PRESERVATION AND SHELF LIFE EXTENSION OF SHRIMPS AND MUSSELS BY HIGH HYDROSTATIC PRESSURE (HHP)

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Approval of the Graduate School of Natural and Applied Sciences.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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ABSTRACT

PRESERVATION AND SHELF LIFE EXTENSION OF SHRIMPS AND MUSSELS BY HIGH HYDROSTATIC PRESSURE (HHP)

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Shrimp and mussel samples were cleaned, washed and exposed to steam before freezing. HHP treatment was performed at combinations of 200, 220 and 250 MPa at 25, 30, 40 and 50°C for 10 and 20 minutes. Microbial analysis were performed by analyzing the effect of treatments on the microbial reduction in the samples. Based on the results of the microbial reduction, the best combinations of HHP treatments were determined as 250 MPa, 50°C, 10 minute for shrimps and 220 MPa, 50°C, 10 minute for mussels where total microbial inactivation was achieved.

Storage analysis was performed on the samples, treated at the selected HHP combinations and stored at room (25°C) and refrigeration temperatures (4°C). For the storage analysis, variations in Total Volatile Bases (TVB-N) and pH were measured. According to the results evaluated, shelf-life of the shrimps were detected as 10 and 16 days for storage at room and refrigeration temperature, respectively as compared to 4 days of untreated sample at 4°C. Similarly shelf-life for the mussel samples were obtained as 12 days for storage at room and 18 day

for storage at refrigeration temperature as compared to 4 days of untreated sample at 4^{o}C.

HHP-at the studied parameters for shrimps and mussels- can be offered as an alternative method for the preservation of shell-fish instead of conventional frozen food technology, which is currently used in the industry, since it gives the opportunity to handle the samples at lower temperatures for the post-production period resulting in both reduction of energy required and operational costs without sacrificing from the quality as measured by microbial reduction, TVB-N and pH.

Keywords: High hydrostatic pressure, Shell-Fish, Mussel, Shrimp, Total Volatile Bases.

ÖZ

YÜKSEK HİDROSTATİK BASINÇ (YHB) İLE KARİDES VE MİDYELERİN KORUNMASI VE RAF ÖMÜRLERİNİN ARTTIRILMASI

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Temizlenmiş, yıkanmış, buhara tabi tutulmuş karides ve midye örnekleri dondurma işleminden önce alınarak 200, 220 ve 250 MPa basınç 25, 30, 40 ve 50°C sıcaklık ve 10 ile 20 dakika zaman kombinasyonlarında YHB işlemine tabi tutulmuştur. Mikrobiyal analizler, işlemlerin örneklerdeki mikrobiyal azalmaya etkisinin incelenmesi ile gerçekleştirilmiştir. Mikrobiyal azalmaların sonuçları baz alındığında, karidesler için 250 MPa, 50°C, 10 dakika kombinasyonu; midye örnekleri için ise 220 MPa, 50°C, 10 dakika kombinasyonu en iyi YHB kombinasyonları olarak belirlenmiştir.

Belirlenen en iyi YHB kombinasyonunda işleme tabi tutularak oda (25°C) ve buzdolabı sıcaklığında (4°C) depolanan örneklerde raf ömrü analizleri gerçekleştirilmiştir. Raf ömrü analizleri için Toplam Uçucu Baz (TUB-A) ve pH değerlerindeki değişimler gözlemlenmiştir. Elde edilen sonuçlara göre, oda ve buzdolabı sıcaklığında depolanan karideslerin raf ömürleri sırasıyla 10 ve 16 gün olarak belirlenmiştir. Benzer şekilde, midye örneklerinin de raf ömürleri oda

sıcaklığında saklandığında 12 gün, buzdolabı şartlarında ise 18 gün olarak belirlenmiştir.

Elde edilen bulgular ışığında-karides ve midye için-, üretim sonrası aşamalarda daha düşük sıcaklıklarda saklayabilme olanağı sağlayarak enerji tüketimini ve işletim maliyetini düşürmesi sebebiyle YHB teknolojisinin -çalışılan kombinasyonlarda-, kabuklu deniz ürünlerinin korunmasında gıda endüstrisinde hali hazırda kullanılan donmuş gıda teknolojisine alternatif olarak sunulabileceği mikrobiyal azalma, TUB-A ve pH değerleriyle ortaya konulmaktadır.

Anahtar Kelimeler: Yüksek hidrostatik basınç, Kabuklu Deniz Ürünleri, Midye, Karides, Toplam Uçucu Bazlar.

to my parents, my friends and beloved…

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CHAPTER 1

INTRODUCTION

1.1 Minimal Processing Technologies and High Hydrostatic Pressure (HHP)

Food processing technologies which are being widely used in the food industry for preservation and shelf-life extension, such as heat treatments, pasteurization and else can cause reduction in the quality of food because of the decrease of nutrients, vitamins, proteins or sensory characteristics such as aroma, flavor, color and else; therefore, over the last decade, there occurred a demand by the consumers for the foods that are minimally or not processed but are compatible with the processed foods in terms of safety, suitability and consumability. This gave rise to the development of minimal processing methods that preserve foods produced by treatments involving reduced or mild degrees of temperature, so as to prevent the loss of nutritional and sensory quality of foods due to the heat applications.

For many years, the traditional preservation methods that need little or no heat treatment such as fermentation, curing or insalination were being used. More recently, research and development studies were focused on several minimal processing methods like pulsed electric fields, high pressure processing, high intensity light and ultrasound, irradiation, ozone treatments, controlled and modified atmosphere. Consequently, those recent processes began to take part for the preservation of several food stuff. However, none of those preservation items were enough by itself for permitting adequate safety and palatability. Novel approach in minimal processing technologies involves the extensions or combinations of one or more minimal processing methods. This also supported the adaptation of the hurdle concept which brings together the combined effect of more than one minimal processing method and each preservation method in this concept constitutes a

hurdle to be beaten by the physical, chemical or microbial agents and other contaminants within the food. The resulting products have higher quality and consumer appeal in markets where the retention of nutritional sensory characteristics can command premium prices (Fellows, 2000).

High Hydrostatic Pressure (HHP) is amongst the emerging technologies that have been investigated to enhance safety and shelf-life of many perishable foods, so as to be proposed as an alternative to thermal processing (Knorr, 1995). It is a 'heatless process' which can reduce the enzymatic activity and the number of microorganisms in the food (Oshima et al., 1993). Structural and morphological changes in the microorganisms by HHP have been reported (Bozoglu et al., 2004; Kaletunc et al., 2004).

1.1.1 History of General Use of HHP in Food Products

Early uses of high pressures involved many industries such as cannons and small arms, processing polyethylene, materials processing and high pressure water jet cutting applications. High pressure treatment to kill bacteria was first described by Roger (Smelt, 1998). The discovery of high pressure as a method to destroy microorganisms leads back to more than a hundred years ago. Experiments were conducted by Hite B.H. in 1899 in the West Virginia University in the USA including the application of high hydrostatic pressures to preserve milk, fruit juice, meat and a variety of fruits. As a result of the experiments, it was reported that microorganisms in these products could be destroyed by pressures of 658 MPa (around 6500 atm) in 10 minutes. However, because of the inadequate conditions in manufacturing, cease of research supporting the results of the early researchers and reports of the researchers about the effect of high pressure on reducing enzymatic activity; those approaches were far from taking the attention of food industry, for over eighty years.

HHP was re-discovered in mid-eighties and since then it has been at the center of food research and development activities. As a result, the emergence of the commercial products manufactured by using HHP began to rise in Japan in 1991. The first commercial products were fruit juices such as orange and grape juices,

fruit jams such as apple, kiwi, strawberry, raspberry; fruit toppings' salad dressings and tenderized meat. In 1995, orange juice produced by HHP was commercialized in France. Following that, in 1999 HHP was discovered to be effective on the shucking of oysters, which initiated the introduction of HHP treated oysters in the US market by Motivatit Sea Foods Inc. as Gold Band Oysters. (Knorr, 1999; Duchene, 2001). The approach was followed by Nisbet Oyster Company in 2001 by introducing pressurized oysters (Kuriloff, 2003). HHP application was not only sustaining the microbial preservation and the retaining of nutrients within the muscle, but also was beneficial in its shucking out of the shell. The application of the new system resulted in a 20-50 % increase in the yield of Motivatit Company, depending on the harvesting time, and a 20 % drop in the labor costs (Duchene, 2001). The company was awarded with FINesse award for sea food industry by the Natural Fisheries Institute (NFI) in 2001.

Figure 1.1 Goose Point Oysters produced by Nisbet Oyster Company (http://www.goosepoint.com/aboutFUP.html).

The emphasis in HHP applications was mainly stated on the preservation of foods and assurance of food safety with minimum impact on the nutritional and sensory characteristics of foods, by means of eliminating the submerged effects of heat

applications. Whereas, the drawback of these products was them to be three to four times more expensive than the conventional products. The subsequent research applications forced HHP to become one of the most promising technique for the preservation of food, especially for the preservation of shell-fish such as oysters (He, 2002).

1.1.2 General Principle and Mechanism of HHP

Pressure primarily affects the volume of the system (Knorr, 1999). The former studies on the mechanism of the relation between pressure and temperature showed that throughout the adiabatic compression of water, its temperature increases by around 3°C per each 100 MPa increase in pressure.

Two principles describe 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 to the food independent of its size and geometry i.e., the food will be compressed by a uniform pressure from every direction and then return to its original shape when the pressure is released. This is known as isostatic pressure (Marquis, 1976; Farr, 1990; Gaucheron et al., 1997; Palou et al., 1997; Smelt, 1998; Gervilla et al., 1999; Knorr, 1999; Tewari et al., 1999; Alpas, 2000; Trujillo et al., 2000).

At present it is known that high pressures up to 300-400 MPa only affect non-covalent chemical bonds. (i.e. ionic, hydrogen and hydrophobic bonds), leaving covalent bonds intact (Alpas et al., 2003). The reason is encountered due to the fact that pressure enhances reactions that are accompanied by a decrease in volume and inhibits reactions related with a decrease in volume (Marquis, 1976; Farr, 1990; Hendrickx et al., 1998; Patterson et al., 2005), where the latter is associated with the breaking of the covalent bonds (Marquis, 1976; Knorr, 1999). This permits destruction of microbial activity without significantly affecting food molecules that contribute to the texture or flavor of the food (Fellows, 2000). Consequently, the destruction of the ionic bonds is associated with decreases in volume (Heremans,

1995). HHP application is also considered as energy efficient, since it requires no additional energy input once the required pressure is maintained (Farr, 1990.)

1.1.3 HHP Equipment and Operation

HHP equipment is constructed by four main parts which are pressure vessel, pressure generating system, temperature control device and materials handling system.

Most pressure vessels are made from a high tensile steel alloy 'monoblocks' (forged from a single piece material), which can withstand pressures of 400-600 MPa. For higher pressures, pre-stressed multilayer or wire-wound vessels are used (Mertens, 1995). On the top of the vessel, a threaded steel closure is generally located which provides loading and emptying of the vessel before and after the process and obstructs a closed system throughout the operation for sustaining a constant temperature and pressure. Pressurization of foods is performed within the pressurization fluid which is either water or oil. The air inside the vessel is removed before the operation. Hydrostatic pressurization is operated maintained either in static pressure seals which also known as indirect compression or by compressing the fluid by a piston which is termed as direct compression. Temperature control device is necessary for sustaining a constant temperature throughout the process. This is usually achieved by using a pump system that recycles a heating/cooling medium through the jacket surrounding the vessel. Two methods are available for the processing of foods in high pressure vessels: in-container processing and bulk processing. The former is generally performed as a batch process while the latter provides a semi-continuous processing. In bulk processing, the food is elevated by pumps and pipes through the pressure vessel.

1.1.4 Effect of HHP on Microorganisms

HHP is used effectively for the inactivation of most vegetative pathogens and spoilage bacteria that are commonly found in foods. Inactivation of bacteria can be performed at moderate pressure levels such as 250 to 350 MPa at room temperature. Several studies were performed for the enhancement of the effect of HHP on food borne pathogens (Kalchayanand et al., 1998a; Kalchayanand et al.,

1998b; Alpas et al., 1999; Alpas, 2000;). The efficacy of HHP for the inactivation of microorganisms depends mainly on the magnitude of pressure, pressurization time, temperature of the process, type of the microorganism, and also on the factors such as cell growth phase, type of food material, suspending media and the presence of antimicrobial agents (Palou et al., 1997; Farkas and Hoover, 2000; Ulmer et al., 2000; McClements et al., 2001; Karatzas and Bennink, 2002;).

HHP involves the combined effect of three parameters which are pressure, temperature and time. The research showed that in general among those parameters magnitude of pressure is the most significantly effective on the inactivation of microorganisms, followed by temperature of pressurization and operation time. It is also reported that different types of microorganisms show different resistances to pressurization (Hoover, 1993; Cheftel, 1995; Alpas et al., 1999). For example, Gram-positive bacteria are more resistant to pressure than Gram-negative bacteria. Also some strains of microorganisms may be more resistant to pressurization (Alpas et al. 1999). For instance, the studies put forward some resistant strains of *Escherichia coli* (Benito et al., 1999). Alpas et al. (1999) also found out the relatively pressure resistant strains among different *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella enteritidis* strains. The general order of pressure sensitivity to HHP from highest to the lowest is given as gram-negative bacteria, yeast, gram-positive bacteria and bacterial spores (Hoover et al., 1989). It is also stated that microorganisms become more stable in foods when compared with the laboratory conditions depending on their intrinsic factors.

