

THE EFFECTS OF HIGH HYDROSTATIC PRESSURE (HHP) TREATMENT ON
SHELF LIFE AND QUALITY PARAMETERS OF CONVENTIONALLY
PRODUCED BOZA

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TREATMENT ON SHELF LIFE AND QUALITY PARAMETERS OF
CONVENTIONALLY PRODUCED BOZA**

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ABSTRACT

THE EFFECTS OF HIGH HYDROSTATIC PRESSURE (HHP) TREATMENT ON SHELF LIFE AND QUALITY PARAMETERS OF CONVENTIONALLY PRODUCED BOZA

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Nowadays, fermented products constitute an important part of the ready to eat products all around the world. A traditional fermented beverage Boza is produced with different formulations and techniques in different countries. Boza is mostly produced and consumed during winter in Turkey due to high energy and nutritional value. Traditionally produced Boza with no thermal process applications is sold in market, therefore, has a short shelf life even kept refrigerated. The aim of this study is to investigate effect of HHP treatment (150, 250, 350MPa; 5°C; 5 minutes) on the keeping parameters of Boza. Viscosity, pH, titratable acidity and total mesophilic

aerobic count and total yeast and mold count were investigated to be compared with the control. HHP combination of 350MPa at 5°C for 5 minutes was sufficient to decrease the bacterial load around 3.0 log cycles and all combinations of HHP treatment were effective on total destruction of yeast and mold. Also, HHP treatment at 350MPa at 5°C for 5 minutes had little impact on pH change during storage. All combinations of HHP treatments showed little differences for viscosity and titratable acidity.

Keywords: Boza, HHP, Shelf life

ÖZ

YÜKSEK HİDROSTATİK BASINÇ (YHB) UYGULAMASININ GELENEKSEL OLARAK ÜRETİLMİŞ BOZANIN RAF ÖMRÜ VE KALİTE PARAMETRELERİ ÜZERİNDEKİ ETKİSİ

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Fermente ürünler, bütün dünya çapında günümüzde tüketilen tüm gıdaların önemli bir kısmını oluşturmaktadır. Geleneksel fermente tahıl ürünlerinden biri olan Boza, farklı ülkelerde, farklı formülasyon ve yöntemlerle üretilmektedir. Yüksek besin ve enerji içeriği sebebiyle boza, Türkiye’de de kış günlerinde bolca tüketilmektedir. Geleneksel boza üretiminin sonunda raf ömrü arttırılması için herhangi bir işlem uygulanmamaktadır. Bu çalışmanın amacı, yüksek hidrostatik basınç (YHB) işleminin (150, 250, 350MPa; 5°C;5 dakika) etki ve sonuçlarının geleneksel olarak üretilmiş bozanın bazı kalite özellikleri üzerinden karşılaştırılmasıdır. Viskozite, titre

edilebilir asitlik, raf ömrü analizler ve toplam mezofilik aerobik bakteri ve toplam maya küf sayısı da mikrobiyolojik analiz olarak incelenmiştir.

350MPa 5°C 5 dakika YHB kombinasyonu bozada bakteriyel yükü yaklaşık olarak 3.0log azaltmıştır ve bütün YHB kombinasyonlarında maya ve küfler tamamen yok edilmiştir. Ayrıca, 350MPa 5°C 5 dakika YHB işlemi pH değişim analizlerinde de az bir etki göstermiştir. Bütün YHB işlemlerinde, viskozite ve titre edilebilir asitlikte az miktarda bir değişim gözlenmiştir.

Anahtar Kelimeler: Boza, YHB, Raf ömrü

Dedicated to My Family

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LIST OF ABBREVIATIONS

CFU: Colony Forming Units

HHP: High Hydrostatic Pressure

HIL: High Intensity Light

LAB: Lactic Acid Bacteria

MW: Molecular Weight

NaOH: Sodium Hydroxide

PCA: Plate Count Agar

PDA: Potato Dextrose Agar

PEF: Pulsed Electric Field

PHP: Phenolphthalein

TMAB: Total Mesophilic Aerobic Bacteria

TTA: Total Titratable Acidity

US: Ultrasound

CHAPTER 1

INTRODUCTION

1.1 Boza

Boza dates from the ancient cultures of Mesopotamia and Anatolia. It is said that Ottomans takes the recipe of boza from these ancient populations and distributes in the countries they conquer. According to Xenophon, the Greek historian, the origin of boza is eastern part of Anatolia in 400 BC which are stored in jars that were kept in underground. After Turk invasion to Anatolia, Turks accepted this nutritious drink and spread it to everywhere they go, under the name of boza. The boza is derived from a Persian word “buze” which means millet. During Ottoman Empire, boza became more popular and it had its golden age (Leblanc and Todorov, 2011). Yeğin and Labore (2012) claim that with the help of Turk immigration, the consumption and production of this beverage spread to where Turks go. After that, it was concluded as a traditional drink of Caucasus Countries and Balkans such as Bulgaria, Romania, Albania, Serbia, Montenegro, and Macedonia. Despite of the production of these Balkan countries, some differences exist between the recipe of boza because of the type of cereals and proportions of them. Generally, cereals are used which are rich in these regions. There are a lot of recipes for boza all around the world. In Albania, boza is made from maize, wheat, millet, barley and chick peas fermented, in Turkey wheat and corn and in Bulgaria and Romania wheat and millet (Alsancak et al., 2009). It has yellowish color and thick consistency with creamy structure (Petrova and Petrov, 2011).

In Table 1.1, chemical properties of boza are given with different ingredients according to TS 9778 (Anonymous, 1992).

Table 1.1: Chemical Properties of Boza with Different Ingredients (Pamir, 1961)

Analyses	Cracked Wheat Boza (%)	Corn + Wheat Boza (%)	Millet + Corn Boza (%)
dry matter	29.93	25.20	23.65
total sugar	17.10	17.10	11.60
Protein	1.66	1.14	0.88
Ash	0.17	0.12	0.16
crude fiber	0.00	0.00	0.02
Lipid	-	0.21	0.27

Boza is a very valuable product which contains vitamins A, C, E and four types of vitamin B, which make people more active and energetic. It is also suitable for vegetarians because it is originated from plants and it is a very nutritious drink. Therefore, it is a good substitute for vegetarians (Petrova and Petrov, 2011).

Boza is rich in nutritious contents and has energy value (Birer, 1987), and they are given in Table 1.2 however; its main reason for consumption is its relieving effect rather than nutritional values. The relieving effect of boza is because of carbon dioxide which is partially dissolved. The sour taste and flavor is sensed when boza is consumed (Pamir, 1961).

Table 1.2: Nutritive Value of Boza (Birer, 1987)

Nutritive value (approx. per serving)	
Energy	242 cal
Protein	3.5 g
Fat	0.5 g
Carbohydrate	5705 g
Calcium	29 mg
Iron	1.3 mg
Phosphorus	97 mg
Zinc	1 mg
Sodium	1 mg
Thiamin (B ₁)	0.09 mg
Riboflavin (B ₂)	0.05 mg
Niacin	1.16 mg
Cholesterol	1 mg

1.2 Traditional Boza

Boza is a kind of malt drink. It is a fermentation product of millet, maize, wheat, barley, chick peas, and corn by yeast and LAB (lactic acid bacteria). From the microflora of boza *Leuconostoc oenos*, *Leuconostoc paramesenteroides*, *Leuconostoc mesenteroides subsp. dextranicum*, *Leuconostoc mesenteroides subsp. mesenteroides*, *Lactobacillus fermentum*, *Lactobacillus confusus*, *Lactobacillus sanfrancisco*, *Lactobacillus coryniformis* etc. are isolated as lactic acid bacteria (LAB) (Evren et al., 2011). Leblanc and Todorov (2011) state the reason of high nutritional value of boza that LAB and most probably yeast can produce a number of hydrosoluble vitamins. Most of the LAB isolated from boza produce antimicrobial compounds, including bacteriocins, very useful for the extension of shelf life of the product and possibly demonstrate health benefits.

Combination of cereals can be changed according to recipe. Boza has a typical sour-sweet taste depending on its acidity, ingredients and also the method of preparation.

