been reported in lager breweries probably due to their stringent carbohydrate requirements [30].

The Gram-negative, aerobic acetic acid bacteria, especially *Gluconobacter oxydans*, *Acetobacter aceti* and *A. pasteurianus* have during the history of brewing been paid a great deal of attention. They are able to convert ethanol into acetic acid and thereby change the flavor of the beer significantly, resulting in vinegary off-flavors.

The diversity of wild yeasts in terms of beer spoilage means that no general description can be given, however, wild yeasts are generally divided into *Saccharomyces* and non-*Saccharomyces* wild yeasts [28, 30-32]. By definition, a wild yeast is any yeast other than the brewing strain(s) which is found in the brewing process [28]. Often the most severe infections will be caused by *Saccharomyces* spp. which, once isolated, can often be distinguished from lager yeasts by cell morphology and spore formation [30, 31].

Among the *Saccharomyces* wild yeasts, most isolates belong to *Saccharomyces cerevisiae* with a predominance of strains previously described as *Sacch. diastaticus*, *Sacch. pastorianus*, *Sacch. ellipsoideus* and *Sacch. willanus* [30].

The most important non-*Saccharomyces* wild yeasts are *Pichia membranefaciens* and *Hansenula anomala* as well as a number of species belonging to the genera *Torulopsis*, *Schizosaccharomyces*, *Brettanomyces*, *Kloeckera* and *Candida*. Among the *Candida* spp., *C. mycoderma* and *C. krusei* have been reported as capable of beer spoilage [30].

In a brewery specific spoilage organisms may be defined as any organism which is not deliberately introduced and which is able to survive and proliferate in the environment i.e., in wort, fermenting wort, beer after filtration or in packaged beer [30].

1.2.4 Finishing Processes

1.2.4.1 Filtration

Filtration is a separation process in which the yeast cells and colloidal particles (responsible for haze) still present in the beer are removed from the beer [24, 33]. At the same time substances are removed which would, in the course of the next few weeks or months, themselves precipitate and make the beer turbid [24]. In order for filtration to have its maximum effect it is necessary to chill beer as much as is practicable. The lower the temperature, the more cold trub and chill haze will form; fine filtration will remove this material providing that the beer temperature does not rise in the filter itself [22].

The purpose of filtration is to make beer so stable that no visible changes occur for a long time and so the beer looks the same as when it was made [24].

1.2.4.2 Chill Stability

Beer that remains brilliantly clear when chilled to 4°C or below is a necessary quality [27]. Beer haze may arise because of the presence of microorganisms and these are normally removed by filtration. With respect to non-biological hazes, the majority of hazes formed in finished beer are due to protein-tanin complexes [34]. Beer, if chilled, often produces what is called “chill haze”.

Such haze will normally disappear when the beer is warmed; cyclic warming and cooling soon causes a permanent haze which will not disappear on warming [27, 34]. Beer held in a package for a long time will produce a permanent haze which is stable at ambient temperature [34]. Chill hazes are obviously a more serious problem with lager beers which are served at a lower temperature than ales [22]. Brewers strive to remove the haze-forming material in the brewery. Prolonged cold storage at –1 or –2°C precipitates the haze-forming complexes which can be removed by filtration of the very cold beer. Additional chilled-proofing treatments however are commonly used. These attack the chill haze components. Those attacking the proteinaceous portion include proteolytic enzymes (e.g. papain, though not now much used), silica hydrogels and special preparations of tannic acid; the tanin components are removed by PVPP (polyvinylpolypyrrolidone) or gelatin. Though tannic acid is used by one very large brewery the insoluble adsorbents (silica gels and PVPP) are most popular. These materials act almost instantaneously and so can be injected into a beer stream on the way to the filter or incorporated into filter sheet material [27].

1.2.4.3 Pasteurization

Clarification is usually followed by pasteurization. Pasteurization is necessary to ensure the microbiological stability of the final product. A stability of 3-6 months at room temperature can be ensured when the retention of beer spoilage organisms (bacteria, yeast) is obtained [35]. The most reliable method for achieving this is pasteurization of the filled bottles or cans in a tunnel pasteurizer. However, tunnel pasteurization of bottles is not customary nowadays and it has largely been replaced by flash pasteurization or sterile filtration. In flash pasteurization the beer is heated by a plate heat exchanger to at least 68 to 72°C and held at this temperature for about 50 seconds. Then it is cooled down again. In the tunnel pasteurizer the filled cans (or bottles) are heated in stages, subjected to pasteurization temperature for a fixed time, and then cooled again [24].

1.3 HHP Applications on Beer

Studies on effect of HHP on alcohol-free beverages such as fruit juices are abundant in literature, however few data were reported about the use of HHP on beer.

Fischer et al. [36] investigated the effects of HHP on the brewing process and the beer. To serve for this purpose, mash, wort and beer samples were treated with HHP. HHP treatments were applied at 3000, 5000 and 7000 bar (300, 500 and 700 MPa, respectively) for 5 min. Additionally a sample was HHP treated at 7000 bar for 30 min. HHP treatment changed the viscosity and the protein fractions of the mash; viscosity seemed to be decreased as the pressure increased. Insoluble proteins were recognized as soluble after the pressure treatments. No changes were determined for pH value. Bitterness, α-acids and iso α-acids were measured for wort. Among the HHP treated samples the best isomerization obtained at 7000 bar for 30 min which could be compared with 30 min cooking. For the treatment of beer, the HHP aplications and heat pasteurization (60°C, 20 min) did not show significant differences with color and foam durability. Turbidity was affected; the turbidity increased with increasing pressure, however up to 3000 bar no increase in turbidity was determined.

Castellari et al. [37] also treated two pale ales and a mild ale with HHP (600 MPa, 5 min) or heat pasteurized (60°C, 10 min). No filtration or fining was done, so the beers were very cloudy and particularly high in suspended matter. The treatments (HHP and heat pasteurization) did not affect pH, ethanol, extract, bitterness or phenolics in comparison with untreated beers; HHP beers retained permanent haze, similar to untreated samples. Both HHP and heat pasteurization reduced total aerobic, yeast and molds count about 4 log cycles. No lactic acid bacteria was detected in either of the stabilized beer samples. Thus, the microbiological stability of HHP beers was comparable with heat-treated beers and

the development of yeast and lactic acid bacteria was inhibited for 49 days of storage.

Inhibiting a specific beer-spoilage microorganism by use of HHP was also studied. Ganzle et al. [38] investigated the effect of HHP for inactivation of *Lactobacillus plantarum* in a model beer. Ethanol (5 and 10 %) and hop extract (50 and 100 ppm) concentration effects were investigated together with pressure. Ethanol enhanced pressure effects on *L. plantarum* whereas hop extract was less effective. In contrast, hop extracts killed pressurized cells during subsequent storage in beer but ethanol did not.

1.4 Objectives of the Study

As few data were reported about the effect of HHP on beer the main objective of this study was to investigate the effects of HHP on quality parameters and shelf life of lager beer in comparison with heat pasteurization. The parameters studied were bitterness, ethanol content, pH, density, apparent, real and original extract, fermentation degree, chill haze or haze after chilling (HAC), protein sensitivity and color. The effect of HHP on some important beer spoilage microorganisms was also investigated.

To serve for this purpose, this study was divided into three main parts:

1. *Filtered, unpastuerized beers*; the effects of pressure (200, 250, 300, 350 MPa) and pressurization time (3 and 5 min at 20°C) on properties of beer and on beer spoilage microorganisms were investigated. Some of the beer samples were heat pasteurized (60°C for 15 min) and untreated samples were used as controls. The comparisons were made among these three (pressurized, heat pasteurized, untreated).

2. *Unfiltered, unpasteurized beers*; pressure range was again between 200-350 MPa (for 5-10 min at 10-20°C) and comparisons were made with unfiltered, pasteurized beers. This part of the study mainly focused on microbiological analysis. The properties of unfiltered and filtered beers were also compared to understand the different effects of HHP on these beers.

3. *Shelf-life Analysis for filtered beer*; pH, bitterness, color, chill haze and protein sensitivity of heat pasteurized and pressurized (350 MPa for 3 and 5 min at 20°C) beer samples were compared during a storage period of 8 weeks (56 days at 20°C). At 1, 7, 14, 28, 42 and 56 days of storage samples were taken from each of the treatments and analyzed.

CHAPTER 2

MATERIALS AND METHODS

2.1 Beer Samples

Beer samples were obtained from “Anadolu Efes Biracılık ve Malt Sanayi A.Ş. Ankara Factory”. Homogeneous samples of crude beer were taken directly from the storage tanks, using 6 ml sterile cryovials with silicone seals and 100 ml sterile glass bottles. After packaging the samples were stored at 4°C and processed (heat pasteurization or HHP) within 24 hours.

2.2 Stabilizing Treatments

HHP treatment of the beer samples is summarized in Figure 2.1. Untreated samples were used as controls.

Heat pasteurization was made by immerging bottles in a water bath at 60°C for 15 minutes and then cooling samples at room temperature with water. Bottles were continuously stirred during pasteurization to improve heat transfer.

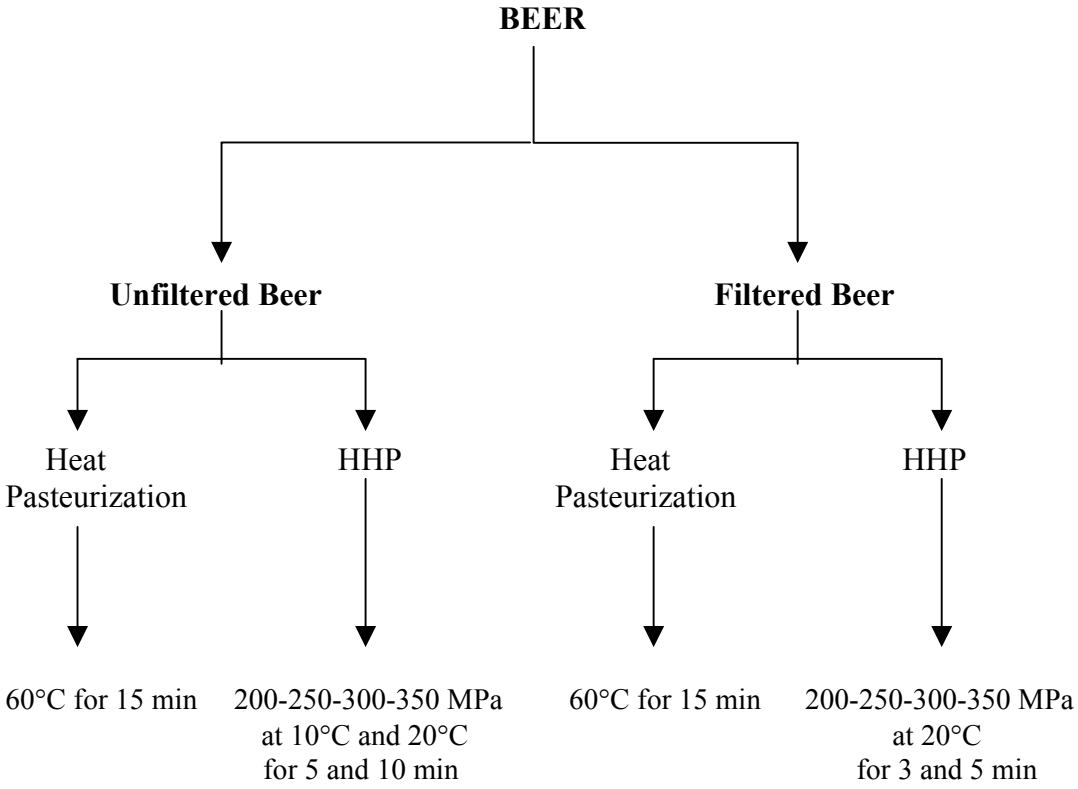

Figure 2.1. Experimental Design

High pressure treatments were made with a laboratory scale pressure system equipped with 10 ml pressure vessel (Figure B.1). The main parts of the HHP equipment are a cylindrically designed pressure vessel, a pressure pump, temperature control device and other system controls.

Temperature inside the vessel is controlled at the intervals of 10-90°C by the temperature control device. The liquid inside the vessel is warmed prior to pressurization to the desired temperature by an electrical heating system around the chamber. A pressure up to 450-500 MPa is created in the pressure vessel. The rate of pressure increase is about 140 MPa/min, and pressure come-down time is less than 2 min. An automatic device sets the pressure level and the temperature of pressurization.