The kinetics of the inactivation was explained by the development of the three-state inactivation model (Heinz and Knorr, 1996) which insists on that under isobaric conditions microorganisms first perform transition from stable to metastable state followed by inactivation. The mechanism of inactivation is a rather complex issue including metabolic changes and membrane effects (Palou et al., 1999; Kaletunc et al., 2004). It is reported that primary pressure damage occurs at the pressures of 400 MPa or higher but the damage of ribosomal units were also observed on pressures lower than 400 MPa (Kaletunc et al., 2004). The effect of HHP on microorganisms can be repairable since injured microorganism can grow after the

process by the occurrence of the suitable conditions throughout the post-processing session. Therefore, HHP application is more effective in high acid foods since the repair of injury cells are retarded or hindered by the effect of acid. The combined effect of HHP and acidity or other antimicrobial agents reduce the resistance of microorganisms against pressurization (Hauben et al., 1997; Garcia-Graells et al., 1998; Alpas , 2000).

1.2 Sea Food Processing

Sea food products were being proposed to human consumption for many years. They are good sources of proteins, lipids, carbohydrates, vitamins and minerals which are vital for growth, development and health. Sea foods are usually preferred for their low energy content, when compared with other meat products (Holland, 1986).

1.2.1 General Information on Sea Food Processing

Because of the complexity of their microbial flora, sea foods are among the highly perishable food items which limit the transportation and therefore the sector mainly refers to the local markets for fresh consumption. The nature of the sea foods allows many microorganisms to grow, including pathogenic bacteria which may cause diseases or poisoning due to consumption. Vast amount of sea foods, especially shell-fish products, are traditionally being consumed as raw, or partially cooked; which by doing so, may give way to the likely occurrence of several related food poisoning outcomes (CDC, 1989). Shellfish are amongst sea foods that act like filter feeders, passing large volumes of water through their gills so as to obtain food and oxygen (Kelly et al., 2005). Throughout this process, they also filter microorganisms, which give way to the accumulation of several bacteria and viruses within their body (Cliver, 1995). Since they are to be consumed raw, inefficient depuration of hazards due to microorganisms is compounded (Lees, 2000). Consequently, the likely occurrence of several related food poisoning outcomes can blow up due to their consumption (CDC, 1989; Potasman et al., 2002). For instance, *Vibrio vulnifucus* and *Vibrio parahaemolyticus* were both implicated in illness outbreaks in the Southeast and the Pacific Northwest in the United States (Klontz et

al., 1993; Kaysner, 1998). In 1999, Centers for Disease Control (CDC) reported 342 cases of non-cholera Vibrio infections and among those, 33 cases were related with *Vibrio vulnifucus*, 31 of which was accounted for death (Duchene, 2001). Several cases involving Vibrio and other pathogens retained from shell-fish confirmed that, in case of inadequate monitoring, of preparation and distribution, serious infectious diseases are susceptible to be addressed in the industrialized countries (d'Oro et al., 1999).

Freezing technology is currently used in sea food industry, which involves freezing the products at temperatures down to -40 °C. Whereas, frozen food technology requires high operational costs because of the necessity of sustaining the cold chain handling of the final product at -18 °C throughout storage, distribution and marketing until the consumption involving the high rates of energy consumption. Therefore, the necessity of an alternative technology that can possibly lower the energy consumption by reducing the extreme reliance on low temperature storage is a fact, since this type of process would be more economical to operate (Alpas, 2000).

In order to asses the quality of sea foods, so as to detect spoilage or their susceptibility for consumption, a number of analysis are being conducted by the manufacturers. These are total microbial count, total volatile bases, amount of trimethylamines (TMA), dosages of hypoxanthine, thiobarbituric acid (TBA), indole; total volatile reducing substances, physical factors such as pH or moisture content, sensory evaluations, refractive index of the eye fluid (Özoğul and Özoğul, 2000). Most of these methods are good indicators for quality assessment, as long as they are implemented carefully. Among those quality factors;

- Total Volatile Bases
- pH value

are commonly being used in the food industry, they are quick and easy to perform.

The production of the Total Volatile Bases or specifically Total Volatile Basic Nitrogen (TVB-N) is a good indication of microbial spoilage, which is amongst the most widely

used chemical analysis method, together with the measurement of trimethylamines (Erdem and Bilgin, 2004). The bacteria growing on the surface of the fish tissue, cause amine containing compounds such as dimethylamines, trimethylamines and ammonia; which in total, forms total volatile bases. Thereby, total volatile bases are the combined exposition of ammonia and amines like dimethylamine and trimethylamine. The increase in the amount of total volatile bases can be due to the enzymatic degradation as well as microbial activity (Özoğul and Özoğul, 2000). The increase in the amount of TVB-N also increases the amount of trimethylamine content (TMA); however, the determination of the former is faster and cheaper when compared with the latter. The formation of ammonia is possibly seen in very fresh fish and sea food (Özoğul and Özoğul, 2000). Each volatile compound contains one basic nitrogen atom per molecule. It is one of the oldest chemical methods which was first described in the beginning of 1930's by the micro diffusion method of Conway and Bryne (European Commission Decision, 1995). The method is based on the measurement of the volatile bases, generated by distillation of the extract of a sample muscle made alkaline, by titration with standard acid. The results are expressed as milligrams of nitrogen in 100 grams of muscle tissue.

European Community (1995) summarizes the routine methods for the measurement of total volatile bases as;

- Micro diffusion method described by Conway and Bryne (Conway, 1968),
- Direct distillation method described by Antonacopoulos (1968),
- Distillation of a protein-free extract prepared by trichloroacetic acid described in Codex Alimentarius Committee on Fish and Fishery Products (1968).

The TVB-N values show tendency to increase during the storage (Rehbein and Oehienschiaeger, 1982). Based on the results of the studies on the freshness of sea foods, the suitability limits of Total Volatile Basic Nitrogen (TVB-N) values were fixed as follows (Schormuller, 1968,; Lang, 1979; Varlık et al., 2000; Erdem et al., 2004; Turan and Koyuncu, 2004):

- TVB-N < 25mg N/100 g very good
- 25mg N/100g < TVB-N < 30 mg N/100g good

- 30mg N/100g < TVB-N < 35 mg N/100g marketable
- TVB-N > 35 mg N/100g spoiled

Generally, TVB-N content is not considered as an indicator for freshness by itself, which in fact must be enhanced by the sensory tests. But it is an effective way for the determination of fitness for human consumption and a good indicator to detect spoilage (Horner, 1997).

The detection of pH is one of the most frequently used physical quality control methods for the sea food products, which is affected by the changes in the concentrations of free hydrogen and hydroxyl ions due to the shifts in the oxidation-reduction balance of the food by the activity of microorganisms or enzymes (Varlik et al., 2000). In several literature surveys, pH values were reported as between 7 to 8 for the shellfish products that are suitable for consumption (Schormuller, 1968; Ludorff and Meyer, 1973; Varlik et al., 1993). Sentürk (1994), detected the pH values of the fresh shrimps as 7.2, while the shrimps were well remained as good in quality, with a pH value of 7.7 and below. In another study performed by Shamshad et al. (1990), untreated fresh shrimps were stored at different temperatures between 0 to 35 °C and the pH was reported to increase from 7.05 to 8.25 in a 16-day-period. Varlik et al. (2000) reported an increase of pH from 6.73 to 7.81 in raw, unprocessed shrimps within 4-day-storage under 4 ˚C±1.

1.2.2 Applications of HHP to Sea Foods

Recently, HHP technology is taking increased attention of the fish and shell-fish industry since it proposes the elimination of pathogenic or spoilage microorganisms at or near room temperature requiring small amount of energy to compress a solid or liquid sea food product as compared to heating up to 212 °F (100 °C) (Flick, 2003). Most shellfish are encountered with several food borne diseases not only because they are filter feeders and contain vast amount of pathogenic bacteria and viruses throughout filtration processing, but also they are susceptible to consumer preference to be consumed as whole including the intestinal tract and raw or with a mild heat treatment (Gram and Huss, 2000; Lees, 2000; Patterson et al., 2005). Traditional preservation methods such as heat may have detrimental effects on the

sensory characteristics of shellfish, which may be unacceptable to many consumers (Patterson et al., 2005). Therefore HHP application can be regarded as an alternative method to study the preservation of shellfish (Patterson et al., 2005) since it does not have an adverse effect on the flavor, texture and nutritional qualities of the product (Hoover et al., 1989; Smelt, 1998).

Beyond its role as a preservation method, HHP is also encountered as a potential for increasing the shelf life of sea food products (Hurtado et al., 2000; Lopez-Caballero et al., 2000; Mermelstein, 2000; Patterson et al., 2005).

Recent research applications put forward the substantial effect of HHP on the preservation of oysters. HHP application at 400 MPa and 7˚C resulted in 5-log reduction of target microorganisms in oysters and enabled 41 days of storage at 2˚C (Lopez-Caballero et al., 2000). He et al. (2002) investigated the effect of HHP treatment applied at different pressures from 207 to 310 MPa for 0,1 and 2 minutes on oysters and evaluated a 2-3 logs and counts remained at a reduced level throughout storage under 4°C. Optimum HHP conditions for reduction of *Vibrio parahaemolyticus* to non-detectable levels in pacific oysters was reported as 345 MPa and 90 seconds (Calik et al., 2002). Oysters are also being commercially produced in different countries, especially in USA. Besides microbial preservation and shelf-life extension, HHP brings out the shucking of oysters out of their shells which complies with the consumer preference.

Study performed on the mussel samples treated with HHP under the conditions of 500 MPa or higher and stored under 2 °C showed that, the extension of shelf-life was evolved up to 14 days, when psychotropic count of 10^6-10^7 cfu/ml was assumed as the level of spoilage (Patterson et al., 2003). Patterson et al. (2003) also stated that fresh and untreated samples were most acceptable, while pressure treated samples with 600 MPa were also acceptable after 2 weeks of storage based on the sensory analysis of the cooked mussels. Altinier et al. (2002) found that high pressure process of 500 MPa for 3 minutes completely inactivated an artificial contamination of 10^5 cfu/g of *Salmonella derby* and *Listeria innocua* and reduced *Escherichia coli,* by 4 logarithmic units, while a shorter treatment applied at 500 MPa for 1 minute conditions completely inactivated *S. derby* and reduced *E. coli* and

L. innocua contamination by 3 logarithmic units. Furthermore, a reduction of the initial total viable count of a natural contaminated sample of mussels, from 4 x 10^5 to 7 x 10^2 CFU/g was observed with an HPP treatment of 700 MPa for 5 minutes (Altinier et al., 2002).

1.2.3 Mussel Processing

Mussels are amongst the most abundantly collected shell food throughout the world. They also have high nutritional value and traditionally they are preferred to be consumed as raw. Because of their characteristic aroma and flavor, mussels are amongst the most demanded food products in many countries. Besides all, mussels are amongst those shell-fish that act as filter feeders, that is, they obtain nutrients and oxygen by pumping water through their complex gill systems, which in turn, allows them to have high contamination with microorganisms and several other impurities (Hicks, 1990). Consequently, the necessity of a processing technology has become vital which can provide preservation and shelf-life extension of mussels with a minimum effect on their nutritional content and the sensory characteristics.

1.2.4 Shrimps Processing

Shrimps have high nutritional value and they are also good sources of proteins. Because of the lack of bond tissues, they can be easily digested when consumed but because of their large microbial contamination they are suitable for spoilage in short times. Depending on the storage conditions, flavor and odor shifts raise out which in parallel, causes the alteration of the characteristic odor of shrimps with the odor of ammonium (Schormuller, 1968). Research analyzing the effect of temperature on pacific shrimps showed that the samples remained fresh and consumable up to 6^{th} and 11^{th} day of storage, performed under 5 different temperature conditions between 0 and 5.6°C; respectively (Matches, 1982). Similarly, Varlik et al. (2000) reported that unprocessed shrimps stored at +4°C±1 were spoiled after 2-day storage. The results of the research show that the minimal processing methods can be performed for the preservation of shrimps without causing an adverse effect on their sensory characteristics and nutritional values. Several cases of poisoning outbreaks were reported, including virutic outcomes such as white spot syndrome

virus (WSSV) and yellow head virus (APHIS-Services for the Aquaculture Industry, 1999), as well as diseases related with the pathogenic bacteria such as *Vibrio cholera* (d'Oro et al., 1999).

The shell-fish samples are selected as shrimps and mussels because of their economic and nutritional value. The samples were supplied from AyFrost Frozen Foods Company which is located in Gölbaşı, Ankara. The freezing technology used currently requires handling and storage of the products via cold chain conditioned to -18 °C, throughout the post-processing steps up to the consumption. Therefore the products could only be exported using cold chain by trucks which results with a great amount of energy requirement during storage, transportation, distribution to the retailers and marketing stages. The shrimp and mussels samples were taken as cleaned, washed, steam treated; just before freezing to be representative of the actual production. Accordingly, the system requires high operation costs and the application is rather expensive. On that purpose, the objective of the second part of this study mainly focused on the reduction of the storage temperatures to elevated degrees or room temperatures if possible with still keeping the same or better quality as measured by TVB-N and pH values. The results of this study will be informative on whether presenting HHP for the processing and preservation of mussels and shrimps as an alternative to the frozen food technology or not.

1.3 Objectives of the Study

The study consists of two parts:

- apply HHP to mussel and shrimps- in order to obtain the best pressure, time and temperature combination based on total microbial inactivation;
- study the shelf-life of pressurized samples during storage at refrigeration (4°C) and room temperatures (25°C)

The main objective of the first part of this study was to detect the best combination amongst the applied pressure (200 to 250 MPa), temperature (25 to 50°C) and time (10 and 20 min) combinations. The treatments were chosen according to the

literature on the microbial inactivation of seafood by HHP and considering the economical aspects of HHP processing.

For the second part of the study, HHP was applied on the selected samples and a shelf life study was performed by measuring the changes in the Total Volatile Bases (TVB-N) and pH values -as the main quality parameters -of the samples throughout their storage. The storage conditions were arranged so as to achieve refrigeration handling (4 °C) and room temperature storage (25 °C). A shelf-life estimation was to be performed according to the data obtained.