Traditional boza production (Figure 1.1) has six main steps (Yeğın and Labore, 2012):

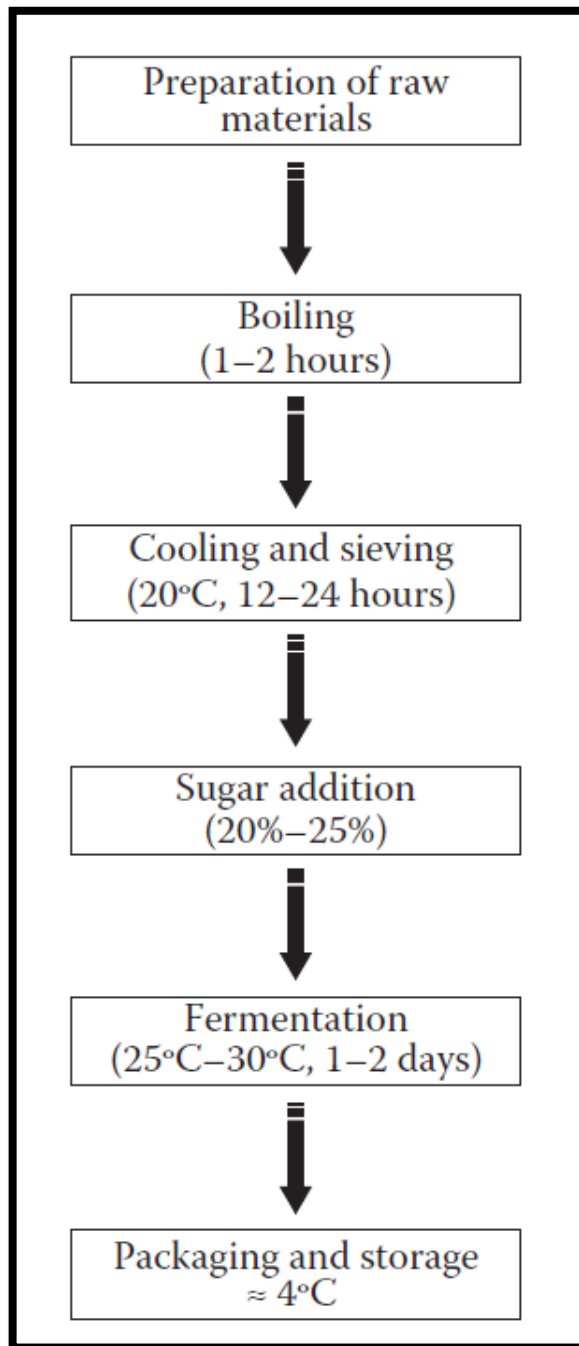


Figure 1.1: Steps of Boza Production (Yeğın and Labore, 2012)

1.2.1 Preparation of Raw Materials

Traditional boza consists of different type of cereals such as millet, wheat, maize, rice and corn. Millet is generally used to get best quality and taste. Firstly, broken raw materials passed through sieves and cleaned from all materials .After that, they are milled to produce semolina or floor (Yeğın and Labore, 2012; Basaran, 1999).

1.2.2 Boiling

After preparation of raw materials, mixture of one volume of the ingredients and water with 4 to 6 volume is mixed with continuously stirring. After mixing, open or steam- jacketed boiler is used to boil mixture. To prevent aggregation and to get proper mixture, stirring is a crucial. Stainless steel boiler is used with different sizes changing to capacity of production facility. During boiling, water addition is needed because hot mixture absorbs water. After about 1 to 2 hours of stirring depending on the temperature, obtained product is called as a mash (Arıcı and Dağlıođlu, 2002).

1.2.3 Cooling and Sieving

After boiling, mash is kept at room temperature to cool down to 20°C. Generally, cooling time changes 12 hours to 24 hours. Traditionally, to make quick cooling, mash is stored in the marble vessels. Dilution of mash is done with water under continuous stirring until the reach of desired temperature. When dilution step is finished, product goes to further sieving process to eliminate undesirable particles (Yeğın and Labore, 2012).

1.2.4 Sugar Addition

After sieving of boza, it needs to be sweetened after processes because it is the necessary substrate for LAB and yeast. Generally, addition of 20%- 25% sugar (saccharose powder) is enough for boza process (Yeğın and Labore, 2012).

1.2.5 Fermentation

Previously fermented boza is kept an inoculum when the season of production starts. Also, sourdough and yoghurt can be considered as inoculants like boza when boza is not available. The ingredients and fermentation temperature affect the amount of starter culture supplied for a good fermentation. Temperature of fermentation could change between 25-30°C. Fermentation time is about 2 days, relying on temperature of fermentation environment. 2% inoculated raw materials can be consumed after a 1 day incubation period at room temperature (25°C) (Yeğın and Labore, 2012).

Çopur and Tamer (2003) reported that boza fermentation has two types: lactic acid fermentation and alcoholic fermentation. They occur at the same time. Boza fermentation is carried out by natural fermentation of yeasts and LAB. During traditional processing, microorganisms are not produced controlled, therefore variations can be occurring. LAB is always dominant. Because of lactic acid fermentation, decrease of pH causes to obtain natural acidic and sourish taste of boza. Lactic acid fermentation helps in increasing the nutritional value, the safety, and acceptability of a wide range of cereal-based boza (Yeğın and Labore, 2012).

Alcohol fermentation is also very crucial for to get natural odor and mouth feel. It produces CO₂ gas therefore volume increase is observed (Yeğın and Labore, 2012).

During boza fermentation, there are some changes are observed which are: physical and biochemical. During the fermentation process of boza for 1day, Hancıoğlu and Karapınar (1997) observed some changes in values of LAB and yeast. There were some rises during this period, LAB and yeast rose from 7.6×10^6 cfu/mL and 2.25×10^5 cfu /mL to 4.6×10^8 cfu/mL and 8.1×10^6 cfu/mL, respectively. According to Gotcheva et al. (2001) observed some changes during fermentation of different kind of Bulgarian boza. They reported that LAB and yeasts amount changing from sample to sample but the average amount is about 10.5×10^7 cfu/mL and of which approximately 70% were LAB. Turkish boza had ten times more microbial load than Bulgarian ones. These differences concluded as because of the differences between microflora.

1.2.6 Packaging and Storage

Before packaging boza cools down to 4°C and put in containers after 24-48 hours, after fermentation. It is needed to consume within 3-5 days after putting packages. The shelf life is limited for boza and its maximum shelf life is less than one week. Because of the acidity of boza, temperature control is very crucial to extend. Gotcheva et al. (2001) worked on different kinds of Bulgarian boza to determine product stability for ten days. It is concluded that boza could be stored at 4°C for eleven days, this temperature considered as a good temperature for storage (Yeğin and Labore, 2012).

1.3 Microbial Flora of Boza

Boza is a cereal beverage produced through lactic acid and yeast fermentation. Pamir (1961) isolated bacteria and yeasts from boza ready for consumption, with the raw materials, maize, rice and millet regards flours. *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Candida mycoderma* and, *Torulopsis candida* were identified as the major microflora of boza. The latter two are responsible for alcoholic fermentation. The others are not desired yeasts since they result in undesirable changes under extreme growth.

In a study carried out by Hancıoğlu and Karapınar (1997), seventy seven isolates of LAB and seventy yeasts were isolated from boza fermented in the laboratory with the raw materials wheat, rice and maize flours in the ratio of 1:1:2, respectively. The inoculum was bought from a local market in İzmir. LAB isolated from boza included *Leuconostoc paramesenteroids* (18.6%), *Lactobacillus sanfransisco* (21.9%), *Leuconostoc mesenteroides subsp. dextranicum* (7.3%), and *Lactobacillus fermentum* (6.5%), *Leuconostoc oenos* (3.7%). The yeasts isolated comprised *Saccharomyces uvarum* (83.0%) and *Saccharomyces cerevisiae* (17.0 %).