2.2.1 HHP Treatment of Beer Samples

Cryovials were kept at 4°C prior to pressurization, which did not exceed 1h. Cryovials, which were filled with beer, were placed inside the cylindrical vessel of HHP equipment and then the chamber was closed. Air bubbles were removed as much as possible. Before the pressurization, the samples were kept in the chamber for 1-2 minutes for the temperature equilibration. These temperature and time relations for equilibration were determined earlier.

The filtered beer samples were pressurized at 200, 250, 300, 350 MPa at 20°C for 3 and 5 minutes. The unfiltered beer samples were pressurized at 200, 250, 300, 350 MPa at 10°C and 20°C for 5 and 10 minutes. After pressurization the vials were immediately removed from the system and put in an ice bath.

For the shelf-life analysis, beer samples were pressurized at 350 MPa at 20°C for 3 and 5 min. Then HHP treated, heat pasteurized and untreated beer samples were stored at 20°C in the dark for two months.

2.3 Chemical and Physical Analyses

2.3.1 Beer Bitterness Analysis

The measurement of the bitterness of beer was done by spectrophotometry (Figure B.2) of an iso-octane extract of beer. First of all beer was degassed without loss of foam. 2 ml of the sample was pipetted into 10 ml centrifuge tube, and 0.1 ml of 6 N hydrochloric acid was added, followed by 4 ml iso-octane and 2 or 3 glass balls. The tube was screwcapped and shaken for 15 minutes at 20°C using a shaker set at 130 rpm. The tube was then centrifuged for 3 minutes at 3000 rpm.

The extinction of iso-octane layer in a 1 cm cell was measured at 275 nm, using pure iso-octane in the reference cell [39, 40].

2.3.2 Color Measurement

Color measurement was done by spectrophotometer at a wavelength of 430 nm. The sample was held in the customary 10 mm cell. The absorbance obtained was multiplied by 25 to yield European Brewery Convention (EBC) units of color.

2.3.3 pH Measurement

The pH values of samples were measured by a pH-meter (WTW 537 pH meter).

2.3.4 Density, Extract, Ethanol Content and Fermentation Degree Measurements of Beer Samples

Density, Real, Apparent and Original Extract, Fermentation Degree and Ethanol content were measured by Anton Paar Beer Analyzer (Figure B.3). Degassed beer was filtered through a folded paper filter and filtered beer was filled into a cylindrical cuvette, which was then placed into Beer Analyzer.

The density was calculated from the oscillation period of the density measuring cell thus:

Density = $A \times P^2 - B$

where, A and B are calibration constants at 20°C and P is the oscillation period of the density measuring cell.

The calibration constants A and B of the density measuring cell could be evaluated by measuring the periods of oscillation P_{air} and P_{water}. By using the stored values for rho_{air} and rho_{water}, A and B could be calculated as follows [41]:

$$A = \frac{rho_{water} - rho_{air}}{P_{water}^2 - P_{air}^2},\ B = \frac{P_{air}^2 \times rho_{water} - P_{water}^2 \times rho_{air}}{P_{water}^2 - P_{air}^2}$$

% Alcohol (w/w) was calculated using a specific function of density and sound velocity number.

The sound velocity ν could be calculated from the period of oscillation of the sound velocity measuring cell thus:

$$\nu = \frac{originallength \times (1 + 1.6 \times 10^{-5} \times temp.)}{(PS/divisor - \tau)}$$

where, temperature is 20°C, PS is the oscillation period of sound velocity measuring cell, τ is the calibration constant for sound velocity, divisor is 512 or 256 and original length of the sound velocity cell can be read from a label at the rear of the analyzer.

The calibration constant τ of the sound velocity measuring cell could be evaluated as:

$$\tau = \frac{PS_{water}}{divisor} - \frac{originallength \times (1 + 1.6 \times 10^{-5} \times temp.)}{V_{water}}$$

where, V_{water} is the sound velocity in water.

The density number (d) is equal to the relative density deviation of the sample from water:

density number (d) $= \frac{density - d_{water}}{d_{water}}$, where d_{water} at 20°C = 0.998204 g/cm^3.

The sound number (s) is equal to the relative sound velocity deviation of the sample from water:

sound number (s) $= \frac{soundvelocity - soundvelocity_{water}}{soundvelocity_{water}}$, where sound velocity at 20°C = 1482.657 m/s.

Calculation of Real Extract (% E real) was done by using a function of density and sound number.

% A (v/v) = $\frac{\%A(w/w) \times density_{BEER}}{density_{alcohol}}$, where $d_{alcohol}$ = 0.78924 g/cm^3.

$$\%\ \text{E original} = \frac{\left[(\%A(w/w) \times 2.0665 + \%Ereal) \times 100\right]}{(100 + \%A(w/w) \times 1.0665)}$$

% E apparent was calculated from the density of the sample.

Degree of fermentation (% Ferm) = [(% E orig- % E app)/ % E orig]×100

2.3.5 Haze After Chilling (Chill Haze) and Protein Sensitivity Measurements of Beer Samples

Chill Haze and Protein Sensitivity were measured by a device called Tannometer (Figure B.4). Fully decarbonated beer was filtered with a plaited filter (Schleicher+Schull, No. 595, ½ dia. 125 mm or comparable) before being analyzed with Tannometer.

Gallic tannin at a concentration of 0.1 g dry matter/l was used as a solution for protein sensitivity measurements (Figure B.5). Sensitive proteins were precipitated by this tannin solution. A precise dosing device makes continuous dosing of solution at flow rates of 0.1 to 5 ml/h possible. The sample was filled in a

fluorescence cuvette with optical layer thickness of 2 cm. The integrated magnetic agitators ensured that the reagent and the sample were well-mixed in the cuvette. Transmitted light was measured at a wavelength of 510 nm.

In order to measure chill haze (Figure B.6) a 4 ml of sample was pippetted into a clean cuvette. The addition of ethanol (up to 6 %) decreases the solubility of the Protein-Tannin complex and enhances the chill haze.

2.4 Microbiological Analyses

For the microbiological analysis of filtered beer; wild yeasts, lactic acid and acetic acid bacteria counts were made by membrane filtration method (Figure 2.2). Wild yeast and acetic acid bacteria counts were determined on Wort Agar. Lactic acid bacteria counts were made using NBB (Nachweismedium für biershädlich Bacterien) Agar. All plates were made in duplicate for each sample and incubated at 28°C ± 1°C for 96 hours. After the incubation period direct microscopic examination was applied for all of the plates.

For the microbiological analysis of unfiltered beer; total aerobic, total yeasts and lactic acid bacteria counts were made by spread plate technique. 1% peptone solution was used to dilute the samples. Total aerobic counts were determined on Plate Count Agar. Total yeasts were determined on Potato Dextrose Agar (acidified with 50 % citric acid to pH 4.00). Lactic acid bacteria counts were made using MRS agar (containing 10 mL/L of cycloheximide 1 %). All plates were made in duplicate for each sample and incubated at 30°C for 72 hours. Compositions of all media used are given in Appendix C.

Figure 2.2. Membrane filtration. (Adapted from Ref. 27.) (a) Filtration unit comprising a Buncher flask connected to a vacuum line (V) and a glass filtration unit containing a sinter (S) with a small-pore (0.46 μm or less) membrane on top. The upper part of this unit contains the sample (SA, hatched area). The upper and lower parts are clamped together (clamp not shown). (b) After filtration the membrane is placed on the surface of medium (AG) solidified with agar in a Petri dish (P). After suitable incubation, microbes present in the sample grow to form colonies on the surface of the membrane.

2.5 Analysis of Results

Results of the first two trials (treatments on filtered and unfiltered beer) were submitted to a one-way ANOVA. A three-way ANOVA was made in the shelf-life analysis, with treatments, replication of the experiments and storage time as factors. Significant differences between means were tested using a Duncan's Multiple Range test with a probability level fixed at $p<0.05$. Statistical treatments were carried out with SPSS 10.0 for Windows.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Effect of HHP on Color

The Institute of Brewing use the EBC method for malts but have adopted a photometric method for worts and beers based on measurements at 430 nm. The result is expressed directly in EBC units of color.

The American Society of Brewing Chemists (ASBC) also use a spectrophotometric method and define beer color as 10 times the absorbance of beer measured in a 12.7 mm (0.5 in.) cell with monochromatic light of wavelength 430 nm. The absorbance at 430 and 700 nm is measured. If the absorbance at 700 nm is equal to or less than 0.039 times the absorbance at 430 nm, the beer is judged free of “turbidity” and the color calculated from the reading at 430 nm. Otherwise the sample must be clarified before the color can be measured.

In Figures 3.1 and 3.2, it can be seen that color was affected by both HHP and conventional heat treatment. As the magnitude of pressure and pressurization time increased, the absorbance of beer at 430 nm also increased. This may be explained by the effect of HHP on beer proteins, turbidity and haze (Figure 3.3, Figure 3.4, Figure 3.5 and Figure 3.6). Fischer et al. [36] found that HHP treatment of beer had no considerable differences for color. On the other hand, Castellari et al. [37] claimed that different stabilization process (heat pasteurization and HHP)

had no influence on the lightness, but all the other color indices were higher in heat processed samples.

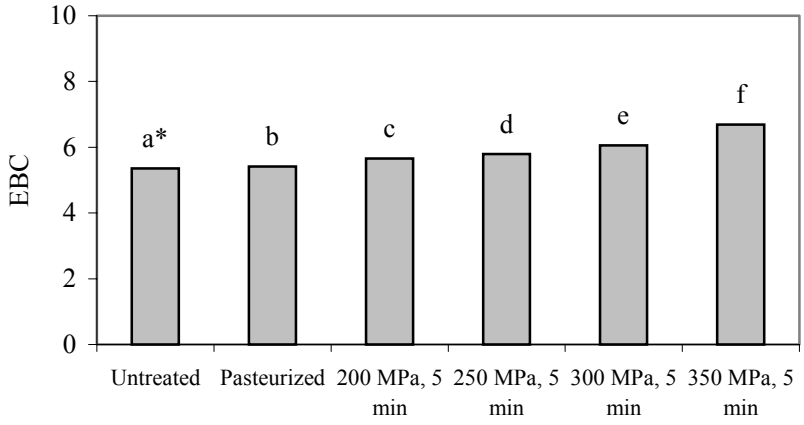

Figure 3.1. Variation of Color in EBC units with conventional heat and 5 min pressure treatments

*Different letters indicate means separation at $p<0.05$

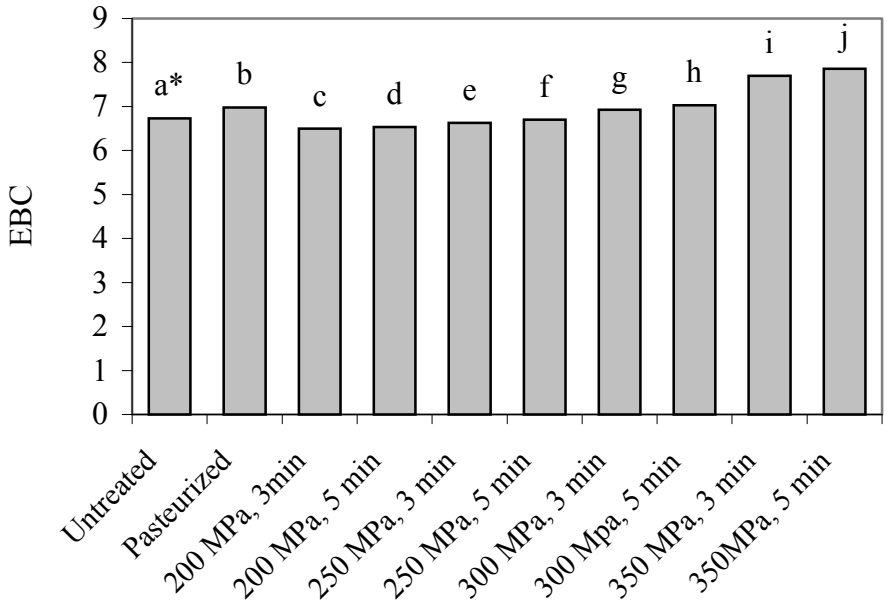

Figure 3.2. Variation of Color in EBC units with conventional heat and HHP treatments

*Different letters indicate means separation at $p<0.05$

3.2 Effect of HHP on Protein Sensitivity and Chill Haze

Early investigations establish that hazes occurred in products rich in proteins: indeed they were originally called protein hazes, but later they were also shown to contain phenolic constituents, carbohydrates and metallic elements. It soon appeared that both protein and polyphenol was essential for haze formation. The EBC Haze group set up collaborative studies of a series of chill hazes, the results of which were reported in 1965. The hazes contained 45.5-66.8 % protein and revealed similar amino acid patterns on hydrolysis [22].