The main goal is to clarify whether HHP technology can be used as an alternative for the preservation and shelf life extension of shell-fish. Shelf-life study is significant for the evaluation of handling conditions for shellfish, with reduced operational costs.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples

Mussels (*Venus gallina*) and shrimps (*Parapenaeus longirostris*) were obtained from AyFrost Frozen Foods Company (Gölbaşı, Ankara, Turkey). They were all collected in the beginning of the season in September (Antalya, Turkey). The collected samples were washed, cleaned, and exposed to steam. For the steam exposion, superheated steam at 80 °C and 10 bar was used. Before freezing, the samples were packaged with vacuum sealing and cooled to -18°C, without being shocked. The samples were kept at -18°C in deep freeze in METU, untill being used in the experimental study, in order to prevent microbial growth and sustain the initial microbial load at a proper level for the evaluation of a justified data. Throughout the HHP treatments and the shelf life analysis, samples from the same main sample were used so as to avoid the possible mistakes that can occur due to differing initial conditions.

2.2 Sample Preparation and Processing

The samples were taken from the deep freeze, 1 day before the treatment and stored in the refrigerator. They were taken out 4 hours before the treatment.

For microbial analysis, 10 g of sample was mixed with peptone water in 1:10 ratio, and blended in the stomacher (Seward Medical Co., England) for 30 minutes in sterile bags. After blending, the aliquot is filtered through pre-sterilized filter paper and filled into 4 ml cryovials. The HHP treatment was designed and performed on a model system. The results of the microbial analysis were given in log cfu/ml, since a fluidized sample was obtained via mincing with stomacher and through filtration.

The sterilization of the equipments used for filtration is performed with %60 ethanol followed by rinsing with sterilized water. The equipments were stored at 100°C for 1 hour before the experiments.

Throughout the shelf-life analysis, the samples were stored in the refrigerator at 4 °C and conditioned at 25 °C, respectively according to the analysis. Both samples shrimps and mussels were kept at the sustained storage conditions, before performing the necessary measurements. For the determination of total volatile bases, 100 g of sample was mixed with 200 ml %7.5 trichloroacetic acid solution (Merck, Germany) and blended in a hand blender (Simbo, Turkey) for 1-2 minutes. A 25 ml of aliquot is taken and filtered through pre-sterilized filter paper and prepared according to the procedure, detailed in section 2.5.1. For the determination of pH, the samples were blended for 1-2 minutes and filtered through pre-sterilized filter paper.

2.3 Treatments

The samples were treated with HHP for the determination of the best combination amongst the detected HHP conditions. For the shelf life analysis, HHP treated samples were stored at room and refrigeration temperatures. Several chemical analysis were performed for monitoring the quality changes.

2.3.1 HHP Application

HHP equipment in the Middle East Technical University Non-Thermal Food Processing Laboratory with the capacity of 30 cm^3 and maximum pressure level of 350 MPa was used for the pressure treatments (Fig. 2.1). Increase and release times of pressure were detected approximately as 5 and 10 seconds for the designed system, respectively. Water was used as the pressure transmitting medium. The equipment consists of 4 main parts:

- Pressure chamber,
- Pressure pump,
- Hydraulic unit,

- Temperature control device.

Pressure chamber is a cylindrical vessel, equipped with two end closures for loading and unloading and a means for restraining the end closures. The vessel is made up of hot galvanized carbon steel. Before the HHP treatment, the vessel is filled with the pressure transmitting fluid where the samples were exposed to high pressure and the air is retained out of the vessel. The pressure pump controls the hard chrome plated piston, polished to mirror finish (steel type heat treated special K). Hydrostatic pressure is supplied mechanically by the compression of the pressure transmitting fluid via piston. The hydraulic unit is for the generation of the high pressure by system compression. For sustaining constant temperature throughout the treatment, a temperature control device is connected to the equipment. The pressure vessel and the chrome plated piston were processed into the required sizes at the Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. The pressure transmittance fluid within the vessel was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Pressurization time reported in this study did not include the pressure increase and release times.

Figure 2.1 HHP unit

2.3.2 Experimental Design

The experimental design was consisting of two parts. Those were the HHP treatment and the shelf life analysis. For the HHP treatment, the treatment conditions were determined and for the shelf life analysis the storage conditions were detected, based on the literature knowledge, economy and the specifications of the equipment.

2.3.2.1. HHP Treatment

Samples were pressurized at 200, 220 and 250 MPa at 25, 30, 40 and 50°C for 10 and 20 minutes. The treatments employed in this study were chosen according to total microbial inactivation of the samples studied, and the results reported in literature on the application of HHP on sea foods. The samples prepared were dispensed in 4 mL portions in sterile cryovials (Simport Plastic, Canada), avoiding as much air as possible and placed inside the pressurization chamber for the HHP application. The chamber was fully filled with water and kept for 1-2 minutes for temperature equilibration before pressurization. Untreated samples were used as controls. Experiments and measurements were duplicated on separate days, in order to justify the data obtained.

The experiments and the measurements were performed on the samples minced with stomacher and filtered, in order to obtain a homogenous medium for the calculations. Therefore, the study is a representation of a model system for the effect of HHP treatment on the preservation and shelf life extension of shellfish samples.

2.3.2.2. Storage

Duplicate samples of shrimps and mussels were pressurized at 250 MPa, 50^{o}C 10 min and 220 MPa, 50^{o}C 10 min; respectively. HHP treated samples were stored at 4 and 25^{o}C in the dark up to 20 days. The samples were analyzed with 2 day

intervals. New cryovials were opened each time. Untreated samples were used as controls.

HHP treatments conducted and the parameters analyzed at the selected conditions based on microbiological analysis are given in tables 2.1 and 2.2, respectively. According to table 2.1, total microbial count in log cfu/ml was detected for each process condition in order to determine a best combination. In table 2.2, the shelf life analysis was performed with the criteria on TVB-N and pH values.

Table 2.1 HHP Treatments

Parameters Studied	HHP Treatment							
	200 MPa							
	25°C		30°C		40°C		50°C	
	10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
Total Microbial Count	+	+	+	+	+	+	+	+
	220 MPa							
	25°C		30°C		40°C		50°C	
	10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
Total Microbial Count	+	+	+	+	+	+	+	+
	250 MPa							
	25°C		30°C		40°C		50°C	
	10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
Total Microbial Count	+	+	+	+	+	+	+	+

Table 2.2 The treatment conditions for shelf life analysis

	HHP Treatment for Shrimps	HHP Treatment for Mussels
	50°C	50°C
	250 MPa	220 MPa
Parameters Studied	10 min	10 min
Total Volatile Bases	+	+

(TVB-N)		
pH Variations	+	+

2.4 Microbiological Analysis

For the microbiological analysis, the treated samples, prepared as given in section 2.1 were diluted in 0,1% peptone (Merck, Germany) water, up to the desired dilution which was determined by preliminary studies. The initial loads for coliforms and total microbial counts were checked. For the determination of coliforms, Violet Red Blue Agar (VRBA) (Merck, Germany) was used. For both mussel and shrimp samples, the amount of coliforms were below detectable levels (data not shown). Therefore coliforms were not taken into consideration as a parameter for the detection of the best combination for the detected HHP conditions.

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

The sample is transferred into two separate cryovials and treated with HHP (200-250 MPa, 25-50 °C, 10-20 minutes). Serial dilutions were prepared by taking 1 ml of HHP treated sample into the tube, mixed with % 0.1 peptone water up to 10 ml. The cultivations were performed from each tube, into the Tryptic Soy Agar plates. By that means, from two separate cryovials from 1 sample, 2 separate agar plates were obtained, which contributes to 4 parallel set-up (Fig 2.2). For the detection of microbial counts on 10^0 dilutions, cultivations were performed on 3 different agar plates by taking 0.3, 0.3 and 0.4 ml of samples, which in total gives 1 ml of sample.

2.5 Physical and Chemical Analysis

The physical and chemical analysis were encountered by taking two quality parameters into consideration. Those were the determination of the total volatile bases (TVB-N) and the pH value of the shellfish samples. The measurements were based on single HHP treatment performed with the best combination conditions detected seperately for each shellfish sample. For the measurement of TVB-N,

sample collection was done by performing series of HHP treatments untill adequate amount of sample is obtained, which was clarified as 100 ml in the procedure defined in section 2.5.1. From the sample collected, duplicate measurements were performed.

2.5.1 Determination of the Total Volatile Bases

For the determination of the Total Volatile Bases, steam distillation of an extract deproteinized by trichloroacetic acid extraction, suggested by Male and Poumeyrol, was used (1989). The principle of the method is based on the determination of the volatile nitrogenous compounds obtained from the protein-free extract prepared by trichloroacetic acid via titration with standard acid (Masette, 2005; Suvanich et al., 2000). The reagents used in the method are:

- 7.5 % Trichloroacetic Acid (TCA)
- 10 % Sodium Hydroxide Solution (NaOH) (Merck, Germany)
- 4 % Boric Acid Solution (Merck, Germany)
- Screened Indicator: 2:1 mixture of methyl red (Merck, Germany) and bromocresol green (Merck, Germany)
- 0.25 N Sulphuric Acid (H_2SO_4) (Merck, Germany)

The following procedure was followed for the determination of the TVB-N values (Masette, 2005)

1. 100 g of sample is weighed.
2. Mixed with 200 ml of TCA solution and blended for 1-2 minutes.
3. Filtered through presterilized filtration paper.
4. 25 ml of aliquot is taken and mixed with 10 ml of boric acid, 6 ml of NaOH solution
5. Distilled at a rapid rate, till final volume within the beaker reach up to 50 ml (40 ml distillate) within 5 minutes.
6. The collected distillate was titrated with the standard H_2SO_4 solution

The experiments for the determination of the total volatile bases were performed in Tınaztepe Flour and Seed Company located in Tınaztepe, Afyon (Turkey). Kjeldahl apparatus (Gerhardt Vapodest 20, Germany) was used for the distillation of the aliquot, where the distillation head was used with the conical distillation flask of 250 ml. All the reagents used were the standard solutions. Standard sodium hydroxide (Merck, Germany) solution of 10 % by weight was prepared. Boric acid (Merck, Germany) solution of 4 % was prepared by the addition of 20 g of boric acid into 500 ml of distilled, hot water and the mixture is stirred until dissolved. As the indicator, the mixture of 0.1 g of bromocresol green (Merck, Germany) and 0.2 g of methyl red (Merck, Germany), dissolved in 100 ml of distilled water was used. The prepared indicator can be used at pH values around 4.5. Also a few drops of silicone anti-foam agent were used before the distillation in order to prevent foaming of the mixture.

The protein free extract was prepared by mixing sample muscles with 7.5 % trichloroacetic acid solution. Sodium hydroxide solution of 10 % was used for making the mixture alkaline. Boric acid solution of 4 % was taking role in trapping the distilled free bases. 0.25 N of sulphuric acid was used for titration of the distillant.

The calculations were performed on the basis that 1 ml of 0.25 N standard acid is equivalent to 0.35 mg of nitrogen. Assuming that the muscle have a moisture content of around 0.8 w/w, which contributes to that TVB obtained from the 300 ml of extract. The calculations were performed according to the following formula:

(1) $C = T * 14 (mgN/mol) * (0.25 N) * 100 / V * 1000$

(2) $C = T * 0.35 * 300/V$

Where;
C is the concentration of TVB in mg nitrogen/100g
T is the volume of the 0.25 N standard acid in ml
V is the volume of the aliquot taken in step 4 of the procedure, which is 25 ml in this calculation.

The results were obtained in duplicate measurements in order to justify the data obtained. Determination of the TVB-N content of the HHP treated samples were performed for both mussels and shrimps, stored at room temperature and refrigeration temperature after the treatment. The measurements were also repeated on the untreated samples for the detection of the control values.

2.5.2 Determination of pH

The pH values of the samples was determined by pH-meter (WTW 537 pH-meter) at 25°C. The samples were prepared for the pH determinations by blending for 1-2 minutes, followed by filtration and mixing. Mixing was done by magnetic stirrer in order to make the sample homogenized. The prepared mixtures were filled into 4 ml of cryovials and treated with the determined HHP application. The untreated samples were used for the control measurements. The pH of the homogenized, treated and untreated samples was measured by pH-meter (Varlık et al., 2000). Both treated and untreated mussel and shrimp samples were stored at room and refrigeration temperature throughout the shelf-life analysis, and pH was measured in a frequency of 2-day-periods. Duplicate measurements were done in order to justify the data obtained.

2.5.3 Statistical Analysis

The results of the microbiological study were analyzed by Analysis of Variance (ANOVA). The data evaluated for the microbial reduction of the HHP treated shrimps and mussels were analyzed with one-way ANOVA with a probability limit of $p<0.05$. Throughout the analysis, differences at $p<0.05$ were considered as significant. Also analysis of Duncan's Multiple Range test was used as the post-hoc tests, with a probability level of $p<0.05$. The data obtained for the analysis of the shelf-life extension was performed with the regression analysis. Throughout the statistical analysis, Microsoft Excel 2000 and SPSS 12.0 for Windows were used.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Microbiological Analysis

Microbial analysis were based on the amount of coliforms and total microbial counts. Before the analysis, the initial microbial loads for both and shrimp samples were measured from the untreated samples. For the microbial analysis, both the coliforms and the total microbial load of the samples were taken into consideration and checked. However, the initial load for coliforms that was checked for shrimps and mussels, were measured at the non-detectable levels (data not shown). Therefore, coliform content was not taken as a parameter for the determination of the best HHP combination amongst the detected HHP conditions. The critera was based on the effect of HHP treatment on total microbial counts for both shrimp and mussel samples.

The results of the effect of HHP applied at the determined pressure-time and temperature combinations for shrimp and mussel samples were analyzed in sections 3.1.1, 3.1.2, 3.1.3 and 3.1.4. The calculations and the statistical analysis were given in the Appendix A.