1.4 Shelf Life of Boza

Production of traditional boza contains an initial boiling process with no thermal treatment or pasteurization after fermentation. Because of that reason, shelf life of traditional boza is 2-3 days at room temperature and slightly increases under refrigeration. Heat treatment has many undesirable effects on quality parameters of boza. It causes browning reactions, following browning reactions; taste, flavor and sourness occur. Researches reveal that to extend shelf life of boza, storage temperature is one of the important parameters. Storage of boza at 4°C helps to extend shelf life of traditional boza. However, at room temperature boza can be stored only for 2-3 days (Köse and Durak, 1998), because of that boza is not

preferred in hot summer days, so shelf life limitation due to high temperature of summer days makes boza a winter drink (Yeğın and Labore, 2012).

In the food industry, except boza, for longer shelf life and high nutritious value maintaining different treatments are applied to food products. Treatments are separated to two types according to the use of heat treatment or not. First one is thermal treatment and second one is non-thermal treatment (Petrova and Petrov, 2011).

1.5 Standards of Boza

Boza, one of the traditional fermented cereal products, is defined in Standards of Turkey TS 9778 as a drink which is prepared from alcoholic and lactic acid fermentation of cleaned and selected semolina and flour of millet, rice, wheat, corn etc. (Anonymous, 1992).

Boza is divided into two classes through the amount of acid produced during fermentation according to the Standards stated by the Institute of Turkish Standards: sweet and sour. According to TS 9778, standards of boza are given in the following tables: Organoleptic Properties (Table 1.3), Microbial Properties (Table 1.4), Chemical Characteristics (Table 1.5) and Categories of Boza (Table 1.6) (Anonymous, 1992).

Table 1.3: Organoleptic Properties of Boza (Anonymous, 1992)

Color	pale or dark cream color
Appearance	homogeneous, unique, thick fluid consistency
Viscosity	high concentrate fluidity
taste and flavor	unique taste and flavor, dough taste foreign taste and flavor, mold flavor not to be detected
visible impurity, foreign materials and cereal glumes	not to be detected

Table 1.4: Microbiological Properties of Boza (Anonymous, 1992)

Properties	Limits (cfu/g)
Coliform bacteria	maximum 10
Fecal coliform	-
<i>Salmonella</i>	-
<i>Staphylococcus aureus</i>	-
Mold	maximum 20

Table 1.5: Chemical Properties of Boza (Anonymous, 1992)

Properties	Limits (% w/w)
Total dry matter	minimum 20
Total sugar (in terms of sucrose)	minimum 10
Ethyl alcohol	maximum 2
Ash not dissolved in 10% HCl	maximum 0.2

Table 1.6: Categories of Boza (Anonymous, 1992)

Properties	SWEET	SOUR
<i>Organoleptic</i>		
Taste	unique sweetness	unique sourness
<i>Chemical</i>	LEVELS (g/100g)	
total acidity (lactic acid)	0.2-0.5	0.5-1.0
volatile acidity (acetic acid)	max 0.1	max 0.2

1.6 Food Preservations and High Hydrostatic Pressure (HHP)

In the last years, consumers prefer food products with minimal processing with natural color and flavor and enough shelf life for distribution and reasonable home storage before they consume products. These desires are only achieved by some processing of products because only they keep their nutritional value and their organoleptic properties with little heat treatment or none. Traditional methods are one of these ways, also irradiation as preferred in most of the countries as a minimal processing method. Apart from those, chilling and controlled and modified atmospheres are used to eliminate microbial growth. Also, some combinations of these novel methods are done to achieve mild preservation. For example, pulsed electric fields (PEF), HHP, high intensity light (HIL) and ultrasound (US) destroy

microorganisms and enzyme without the addition of heat. Because of little damage on pigments, vitamins and compounds, the natural and organoleptic properties of products are kept in minimal processing. These products have consumer appeal in markets and higher quality due to keeping of nutritional and sensory properties of the products with high prices (Fellows, 2000).

1. 6.1 History of HHP

HHP is a process which is pressurizing of foods in the range of 300-800 MPa for a predetermined time resulting inactivation of microorganisms and enzymes without any change in the flavor and nutrients of the food product when compared to thermal processing (Ohlsson and Bengtsson, 2009).

Hite (1899) were the person who first reported the persuasive result of HHP on the microorganisms, and he extended shelf life of milk with HHP. Also, Hite et al. (1914) reported that they observed little or no-growth on non-spore forming *Bacillus prodigious* (now called as *Serratia marcescens*), and vegetative cells of *Bacillus subtilis*, yeasts, as well as pathogens like *Bacillus typhosus*, *Bacillus diphtheriae*, anthrax, tuberculosis and bubonic plague. Cruess (1924) did some predictions about usage of HHP on fruit juices.

Macfarlane (1973) used HHP with meat and he demonstrated usage of tenderization of meat with pressurization. Zipp and Kauzmann (1973) investigated metmyoglobin denaturation under high pressure and the effect of ionic environment.

By 1990, the first products produced with HHP released on market. Pressurized jams such as apple, kiwi, raspberry, and strawberry put in flexible plastic jars and sold by one company. Apart from this company, two other companies are decided to produce juices such as orange and grape fruit juices. These jams had two months of shelf life at refrigeration temperatures and other products like fruit jellies, fruit yoghurts, salad dressing and sauces. However, these products cost too much than conventionally

produced ones. Therefore, they are sold three or four times expensive. But they had high quality, more ensured and good flavor and texture (Tewari et al., 1999).

In the table below (Table1.7), some current application of HHP is given (Ohlsson and Bengtsson, 2009).

Table 1.7: Current Applications of HHP (Ohlsson and Bengtsson, 2009)

Product	Manufacturer	Process Conditions
Jams, fruit dressing, fruit sauce topping, yoghurt, fruit jelly	Meidi-ya Company, Japan	400 MPa, 10-30 min, 20°C
Grapefruit juice	Pokka Corp., Japan	120-400 MPa, 2-20 min, 20°C
Mandarin juice	Wakayama Food Ind., Japan	300-400 MPa, 2-3 min, 20°C
Non-frozen tropical fruit's	Nishin Oil Mills, Japan	50-200 MPa (freeze at -18°C)
Tenderized beef	Fuji CikuMutterham, Japan	100-500 MPa, 30-40 min, 20°C
Avocado	Pokka Corp., Japan	700 MPa, 600-800 L/h
Orange juice	Ultifruit, France	500 MPa, 5-10 min cycles

1.6.2 General Principle and Mechanism of HHP

HHP is concluded as a non-thermal process even if rise in pressure causes a little increase in the temperature. All reactions and structural changes which contain volume change and gelation of proteins and starch are affected by high pressure (Ohlsson and Bengtsson, 2009). According to University de Lleida, volume reduction is at 100 MPa, 200 MPa and 400 MPa reported as 4%, 7% and 11.5% respectively during pressurization (Anonymous, 2005-2006).

Two types of pressure can be discussed in HHP process:

- Static pressures where the value of pressure can be kept during process. Among these static pressures two different categories exist:

Isostatic pressures occur when pressure is the same in all directions. This is in particular the case in water (hydrostatic pressure).

Non-isostatic pressures where a pressure gradient is induced versus the structure of the equipment generating the pressure or versus the non-homogeneous compressibility of the medium (in particular in the case of solids with an anisotropic structure).

- Dynamic pressures concern super-high pressures developed during a short-time and associated in the main cases with temperature. Shock-waves are mainly used for generating such pressures (Buzrul, 2008).

The mechanism of microorganism inactivation is combination of permeabilisation of the cell membrane and the breakdown of non-covalent bonds and possible effect on DNA and RNA of the cell. For inactivation of vegetative cells the hydrostatic pressure must be around 3000 bars (300 MPa) at room temperature, however inactivation of spores requires 6000 bars (600 MPa) with combination of temperature (Ohlsson and Bengtsson, 2009).

Another important parameter in this technology is moisture, for effective pressurizing moisture levels of products are needed to be higher than 40% (Ohlsson and Bengtsson, 2009).