As the pressure and pressurization time increased, the protein sensitivity and chill haze of the samples also increased (Figure 3.3, 3.4, 3.5 and 3.6). Fischer et al. [36] stated that as the pressure increased the turbidity also increased, however up to 3000 bar (300 MPa) no increase in turbidity was determined. Castellari et al. [37] also found that HHP increased both permanent and chill haze when compared with pasteurized ale beers.

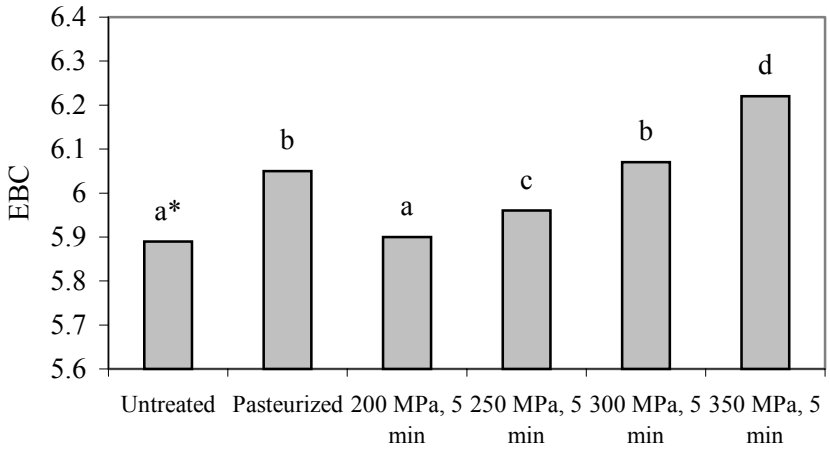

Figure 3.3. Variation of Protein Sensitivity in EBC units with conventional heat and 5 min pressure treatments

*Different letters indicate means separation at $p<0.05$

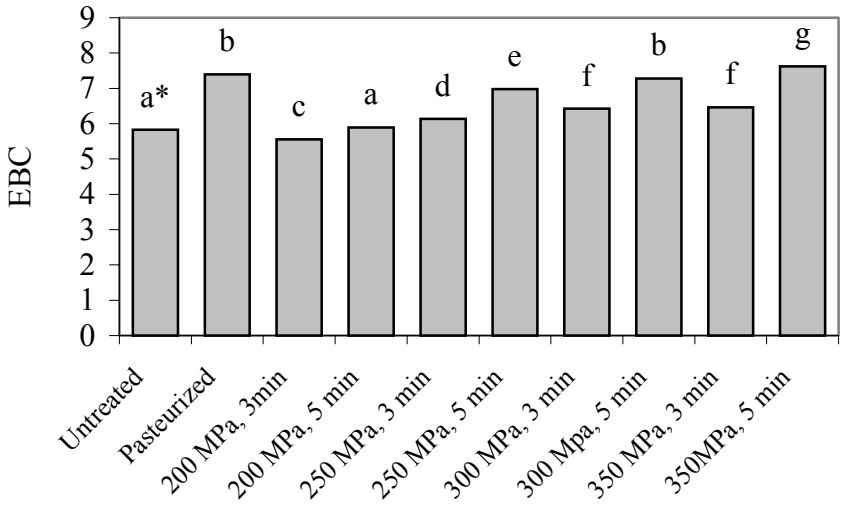

Figure 3.4. Variation of Protein Sensitivity in EBC units with conventional heat and HHP treatments

*Different letters indicate means separation at $p<0.05$

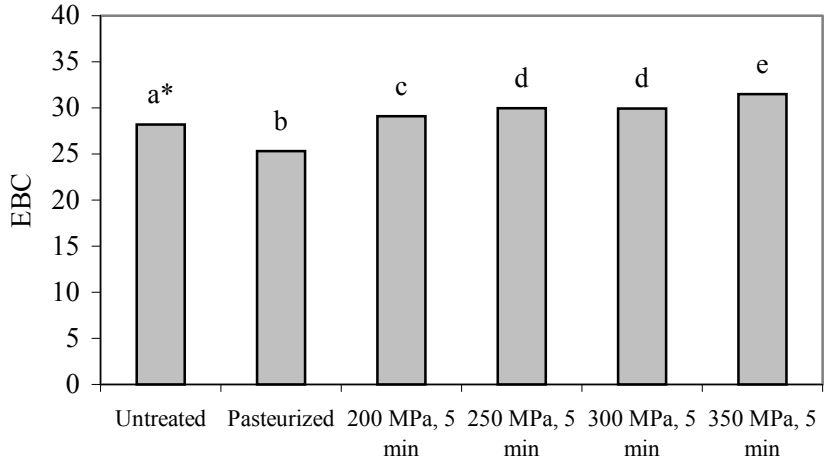

Figure 3.5. Variation of Chill Haze in EBC units with conventional heat and 5 min pressure treatments

*Different letters indicate means separation at $p<0.05$

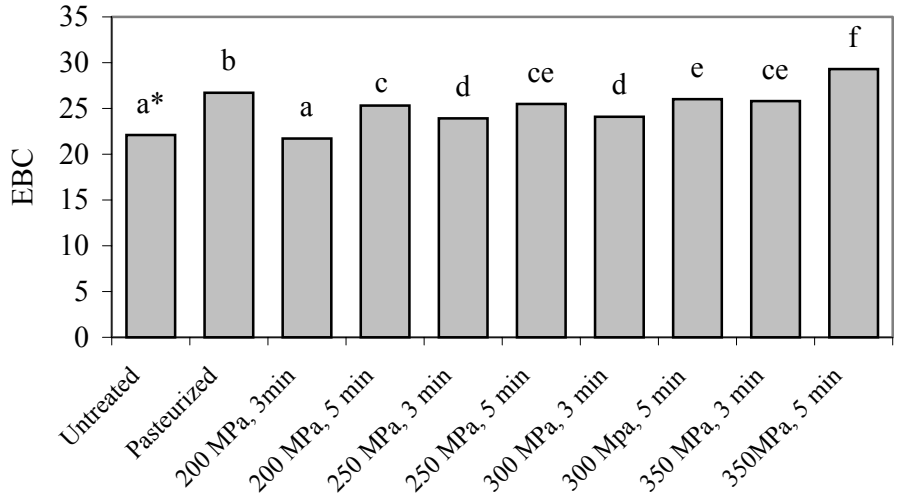

Figure 3.6. Variation of Chill Haze in EBC units with conventional heat and HHP treatments

*Different letters indicate means separation at $p<0.05$

3.3. Effect of HHP on Bitterness

Bitterness in beer arises when the α-acids in hops are isomerized during boiling with wort. The formation of iso- α-acids or isohumulones has been widely reported [45].

Although the bitterness units (BU) of the samples were increased by conventional heat pasteurization, some of the HHP treated samples had similar bitterness units when compared with controls (Figure 3.7 and 3.8), showing the effect of heat on α-acids in beer. Fischer et al. [36] had similar results; untreated and pressurized wort samples had lower bitterness units than the 30 and 60 min cooking. Fischer et al. [36] also reported the effect of HHP and heat on isomerization of α-acids in wort. HHP at 3000 and 5000 bar (300 and 500 MPa) for

5 min had very little effect on isomerization, however among the HHP treated samples best isomerization could be obtained at 7000 bar (700 MPa) for 30 min and this could be comparable with 30 min cooking.

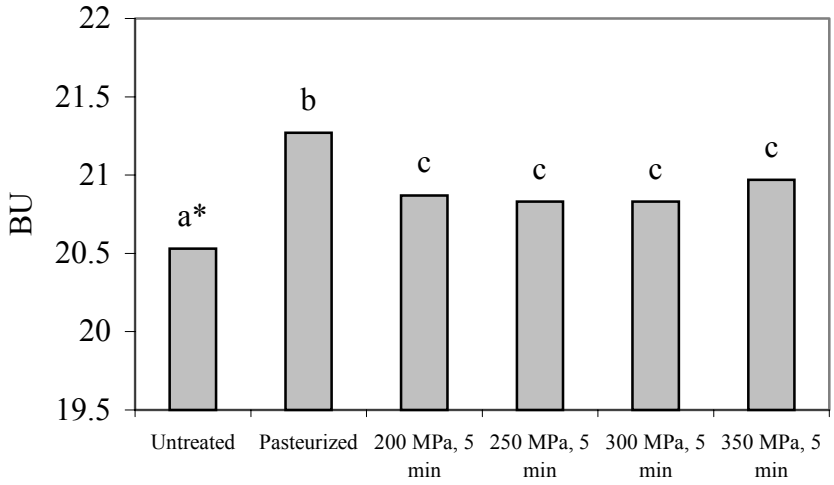

Figure 3.7. Variation of Bitterness in BU with conventional heat and 5 min pressure treatments

*Different letters indicate means separation at $p<0.05$

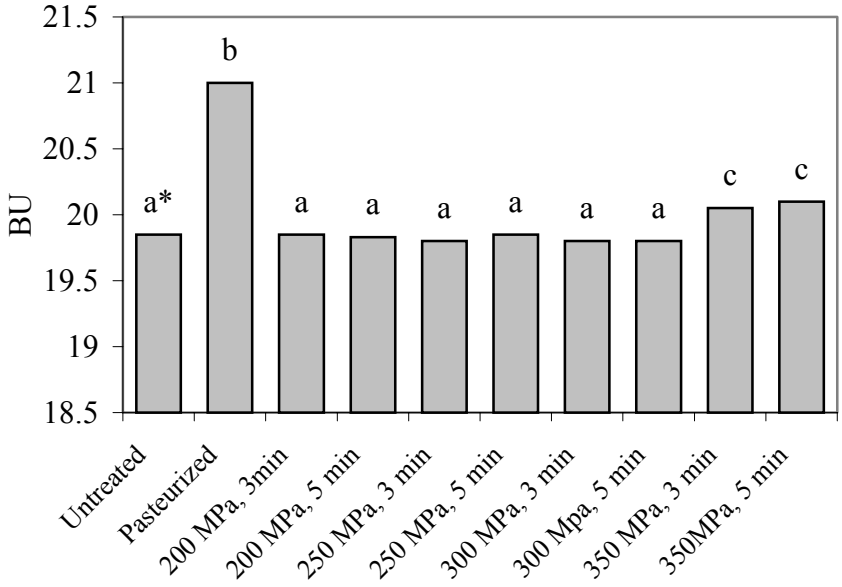

Figure 3.8. Variation of Bitterness in BU with conventional heat and HHP treatments

*Different letters indicate means separation at $p<0.05$

3.4 Effect of HHP on Ethanol, Extract, Density, Fermentation Degree and Microorganisms in Beer

The comparisons with untreated lager beer samples in terms of ethanol, extract, fermentation degree, density and pH showed that these parameters were not significantly affected ($p<0.05$) by the HHP treatment (at the range studied; 200-350 MPa) and heat pasteurization (Table 3.1 and Table 3.2). Castellari et al. [37] also reported that HHP (600 MPa for 5 min) had no significant affect on ethanol, extract and pH of the pale ale beer. Although the treated beers were different (ale and lager), these results showed that lower pressures (the range studied; 200-350 MPa) had the similar effect on ethanol, extract and pH as high pressure (600 MPa).

Table 3.1* Effect of 5 min HHP treatment on Ethanol, Extract, Density, Fermentation Degree and Microorganisms in Beer

	Units	**Untreated**	**Heat Past. 60°C, 15 min**	**HHP 200 MPa, 5 min**	**HHP 250 MPa, 5 min**	**HHP 300 MPa, 5 min**	**HHP 350 MPa, 5 min**
Ethanol	v/v	5.04[a]	5.05[a]	5.05[a]	5.05[a]	5.06[a]	5.06[a]
Density	g/ml	1.005743[a]	1.005697[b]	1.005690[b]	1.005647[c]	1.005680[bd]	1.005653[cd]
Real Extract	w/w	3.73[a]	3.74[a]	3.74[a]	3.73[a]	3.73[a]	3.73[a]
App. Extract	w/w	1.92[a]	1.93[a]	1.92[a]	1.92[a]	1.91[a]	1.92[a]
Orig. Extract	w/w	11.43[a]	11.43[a]	11.44[a]	11.43[a]	11.43[a]	11.44[a]
Fer. Degree	%	83.21[a]	83.20[a]	83.21[a]	83.21[a]	83.20[a]	83.20[a]
pH	20°C	4.33[a]	4.32[a]	4.32[a]	4.33[a]	4.32[a]	4.33[a]
LAB**	cfu/50ml	7	n.d.	n.d.	n.d.	n.d.	n.d.
AAB**	cfu/50ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WY**	cfu/50ml	3	n.d.	n.d.	n.d.	n.d.	n.d.