3.1.1 Microbiological Analysis for Shrimps

In the first part of the study, best combination of pressure, temperature and time was evaluated based on the microbial analysis. Total microbial counts were monitored and Log_{10} reduction values were calculated in cfu/ml. For shrimps, the initial microbial load of the untreated samples was calculated as 7.60 log cfu/ml.

The results of the HHP treatment on shrimps at 200 MPa are represented in figure 3.1.

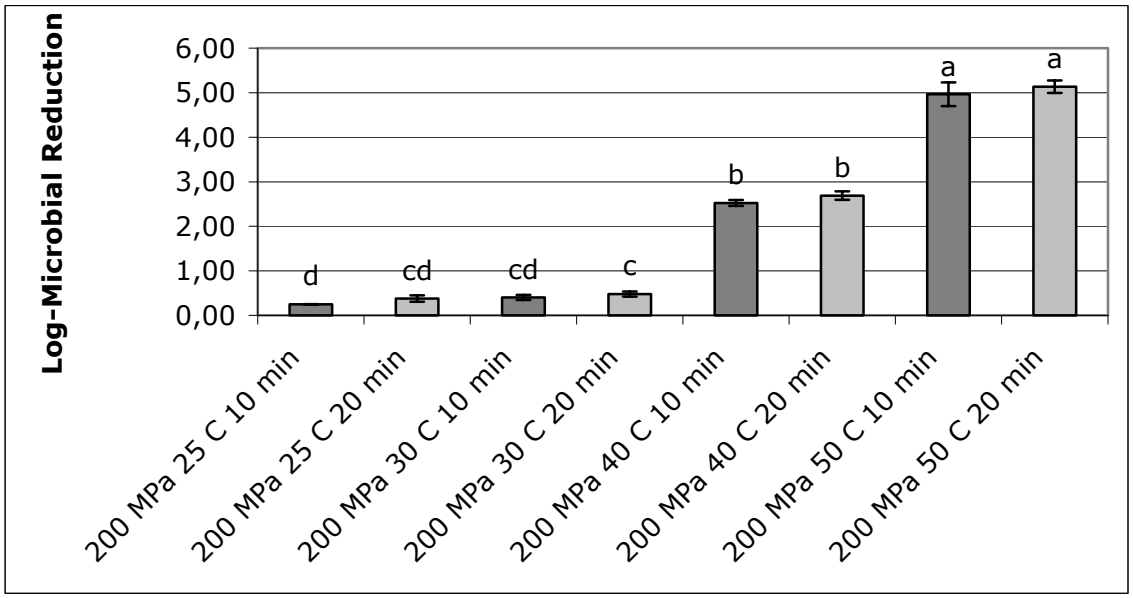

Figure 3.1 Total microbial reduction in log cfu/ml for shrimps treated at 200 MPa for 10 and 20 minutes by temperature change from an initial count of 7.60 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of the 200 MPa HHP application show that at 25 and 30°C microbial reduction is below 1 log cfu/ml. As given in the figure 3.1, in all temperature values, a slight increase in reduction values is observed with increments in time. At 40°C, microbial reduction is observed around 2.5 log cfu/ml. The highest microbial reduction at 200 MPa HHP treatment is observed at 50°C, 20 min application, where 5.14 log cfu/ml of reduction was detected.

In figure 3.1, it was stated that at 200 MPa application, 50°C, 10 and 20 minutes applications were statistically the most significant amongst the given temperature and time conditions at this pressure ($p<0.05$). At 40°C, there were no significant difference among 10 and 20 minutes variations ($p>0.05$). Amongst the rest of the treatment conditions, 30°C and 20 minutes application was significantly different

from 25°C and 10 minutes application ($p<0.05$), while both remain within the same statistical group with; 25°C, 20 minutes and 30°C, 10 minutes applications ($p>0.05$).

The results of the HHP treated shrimps at 220 MPa is given in figure 3.2. The bars indicate the total microbial reduction in log cfu/ml.

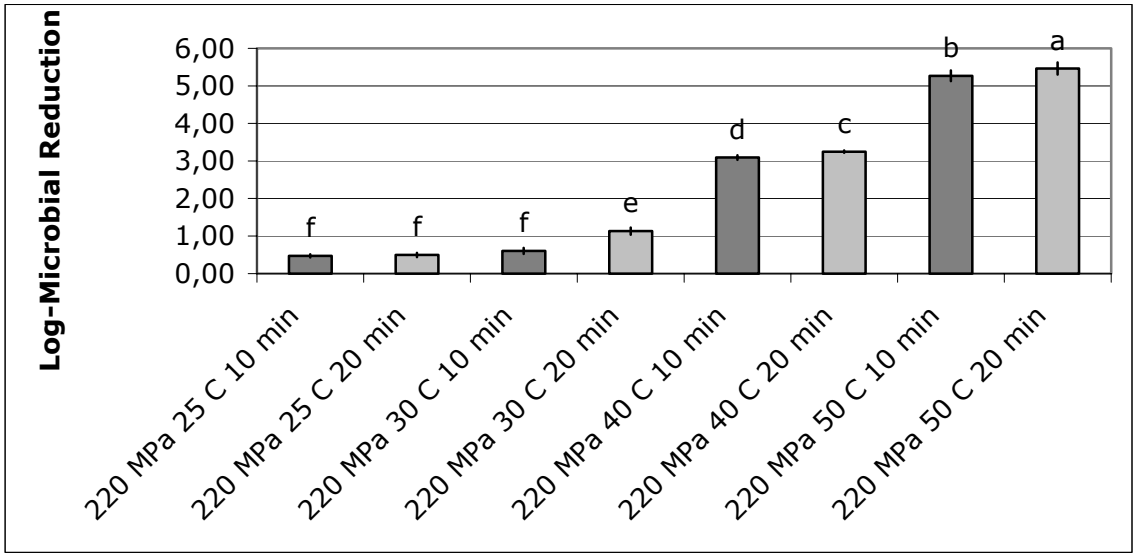

Figure 3.2. Total microbial reduction in log cfu/ml for shrimps treated at 220 MPa for 10 and 20 minutes by temperature change from an initial count of 7.60 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

At 220 MPa less than 1 log cfu/ml reduction was detected at 25°C, 10 and 20 minutes and 30°C 10 minutes applications. Microbial reduction was observed as 1.13 log cfu/ml at 30°C for 20 minutes HHP treatment. At 40 and 50°C applications, microbial reductions exceeded 3 and 5 log cfu/ml, respectively. The highest microbial reduction was observed at 50°C, 20 minute application where a microbial reduction of 5.46 log cfu/ml were detected.

According to the statistical analysis for HHP treatment on shrimps at 220 MPa, it was stated in figure 3.2 that there were no significant difference within 10 and 20

minutes applications at 25 °C and 10 minutes application at 30°C ($p>0.05$). At 50°C, 10 and 20 minutes applications were found to be significantly different ($p<0.05$)

Figure 3.3 gives the results of the HHP treatment on shrimps at 250 MPa for 10 and 20 minutes.

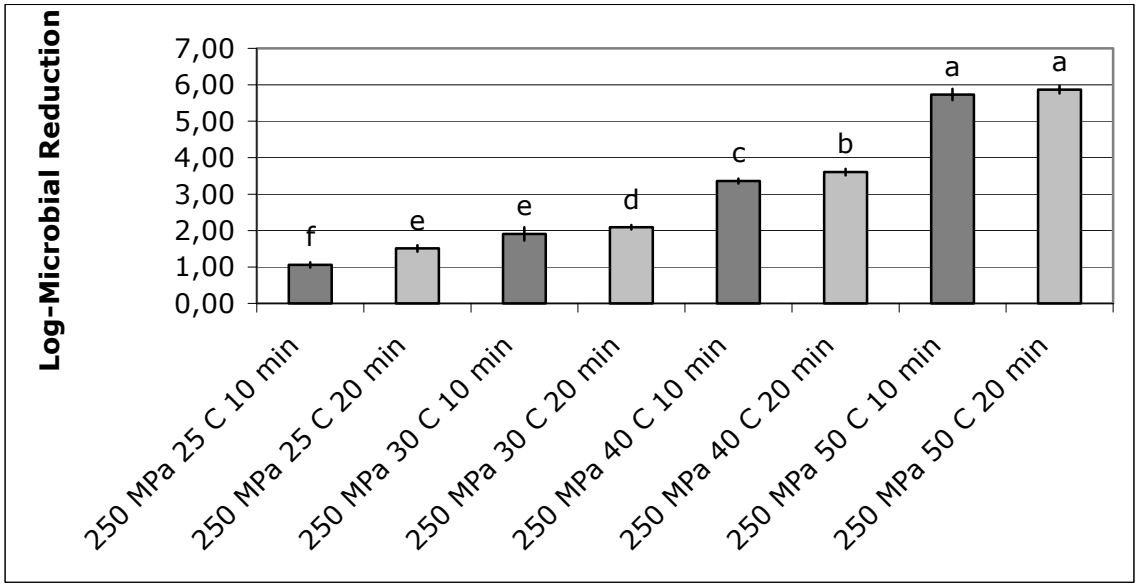

Figure 3.3 Total microbial reduction in log cfu/ml for shrimps treated at 250 MPa for 10 and 20 minutes by temperature change from an initial count of 7.60 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

According to the results of the 250 MPa HHP treatment it is obvious that at all temperatures the degree of microbial reduction were to exceed 1 log cfu/ml for both 10 and 20 minutes application. However the degree of reduction is close to 2 log cfu/ml at 30°C for 10 and 20 minutes. At 40°C the microbial reduction was reported to be exceeding 3 log cfu/ml. The microbial reduction was 5.73 and 5.87 log cfu/ml for 10 and 20 minutes, respectively at 50°C HHP treatment, where the viable cell counts are pulled down below 2 log cfu/ml within the samples.

The difference between 10 and 20 minutes applications were insignificant ($p>0.05$), however, both conditions statistically differs from the rest at 250 MPa ($p<0.05$).

Also at 40°C, 10 and 20 minutes applications were found to be significantly different with the given pressure condition ($p<0.05$)

3.1.2 Summary of Effective Microbiological Analysis for Shrimps

The results of the effective HHP application on shrimps were summarized in Figure 3.4.

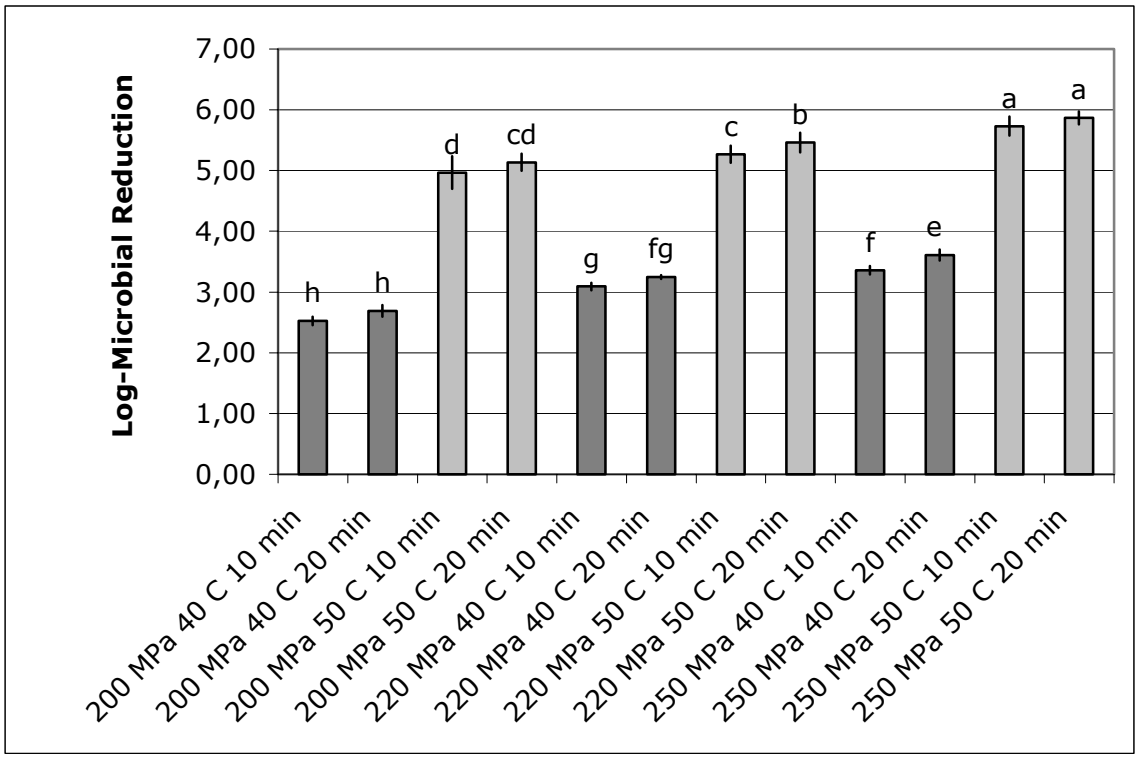

Figure 3.4 Total microbial reduction in log cfu/ml for shrimps treated at 40 and 50°C for 10 and 20 minutes. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

In figure 3.4, HHP treatments at only 40 and 50°C are given, since microbial reductions at 25 and 30°C were reported below 2.09 log cfu/ml for all pressure and time values studied. The highest microbial reduction with the HHP treatment was achieved at 250 MPa and 50°C.

The results clarifies that increasing the pressure, temperature or process time in an HHP treatment on shrimps also increase the reduction of the total microbial count of the samples. Increasing pressurization value have a significant effect on the microbial reduction ($p<0.05$).