1.6.3 HHP Equipment and Technology

HHP system has four main components:

1. a pressure vessel and its closure
2. a pressure generating system
3. a temperature control device
4. a materials handling system

Pressure vessels are generally made of monoblocks which are produced from single pieces of material. These materials can withstand high pressure values between 400 - 600MPa. For elevated pressure values, wire-wound or pressurized multilayer vessels are preferred (Mertens, 1995). Threaded steel closure seals vessels. This closure has an interrupted thread, which can be removed more quickly, or by a sealed frame that is positioned over the vessel. During the operation, although all air has been removed, a pressure transmitting medium (oil or water) is pumped from reservoir into the pressure vessel which is used with a pressure intensifier till the vessel is reached. This is known as “indirect compression” as well as it needs static pressure seals. Contrary to first method, the second method termed as “direct compression”. There is a piston to compress the vessel. Also, dynamic pressure is needed to seal internal vessel surface to the piston. These are forged wearing and are not used in commercially (Fellows, 2000).

Pumping a heating/cooling medium can achieve temperature control in commercial operations, pressure vessel surrounded by a jacket. It is necessary almost all of the applications as a permanent temperature. However, for the very huge temperature change, the large thermal inertia of the vessel and relatively small heat transfer area

make this type of temperature check too slow to respond to changes. In such situations, an internal heat exchanger is fitted (Raghubeer, 2008).

In container processing and bulk processing are methods of processing of foods in high pressure vessels. Since foods decrease in volume at the very high pressure which is used in processing (to illustrate, water reduces in volume by approximately 4% at 100MPa, 7% at 200MPa, 11.5% 400MPa and 600MPa at 22°C), there is serious stress and distortion to the package and the seal. While in container processing is used as the pressure is uniform. Bulk handling is similar which needs only pipes, pumps and valves.

Practically, the stability of pressure chambers has 600 MPa limitations because of food processing and cost reasons. However, this limitation is enough for most applications. Although semi-continues production may be achieved, units are batch systems due to technical reasons (Jongen, 2002). Semi-continuous processing is very efficient the in terms of energy, but the capital costs of equipment are high. Also, liquid foods can be used as the pressurizing fluid by direct pumping with high pressure pumps; thus, the capital cost of a pressure vessel would be reduced and material handling can be simplified. If liquids were speedily decompressed towards a small orifice, the shearing forces on microorganisms would be increased by the turbulent flow and high velocity. This ascends the destruction rate of microorganism (Earnshaw, 1992). Knorr (1995) said that developments contain high pressure blanching, freeze concentration and pressure freezing. According to initial results, the fruits, which are blanched by pressure, are dried faster than conventional hot water blanched fruits.

1.6 Aim of This Study

The main aim of this study was to get the potential of HHP application to extend the shelf life of boza; since heat treatment is not effectively applied to boza due to its high sugar content in industrial processes. Besides, HHP is well documented as a non-thermal treatment that affects quality parameters of many foods at minimum levels. As a conclusion, in this study comparison of effects for the followings on shelf-life and quality parameters: i) HHP treated boza at different pressure, temperature and time combinations and ii) fresh boza is planned.

CHAPTER 2

MATERIALS AND METHODS

2.1 Material

2.1.1 Supplying of Samples

Boza samples were supplied by Balaban Boza at Lüleburgaz, Kırklareli. The samples were freshly supplied every time for each assay because of limited shelf-life of boza. The samples were sent to our laboratory with sugar-free formulation to avoid quick decay of boza during transportation. After addition of predetermined amount of sugar, boza was kept for about one hour to form its required properties. Production of boza is generally in winter season therefore there is a negligible increase of microbial load during transportation due to cooler weather conditions that makes boza stable for some times.

2.1.2 Reagents

Chemicals used were of analytical grade. The materials (biological and chemical) that were not specified were bought from Merck (Darmstadt, Germany). Before

usage, all of the equipment used were sanitized with %60 ethanol (Merck, Germany) and rinsed with sterile distilled water thereafter.

2.2 Method

2.2.1 Preparation of Samples

The samples were taken just before experiment and kept in refrigerator till the experiments. The samples are loaded into the scintillation bottles and sealed. These bottles are used because of their plastic composition, suitable dimensions and pressure resistance; they are suitable for HHP treatment. Boza can be easily loaded into these bottles and stored in the refrigerator. Samples are loaded in these tubes just before each treatment.

For the shelf life analysis, the samples were kept at room temperature until the analyses.

2.2.2 HHP Treatment

In the experiments, High Hydrostatic Pressure was performed with 760.01 type high pressure equipment supplied by SITEC-Sieber Engineering AG (Zurich, Switzerland) in the Food Engineering Department of Middle East Technical University. Vessel is with 100 ml volume and with diameter of 24mm and length of 153mm. Ethylene glycol is used as an agent and it was circulated around the jacketed pressure vessel. 7000 bar (700 MPa) is a maximum design pressure and temperatures range is between -10 – 80°C. Thermocouple which is used to keep and monitor the required temperature, a built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used. In order to measure the inner

temperature of the vessel during treatment, upper plugged is designed. Water (distilled) is used as transmitting medium to transmit pressure. Pressure applied and release times were designed as less than 20 seconds for each of pressurizations.

In this study, pressurization time reported did not include the pressure apply and release times. During the time period of pressurization to 400 MPa, temperature increase due to adiabatic heating was reduced to 4-5°C. Temperature in the study is the actual process temperature during holding time at applied pressure levels.

Fresh boza samples were loaded into 20ml scintillation bottles (LP Italiana SPA) and placed into pressurization vessel. After that, samples were pressurized at 150, 250 and 350MPa at 5°C for 5minutes. After pressurization, the vials were taken and cooled in an ice bath, immediately. As control sample, unpressurized boza was used.

2.2.3 Analyses

2.2.3.1 Microbial Analyses

Preparation of 10 ml of samples were done under sterile conditions and then these samples were homogenized in sterile stomacher bags (Seward Medical, England) with a stomacher (Seward Laboratory Blender Stomacher 400, England) in 90 ml sterile peptone water solution. Subsequently, decimal dilution series of the homogenate were performed in peptone water. Count of total bacteria, yeast and mold were targeted. Microbial counts were performed on unprocessed and HHP treated samples at room temperature after 24, 48 and 72 hours.

On the non-selective plate count agar (PCA) were used for the enumeration of total mesophilic aerobic bacteria with spread plate technique was used. 100 µl aliquot was poured into plates, spread plated and incubated at 37°C for 48 hour. For the enumeration of mold and yeast, spread plate technique was used on the potato dextrose agar (PDA). 100 µl aliquot was poured into plates, spread plated and

incubated at 37°C for 48 hour. All of the experiments were done as two parallel measurements. At the end of incubation, plates with 30-300 colonies were considered for enumeration. All counts were expressed as log₁₀ colony forming units (CFU)/ml boza sample. It was calculated by using the formula below (2.1) (Harley and Prescott, 1997):

$$\frac{\text{cfu}}{\text{ml boza}} = \frac{\text{number of colonies} \times \text{dilution factor of boza}}{\text{volume of culture plate}} \quad (2.1)$$

2.2.3.2 Physical Analyses

2.2.3.2.1 pH Analyses

For the analyses of the acidity, 10 ml of the sample is diluted with 25 ml distilled water and pH is measured by pH meter (Mettler-Toledo MP220, Schwerzenbach, Switzerland); experiments were performed as three parallel measurements.

2.2.3.2.2 Viscosity Analyses

This experiment was performed by using rheometer (TA Instruments AR2000). Viscosity profiles of untreated and treated boza samples were obtained versus shear rate (1/s). Viscosity values were measured in Pa.s.

This equipment is suitable for most of the applications such as characterization of delicate structures in different viscosity fluids, polymer melts, and reactive materials.

For constant amount of samples, three parallel measurements were done with rheometer at 20°C with sheer rate between 1 and 30.