* Different letters following the numbers on the same line indicate means separation at $p<0.05$.

** Microbiological analyses were done by membrane filtration method on filtered beer.
n.d., not detected; no cfu were detected in 50 ml of beer sample.

Table 3.2* Effect of 3 and 5 min HHP treatment on Ethanol, Extract, Density, Fermentation Degree and Microorganisms in Beer

	Units	Untreated	Heat Past. 60°C, 15 min	HHP 200 MPa		HHP 250 MPa		HHP 300 MPa		HHP 350MPa	
				3 min	5 min	3 min	5 min	3 min	5 min	3 min	5 min
Ethanol	v/v	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]
Density	g/ml	1.00579[a]	1.00579[a]	1.00578[a]	1.00578[a]	1.00580[a]	1.00579[a]	1.00578[a]	1.00578[a]	1.00577[a]	1.00577[a]
Real Ext.	w/w	3.82[a]	3.82[a]	3.82[a]	3.82[a]	3.82[a]	3.81[a]	3.81[a]	3.8[a]	3.81[a]	3.82[a]
Ap. Ext.	w/w	2.03[a]	2.03[a]	2.03[a]	2.03[a]	2.02[a]	2.03[a]	2.02[a]	2.03[a]	2.02[a]	2.02[a]
Or. Ext.	w/w	11.49[a]	11.48[a]	11.49[a]	11.49[a]	11.48[a]	11.48[a]	11.48[a]	11.48[a]	11.49[a]	11.48[a]
Fer. Deg.	%	82.31[a]	82.30[a]	82.31[a]	82.32[a]	82.31[a]	82.30[a]	82.31[a]	82.31[a]	82.31[a]	82.31[a]
pH	20°C	4.37[ab]	4.37[a]	4.37[a]	4.37[ab]	4.38[ab]	4.37[a]	4.37[a]	4.37[a]	4.37[a]	4.38[b]
LAB[1]	cfu/50ml	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AAB[1]	cfu/50ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WY[1]	cfu/50ml	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* Different letters following the numbers on the same line indicate means separation $p<0.05$.

** Microbiological analyses were done by membrane filtration method on filtered beer.
n.d., not detected; no cfu were detected in 50 ml of beer sample.

3.5 Shelf-life

The shelf-life of beer is largely determined by its colloidal stability, although flavor changes may develop prior to any haze formation. Off-flavors due to beer staling are as varied as the beer flavors themselves.

Only five parameters (pH, bitterness, color, protein sensitivity, chill haze) were studied in the shelf-life analysis (Figures 3.9, 3.10, 3.11, 3.12 and 3.13).

At the end of the eight weeks (56 days) pH and color of the samples were not significantly different (except for the untreated samples). Untreated samples demonstrated a sharp decrease in pH at the end of the fourth week (Figure 3.9), this may be explained by the activity of microorganisms in untreated beers. However, very slight changes in pH were observed for heat pasteurized and HHP treated samples.

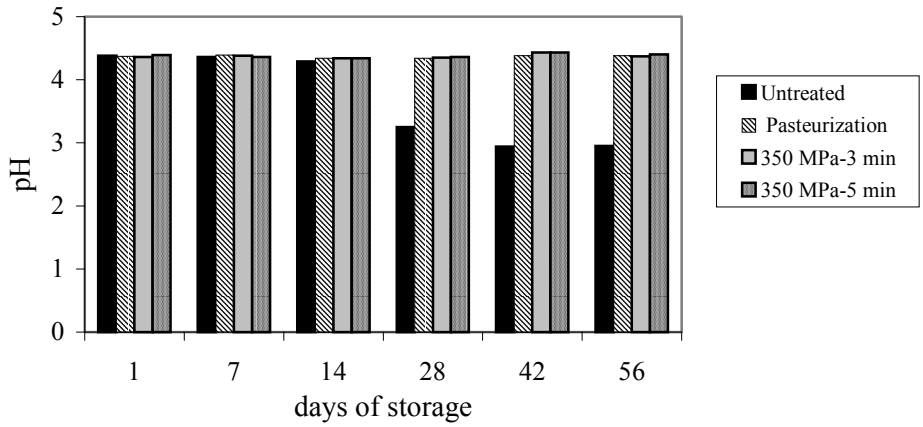

Figure 3.9. Variation of pH during a period of 8 weeks

Color (EBC) of untreated samples was the highest during storage period except from the first week. Since the turbidity increased in untreated beer samples, color (EBC) also increased (Figure 3.10).

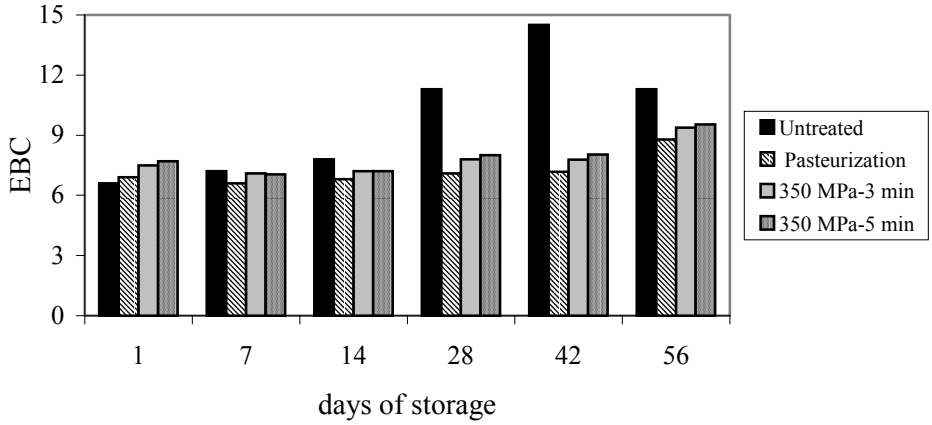

Figure 3.10. Variation of color during a period of 8 weeks

Bitterness was higher for the heat pasteurized samples than the pressurized beers and it was not significantly different ($p<0.05$) for both of the pressurized samples (350MPa for 3 and 5 min) at the end of the storage period (Figure 3.11).

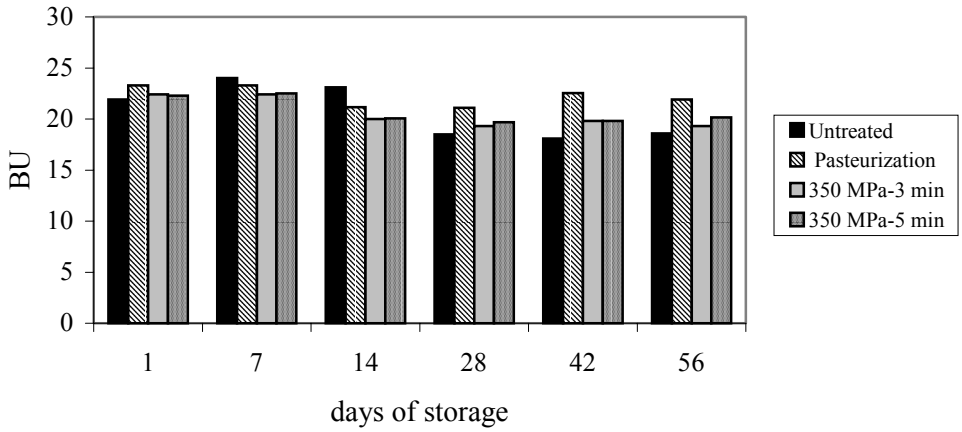

Figure 3.11. Variation of bitterness during a period of 8 weeks

It has been claimed that beer bitterness decreases with age while sweetness increases, thus shifting the balance towards a sweeter flavor for all beer types is expected [44, 45]. According to Pangborn et al. [46], this decrease in bitterness is

accompanied by a change in its nature: fresh-bitterness is related to analytically-determined isohumulone concentrations expressed in bitter units whereas aged-bitterness is not therefore decrease in bitterness could be seen.

Walters et al. [48] measured a 71 % decrease in total iso-α-acids (from 15.3 to 4.5 mg/L) when the beer they studied was stored 156 days at 40°C. The same beer stored 220 days at 0 or 25°C showed no loss of total iso-α-acids. However, in this study it was observed that very slight decreases occurred in bitterness units of the stored beers (at 20°C for 56 days); heat pasteurized beers had a loss of 1.4 BU, HHP treated beers had losses of 3.1 and 2.15 BU, respectively at the end of storage period.

Beers infected with bacteria or wild yeast will rapidly go turbid and develop a biological haze but with the widespread use of pasteurization and sterile filtration such infections are fairly rare. However, uninfected beers when stored for any length of time, usually in bottle, also become cloudy and deposit a haze. Such beers are usually unacceptable and the rate of development of this non-biological haze determines the shelf-life of bottled beer. Before a beer shows any permenant haze at room temperature it may form a chill haze if suddenly cooled to 0°C. Such hazes redissolve when the beer is warmed up again to room temperature (20°C).

The HHP treated beers had lower protein sensitivity and higher chill haze than the pasteurized samples (Figures 3.12 and 3.13). Protein sensitivity of untreated beer samples increased up to the 28^{th} day and then decreased; this may be due to activity of microorganisms that are exposed to other proteins and peptides causing decrease in protein sensitivity. On the contrary; chill haze values of untreated samples was nearly zero from the 28^{th} day until the end of 56^{th} day. Since untreated beers were already hazy, no additional haze were detected when these beers were further chilled.

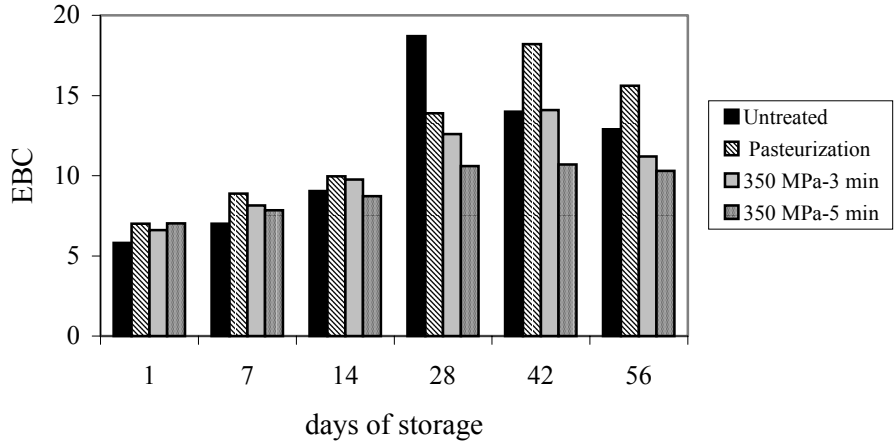

Figure 3.12. Variation of protein sensitivity during a period of 8 weeks

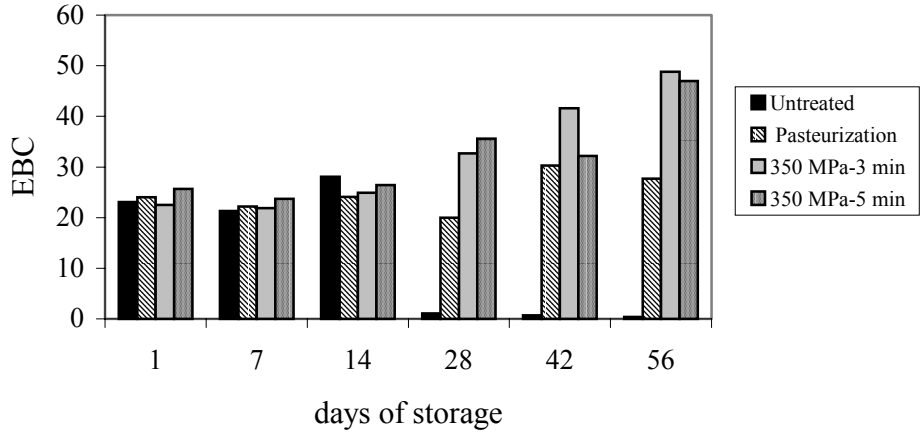

Figure 3.13. Variation of chill haze during a period of 8 weeks

The microbiological experiments done on shelf-life analysis showed that untreated beers had 2 cfu of lactic acid bacteria and 3 cfu of acetic acid bacteria in 50 ml of beer sample at the first day of storage period. At 1, 28 and 56 days of storage one sample from each of the treatments (heat pasteurization, 350 MPa at 3 and 5 min) were analyzed and no cfu were detected in 50 ml of samples.