He et al. (2002) studied with whole pacific oysters processed with HHP from 207 to 310 MPa at 0,1 and 2 minutes followed by storage under 4°C. With the given conditions, a reduction of 2 to 3 log cfu/g was reported for both aerobic, anaerobic counts and coliforms, after 27 days of storage. He et al. (2002) also reported that the highest microbial reduction was achieved with the HHP treatment of 311 MPa 0 minutes, where 0 minutes application indicates the time the samples are brought to pressure followed by immediate decompression. In another study, optimum HHP conditions amongst 241, 276, 310 and 345 MPa treatments for reduction of *Vibrio parahaemolyticus* to non-detectable levels in pure cultures and pacific oysters was reported as 345 MPa for 30 and 90 seconds, respectively (Calik et al., 2002). Lopez-Caballero et al. (2000) analyzed the effect of 10 minutes of continuous pressure and two 5-minute steps at 400 MPa and 7°C on microbial flora, total volatile bases (TVB-N), pH and texture of purified and unpurified oysters. In this study HHP treatment was reported to reduce the number of all target microorganisms in some cases by around 5-log units, while with the given conditions step-pulse treatment was slightly effective on the microbial reduction than continuous pressurization, but the effect was not significant ($p<0.10$). Kelly et al. (2005) compared the resistance of *Vibrio mimicus, Escherichia coli, Listeria innocua* and *Listeria monocytogenes* in oysters and phosphate buffered saline (PBS), with an initial load of 10^6-10^8 cfu/g, against HHP at 200-700 MPa, 5-20 min at 20 °C. In this study, Kelly et al. (2005) reported that at pressures above 400 MPa, inactivation of all bacteria were less in oysters.

In all HHP applications (200, 220 and 250 MPa), the total microbial inactivation was below 2.09 log cycles up to 40°C. The additional microbial reduction was maximum of 0.63 log cycle at 220 MPa for 20 min as a result of pressurization temperature increase from 25 to 30°C among studied parameters. In contrast, at 250 MPa, increasing pressurization temperature from 30 to 40°C and then to 50°C at 20 min were contributing an additional of at least 1.52 and 2.26 log cfu/ml. The possible reasons can be due to microorganisms' transition state, which is around 40°C, where their resistances to stress reduce, and selecting little increments in pressure

when compared with temperature. Marquis (1976) states that high pressure inactivation rates can be improved with treatments performed above or below optimum growth temperature. In previous research about the effect of HHP on microbial reduction of foods, a significant increase was reported after 37°C where the microorganisms grow at the optimum rate. (Alpas et al., 1999). The reason is probably due to the phase transition of the membrane lipids at that temperature (Kalchayanand et al., 1998a). In terminology, effect of treatment conditions was effective on the microorganisms as time, temperature and pressure, in an increasing manner. However, within the scope of this study, pressurization temperature was found to be statistically more effective than pressure for the reduction of the microbial load. The reason can be the study range of the treatment conditions selected for the HHP processing of shrimps.

According to the results evaluated, the combination of 250 MPa, 50°C and 10 minutes application was selected as the best combination of HHP treatment for shrimps. The criterion in selecting the best combination was based on the microbial reduction together with statistical analysis and economical measures. With the given treatment conditions, temperature was a significant factor among the dependent parameters ($p<0.05$). Since time was an insignificant factor on the microbial reduction at 250 MPa, 10 minute application was taken as the HHP processing for shrimps instead of 20 minute exposure ($p>0.05$). This case is also advantageous when evaluated in industrial scale, since there is a reduction in time which would reduce the cost of the operation and the process.

3.1.3 Microbial Analysis for Mussels

The same pressure, temperature and time combinations were applied on mussel samples in order to determine the best combination of pressure, temperature and time, based on the microbial analysis. Total microbial counts were monitored and Log_{10} reduction values were calculated. For mussels, the initial microbial load of the untreated samples was calculated as 5.72 log cfu/ml.

The results of the HHP treatment on mussels for microbial reduction at 200 MPa are given in figure 3.5.

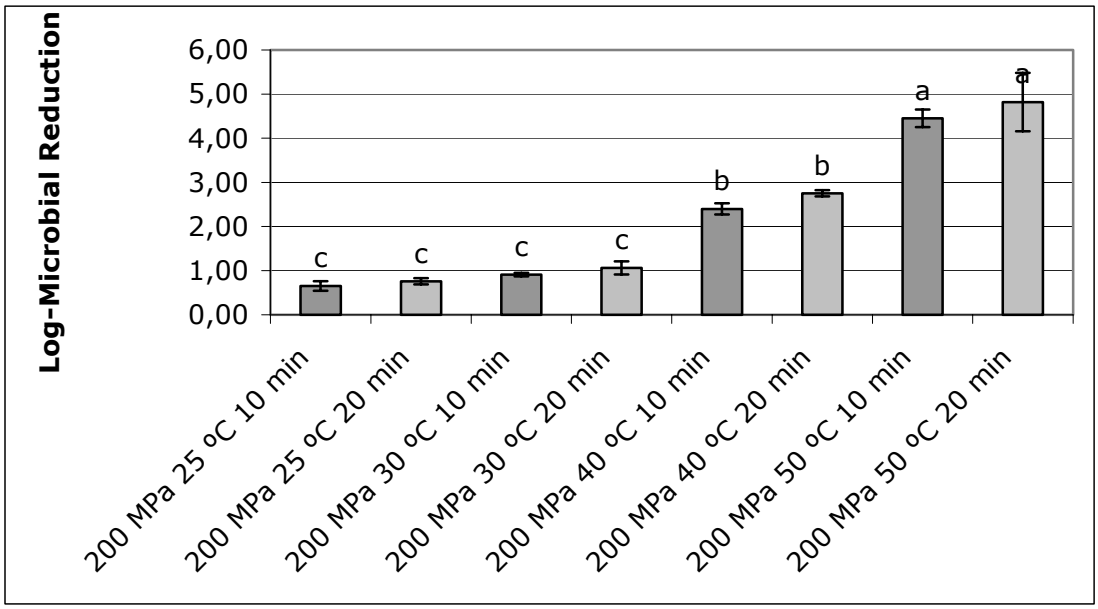

Figure 3.5 Total microbial reduction in log cfu/ml for mussels treated at 200 MPa for 10 and 20 minutes by temperatures change from an initial count of 5.72 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

The results of 200 MPa HHP application shows that at 25 and 30˚C microbial reduction is below 1.06 log cfu/ml. As given in the figure 3.5, in all temperature values, a slight increase in reduction values was observed with increments in time which is statistically insignificant ($p>0.05$). Increasing the pressurization temperature was significant ($p<0.05$), except from 20 to 30 ^{o}C ($p>0.05$). The highest microbial reduction was observed at 200 MPa, 50˚C, 20 min application, where 4,82 log cfu/ml of reduction was detected. At 250 MPa and 50°C, 10 and 20 minutes applications were statistically the same ($p>0.05$)

The results of the microbial reduction by HHP treated mussels at 220 MPa is given in figure 3.6. The bars indicate the reduction in the total microbial counts in log cfu/ml.

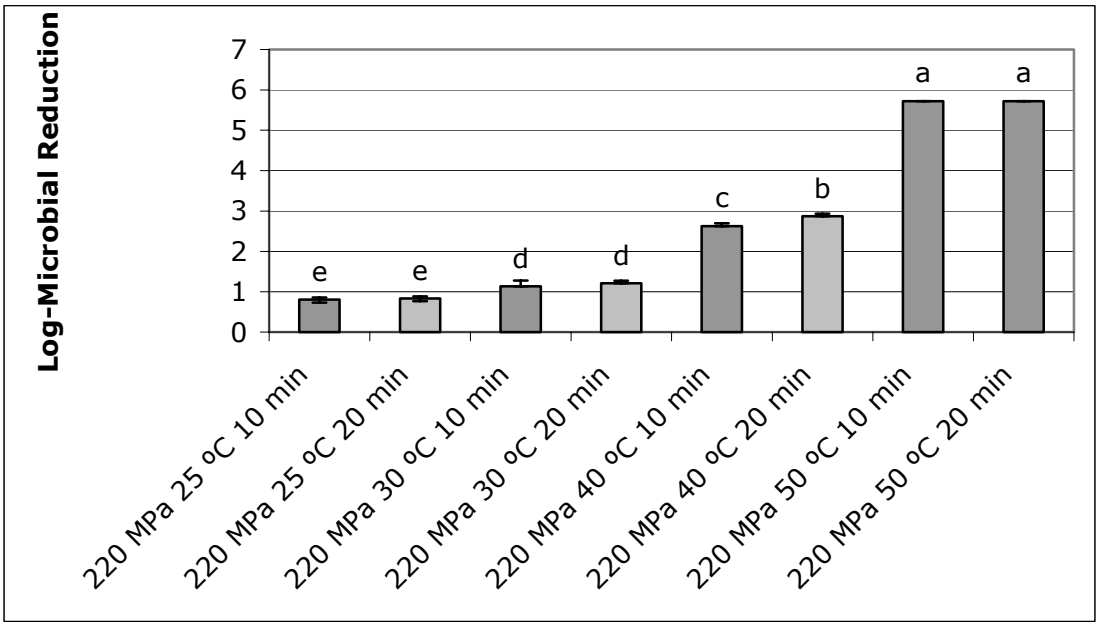

Figure 3.6 Total microbial reduction in log cfu/ml for mussels treated at 220 MPa for 10 and 20 minutes by temperatures change from an initial count of 5.72 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

At 220 MPa less than 1 log cfu/ml reduction was detected at 25°C. Microbial reduction was observed as 1.14 and 1.21 log cfu/ml at 30°C for 10 and 20 minutes of HHP treatment, respectively. Pressurization time was a significant parameter only at 40°C ($p<0.05$), whereas increasing the pressurization temperature (25 to 50°C) was significantly affecting microbial reduction at this pressure range ($p<0.05$). At 50°C, with the HHP treatment for 10 and 20 minutes no viable cells were detected, which means that the load of the total microbial count was pulled down to the non-detectable levels with the given HHP treatment. Consequently, 220 MPa, 50°C, 10 and 20 minutes applications were statistically the same ($p>0.05$).

Figure 3.7 implies the microbial inactivation results of the HHP treatment on mussels at 250 MPa for 10 and 20 minutes. The bars were representing the reduction in the total microbial count in log cfu/ml.

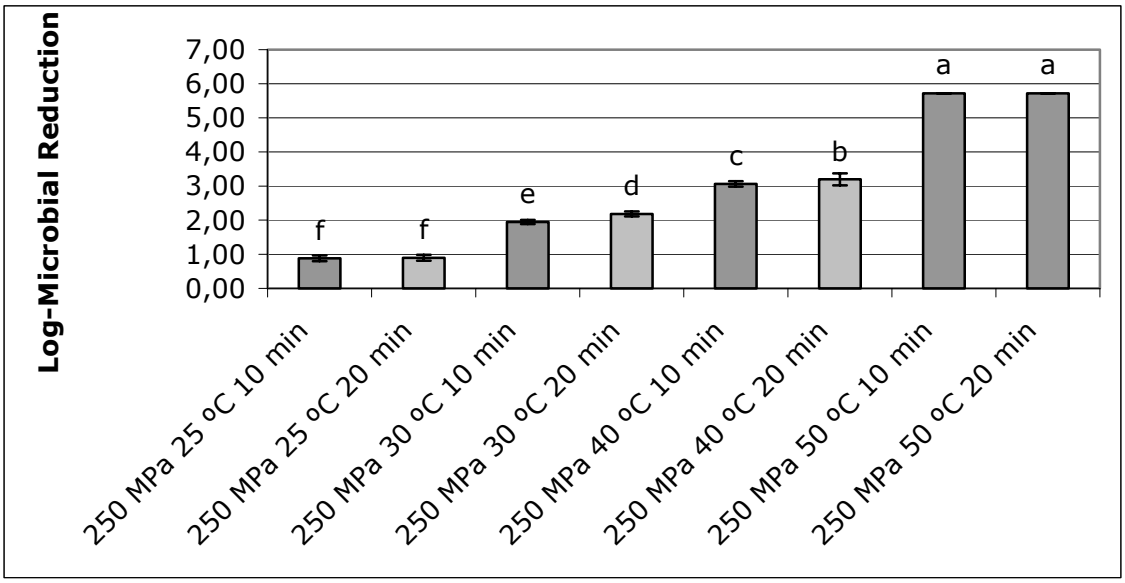

Figure 3.7 Total microbial reduction in log cfu/ml for mussels treated at 250 MPa for 10 and 20 minutes by temperatures change from an initial count of 5.72 log cfu/ml. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

According to the results of the 250 MPa HHP treatment it is obvious that at all temperature ranges, except for pressurization at 25°C more than 1 log cfu/ml microbial reduction was obtained. Pressurization time was a significant parameter at 30 and 40°C whereas pressurization temperature was a significant parameter at all temperature ranges at this pressure ($p< 0.05$). The microbial reduction was 5,72 log cfu/ml for 10 minutes at 50°C HHP treatment, as no viable cell colonies were detected.

3.1.4 Summary of Effective Microbiological Analysis for Mussels

The results of the HHP application on the microbial inactivation of mussels were summarized in figure 3.8. In the figure, only HHP treatments at 40 and 50°C were taken as data, while 25 and 30°C applications were omitted, in order to provide an effective assesment of the data.