2.2.3.3 Chemical Analyses

2.2.3.3.1 Titratable Acidity Analyses

Titrateable acidity is determined according to Thyagaraja et al. (1992). 10 grams of samples were transferred to 100 ml beakers and 25 ml of distilled water and few drops of phenolphthalein (PHP) are added. The samples are mixed well and titrated with 0.1 N NaOH to a pale rose color. Acidity was expressed as percentage of lactic acid (w/w) according to the formula given below (2.2) (Gallander, 1987):

$$\%acid = \frac{N \text{ NaOH} \times \text{ml NaOH} \times \text{MWlactic acid}}{\text{sample weight}} \times 100 \quad (2.2)$$

2.2.3.4 Shelf Life Analyses

For shelf life analyses, treated and untreated samples were kept at room temperature for 15 days and during this time measurements for microbial load, pH and titrateable acidity changes are done. Microbial load, pH and titrateable acidity measurements are done for the first three days. After three days, only pH measurements were done for shelf life studies for the remaining 12 days. New sample tubes were used for each experiment.

2.2.3.5 Statistical Analyses

In order to draw graphs Microsoft Excel 2010 was used.

CHAPTER 3

RESULTS AND DISCUSSION

Boza is defined as a dense product prepared by fermentation of a solution containing mixtures of different cereals- in the form of flour or semolina- water and sugar. In boza production, sugar addition is done after obtaining maize and after fermentation process, alcohol and lactic acid are produced (Anonymous, 1992). Limited shelf life of boza is the main drawbacks of the boza industry. Heat treatments of boza in order to increase the quality during storage have some undesirable effects therefore pasteurization of boza to increase the shelf life is not very practical. HHP was introduced as a minimal food processing technique and an alternative method to heat treatment (Knorr et al., 2002). The aim of this study was to investigate the effects of HHP treatment of boza and evaluation of the use of HHP for shelf-life extension of traditionally processed boza.

3.1 Assessment of Pressure, Temperature and Time Combinations

3.1.1 Results of Microbial Analyses

HHP has been considered as an alternative non-thermal technology, which is effective on inactivation of microorganisms (Linton et al., 1999; Parish, 1998; Reyns et al., 2000; Teo et al., 2001; Zook et al., 1999). Mechanism of HHP treatment in order to destroy microorganism is inactivation of microorganisms (Norton et al., 2008).

Tables for microbial load of initial and final samples are given In Appendix A.

In Figure 3.1, Results of mean total mesophilic aerobic bacterial load of HHP treated boza samples are given. Initial load of samples are 8.27log cfu/ml. The most effective reduction is observed for HHP of 350MPa at 5°C for 5 minutes. And after that, HHP of 250MPa at 5°C for 5 minutes follows as the next most effective reductions.

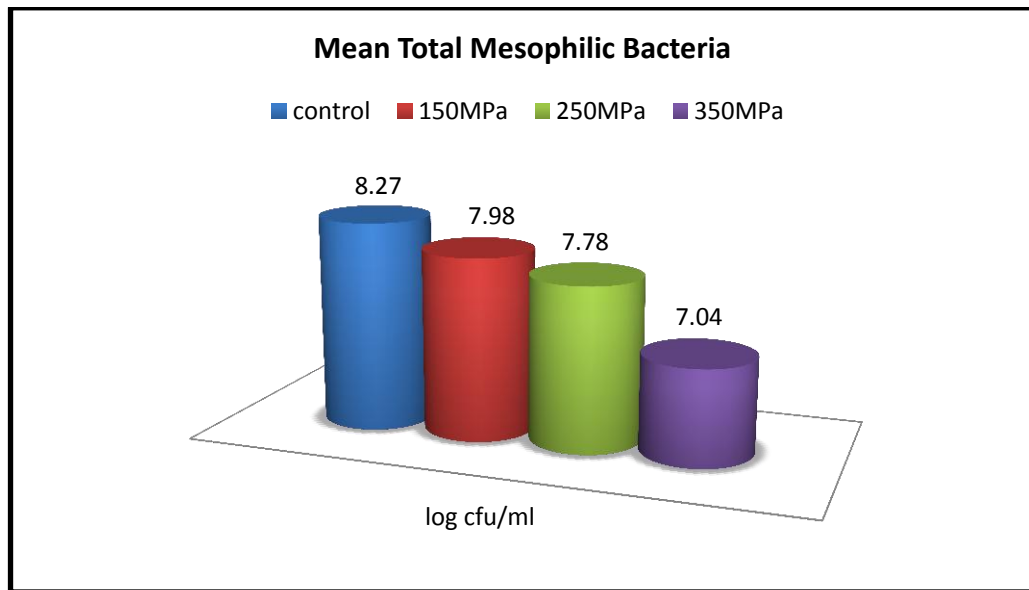


Figure 3.1: Mean Total Mesophilic Bacteria (log cfu/ml) of HHP Treated and Control Boza Samples (**control** for unprocessed boza, **150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, and **350MPa** for 350MPa at 5°C for 5 minutes).

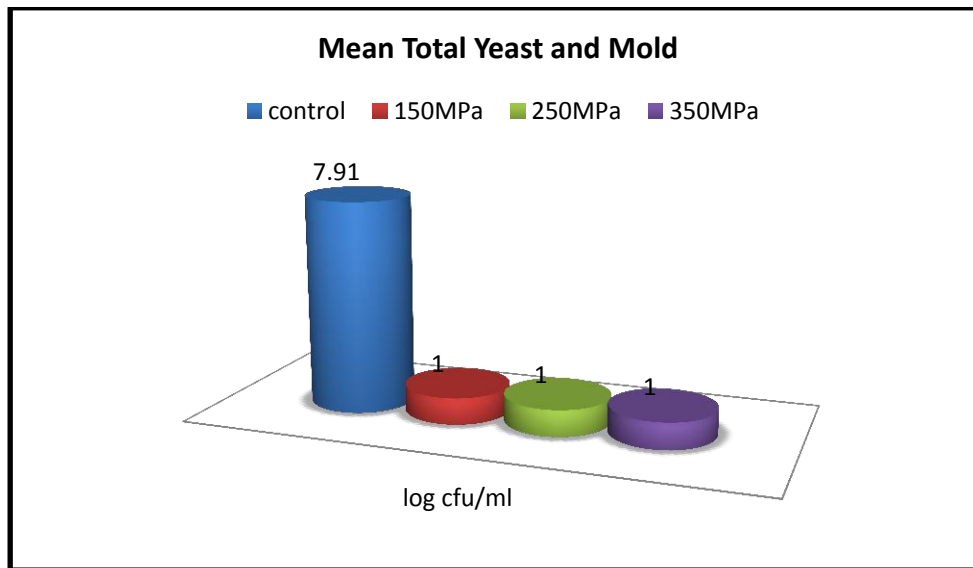


Figure 3.2: Mean Total Yeast and Mold Population (log cfu/ml) of HHP Treated and Control Boza Sample (**control** for unprocessed boza, **150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, and **350MPa** for 350MPa at 5°C for 5 minutes).

Yeast or molds are reported to be highly inactivated at 400 MPa (Raghubeer, 2008). After HHP treatment, there is total destruction in mold and yeasts (Figure 3.2). Molds and yeasts are highly affected by HHP and it has detrimental effect on their population.

3.1.2 Results of pH Analyses

Turkish Standards for Boza, TS 9778 does not give pH or pH range for boza and that makes very hard to get best results boza producers in order to prepare boza ready to drink. Also, there are too many different pH levels for boza because of very different recipes. Yücel and Köse (2002) collected ten samples from İzmir market and these raw boza samples had pH within the range of 4.6-6.7 just before fermentation. After fermentation, pH levels drops below 4.0, pH ranges were between 3.22 and 3.82 with an average pH of 3.5 ± 0.1 . Pamir (1961) reported average pH levels for boza samples

as 3.66 ± 0.1 and Kozat (2000) collected 6 samples from market and pH of the samples ranged between 2.8 and 3.3 and the average was calculated as 3.1 ± 0.1 . pH change is observed after 30 h fermentation for Boza prepared with combination of rice and wheat flour and pH level decreases from 5.8 to 3.5 (Hayta et al., 2001). Until pH of boza of reached 3.5, it is acceptable as suitable for consumption at every levels of fermentation process (Gotcheva et al., 2000).