The behavior of HHP samples must be evaluated taking into account that every beer contains a considerable amounts of haze-active proteins. Denaturation of these proteins expose additional hydrophobic binding sites and this leads to strong protein-phenolics binding developing haze [37, 43]. The different degree and modality of protein denaturation may influence the interaction between colloidal substances and could be the basis of observed effects on beer protein sensitivity and chill haze

The effects of variables are reported in Table 3.3. Except from the ineffectiveness of replication on color, all the parameters were affected by treatment, time of storage, replication and interaction between treatment and time of storage. Interaction between treatment and replication affected color and bitterness. However, interaction between time of storage and replication had no influence on the parameters studied.

Table 3.3.* Effects of variables

	treatment (a)	**time of storage (b)**	**replication (c)**	**a×b**	**b×c**	**a×c**
pH	√	√	√	√		
Color (EBC)	√	√		√		√
Bitterness (BU)	√	√	√	√		√
Prt. Sens. (EBC)	√	√	√	√		
HAC (EBC)	√	√	√	√		

* significance at $p<0.05$
√ means the parameter is effected by the variable

3.6 Comparison of Unfiltered and Filtered Lager Beers

The comparison of filtered beer with unfiltered beer in terms of bitterness, original extract, fermentation degree and pH were not significantly affected ($p<0.05$) by the filtration treatment. However, ethanol content, density, color, real and apparent extract values were higher in unfiltered beers than in filtered beers (Table 3.4).

Table 3.4.* Comparison of unfiltered and filtered lager beers

	Units	**Unfiltered**	**Filtered**
Bitterness	BU	20.85[a]	20.72[a]
Ethanol	v/v	5.12[a]	5.05[b]
Density	g/ml	1.00699[a]	1.00598[b]
Real Extract	w/w	4.09[a]	3.78[b]
Apparent Extract	w/w	2.26[a]	1.96[b]
Original Extract	w/w	11.60[a]	11.49[a]
Fermentation Degree	%	82.20[a]	82.38[a]
pH	20°C	4.33[a]	4.29[a]
Color	EBC	7.63[a]	6.1[b]

*Different letters following the numbers on the same line indicate means separation at $p<0.05$

When filtering storage beer through a 0.22μm polysulfone membrane, Ryder et. al. [47] found decreases in original gravity (12.30-11.73°P), alcohol (3.94-3.78 wt. %), protein (0.72-0.59 wt.%) and bitterness (15.1-13.5 BU). Therefore, it was not surprising that the filtered beer had lower bitterness, ethanol and original extract values than the unfiltered beer in this study.

3.7 Microbial Inactivation of Unfiltered Lager Beer by HHP

Unfiltered beer samples had 7. 48, 7.15 and 2.64 $\log_{10}$cfu/ml of total yeasts, total aerobic and lactic acid bacteria counts, respectively. No colony formation of lactic acid bacteria was observed when the samples were pressurized at pressures equal to or higher than 300 MPa at 10 and 20°C for 5 and 10 min (Figure 3.14 and 3.15).

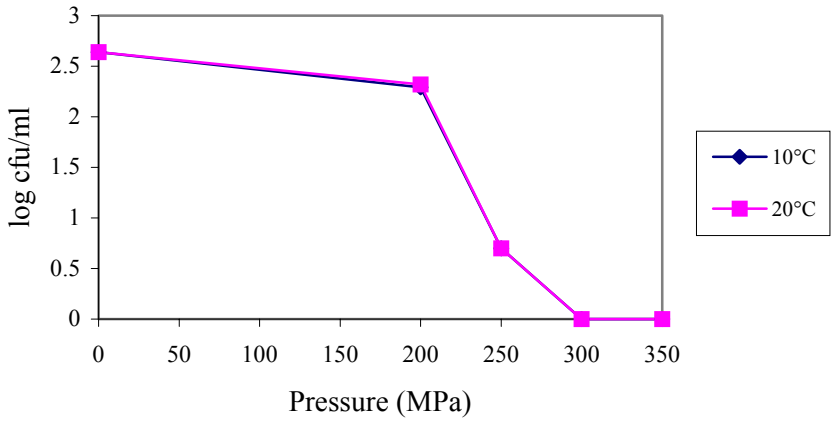

Figure 3.14. Effect of HHP on lactic acid bacteria in unfiltered lager beer (pressure for 5 min).

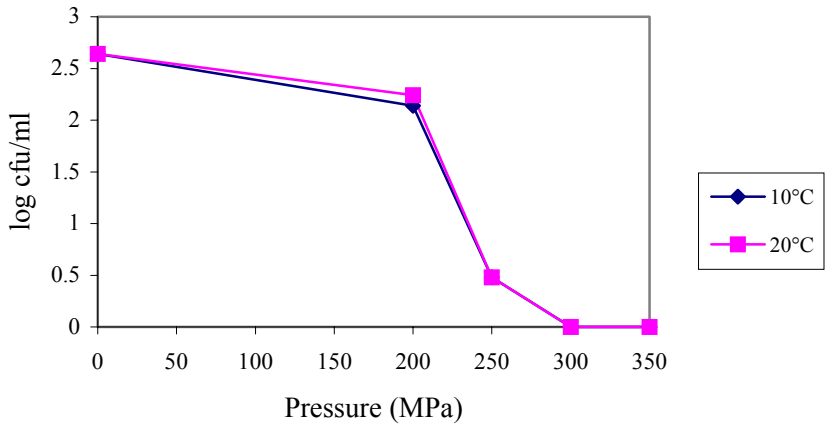

Figure 3.15. Effect of HHP on lactic acid bacteria in unfiltered lager beer (pressure for 10 min).

Total yeasts and total aerobic counts demonstrated more than 7 and 6 log-cycle reduction when pressurized at 350 MPa at 10 and 20°C for 10 min, respectively. Heat treatment gave similar results in terms of log reductions (Figure 3.17 and Figure 3.19).

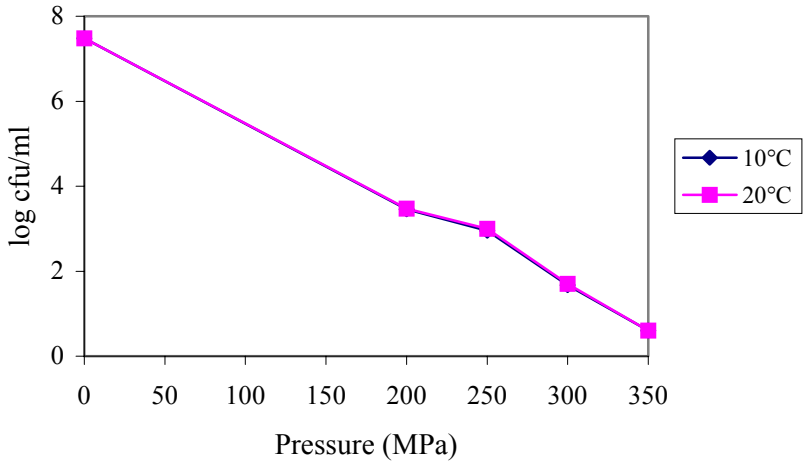

Figure 3.16. Effect of HHP on total yeasts in unfiltered lager beer (pressure for 5 min).

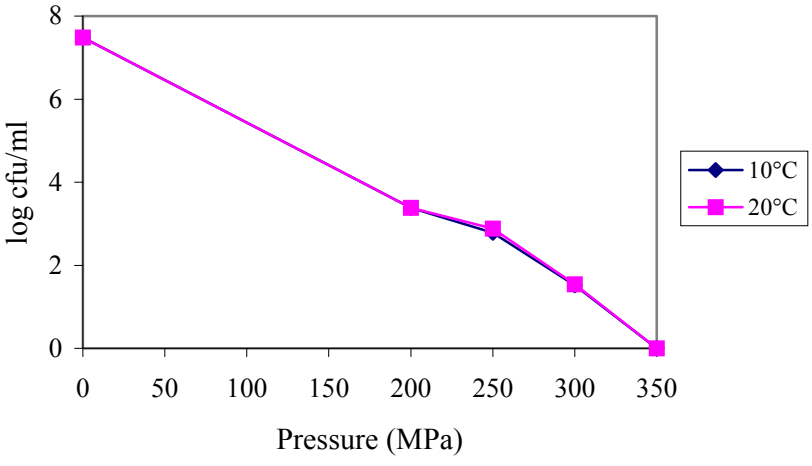

Figure 3.17. Effect of HHP on total yeasts in unfiltered lager beer (pressure for 10 min).

Both HHP and heat treatment gave comparable ($p<0.05$) microbial inactivation. However, application of higher pressures (≥ 300 MPa) and longer pressurization times (> 5 min) gave better results in terms of viability loss. Effect of time could be seen clearly for total yeasts and total aerobic counts from the comparisons of Figure 3.16 with Figure 3.17 and comparisons of Figure 3.18 with Figure 3.19, respectively.

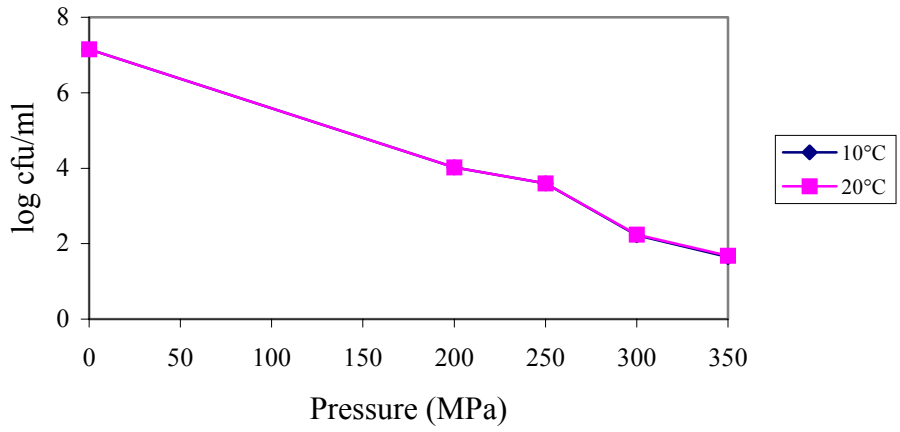

Figure 3.18. Effect of HHP on total aerobic count in unfiltered lager beer (pressure for 5 min).

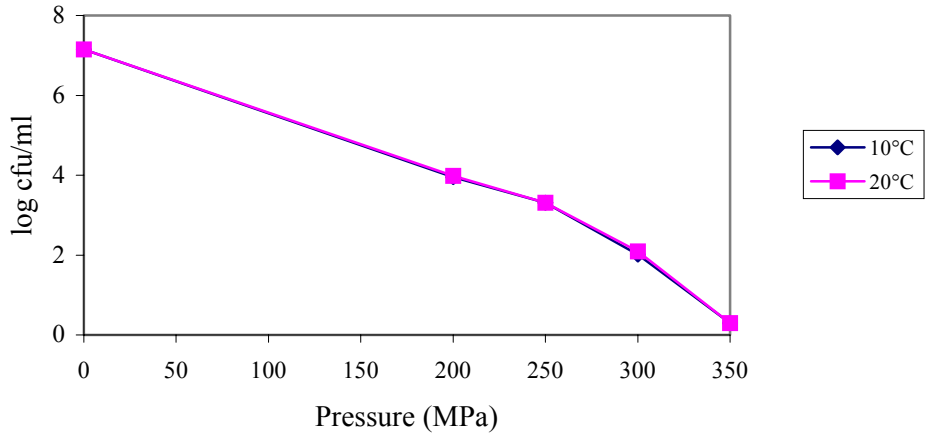

Figure 3.19. Effect of HHP on total aerobic count in unfiltered lager beer (pressure for 10 min).

Probably the acidic medium, the carbon dioxide, the hop components and ethanol content increased the efficacy of HHP [49, 50].

The results in this study are only partially in agreement with those of Fischer et al. (1998) and Castellari et al. (2000) but differences between experimental procedures must be taken into account.

CHAPTER 4

CONCLUSIONS

Beer drinking is a widely established habit in many countries. In recent years, there has been an increase in consumption, even in nations were alcoholic beverages are not traditional.

Prior to packaging, beer conventionally undergoes two processing steps. Firstly, it is filtered to remove any haze causing material, resulting in a bright beer. Secondly, the shelf-life of the product is increased by pasteurization. Currently, heat treatment is either done by flash pasteurization (with plate heat exchangers) or by tunnel pasteurization. Beer is packaged (usually into metal kegs) after it has been treated by the flash pasteurization process. However, in tunnel pasteurizers beer is first filled into glass bottles and then pasteurized.