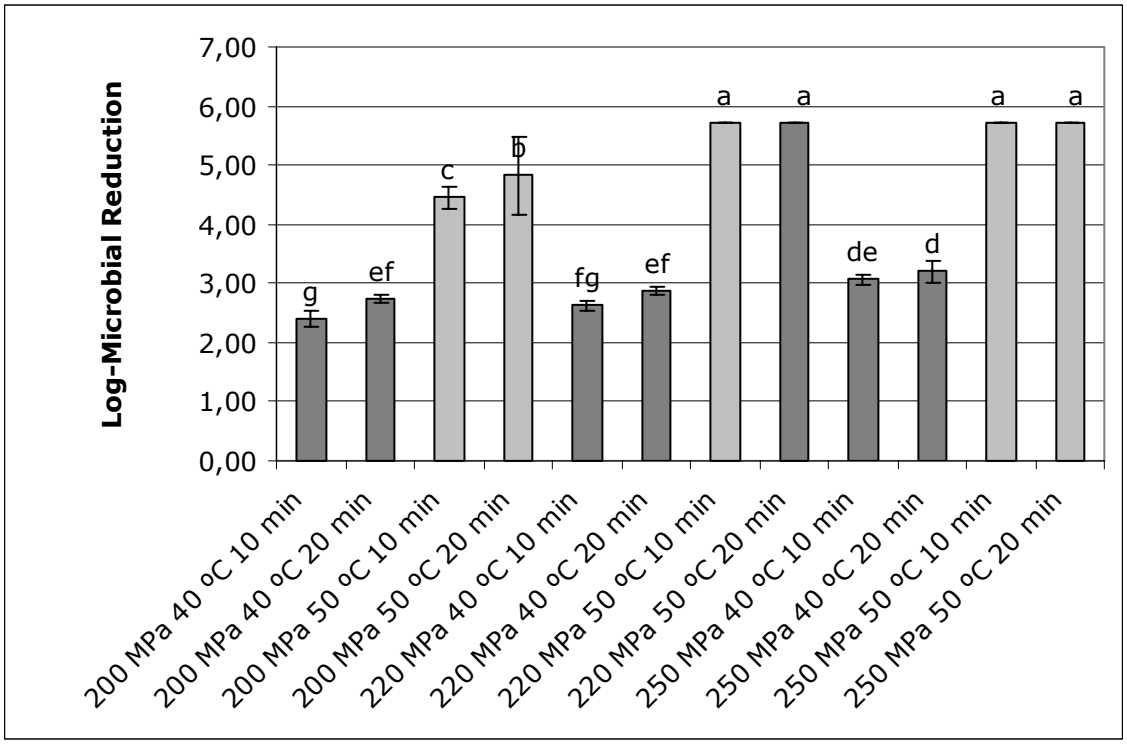

Figure 3.8 Total microbial reduction in log cfu/ml for mussels treated at 40 and 50°C for 10 and 20 minutes. The error bars indicate the standard deviations of the measurements. Different letters imply significant changes ($p<0.05$).

In figure 3.8, HHP treatments at 40 and 50°C are given, since microbial reductions at 25 and 30°C treatments were reported below 2.18 log cfu/ml. The amount of microbial reduction increases with increasing pressure, temperature and time. The results obtained indicated that all of the microbial load (5.72 log cfu/ml) within the mussel samples was inactivated via HHP treatment at 220 MPa, 50°C 10 min.

As can be seen from figures 3.5, 3.6 and 3.7, increasing pressurization also increased the microbial reduction. The statistical analysis of the results of HHP treated mussels gives that, changes in pressurization time did not have a significant effect on the microbial inactivation except at 220 MPa 40^{o}C, 250 MPa 30 and 40^{o}C combinations ($p<0.05$) while above 200 MPa 30^{o}C increasing the pressurization temperature is significantly affecting the microbial reduction ($p<0.05$).

According to the results, the combination of 220 MPa, 50°C and 10 minute was selected as the best combination of HHP treatment for mussel samples in this study.

3.2 Shelf-Life Analysis of Shrimps and Mussels

For the shelf-life analysis, HHP treated shrimps and mussels were stored at 4 and 25°C in dark and the samples were analyzed with 2 day intervals until they reach to the critical or unacceptable level in terms of pH (above pH = 8.0) (Schormuller, 1968; Ludorff and Meyer, 1973; Varlik et al., 1993) or Total Volatile Bases (TVB-N > 35 mg N/100 g sample) (Schormuller, 1968; Lang, 1979; Varlık et al., 2000; Erdem et al., 2004; Turan and Erkoyuncu, 2004). Experiments were performed twice on separate days and the average results are presented.

3.2.1 Total Volatile Bases

In sea food samples a TVB-N value below 25mg N/100g sample is an indication of "Very Good" quality. A value between 25 to 30 mg N/100g sample is "Good" and between 30 and 35 mg N/100g sample is considered as "Acceptable-Marketable" product. Any value above 35mg N/100g sample is an indication of an unacceptable (spoiled) product (Schormuller, 1968; Lang, 1979; Varlık et al., 2000; Erdem et al., 2004; Turan and Erkoyuncu, 2004).

Table 3.1 gives the variation of TVB-N values for untreated and HHP treated shrimps (250 MPa, 50°C and 10 minutes) stored at 4 and 25°C, while table 3.2 shows the results of the TVB-N measurements for untreated and treated mussels (220 MPa, 50°C and 10 minutes) stored at 4 and 25°C.

Th initial TVB-N values were calculated for the untreated shrimp samples before storage as 12,60 mg N/100g, which contributes to very good in quality. The values were given as 0 day measurements in the table 3.1. The measurements were done untill critical limit to spoilage (35 mg N/100g) was exceeded for each storage condition.

Table 3.1 TVB-N values for untreated and HHP (250 MPa, 50°C and 10 minutes) treated shrimps stored at 4 and 25°C.

	Refrigeration Temperature (+4ºC)		Room Temperature (+25ºC)	
Days	**Untreated**	**Treated**	**Untreated**	**Treated**
0	12,60	12,60	12,60	12,60
2	29,19	12,81	39,90	18,69
4	42,84	14,91		21,42
6		19,11		25,41
8		23,52		29,61
10		25,41		33,81
12		27,72		35,70
14		30,24		38,01
16		34,65		
18		38,85		

Table 3.2 TVB-N values for untreated and HHP (220 MPa, 50°C and 10 minutes) treated mussels stored at 4 and 25°C.

	Refrigeration Temperature (+4ºC)		Room Temperature (+25ºC)	
Days	**Untreated**	**Treated**	**Untreated**	**Treated**
0	10,08	10,08	10,08	10,08
2	25,41	10,71	37,17	14,91
4	38,85	12,81		19,11
6		17,01		22,47
8		17,64		23,94
10		19,11		25,62
12		21,84		30,24
14		25,41		36,12
16		29,19		
18		33,60		
20		35,49		

From table 3.1, within the 2-day period, the untreated shrimp samples stored at 25°C exceeded 35 mgN/100g, which is the critical TVB-N value for acceptability. When stored at 4°C, untreated mussel samples remained as good within 2 days of storage and became unacceptable after 4 days of storage. HHP treated mussels remained well within 4 and 8 day-periods, for storage at room and refrigeration temperatures, respectively. In the 10^{th} day of storage at 25 °C, TVB-N value of shrimps were measured as 33,81 mgN/100g, which is above the critical limit of

good (Varlık et al., 2000). In the 12th day, HHP treated shrimps, stored at room temperature were detected to have TVB-N value of 35,70 mgN/100g, which exceeds the critical limit for spoilage. For the refrigerated storage, HHP treated shrimps were to pass up to the good range in 10th day, with the TVB-N value of 25,41 mgN/100g. At 14th day, these shrimps were still within the acceptable range. Spoilage of the HHP treated shrimps stored at 4 °C was detected at the 18th day of the storage.

The data evaluated for shrimps are in agreement with previous results reported in literature. Varlık et al. (2000) reported that fresh shrimps became unacceptable at the second day of the storage at 4°C referring to their TVB-N values. Matches (1982) reported the TVB-N values of untreated shrimps as 39,5 mg/100g at the 3rd day of storage at 0°C and 50.8 mg/100g at the 1st day of storage at 5.6°C. Stockemer and Nieper (1984) detected that the untreated shrimps stored at 7°C became unacceptable at the 4th day of storage, with the TVB-N value of 37,1 mgN/100g. Cheuk et al. (1979) calculated the TVB-N values of untreated shrimps stored in ice as 30 mgN/100g at the 3rd day of storage. Chang et al. (1983) calculated the increase in the TVB-N values of untreated shrimps from 18 mg/100g to 30 mg/100g in the end of 5-day storage period at 4°C. At 0°C, TVB-N values of shrimps reached up to 30 mg/100g after 14 days of storage (Angel et al. 1979). Shamshad et al. (1990) reported the TVB-N values of fresh shrimps as 4.5 mg N/100g in the beginning of the storage and 16.7 mg N/100g at the 13th day of the storage at 0°C and 21,4 mg N/100g at the 9. day of storage under 5°C. Erdem and Bilgin (2004) studied with shrimps as raw and cooked with boiling water at 100 °C for 15 minutes, followed by storage at 4 °C and the TVB-N values were obtained as 33.13 mg N/100g at the 3. day and 35.86 mg N/100g at the 5. day of storage for raw and cooked shrimps, respectively. Erdem and Bilgin (2004) also has detected the pH values of the raw and cooked shrimp samples as 8.06 and 7.86, respectively, at the 5.th day of storage. In their study, they stated that, based on the sensory analysis, the critical limit to the freshness of the shrimps samples were 2 days for the raw and 3 days for the cooked samples As can be followed from the reported literature most of the works on sea foods were on untreated samples and almost all samples has reached the unacceptable level of TVB-N values before 18 days, even though they were stored at or around refrigeration temperatures.

In table 3.2 the TVB-N values for HHP treated (220 MPa, 50°C and 10 minute) and untreated mussels stored at 4 and 25 °C are given. The 0 day calculations contribute to the initial TVB-N values of the mussel samples, which were regarded as the control measures. Similar to shrimps, the initial TVB-N values of the mussel samples, 10,08 mgN/100g, were within the very good range. The untreated samples were exposed to spoilage at the 2^{nd} and 4^{th} day of storage at room and refrigeration temperatures, respectively.

When stored at room temperature, HHP treated mussels were detected to be within the good range in the 10^{th} day of storage, with a TVB-N value of 25,62 mgN/100g. In the 12^{th} day of storage under 25°C, TVB-N of the mussel samples were calculated as 30,24 mgN/100g, which contributes to acceptable level. Spoilage was detected in the 14^{th} day at this storage condition (25°C), where a TVB-N value of 36,12 mgN/100g was measured. At the refrigeration temperature, the treated mussels seemed to pass the acceptable limit to good, in the 14^{th} day of storage, with a TVB-N value of 25,41 mgN/100g. In the 18^{th} day of storage at 4^{o}C, the TVB-N values were still well below the spoilage level, where the samples remained to be accepted for consumption, with a TVB-N value of 33,60 mgN/100g. The spoilage of the HHP treated mussel samples stored at 4°C was detected in the 20^{th} day of storage, where the TVB-N value was calculated as 35,49 mgN/100g.

HHP treatment of mussel samples at 500 MPa or higher followed by storage at 2°C resulted with an extension of the shelf-life up to 14 days, when psychotropic count of 10^6-10^7 cfu/ml was assumed as the level of spoilage (Patterson et al. 2003). Patterson et al. (2003) also reported that, based on the sensory analysis of the cooked mussels, fresh and untreated mussel samples were most acceptable, when compared with the HHP treated samples HHP at 600 MPa, however the latter were also acceptable after 2 weeks of storage. Altinier et al. (2002) reported that high pressure application of mussel samples at 500 MPa for 3 minutes completely inactivated an artificial contamination of 10^5 cfu/g of *Salmonella derby* and *Listeria innocua* and reduced *Escherichia coli,* by 4 logarithmic units, while a shorter treatment at 500 MPa for 1 minute completely inactivated *S. derby* and reduced *E. coli* and *L. innocua* by 3 logarithmic units. Furthermore, a reduction of the initial total viable count of a natural contaminated sample of mussels, from $4x10^5$ to $7x10^2$

CFU/g was observed with an HPP treatment of 700 MPa for 5 minutes (Altinier et al., 2002).

Storage of HHP treated shrimps and mussels have extended the shelf-life to 18 and 20 days, respectively, at refrigerated storage and to 12 and 14 days at 25°C. Considering that both untreated samples were unacceptable after 4 days even stored at 4°C; effect of HHP treatment on extending the shelf-life of shrimps and mussels is obvious.

3.2.2 pH Evaluation

The pH variations of the samples at different storage conditions are given in the tables below. The effect of the storage temperature for treated and untreated samples were tabulated in Table 3.3 and 3.4 for shrimps and mussels, respectively. The pH data exceeding values of 8.00 were omitted. The values are reported as the average of two replicas of measurements.

Table 3.3 pH values for untreated and treated (250 MPa, 50°C and 10 minute) shrimps stored at 4 and 25°C.

	Refrigeration Temperature (4ºC)		**Room Temperature (25ºC)**	
Days	**Untreated**	**HHP Treated**	**Untreated**	**HHP Treated**
0	6,95	6,95	6,95	6,95
2	7,34	6,99	8,04	7,12
4	8,53	7,09		7,28
6		7,17		7,42
8		7,35		7,62
10		7,44		7,85
12		7,71		7,94
14		7,79		8,26
16		7,88		
18		7,98		

In table 3.3, the pH values were given for the HHP treated and untreated shrimp samples, stored at refrigeration and room temperature conditions. The 0 day measurements represent the control values for the shrimp samples. For the

untreated samples, the pH values were reported as 8,53 at the fourth day of storage under 4°C and 8,04 at the second day of storage under 25°C.

Table 3.4 pH values for untreated and treated (220 MPa, 50°C and 10 minute) mussels stored at 4 and 25°C.

Days	Refrigeration Temperature (4ºC)		Room Temperature (25ºC)	
	Untreated	HHP Treated	Untreated	HHP Treated
0	6,80	6,80	6,80	6,80
2	7,22	6,90	8,02	6,95
4	7,72	6,94		7,11
6	8,73	6,98		7,22
8		7,15		7,30
10		7,21		7,36
12		7,36		7,62
14		7,43		7,81
16		7,65		8,33
18		7,72		

Table 3.4 gives the results of the pH values of mussel samples, treated or untreated with HHP, followed by storage at room and refrigeration temperatures. The 0 day calculations gives the control values, which was measured as 6,80. The untreated samples were exceeding pH 8,00 within 2 days of storage at 25 °C. When stored at 4 °C, the pH of the untreated mussel samples was exceeding the pH 8,00 in 6^{th} day of storage.