In our studies, boza samples had initial pH levels between 4.04-4.08 just after production. After three days, pH was dropped to 3.60- 3.65 for the boza samples kept at room temperature. After pH of 3.60, boza has started to sour with very acidulous odor and over-fermented appearance. Therefore, in this study it became very important to measure pH of boza samples to determine shelf life of boza samples for each treatment. In Appendix B.1 pH changes of boza samples are given in detail as unprocessed boza and processed boza samples with HHP. Boza is categorized as non-consumable and/or acceptable after reaching pH to 3.65, the results of shelf life analyses with HHP treatment of 350MPa at 5°C for 5 minutes were observed as the longest.

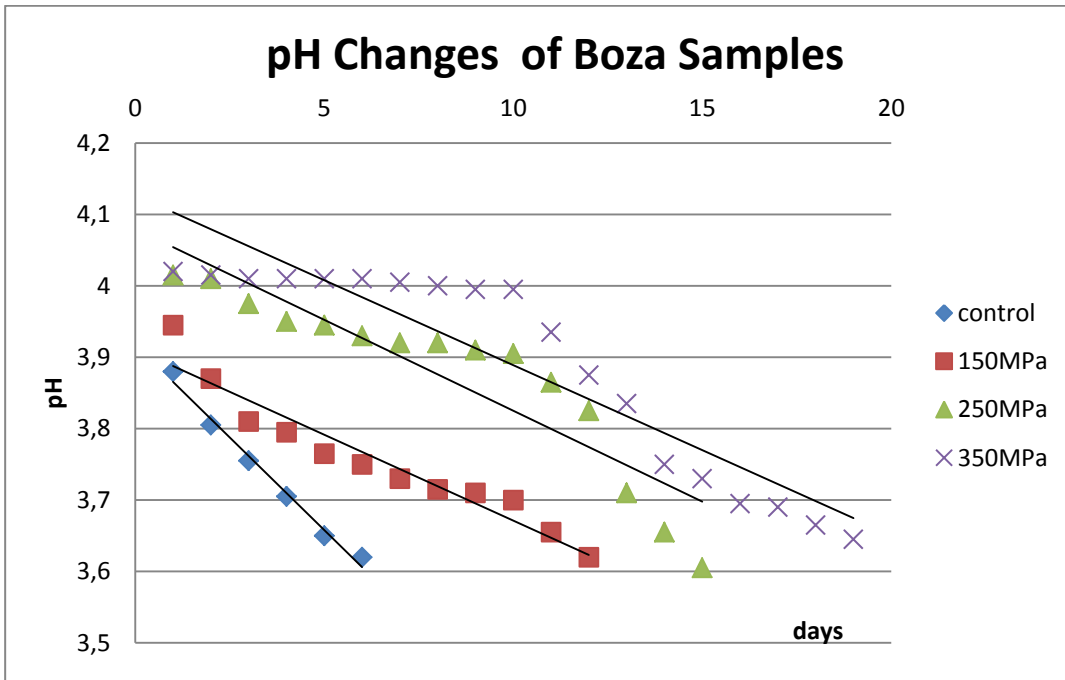


Figure 3.3: pH Changes of HHP Treated and Control Boza Samples by Time (**control** for unprocessed boza, **150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, and **350MPa** for 350MPa at 5°C for 5 minutes).

3.1.3 Results of Viscosity Analyses

Rico et. al. (2010) reported that for the products containing starch, HHP treatment is applied not only to preserve food product but also to help to process of gelatinization which result in decrease in viscosity. As a support, HHP treatment at room temperature can effectively gelatinize/inactivate proteins and polysaccharides which help to decrease viscosity (Hendrix, 2002). Also, a study was published to link rheological behavior of boza with its acceptance during sensory analysis by Genç et al. (2001). Boza has non-Newtonian behaviour that is shown by reduction in viscosity values of Boza and an increase of shear rate. A power law function expresses this behaviour with Boza's pseudo-plastic nature. It is shown that, there is inverse relation between "appearance" and "mouth-feel" to the flow behavior index but there is direct relation between the fluid consistency (K) with mentioned sensory scores. It is concluded that, rheological properties of boza could be used as a key for consumer acceptance. Also, Yeğın and Labore (2012) reported that the bran particles present in the flour cause obtaining a significantly higher viscosity values. It has been concluded a product, which has a higher viscosity and dry matter content, is the result of that the use of wheat flour; as compared to that produced from whole-wheat grains.

In this study, the change in viscosity with shear rate was measured for untreated, HHP treated boza. The measurements were carried at 20°C between shear rates of 1-30.

Boza samples exhibit a rapid decrease in viscosity when the shear rate increases. Therefore, the Power Law Model described the pseudo-plastic properties of boza samples. Rico et. al. (2010) reported that HHP treated samples showed a significant reduction of viscosity parameter compared to non-HHP treated samples regardless of the extension of the HHP treatment (5 or 10 minutes). Also, HHP treated grains had a reduction of viscosity (Yamakura et. al., 2005).

Figure 3.4 indicates that viscosity behavior of boza samples are not affected from HHP treatment and they preserved their pseudo-plastic behavior with a small decrease in viscosity observed during processing.

And in the Appendix C.1, viscosity changes if groups are given in detailed illustrations.

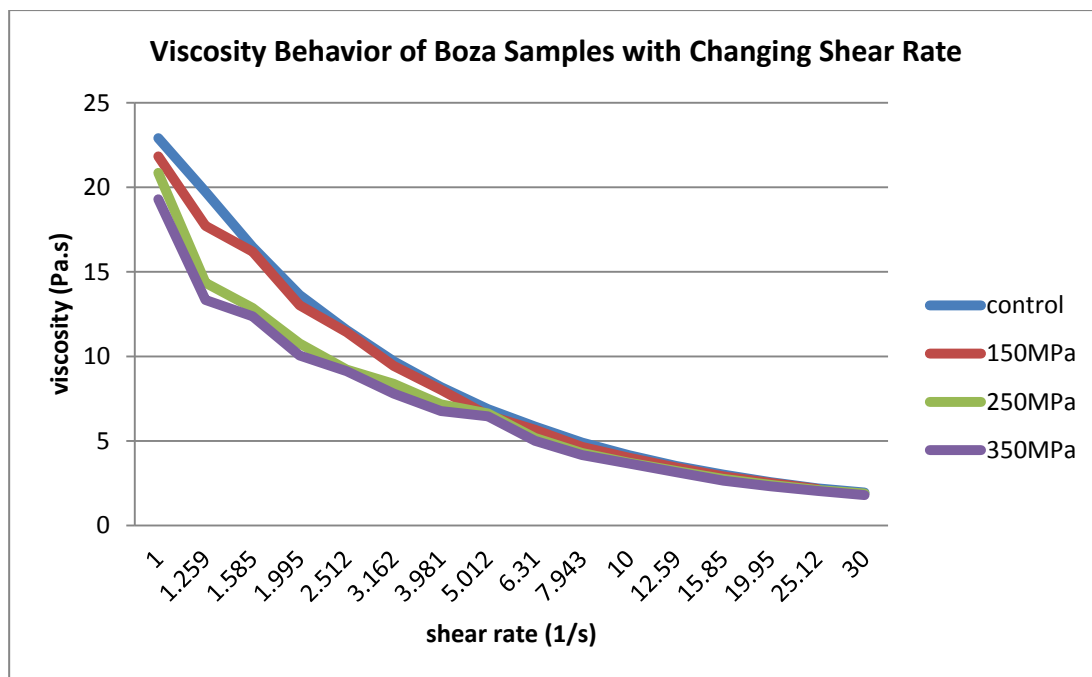


Figure 3.4: Viscosity Behavior of Boza Samples with Changing Shear Rate (**control** for unprocessed boza, **150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, **350MPa** for 350MPa at 5°C for 5 minutes).

3.1.4 Results of Titratable Acidity Analyses

Boza is in the category of sweet type food according to the Boza Standards stated by the Institute of Turkish Standards (Anonymous, 1992). The titratable acidity (% w/w) of the samples studied by Bolat (2000) , varied between 0.32% and 0.64 % (w/w)

and the average was calculated as 0.42%. Also, the titratable acidity of the samples collected from Ankara and İstanbul reported by Pamir (1961) ranged between 0.28-0.46%. The titratable acidity of the various boza produced in our country is within the range to that of boza produced in other countries. As an example, boza of Turkistan origin has titratable acidity in the range 0.2-0.67% (Pamir, 1961). Total titratable acidity (lactic acid) should be 0.2%–0.5% in sweet boza and 0.5%–0.0% in sour boza (Yeğin and Labore, 2012).