Since HHP offers an alternative potential non-thermal preservation method for pasteurization of food products its effects on lager beer was investigated to compare its quality parameters with heat pasteurization process.

HHP was found not to affect main beer attributes (ethanol, extract, fermentation degree, density and pH) in comparison with untreated beers. Both HHP and heat pasteurization produced microbiologically stable products. Bitterness, color, protein sensitivity and chill haze parameters were affected by both of the treatments. Bitterness of the HHP treated beers were similar to untreated beers. This shows that HHP may result more “fresh-like” taste than heat

pasteurization. Protein sensitivity and chill haze parameters increased as the pressure and pressurization time increased therefore lower pressures (200 and 250 MPa) and pressurization times (< 5 min) may give better results in terms of protein sensitivity and chill haze.

A storage period of 56 days showed that HHP and heat pasteurization had similar results in terms of pH and color. However heat pasteurized samples had higher bitterness and protein sensitivity and lower chill haze values than the HHP treated samples which indicates HHP treatment had a positive effect on bitterness and protein sensitivity at the end of the storage period. The microbiological stability (for shelf-life analysis) of HHP treated beers was comparable with heat-treated beers, and the development of both lactic and acetic acid bacteria was inhibited for 56 days of storage.

HHP keeps most of the quality parameters of lager beer as intact, which impresses that HHP treated beers are very similar to untreated beers. These results show that HHP can be alternative process to heat pasteurization of lager beer.

CHAPTER 5

RECOMMENDATIONS

Further research may be done to understand the effect of HHP on different type of lagers or ales such as; dark, low alcohol, high alcohol etc. Also further studies could be done to investigate the effects of HHP on permanent haze of beers, haze-active proteins, beer flavor and foam, phenolic compounds and shelf-life of different beer types.

REFERENCES

[1] Palou, E. 1998. Food Preservation by High Hydrostatic Pressure, Process Variables and Microbial Inactivation. Ph. D. Thesis, Washington State University, Pullman.

[2] Trujillo, A.J., Capellas, M., Buffa, M., Royo, C., Gervilla, R., Felipe, X., Sendra, E., Saldo, J., Ferragut, V., Guamis, B. 2000. Application of high hydrostatic pressure treatment for cheese production. Food Res. Int. 33, 311-316.

[3] Alpas, H. 2000. Effect of temperature, pH and bacteriocins on the viability of selected food pathogens in food systems during high hydrostatic pressure (HHP) processing. Ph. D. Thesis, Middle East Technical University, Ankara.

[4] Tewari, G., Jayas, D.S., Holley, R.A. 1999. High Pressure Processing of Foods: An Overview. Sci. des Aliments 19, 619-661.

[5] Roger, H. 1895. Arch. Physiol. Normale Pathol. 7, 12.

[6] Hite, B.H. 1899. The effect of pressure in the preservation of milk. Agric. Expt. Station Bull. 58, 15-35.

[7] Hite, B.H., Giddings, N.J., Weakly, C.E. 1914. The effects of pressure on certain microorganisms encountered in the preservation of fruits and vegetables. West Virgina Univ. Agric. Expt. Station Bull. 146, 1-67.

[8] Hayashi, R. 1995. Advances in high pressure processing technology in Japan. Food Processing: Recent Developments, (A.G. Gaonkar ed.). Elseiver. London, p. 185-195.

[9] Smelt, J.P.P.M. 1998. Recent advances in the microbiology of high pressure processing. Trends in Food Sci. and Technol. 9, 152-158.

[10] Gervilla, R., Sendra, E., Ferragut, V., Guamis, B. 1999. Sensitivity of *Staphylococcus aureus* and *Lactobacillus helveticus* in Ovine Milk Subjected to High Hydrostatic Pressure. J. Dairy Sci. 82, 1099-1107.

[11] Gaucheron, F., Famelart, M.H., Mariette, F., Raulot, K., Michel, F., LeGraet, Y. 1997. Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. Food Chem. 59, 439-447.

[12] Knorr, D. 1993. Effects of High-Hydrostatic-Pressure Processes on Food Safety and Quality. Food Technol. 47(6), 156-161.

[13] Mertens, B., Deplace, G. 1993. Engineering Aspects of High-Pressure Technology in the Food Industry. Food Technol. 47(6), 164-169.

[14] Bridgmann, P.W. 1912. J. Biol. Chem. 19, 511-512.

[15] Olson, S. 1995. Production equipment for commercial use. High Pressure Processing of Foods (Ledward, D.A., Johnston, D.E., Earnshaw, R.G., Hosting, ed.). Nottingham University Press. Nottingham, p. 167-180.

[16] Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunno, C.P., Ray, B. 1999. Variation in Resistance to Hydrostatic Pressure among strains of Food-Borne Pathogens. Appl. and Environ. Microbiol. 65(9), 4248-4251.

[17] Alpas, H., Bozoglu, F. 2000. The combined effect of high hydrostatic pressure, heat and bacteriocins on inactivation of foodborne pathogens in milk and orange juice. World J. Microbiol. and Biotechnol. 16, 387-392.

[18] Alpas, H., Kalchayanand, N., Bozoglu, F., Ray, B. 2000. Interaction of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure sensitive starins of foodborne pathogens. Int. J. Food Microbiol. 60, 33-42.

[19] Hoover, D.G. 1993. Pressure effect on biological systems. Food Technol. 47(6), 150-155.

[20] Lechowich, R.V. 1993. Food Safety Implications of High Hydrostatic Pressure as a Food Processing Method. Food Technol. 47(6), 170-172.

[21] Dervisi, P., Lamb, J., Zabetakis, I. 2001. High Pressure processing in jam manufacture: effects on textural and colour properties. Food Chem. 73, 85-91.

[22] Hough, J.S., Briggs D.E., Stevens, R., Young T.W. 1982. Malting and Brewing Science. Vol. 2. Chapman & Hall. London.

[23] Agu, R.C., Palmer, G.H. 1998. A Reassesment of Sorghum for lager-beer brewing. Bioresource Technol. 66, 253-261.

[24] Kunze, W. 1996. Technology Brewing and Malting. International Edition. VLB. Berlin.

[25] Bamforth, C.W. 2002. Nutritional aspects of beer — a review. Nutr. Res. 22, 227-237.

[26] Linko, M., Haikara, A., Ritala, A., Pentilla, M. 2000. Recent advances in the malting and brewing industry. J. Biotechnol. 65, 85-98.

[27] Lewis, M.J., Young T.W. 1995. Brewing. Chapman & Hall. London.

[28] Priest, F.G., Campbell, I. 1996. Brewing Microbiology. 2nd Edition. Chapman & Hall. London.

[29] Suzuki, K., Sami, M., Kadokura, H., Nakajima, H., Kitamoto, K. 2002 Biochemical characterization of *hor A*-independent hop resistance mechanism in *Lactobacillus brevis*. Int. J. Food Microbiol. 76, 223-230.

[30] Jespersen, L., Jakobsen, M. 1996. Specific spoilage organisms in breweries and laboratory media for their detection. Int. J. Food Microbiol. 33, 139-155.

[31] Aa Kühle van der A., Jespersen, L. 1998. Detection and identification of wild yeasts in lager breweries. Int. J. Food Microbiol. 43, 205-213.

[32] Hornsey, I.S. 1999. Brewing. The Royal Society of Chemistry. Cambridge.

[33] Kuiper, S., Rijn, C., Nijdam, W., Raspe, O., Wolferen, H., Krijnen, G., Elwenspoek, M. 2002. Filtration of lager beer with microsieves: flux, permeate haze and in-line microscope observations. J. Membr. Sci. 196, 159-170.

[34] Yang, J.I. 2000. Development of a Method for Assessing Haze-active Active Protein in Beer by Dye Binding. Ph. D. Thesis, Cornell University. New York.

[35] Fillaudeau, L., Carrere, H. 2002. Yeast cells, beer composition and mean pore diameter impacts on fouling and retention during cross-flow filtration of beer with ceramic membranes. J. Membr. Sci. 196, 39-57.

[36] Fischer, S., Schoeberl, H., Russ, W., Meyer-Pittroff, R. 1998. Effects of hydrostatic high pressure on the brewing process and on beer. Monatsschrift fuer Brauwissenschaft 51 (7/8), 120-123.

[37] Castellari, M., Arfelli, G., Riponi, C., Carpi, G., Amati, A. 2000. High hydrostatic pressure treatments for beer stabilization. J. Food Sci. 65(6), 974-977.

[38] Ganzle, M.G., Ulmer, H.M., Vogel, R.F. 2001. High pressure inactivation of *Lactobacillus plantarum* in a model beer system. J. Food Sci. 66 (8), 1174-1181.

[39] European Brewery Convention Analytica. 1975. EBC — 3rd edition. E45-E54 (suppl. 1980). E60. Schweiz Brau Kundsch.

[40] Linskens, H.F., Jackson, J.F. 1988. Beer Analysis. Springer-Verlag. Berlin. Heidelberg.

[41] Brautechnische Analysen methoden. 1987. Bd. 2; Methodensammlung der Mitteleuropaischen Brautechnischen Analysenkommission (MEBAK). 2. Auflage. Selbstverlay der MEBAK, D-8050 Freising-Weihenstephan; seite 67 und seite 68.

[42] http://www. wonjiny.co.kr/Pfeuffer/html/Tanno1.htm.

[43] Siebert, K.J., Carrasco, A., Lynn, P.Y. 1996. Formation of protein-polyphenol haze in beverages. J. Agric. Food Chem. 44 (8), 1997-2005.

[44] Dalgliesh, C.E. 1977. Flavour stability. Proc. Congr. Eur. Brew. Conv. 9, 623-659.

[45] King, B.M., Duineveld, C.A.A. 1999. Changes in bitterness as beer ages naturally. Food Quality and Preference 10, 315-324.

[46] Pangborn, R.M., Lewis, M.L., Tanno, L.A.S. 1977. Sensory quantification of bitterness and flavour of beer during storage. J. Inst. Brew. 83, 244-250.

[47] Ryder, D.S., Davis, C.R., Anderson, D., Glancy, F.M., Power, J.N. 1988. Brewing experience with cross-flow filtration. Technol. Q. Master Brew. Assoc. Am. 25, 67.

[48] Walters, M.T., Heasman, A.P., Hughes, P.S. 1997. Comparison of (+)-catechin and ferulic acid as a natural antioxidants and their impact on beer flavor stability. Part 2: extended storage trials. J. Am. Soc. Brew. Chem. 55(3), 91-98.

[49] Haas, G.J., Prescott, H.E., Dudley, E., Dik, R., Hintlian, C., Keane, L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. J. Food Safety. 9(4), 253-265.

[50] Sangronis, E., Pothakamury, U., Ramos, A.M., Ibarz,A., Barbosa-Canovas, G.V. 1997. La alta presion hidrostatica: una alternativa en el procesamiento no termico de alimentos. Alimentaria. 35(283), 33-43.