The shrimps were exposed to HHP treatment of 250 MPa, 50°C for 10 minutes, which was determined as the best combination for the shrimp samples based on the microbial analysis. The pH of the samples remained below 8,00 until the end of the 12^{th} day of storage for both temperature. The pH of the shrimps stored under 25°C were detected to be exceeding 8,00 in the 14^{th} day with the pH value of 8,26. The results for the storage conditioned under 4 °C showed that the pH of the samples were under 7,00 up to the 4^{th} day of the storage. The pH values were also reported to be below 8,00, within 18 days of storage at the refrigeration temperature. The pH of the treated shrimps was 7,98 at the 18^{th} day of the storage.

The mussel samples were treated at 220 MPa, 50°C and 10 minutes; which was detected as the best combination for mussels processing based on the data evaluated via microbial analysis. When stored at room temperature. The pH values were reported below 8,00 within a 14-day period and detected to be exceeding 8,00 in the 16th day of storage. The outcomes of the pH analysis show that within a 18-day period, the pH of the HHP treated mussel samples were under 8,00 with the storage at 4°C.

3.2.3 Summary of the Shelf Life Study

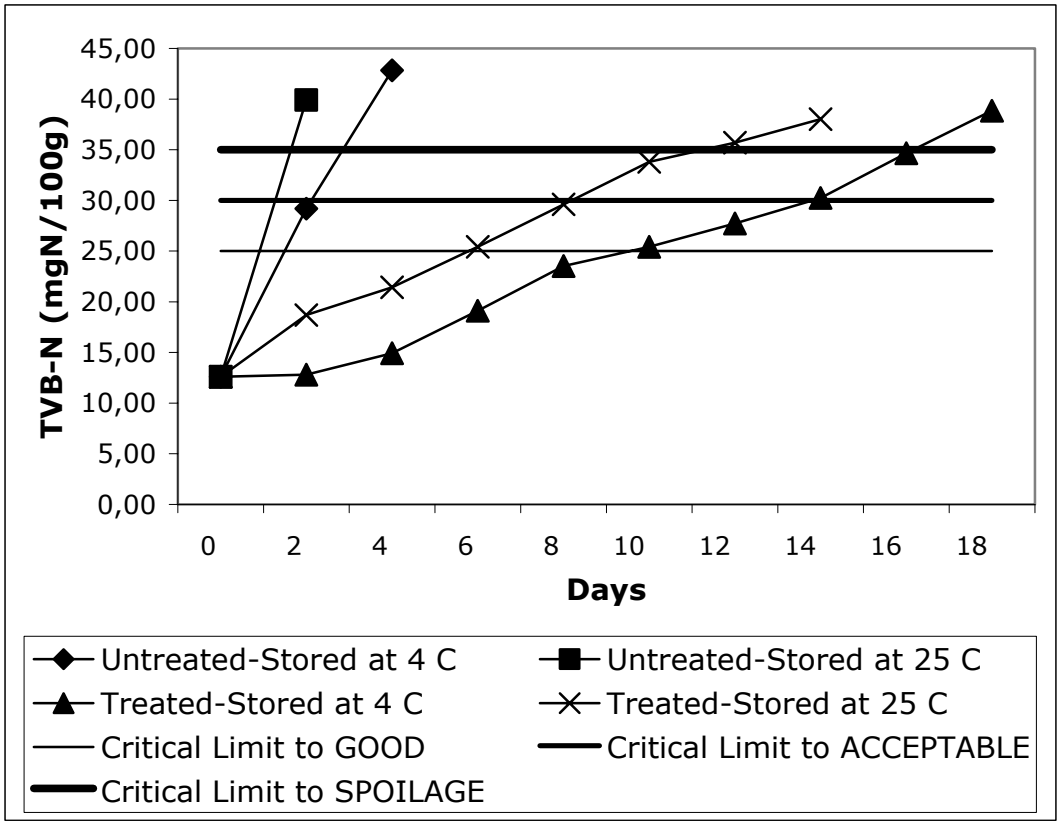

Figure 3.9 TVB-N values of the HHP (250 MPa, 50°C and 10 minute) treated and untreated shrimps at 4 and 25°C storage.

Figures 3.9 and 3.10 summarize the TVB-N values of the shellfish samples stored at room and refrigeration temperature after being treated by HHP (shrimps 250 MPa,

mussels 220 MPa at 50°C and 10 minutes). In general, mussels have lower TVB-N values than shrimps before and throughout the storage period. This is because of the lower initial microbial load of mussels when compared with shrimps.

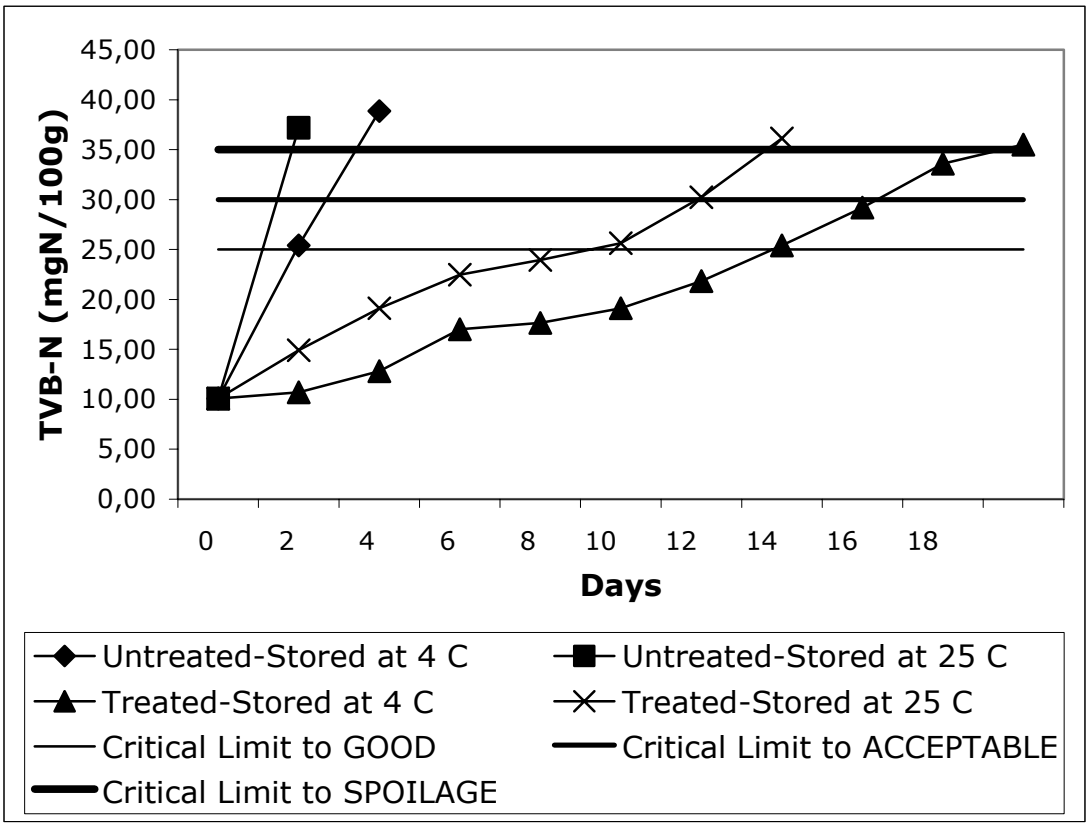

Figure 3.10 TVB-N values of the HHP (220 MPa, 50°C and 10 minute) treated and untreated mussels at 4 and 25°C storage.

In figure 3.9, TVB-N values of shrimps were plotted. For the untreated samples, the shrimps exceeded the critical limit to spoilage before 2nd (R^2=0.997) and 4th day of room and refrigeration storage, respectively. In the graph, it was also shown that the treated samples stored at 25°C exceeded the critical limit to spoilage before 12th day (R^2=0,984). When stored at 4°C, shrimps were detected to be exceeding the spoilage limit before 18th day of storage (R^2=0,983). Also in the graph, it can be detected that the shrimps were exceeding the range of good, before 6th and 10th day of storage conditioned to 25 and 4°C, respectively.

In figure 3.10, the untreated mussel samples stored at room and refrigeration temperature was exceeding the spoilage level before 2nd and 4th (R^2=0,999) day of storage, respectively. Treated shrimps were below spoilage limit till 14th (R^2=0,972) and 20th (R^2=0,973) days of storage under 25 and 4°C. The treated mussels were exceeding the critical limit to good, before the 10th and 14th day of storage at 25 and 4°C, respectively.

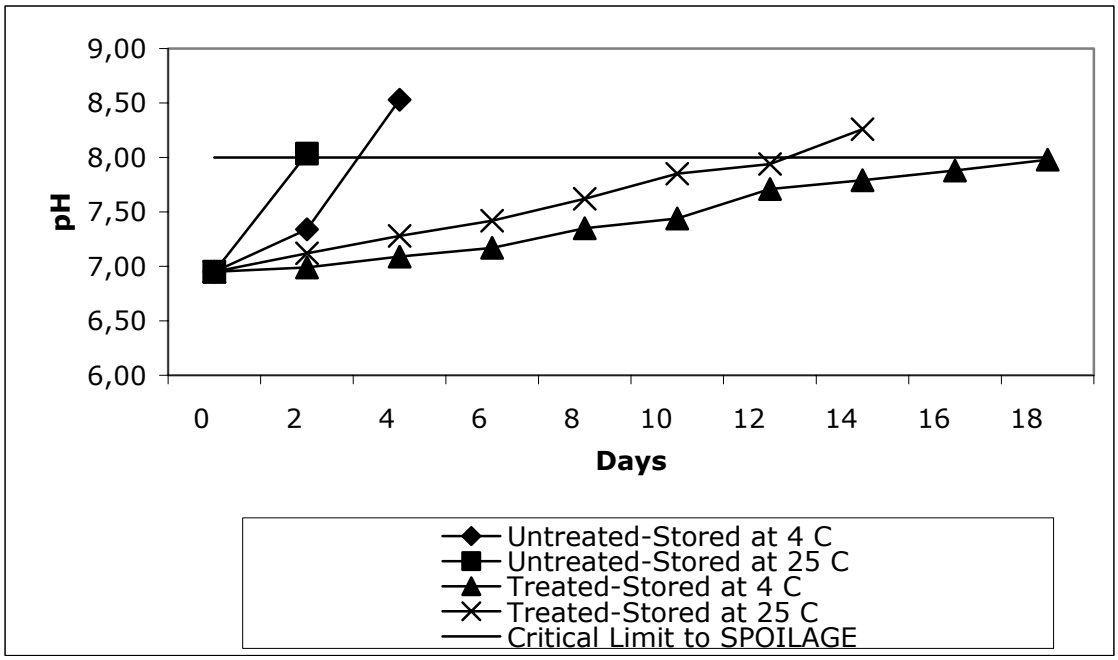

Figure 3.11 pH variations of the HHP (250 MPa, 50°C and 10 minute) treated and untreated shrimps at 4 and 25°C storage.

Lopez-Caballero et al. (2000) observed the TVB-N values for oysters with an initial TVB-N value of 13.3 and 11.2 mgN/100g for non-purified and purified oysters, after treated with HHP at 400 MPa and 7°C for 5 minutes and reported an increase in TVB-N values up to 25-30 mgN/100g in 6 weeks of storage. The reason for such long storage period for TVB-N values may be due to the higher pressure treatment application and the fermentative spoilage of oysters because of their high glycogen content which causes the acidification of the medium and lowers the alkalinity. Similarly, Murata and Shakaguchi (1986) studied with shucked oysters stored in ice, with an initial value of 10.5 mgN/100g, and the result was reported as relatively low

increase followed by a sudden increase of TVB-N up to 25 mg N/100g. The reason for the slow increase in TVB was reported to be due to general acidification of the high glycogen content that is converted to lactic acid (Lopez-Caballero et al., 2000).

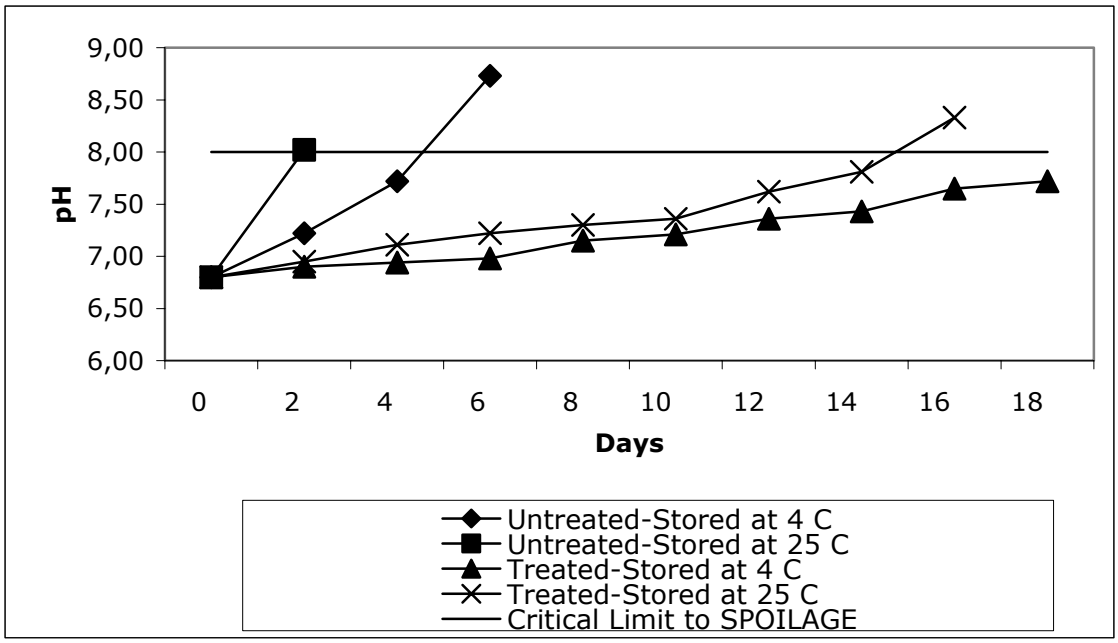

Figure 3.12 pH variations of the HHP (220 MPa, 50°C and 10 minute) treated and untreated mussels at 4 and 25°C storage.