Titratable acidity, in terms of lactic acid, were calculated and given in Figure 3.5.

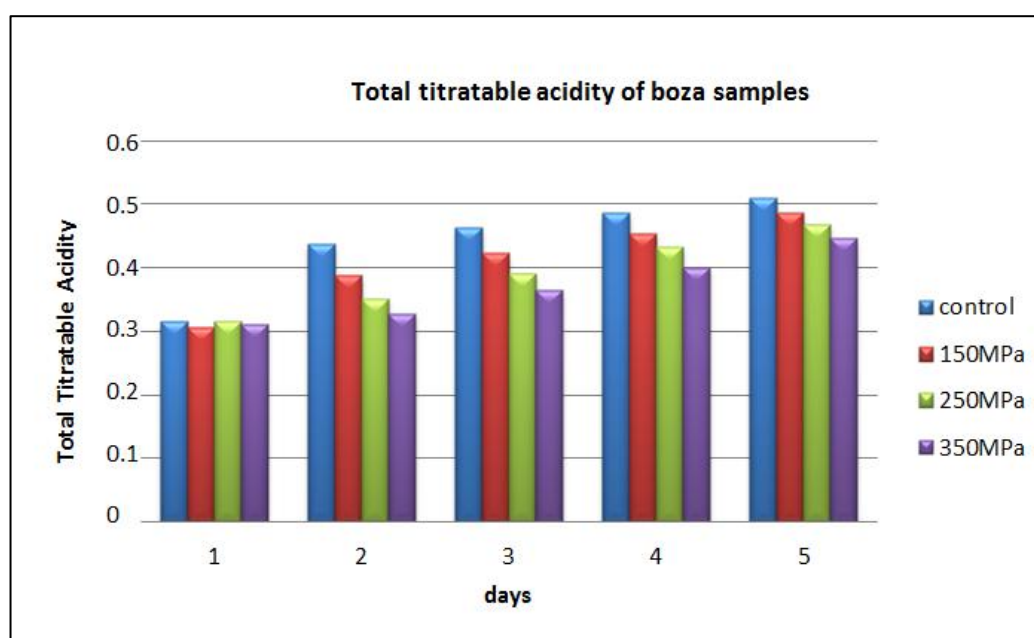


Figure 3.5: Total Titratable Acidity of Boza Samples (**control** for unprocessed boza, **150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, **350MPa** for 350MPa at 5°C for 5 minutes).

For the first days, no difference between mean titratable acidity is seen. However, after five days some changes observed on titratable acidity.

3.1.5 Results of Shelf Life Analyses

In this study, the aim was to investigate shelf life of traditionally produced boza after treatment with HHP. HHP treatments were done at 150MPa at 5°C for 5 minutes, at 250MPa at 5°C for 5 minutes and finally at 350MPa at 5°C for 5 minutes. Effects of HHP treatment on pH, titratable acidity, and microbial load and viscosity properties of traditional boza were studied. Changes in pH and microbial load are the main determinants for the shelf-life.

Shelf-life is expected to be around 6-7 days for traditional boza at room temperature. The shelf life of boza samples after HHP treatments are prolonged. HHP treatment at 350MPa at 5°C for 5 minutes, 250MPa at 5°C for 5 minutes, and 150MPa at 5°C for 5 minutes extended shelf life of boza up to 19,15 and 12 days, respectively.

3.2 Summary of Experimental Results

Stonaya et al. (2005) reported that when fermentation of boza is finished, pH is generally between 3.5. Also, in millet boza (0.32%), total titratable acidity is found to be lowest and in wheat boza (0.61%), total titratable acidity is found to be highest and both of acidities are in terms of lactic acid. Reason of this the high carbohydrate of wheat component compared to other ingredients. The pH of boza after fermentation is between 3.43 – 3.68 depending on the ingredients (Yeğın and Labore, 2012).

Results of mean total mesophilic aerobic bacteria (log cfu/ml) and mean total yeast and mold (log cfu/ml) of HHP treated boza samples are given in Figure 3.1 and 3.2. The most effective reduction is observed at HHP treatment of 350MPa at 5°C for 5 minutes. After HHP treatment, there is total destruction in mold and yeasts.

Initially boza samples had pH between 4.04-4.08 just after production. After three days, non-treated control boza samples pH levels dropped to 3.60- 3.65 at room temperature. After pH of 3.65 all boza samples started to sour with very acidulous

odor, over-fermented appearance. The best result for storage time was with HHP treatment at 350MPa at 5°C for 5 minutes, pH dropped to 3.65 after 19 days.

Viscosity properties of boza samples after treatments were not affected from treatment type and they all preserved their pseudo-plastic behavior. For the first day, there are negligible differences between titratable acidity of boza samples. During the following five days changes on titratable acidity is observed.

CHAPTER 4

CONCLUSION

From our studies, HHP treated boza was found to be stable at room temperature without losing its measured quality parameters up to 19 days with HHP treatment at 350MPa at 5 minutes at 5°C. In the coming years with progress of technology, HHP is likely to be used commercially before its full potential is comprehensively understood. For future boza production, HHP treatment must be considered as an alternative way to prolong its stability. High cost of capital may limit its application initially but this will be offset by lower operating cost due to less use of energy. In the future, with improvement in technology and commercialization, HHP equipment and process will be affordable and HHP processed boza will be available as a safe and nutritious product with extended shelf life and quality and its sensory parameters preserved to all consumers at an affordable cost.

In food industry, some techniques and methods are used in order to produce raw material to food or other kinds for consumption by animals or animals at home or industry to ensure food safety, increase digestibility, extend shelf life , add value and to produces new products. In the food preservation, it is important to minimize microbial activity and control the chemical reactions. The main point is to induce minimum change in food quality and nutritious value with maximum food safety. HHP is one of these major approaches accepted in food industry to produce safe foods with extended shelf life.

CHAPTER 5

RECOMMENDATIONS

The aim of this study was to create a general idea on the effects of HHP treatment on chosen quality parameters and shelf-life of traditionally produced boza that is not stable for long time storage for commercial purposes. These quality parameters were chosen according to the most often used ones in the literature. Results seem to be promising for the use of HHP to increase the shelf-life of boza without decreasing quality of the product. As used in other food materials, HHP and thermal treatment can also be used as a hurdle technology to increase the shelf life of boza for industrial applications. Apart from HHP other minimal processing technologies can also be used alone or they can be combined to check their synergistic or antagonistic effects on traditionally produced boza as Pulsed Electric Field (PEF) or Ultra Sound (US).

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APPENDIX A

TABLES OF MICROBIAL ANALYSES

Table A.1: Mean Total Mesophilic Aerobic Bacteria

Name	microbial load (log cfu/ml)
control	8.27
150MPa	7.71
250MPa	7.78
350MPa	7.04

Table A.2: Mean Total Yeast and Mold

Name	microbial load (log cfu/ml)
control	7.91
150MPa	1.00
250MPa	1.00
350MPa	1.00