[51] http://www.sigmaaldrich.com/img/assets/6840/70196_Wort_Agar.pdf

APPENDIX A

ANOVA and DUNCAN TABLES

Table A.1 ANOVA Table for 5 min HHP treatments

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Bitterness	Between Groups	0.845	5	0.169	33.8	0
	Within Groups	6.00E-02	12	5.00E-03		
	Total	0.905	17			
Ethanol	Between Groups	8.94E-04	5	1.79E-04	1.464	0.272
	Within Groups	1.47E-03	12	1.22E-04		
	Total	2.36E-03	17			
Density	Between Groups	1.82E-08	5	3.64E-09	14.23	0
	Within Groups	3.07E-09	12	2.56E-10		
	Total	2.13E-08	17			
Real Extract	Between Groups	9.44E-05	5	1.89E-05	0.68	0.647
	Within Groups	3.33E-04	12	2.78E-05		
	Total	4.28E-04	17			
Apparent Extract	Between Groups	3.11E-04	5	6.22E-05	0.862	0.534
	Within Groups	8.67E-04	12	7.22E-05		
	Total	1.18E-03	17			
Original Extract	Between Groups	9.44E-05	5	1.89E-05	0.68	0.647
	Within Groups	3.33E-04	12	2.78E-05		
	Total	4.28E-04	17			
Fermentation Degree	Between Groups	1.11E-04	5	2.22E-05	0.5	0.771
	Within Groups	5.33E-04	12	4.44E-05		
	Total	6.44E-04	17			
pH	Between Groups	1.61E-04	5	3.22E-05	0.58	0.715
	Within Groups	6.67E-04	12	5.56E-05		
	Total	8.28E-04	17			

Table A.1 ANOVA Table for 5 min HHP treatments Continued

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Protein Sensitivity	Between Groups	0.24	5	4.79E-02	148.693	0
	Within Groups	3.87E-03	12	3.22E-04		
	Total	0.243	17			
Color	Between Groups	3.695	5	7.39E-01	3325.67	0
	Within Groups	2.67E-03	12	2.22E-04		
	Total	3.698	17			
Chill Haze	Between Groups	67.187	5	13.437	1209.36	0
	Within Groups	0.133	12	1.11E-02		
	Total	67.32	17			

Post Hoc Tests

Homogeneous Subsets

Treatment

Untreated⇒ 1.00

Heat Pasteurization⇒ 2.00

HHP 200 MPa, 5 min⇒ 3.00

HHP 250 MPa, 5 min⇒ 4.00

HHP 300 MPa, 5 min⇒ 5.00

HHP 350 MPa, 5 min⇒ 6.00

Table A.2 Duncan's Multiple Range Table for Bitterness

BITTERNESS

Duncan[a]

		Subset for alpha = .05		
TREATMENT	N	1	2	3
1.00	3	20.5333		
4.00	3		20.8333	
5.00	3		20.8333	
3.00	3		20.8667	
6.00	3		20.9667	
2.00	3			21.2667
Sig.		1.000	.053	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.3 Duncan's Multiple Range Table for Ethanol

ETHANOL

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
3.00	3	5.0433
1.00	3	5.0467
2.00	3	5.0500
5.00	3	5.0533
4.00	3	5.0600
6.00	3	5.0633
Sig.		.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.4 Duncan's Multiple Range Table for Density

DENSITY

Duncan[a]

		Subset for alpha = .05			
Treatment	N	1	2	3	4
4.00	3	1.0056			
6.00	3	1.0057	1.00565		
5.00	3		1.00568	1.00568	
3.00	3			1.00569	
2.00	3			1.00570	
1.00	3				1.00574
Sig.		.619	.064	.248	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5 Duncan's Multiple Range Table for Real Extract

REAL EXTRACT

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
5.00	3	3.7300
1.00	3	3.7333
4.00	3	3.7333
6.00	3	3.7333
2.00	3	3.7367
3.00	3	3.7367
Sig.		.186

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.6 Duncan's Multiple Range Table for Apparent Extract

APPARENT EXTRACT

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
5.00	3	1.9133
1.00	3	1.9167
4.00	3	1.9167
3.00	3	1.9200
6.00	3	1.9200
2.00	3	1.9267
Sig.		.108

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7 Duncan's Multiple Range Table for Original Extract

ORIGINAL EXTRACT

Test: Duncan[a]

TREATMENT	N	Subset for alpha = .05 1
1.00	3	11.4300
2.00	3	11.4333
4.00	3	11.4333
5.00	3	11.4333
3.00	3	11.4367
6.00	3	11.4367
Sig.		.186

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.8 Duncan's Multiple Range Table for Fermentation Degree

FERMENTATION DEGREE

Duncan[a]

TREATMENT	N	Subset for alpha = .05 1
5.00	3	83.2000
2.00	3	83.2033
6.00	3	83.2033
1.00	3	83.2067
3.00	3	83.2067
4.00	3	83.2067
Sig.		.288

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.9 Duncan's Multiple Range Table for pH

pH

Duncan[a]

TREATMENT	N	Subset for alpha = .05 1
3.00	3	4.3200
5.00	3	4.3200
2.00	3	4.3233
1.00	3	4.3267
4.00	3	4.3267
6.00	3	4.3267
Sig.		.339

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.10 Duncan's Multiple Range Table for Protein Sensitivity

PROTEIN SENSITIVITY

Duncan[a]

		Subset for alpha = .05			
TREATMENT	N	1	2	3	4
1.00	3	5.8933			
3.00	3	5.8967			
4.00	3		5.9600		
2.00	3			6.0500	
5.00	3			6.0733	
6.00	3				6.2233
Sig.		.824	1.000	.137	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.11 Duncan's Multiple Range Table for Color

COLOR

Duncan[a]

		Subset for alpha = .05					
Treatment	N	1	2	3	4	5	6
1.00	3	5.36					
2.00	3		5.41				
3.00	3			5.66			
4.00	3				5.79		
5.00	3					6.06	
6.00	3						6.69
Sig.		1.00	1.00	1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.12 Duncan's Multiple Range Table for Chill Haze

CHILL HAZE

Duncan[a]

		Subset for alpha = .05				
Treatment	N	1	2	3	4	5
2.00	3	25.30				
1.00	3		28.20			
3.00	3			29.10		
5.00	3				29.93	
4.00	3				29.97	
6.00	3					31.50
Sig.		1.000	1.000	1.000	.705	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.13 ANOVA Table for 3 and 5 min HHP treatments

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Bitterness	Between Groups	3.682	9	0.409	132.685	0
	Within Groups	6.17E-02	20	3.10E-03		
	Total	3.744	29			
Ethanol	Between Groups	1.87E-04	9	2.10E-05	0.691	0.709
	Within Groups	6.00E-04	20	3.00E-05		
	Total	7.87E-04	29			
Density	Between Groups	2.82E-09	9	3.10E-10	2.608	0.036
	Within Groups	2.40E-09	20	1.20E-10		
	Total	5.22E-09	29			
Real Extract	Between Groups	1.00E-03	9	1.10E-04	0.816	0.608
	Within Groups	2.73E-03	20	1.40E-04		
	Total	3.74E-03	29			
Apparent Extract	Between Groups	5.37E-04	9	6.00E-05	1.491	0.218
	Within Groups	8.00E-04	20	4.00E-05		
	Total	1.34E-03	29			
Original Extract	Between Groups	1.37E-04	9	1.50E-05	0.506	0.853
	Within Groups	6.00E-04	20	3.00E-05		
	Total	7.37E-04	29			
Fermentation Degree	Between Groups	1.28E-03	9	1.00E-04	0.658	0.736
	Within Groups	4.33E-03	20	2.20E-04		
	Total	5.62E-03	29			
pH	Between Groups	5.20E-04	9	5.80E-05	1.333	0.281
	Within Groups	8.67E-04	20	4.30E-05		
	Total	1.39E-03	29			
Protein Sensitivity	Between Groups	14.11	9	1.57E+00	194.838	0
	Within Groups	1.61E-01	20	8.00E-03		
	Total	14.271	29			
Color	Between Groups	5.969	9	6.63E-01	3684.61	0
	Within Groups	3.60E-03	20	1.80E-04		
	Total	5.973	29			
Chill Haze	Between Groups	133.992	9	14.888	114.523	0
	Within Groups	2.6	20	0.13		
	Total	136.592	29			

Post Hoc Tests

Homogeneous Subsets

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.14 Duncan's Multiple Range Table for Bitterness

BITTERNESS

Duncan[a]

		Subset for alpha = .05		
TREATMENT	N	1	2	3
5.00	3	19.8000		
7.00	3	19.8000		
8.00	3	19.8000		
4.00	3	19.8333		
1.00	3	19.8500		
3.00	3	19.8500		
6.00	3	19.8500		
9.00	3		20.0500	
10.00	3		20.1000	
2.00	3			21.0000
Sig.		.343	.283	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.15 Duncan's Multiple Range Table for Ethanol

ETHANOL

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
1.00	3	5.0267
3.00	3	5.0267
4.00	3	5.0267
10.00	3	5.0267
2.00	3	5.0300
5.00	3	5.0300
6.00	3	5.0300
9.00	3	5.0300
7.00	3	5.0333
8.00	3	5.0333
Sig.		.210

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.16 Duncan's Multiple Range Table for Density

DENSITY

Duncan[a]

		Subset for alpha = .05		
TREATMENT	N	1	2	3
9.00	3	1.00577		
10.00	3	1.00577		
7.00	3	1.00577	1.00577	
8.00	3	1.00577	1.00577	
4.00	3	1.00578	1.00578	1.005780
3.00	3	1.00578	1.00578	1.005783
1.00	3	1.00579	1.00579	1.005787
6.00	3	1.00579	1.00579	1.005787
2.00	3		1.00579	1.005793
5.00	3			1.005800
Sig.		.119	.062	.060

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.17 Duncan's Multiple Range Table for Real Extract

REAL EXTRACT

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
8.00	3	3.8033
7.00	3	3.8067
6.00	3	3.8100
9.00	3	3.8133
1.00	3	3.8167
4.00	3	3.8167
5.00	3	3.8167
10.00	3	3.8167
2.00	3	3.8200
3.00	3	3.8233
Sig.		.085

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.18 Duncan's Multiple Range Table for Apparent Extract

APPARENT EXTRACT

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
5.00	3	2.0233
7.00	3	2.0233
9.00	3	2.0233
10.00	3	2.0233
3.00	3	2.0267
8.00	3	2.0267
2.00	3	2.0300
1.00	3	2.0333
4.00	3	2.0333
6.00	3	2.0333
Sig.		.109

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.19 Duncan's Multiple Range Table for Original Extract

ORIGINAL EXTRACT

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
5.00	3	11.4800
2.00	3	11.4833
6.00	3	11.4833
7.00	3	11.4833
8.00	3	11.4833
10.00	3	11.4833
1.00	3	11.4867
3.00	3	11.4867
4.00	3	11.4867
9.00	3	11.4867
Sig.		.210

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.20 Duncan's Multiple Range Table for Fermentation Degree

FERMENTATION DEGREE

Duncan[a]

		Subset for alpha = .05
TREATMENT	N	1
2.00	3	82.2967
6.00	3	82.3000
3.00	3	82.3067
5.00	3	82.3067
8.00	3	82.3067
9.00	3	82.3067
1.00	3	82.3133
7.00	3	82.3133
10.00	3	82.3133
4.00	3	82.3200
Sig.		.108

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.21 Duncan's Multiple Range Table for pH

pH

Duncan[a]

		Subset for alpha = .05	
TREATMENT	N	1	2
2.00	3	4.3700	
3.00	3	4.3700	
6.00	3	4.3700	
7.00	3	4.3700	
8.00	3	4.3700	
9.00	3	4.3700	
1.00	3	4.3733	4.3733
4.00	3	4.3733	4.3733
5.00	3	4.3767	4.3767
10.00	3		4.3833
Sig.		.292	.102

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.22 Duncan's Multiple Range Table for Protein Sensitivity

PROTEIN SENSITIVITY

Duncan[a]

		Subset for alpha = .05						
Treatment	N	1	2	3	4	5	6	7
3.00	3	5.56						
1.00	3		5.83					
4.00	3		5.89					
5.00	3			6.14				
7.00	3				6.43			
9.00	3				6.46			
6.00	3					6.98		
8.00	3						7.28	
2.00	3						7.40	
10.00	3							7.62
Sig.		1.00	.448	1.00	.720	1.00	.117	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.23 Duncan's Multiple Range Table for Color

COLOR

Duncan[a]

		Subset for alpha = .05									
Treatment	N	1	2	3	4	5	6	7	8	9	10
3.00	3	6.5									
4.00	3		6.53								
5.00	3			6.63							
6.00	3				6.7						
1.00	3					6.73					
7.00	3						6.93				
2.00	3							6.98			
8.00	3								7.03		
9.00	3									7.7	
10.00	3										7.86
Sig.		1.0	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.0	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Treatment

Untreated⇒ 1.00
Heat Pasteurization⇒ 2.00
HHP 200 MPa, 3 min⇒ 3.00
HHP 200 MPa, 5 min⇒ 4.00
HHP 250 MPa, 3 min⇒ 5.00
HHP 250 MPa, 5 min⇒ 6.00
HHP 300 MPa, 3 min⇒ 7.00
HHP 300 MPa, 5 min⇒ 8.00
HHP 350 MPa, 3 min⇒ 9.00
HHP 350 MPa, 5 min⇒ 10.00

Table A.24 Duncan's Multiple Range Table for Chill Haze

CHILL HAZE

Duncan[a]

		Subset for alpha = .05					
Treatment	N	1	2	3	4	5	6
3.00	3	21.7					
1.00	3	22.1					
5.00	3		23.9				
7.00	3		24.1				
4.00	3			25.3			
6.00	3			25.5	25.5		
9.00	3			25.8	25.8		
8.00	3				26.0		
2.00	3					26.7	
10.00	3						29.3
Sig.		.189	.505	.123	.123	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.25 ANOVA Table for Shelf-life Analysis