APPENDIX B

RESULTS OF pH ANALYSES

B.1 pH Changes of Boza Samples during Shelf Life

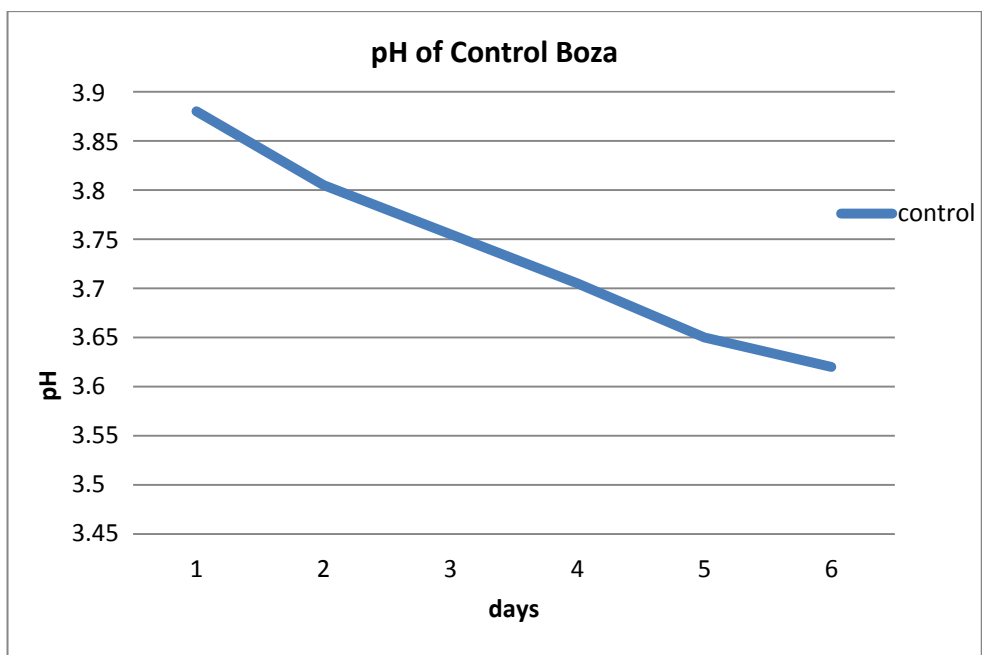


Figure B.1: pH of Traditional Boza during Shelf Life (**control** for unprocessed boza)

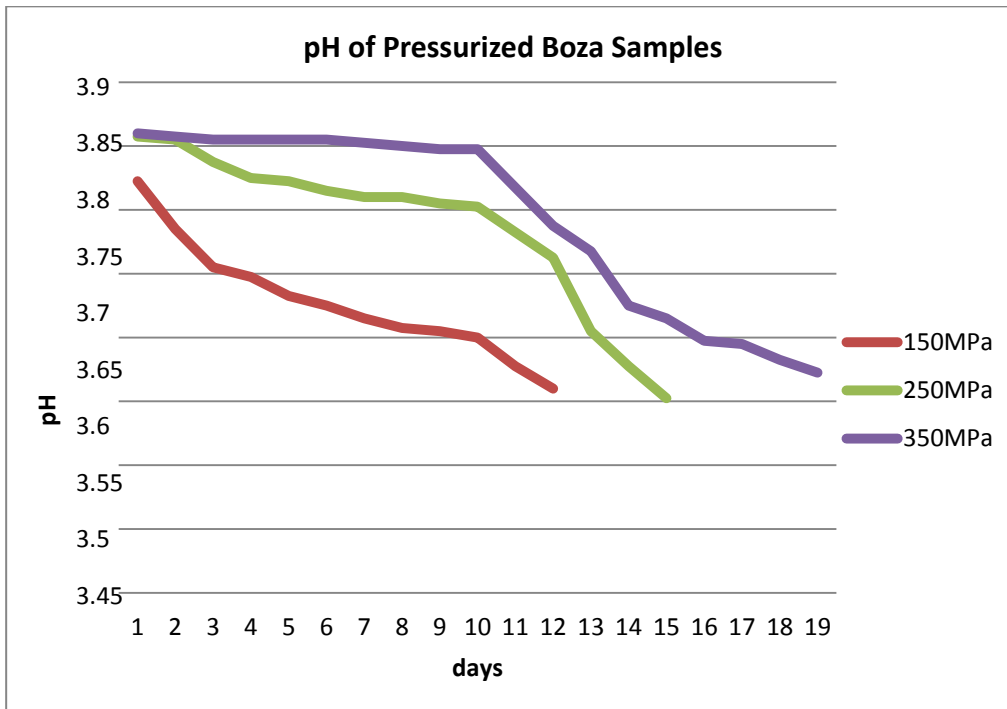


Figure B.2: pH of Pressurized Boza during Shelf Life (**150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes, **350MPa** for 350MPa at 5°C for 5 minutes)

APPENDIX C

RESULTS OF VISCOSITY ANALYSES

C.1 Viscosity Change Curves

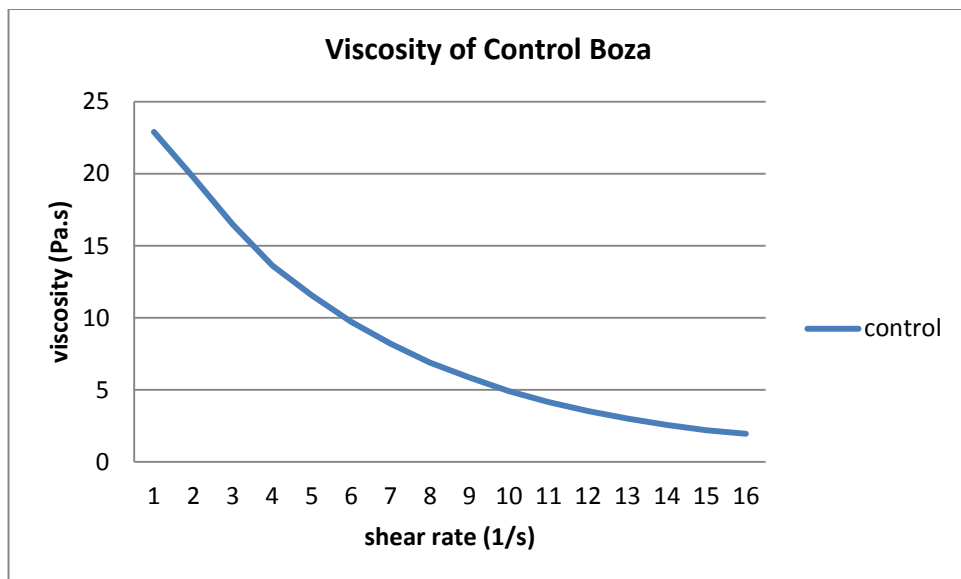


Figure C.1: Viscosity Change of Unprocessed Boza as a Sample (**control** for unprocessed boza)

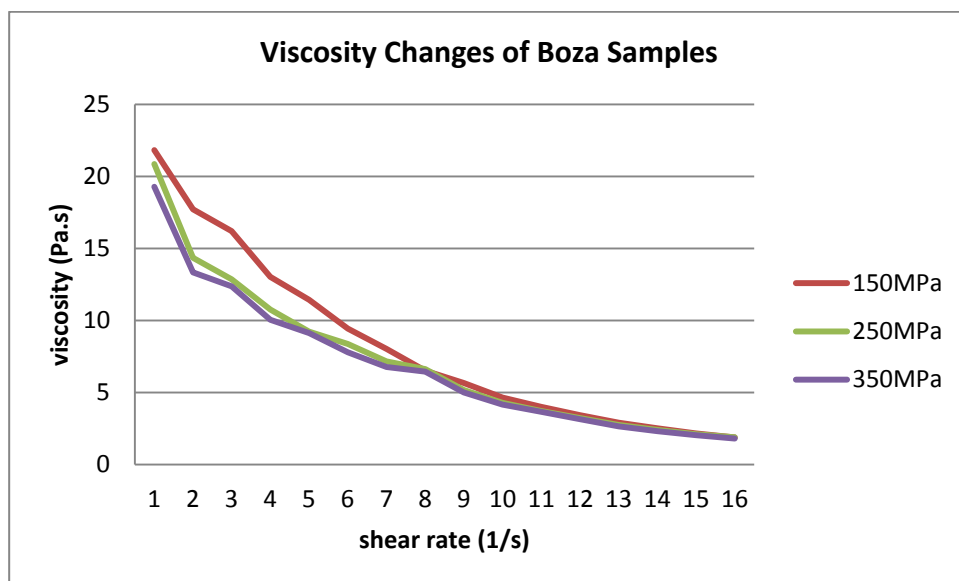


Figure C.2: Viscosity Changes of Boza Samples after Treated with HHP (**150MPa** for 150MPa at 5°C for 5 minutes, **250MPa** for 250MPa at 5°C for 5 minutes and **350MPa** for 350MPa at 5°C for 5 minutes).