Source	D. Var.	SS	df	MS	F	Sig.
Corrected Model	pH	14.003[a]	41	0.342	3424.952	0
	Color	305.994[b]	41	7.463	3.581	0
	Bitterness	211.944[c]	41	5.169	58.971	0
	Protein	887.965[d]	41	21.658	179.484	0
	Chill Haze	10312.8[e]	41	251.532	786.515	0
Intercept	pH	1271.85	1	1271.85	1.30E+07	0
	Color	4819.49	1	4819.49	2312.457	0
	Bitterness	31916	1	31916	364089.8	0
	Protein	8365.5	1	8365.5	69327.58	0
	Chill Haze	45899.5	1	45899.5	143523	0
Treatment	pH	6.193	3	2.064	20701.18	0
	Color	66.352	3	22.117	10.612	0
	Bitterness	33.19	3	11.063	126.209	0
	Protein	90.797	3	30.266	250.82	0
	Chill Haze	4555.16	3	1518.39	4747.84	0
Time of storage	pH	1.759	5	0.352	3527.423	0
	Color	88.118	5	17.624	8.456	0
	Bitterness	120.539	5	24.108	275.016	0
	Protein	628.617	5	125.723	1041.911	0
	Chill Haze	640.131	5	128.026	400.325	0
Replication	pH	2.30E-03	2	1.10E-03	11.407	0
	Color	13.656	2	6.828	3.276	0.05
	Bitterness	1.577	2	0.788	8.994	0
	Protein	3.62	2	1.81	15.001	0
	Chill Haze	13.452	2	6.726	21.031	0

a	R Squared = 1.000 (Adjusted R Squared = .999)
b	R Squared = .830 (Adjusted R Squared = .598)
c	R Squared = .988 (Adjusted R Squared = .971)
d	R Squared = .996 (Adjusted R Squared = .990)
e	R Squared = .999 (Adjusted R Squared = .998)

Table A.25 ANOVA Table for Shelf-life Analysis Continued

Source	D. Var.	SS	df	MS	F	Sig.
Treatment * Time	pH	6.047	15	0.403	4042.23	0
	Color	86.325	15	5.755	2.761	0.1
	Bitterness	53.329	15	3.555	40.558	0
	Protein	161.385	15	10.759	88.164	0
	Chill Haze	5099.11	15	339.94	1062.96	0
Treatment * Replication	pH	1.30E-03	6	2.20E-04	2.214	0.07
	Color	30.302	6	5.05	2.423	0.05
	Bitterness	2.338	6	0.39	4.445	0
	Protein	1.321	6	0.22	1.825	0.13
	Chill Haze	0.476	6	7.90E-02	0.248	0.96
Time * Replication	pH	1.30E-03	10	1.30E-04	1.279	0.29
	Color	21.242	10	2.124	1.019	0.45
	Bitterness	0.97	10	9.70E-02	1.107	0.39
	Protein	2.24	10	0.222	1.843	0.1
	Chill Haze	4.485	10	0.448	1.402	0.23
Error	pH	3.00E-03	30	1.00E-04		
	Color	62.524	30	2.084		
	Bitterness	2.63	30	8.80E-02		
	Protein	3.62	30	0.121		
	Chill Haze	9.594	30	0.32		
Total	pH	1285.85	72			
	Color	5188.01	72			
	Bitterness	32130.6	72			
	Protein	9257.08	72			
	Chill Haze	56221.9	72			
Corrected Total	pH	14.006	71			
	Color	368.518	71			
	Bitterness	214.574	71			
	Protein	891.585	71			
	Chill Haze	10322.4	71			

Table A.26 ANOVA Table for comparison of unfiltered and filtered lager beers

ANOVA

		SS	df	MS	F	Sig.
Bitterness	Between Groups	5.3E-02	1	5.3E-02	.178	.682
	Within Groups	3.003	10	.300		
	Total	3.057	11			
Ethanol	Between Groups	1.8E-02	1	1.8E-02	8.064	.018
	Within Groups	2.2E-02	10	2.2E-03		
	Total	4.0E-02	11			
Density	Between Groups	3.1E-06	1	3.1E-06	22.858	.001
	Within Groups	1.3E-06	10	1.3E-07		
	Total	4.4E-06	11			
pH	Between Groups	4.4E-03	1	4.4E-03	2.936	.117
	Within Groups	1.5E-02	10	1.5E-03		
	Total	1.9E-02	11			
Fermentation Degree	Between Groups	9.7E-02	1	9.7E-02	.315	.587
	Within Groups	3.085	10	.308		
	Total	3.182	11			
Real Extract	Between Groups	.285	1	.285	42.389	.000
	Within Groups	6.7E-02	10	6.7E-03		
	Total	.352	11			
Apparent Extract	Between Groups	.258	1	.258	31.686	.000
	Within Groups	8.1E-02	10	8.1E-03		
	Total	.340	11			
Original Extract	Between Groups	3.5E-02	1	3.5E-02	1.470	.253
	Within Groups	.239	10	2.4E-02		
	Total	.275	11			
Color	Between Groups	7.053	1	7.053	96.182	.000
	Within Groups	.733	10	7.3E-02		
	Total	7.787	11			

APPENDIX B

FIGURES

Figure B.1 High Hydrostatic Pressurization Equipment

Figure B.2 UV-visible Spectrophotometer (UV-1601 Shimadzu)

Figure B.3 Anton Paar Beer Analyzer

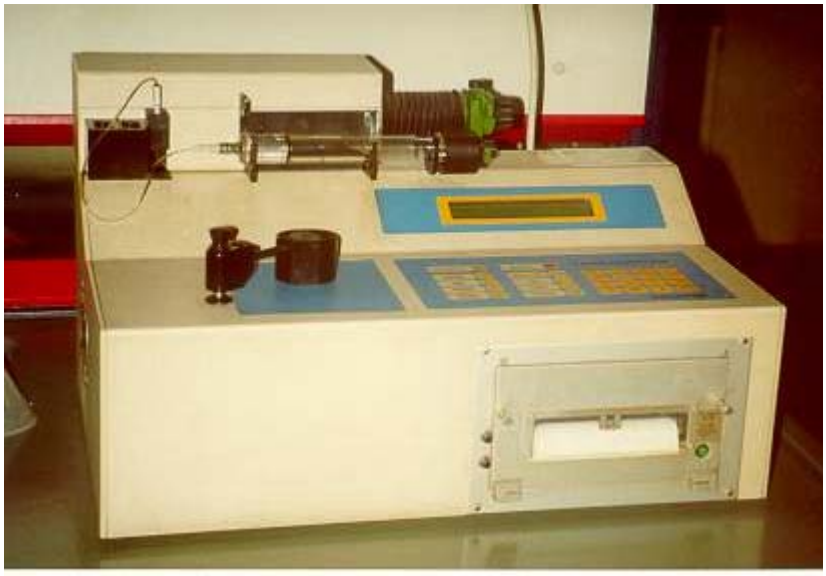

Figure B.4 Tannometer

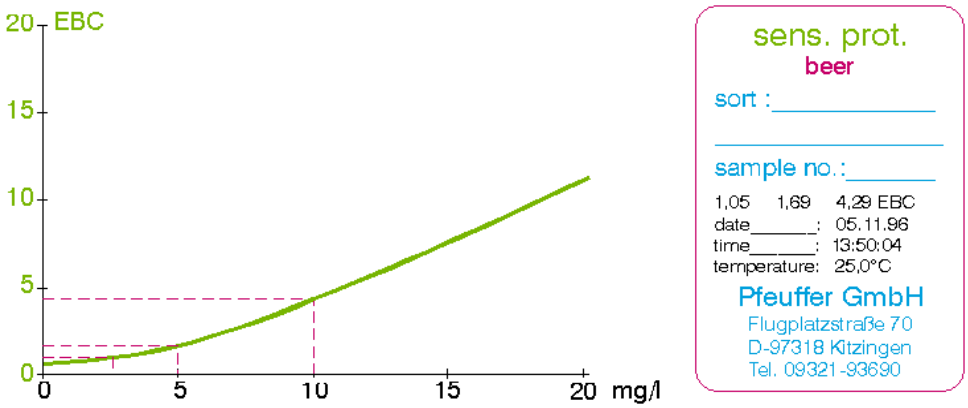

Figure B.5 Protein Sensitivity Measurement. (Adapted from Ref. 42) x-axis indicates mg of tannic acid per liters of beer while y-axis shows European Brewery Convention (EBC) unit.

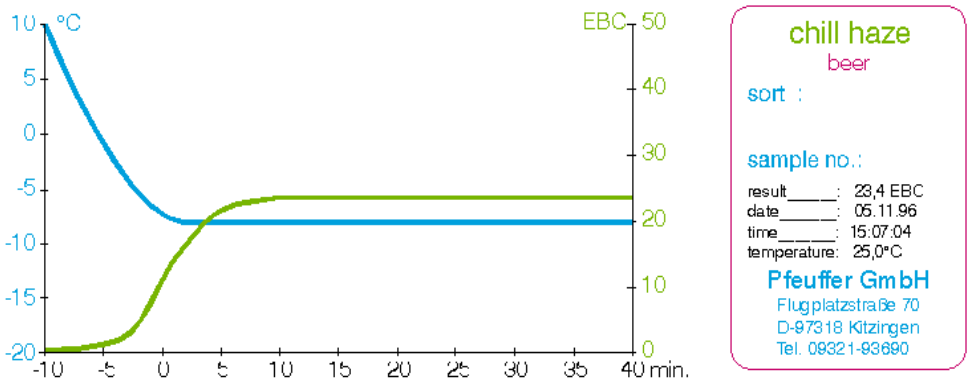

Figure B.6 Measurement of chill haze. (Adapted from Ref. 42)

Blue line indicates the temperature of beer while the green one shows the chill haze in EBC unit.

APPENDIX C

COMPOSITIONS OF MEDIA USED

Table C.1.* Composition of NBB medium

Casein peptone	5.0 g
Yeast Extract	5.0 g
Meat Extract	2.0 g
Tween-80	0.5 ml
Potassium acetate	6.0 g
Sodium phosphate, dibasic	2.0 g
L-Cysteine monohydrochloride	0.2 g
Cholorophenolred	70 mg
Glucose	15 g
Maltose	15 g
L-Malic acid	0.5 g
Agar	15 g
Beer/distilled water (1:1)	1000 ml
Final pH (at 25°C)	5.8±0.1

* Adapted from Ref. 31

Table C.2.* Composition of Wort Agar

Malt Extract	15 g
Peptone (from casein)	1.0 g
D-Maltose	12.5 g
Dextrin	2.5 g
Dipotassium hydrogen phosphate	1.0 g
Sodium phosphate, dibasic	2.0 g
Ammonium chloride	1.0 g
Agar	17 g
Wort (with 12 % extract)	1000 ml
Final pH (at 25°C)	4.8±0.2

* Adapted from Ref. 51

Table C.3. Composition of Plate Count Agar

Yeast Extract	2.5 g
Tryptone	5.0 g
Dextrose	1.0 g
Agar No.1	9.0 g
Distilled Water	1000 ml
Final pH (at 25°C)	7.0±0.2

Table C.4. Composition of M.R.S. Agar

Mixed Peptones	10.0 g
Yeast Extract	5.0 g
Meat Extract	10.0 g
Glucose	20.0 g
Potassium phosphate	2.0 g
Sodium acetate	5.0g
Ammonium citrate	2.0 g
Magnesium sulphate	0.2 g
Manganase sulphate	0.05 g
Tween 80	1.08 g
Agar No.1	15.0 g
Cycloheximide (1%)	10 ml
Distilled Water	1000 ml
Final pH (at 25°C)	6.4±0.2

Table C.5. Composition of Potato Dextrose Agar

Potato Infusion (dehydrated)	4.0 g
Tween 80	5.0 g
Glucose	20.0 g
Lecithin	0.7 g
Agar	15.0 g
Distilled Water	1000 ml
Final pH (at 25°C)	5.6±0.2

APPENDIX D

THE BREWING PROCESS

Figure D.1. An overview of brewing from malt milling to beer disperse.

APPENDIX E

QUALITY STANDARDS

Table E.1. Quality Standards Lager Beer produced by Efes Pilsen A.Ş.

Parameter	Units	Standard
Bitterness	BU	20 - 24
Chill Haze	EBC	max. 40
Color	EBC	5 - 7.5
Original Extract	w/w	11.40 - 11.70
Ethanol	v/v	max. 5.5
Fermentation Degree	%	82 - 88
pH	20°C	4.1 - 4.4
Protein Sensitivity	EBC	max. 15