In figures 3.11 and 3.12, the variations in pH were summarized. Varlık et al. (2000) and Schormuller (1968) reported that, in general, the acceptable pH levels for shellfish must be between 7.00 and 8.00. From the figures, it is understood that spoilage occurs before 14th day of storage for both mussels and shrimps, when stored at 25°C. In the case of storage at 4°C, pH values remain under 8.00 up to 18th day, which was also reported as another critical limit for most shellfish (Schormuller, 1968). The increase in pH values are reliable with the results of the TVB-N values, since increasing TVB-N during the storage turns the medium alkaline and as a result pH increases (Varlık et al., 2000).

Consequently, TVB-N values are convenient for the determination of the shelf-life of samples, since spoilage was detected earlier when based on the data obtained by

the calculations of TVB-N. Therefore the pH values were not taken into consideration for the shelf life prediction. The shelf-life of shrimps treated with the determined HHP combination (250 MPa, 50°C and 10 minutes) is evaluated as 10 days for room temperature storage and 16 days for refrigeration storage. Similarly for mussels, treated with the determined HHP combination (220 MPa, 50°C and 10 minute), the shelf-life is evaluated as 12 days for room temperature storage and 18 days for refrigeration storage.

According to the results, a shelf life enhancement of 10 and 14 days was achieved between untreated and shrimps at room and refrigeration temperature, respectively. The same case was observed for mussels as 12 and 16 days for room and refrigeration temperatures, respectively. Moreover, throughout storage at 4°C rather than 25°C, a shelf life enhancement of 4 days were obtained for both shrimp and mussel samples.

CHAPTER 4

CONCLUSIONS and RECOMMENDATIONS

The study was evaluated and conducted in two parts. In the first part, HHP treatments with the selected pressure, temperature and time combinations were conducted. Among the selected parameters, a best pressure, temperature and time combination was determined, based on the microbial analysis of the total microbial counts. According to the results obtained, and the evaluation of the data collected, the best treatment combinations were determined as 250 MPa, 50°C and 10 minutes for shrimps and 220 MPa, 50°C and 10 minutes for mussels.

HHP treated samples at the determined best combinations specific to shrimps and mussels were stored at room (25°C) and refrigeration temperature (4°C). Throughout the storage period, variations in TVB-N and pH values of the samples - in order to detect the physical and chemical changes due to the spoilage by microorganisms- were monitored. The shelf-life of shrimps treated with the determined HHP combination is evaluated as 10 days at room temperature storage and 16 days for refrigeration storage. Similarly for mussels, treated with the determined HHP combination, the shelf-life is evaluated as 12 days for room temperature storage and 18 days for refrigeration storage. After these storage periods HHP treated shrimps and mussels are still marketable accoding to the TVB-N values.

Freezing is widely used in the food industry, especially in case of shell-fish. For the manufacture of sea foods, loss of sensory and nutritional characteristics is not preferred since these products are usually consumed raw. Due to the requirement of the protection during cold storage throughout the post-production period up to consumption, a vast amount of energy is lost which constitutes a burden for the economy of the companies. Especially, in case of frozen food technology, throughout the post-production period, the final products must be kept under -

18°C, which brings out relatively increased expenditures for the post-production. In contrast, the necessity of the system that will lower the operational costs and expenditures by reducing the energy requirement of the processing during the production, storage, distribution and marketing period, together with it's maintaining the sensory and the nutritional characteristics of the final products was being proposed. HHP technology has been in use for the preservation and shucking of oysters for over 5 years.

The results of this study states that the shelf life of shrimp samples were extended from 4 to 16 days and from 2 to 12 days at 4 and 25 °C storage, respectively by HHP treatment. Similarly, the shelf life of mussels was enhanced from 4 to 20 and from 2 to 14 days, for the storage at 4 and 25 °C, respectively. On this basis the study points out that HHP can be offered as an alternative method to conventional freezing for the preservation of mussels and shrimps, while it provides a shelf life enhancement of additional 10-14 days for untreated and treated shrimps and 12-16 days for untreated and treated mussels. Enabling storage of the final product requires much higher temperatures when compared with frozen food technology and keeping the product properties more fresh-like. Besides its high investment cost, HHP is proposed with a much lower operational cost when compared with the current technology, which can be more economical in long term assessments.

As a recommendation, the research can be sustained by covering more sea foods and shell-fish and higher pressure values with lower temperature and time variations may be performed. Also, for the determination of the shelf-life the results of the physical and chemical analysis can be supported and verified by sensory evaluations during the storage period.

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APPENDIX A

RESULTS AND CALCULATIONS

Table A.1 HHP treatment for shrimps at 200 MPa.

200 MPa for Shrimps		Measurements				Mean	St dev
		1	2	3	4		
200 MPa	counts	220	225	225	225		
25°C	Exp	2,2E+07	2,3E+07	2,3E+07	2,3E+07		
10 min	log cnt	7,34	7,35	7,35	7,35	7,35	0,005
	Log red	0,26	0,25	0,25	0,25	**0,25**	
200 MPa	counts	180	130	190	170		
25°C	Exp	1,8E+07	1,3E+07	1,9E+07	1,7E+07		
20 min	log cnt	7,26	7,11	7,28	7,23	7,22	0,073
	Log red	0,34	0,49	0,32	0,37	**0,38**	
200 MPa	counts	180	160	160	130		
30°C	Exp	1,8E+07	1,6E+07	1,6E+07	1,3E+07		
10 min	log cnt	7,26	7,20	7,20	7,11	7,19	0,059
	Log red	0,34	0,4	0,4	0,49	**0,41**	
200 MPa	counts	140	130	110	150		
30°C	Exp	1,4E+07	1,3E+07	1,1E+07	1,5E+07		
20 min	log cnt	7,15	7,11	7,04	7,18	7,12	0,058
	Log red	0,45	0,49	0,56	0,42	**0,48**	
200 MPa	counts	140	130	110	100		
40°C	Exp	140000	130000	110000	100000		
10 min	log cnt	5,15	5,11	5,04	5,00	5,08	0,067
	Log red	2,45	2,49	2,56	2,6	**2,52**	
200 MPa	counts	100	80	60	90		
40°C	Exp	100000	80000	60000	90000		
20 min	log cnt	5,00	4,90	4,78	4,95	4,91	0,096
	Log red	2,6	2,7	2,82	2,65	**2,69**	
200 MPa	counts	28	73	23	73		
50°C	Exp	280	730	230	730		
10 min	log cnt	2,45	2,86	2,36	2,86	2,63	0,267
	Log red	5,15	4,74	5,24	4,74	**4,97**	
200 MPa	counts	33	41	19	28		
50°C	Exp	330	410	190	280		
20 min	log cnt	2,52	2,61	2,28	2,45	2,46	0,141
	Log red	5,08	4,99	5,32	5,15	**5,14**	

Table A.2 HHP treatment for shrimps at 220 MPa.

220 MPa for Shrimps		Measurements				Mean	St dev
		1	2	3	4		
220 MPa	Counts	130	120	140	150		
25°C	Exp	1,30E+07	1,20E+07	1,40E+07	1,50E+07		
10 min	log cnt	7,11	7,08	7,15	7,18	7,13	0,042
	Log red	0,49	0,52	0,45	0,42	0,47	
220 MPa	Counts	120	110	150	130		
25°C	Exp	1,20E+07	1,10E+07	1,50E+07	1,30E+07		
20 min	log cnt	7,08	7,04	7,18	7,11	7,1	0,057
	Log red	0,52	0,56	0,42	0,49	0,5	
220 MPa	Counts	120	80	90	110		
30°C	Exp	1,20E+07	8000000	9000000	1,10E+07		
10 min	log cnt	7,08	6,9	6,95	7,04	6,99	0,08
	Log red	0,52	0,7	0,65	0,56	0,61	
220 MPa	Counts	25	25	30	40		
30°C	Exp	2500000	2500000	3000000	4000000		
20 min	log cnt	6,4	6,4	6,48	6,6	6,47	0,096
	Log red	1,2	1,2	1,12	1	1,13	
220 MPa	Counts	30	40	30	30		
40°C	Exp	30000	40000	30000	30000		
10 min	log cnt	4,48	4,6	4,48	4,48	4,51	0,062
	Log red	3,12	3	3,12	3,12	3,09	
220 MPa	Counts	220	250	210	220		
40°C	Exp	22000	25000	21000	22000		
20 min	log cnt	4,34	4,4	4,32	4,34	4,35	0,033
	Log red	3,26	3,2	3,28	3,26	3,25	
220 MPa	Counts	22	14	31	22		
50°C	Exp	220	140	310	220		
10 min	log cnt	2,34	2,15	2,49	2,34	2,33	0,142
	Log red	5,26	5,45	5,11	5,26	5,27	
220 MPa	Counts	11	14	23	10		
50°C	Exp	110	140	230	100		
20 min	log cnt	2,04	2,15	2,36	2	2,14	0,162
	Log red	5,56	5,45	5,24	5,6	5,46	

Table A.3 HHP treatment for shrimps at 250 MPa.

250 MPa for Shrimps		Measurements				Mean	St dev
		1	2	3	4		
250 MPa	Counts	30	30	40	40		
25°C	Exp	3000000	3000000	4000000	4000000		
10 min	log cnt	6,48	6,48	6,6	6,6	6,54	0,072
	Log red	1,12	1,12	1	1	1,06	
250 MPa	Counts	100	160	110	130		
25°C	Exp	1000000	1600000	1100000	1300000		
20 min	log cnt	6	6,2	6,04	6,11	6,09	0,09
	Log red	1,6	1,4	1,56	1,49	1,51	
250 MPa	Counts	70	40	30	70		
30°C	Exp	700000	400000	300000	700000		
10 min	log cnt	5,85	5,6	5,48	5,85	5,69	0,184
	Log red	1,75	2	2,12	1,75	1,91	
250 MPa	Counts	30	30	30	40		
30°C	Exp	300000	300000	300000	400000		
20 min	log cnt	5,48	5,48	5,48	5,6	5,51	0,062
	Log red	2,12	2,12	2,12	2	2,09	
250 MPa	Counts	200	140	190	170		
40°C	Exp	20000	14000	19000	17000		
10 min	log cnt	4,3	4,15	4,28	4,23	4,24	0,069
	Log red	3,3	3,45	3,32	3,37	3,36	
250 MPa	Counts	130	100	90	80		
40°C	Exp	13000	10000	9000	8000		
20 min	log cnt	4,11	4	3,95	3,9	3,99	0,09
	Log red	3,49	3,6	3,65	3,7	3,61	
250 MPa	Counts	9	11	5	6		
50°C	Exp	90	110	50	60		
10 min	log cnt	1,95	2,04	1,7	1,78	1,87	0,157
	Log red	5,65	5,56	5,9	5,82	5,73	
250 MPa	counts	4	5	6	7		
50°C	Exp	40	50	60	70		
20 min	log cnt	1,6	1,7	1,78	1,85	1,73	0,105
	Log red	6	5,9	5,82	5,75	5,87	

Table A.4 HHP treatment for mussels at 200 MPa.

200 MPa for Mussels		Measurements				Mean	st dev
		1	2	3	4		
200 MPa 25°C 10 min	counts	90	150	140	100		
	exponents	90000	150000	140000	100000		
	log counts	4,95	5,18	5,15	5,00	5,07	0,11
	Log reduction	0,77	0,54	0,57	0,72	0,65	0,11
200 MPa 25°C 20 min	counts	80	80	110	100		
	exponents	80000	80000	110000	100000		
	log counts	4,90	4,90	5,04	5,00	4,96	0,07
	Log reduction	0,82	0,82	0,68	0,72	0,76	0,07
200 MPa 30°C 10 min	counts	60	70	70	60		
	exponents	60000	70000	70000	60000		
	log counts	4,78	4,85	4,85	4,78	4,81	0,04
	Log reduction	0,94	0,87	0,87	0,94	0,91	0,04
200 MPa 30°C 20 min	counts	40	30	60	60		
	exponents	40000	30000	60000	60000		
	log counts	4,60	4,48	4,78	4,78	4,66	0,15
	Log reduction	1,12	1,24	0,94	0,94	1,06	0,15
200 MPa 40°C 10 min	counts	319	159	188	203		
	exponents	3190	1590	1880	2030		
	log counts	3,50	3,20	3,27	3,31	3,32	0,13
	Log reduction	2,22	2,52	2,45	2,41	2,40	0,13
200 MPa 40°C 20 min	counts	83	79	111	102		
	exponents	830	790	1110	1020		
	log counts	2,92	2,90	3,05	3,01	2,97	0,07
	Log reduction	2,80	2,82	2,67	2,71	2,75	0,07
200 MPa 50°C 10 min	counts	2	1	3	2		
	exponents	20	10	30	20		
	log counts	1,30	1,00	1,48	1,30	1,27	0,20
	Log reduction	4,42	4,72	4,24	4,42	4,45	0,20
200 MPa 50°C 20 min	counts	1	0	1	4		
	exponents	10	0	10	40		
	log counts	1,00	0,00	1,00	1,60	0,90	0,66
	Log reduction	4,72	5,72	4,72	4,12	4,82	0,66

Table A.5 HHP treatment for mussels at 220 MPa.

220 MPa for Mussels		Measurements				mean	st dev
		1	2	3	4		
220 MPa 25°C 10 min	counts	90	70	80	90		
	exponents	90000	70000	80000	90000		
	log counts	4,95	4,85	4,90	4,95	4,91	0,05
	log reduction	0,77	0,87	0,82	0,77	0,81	0,05
220 MPa 25°C 20 min	counts	70	80	90	70		
	exponents	70000	80000	90000	70000		
	log counts	4,85	4,90	4,95	4,85	4,89	0,05
	log reduction	0,87	0,82	0,77	0,87	0,83	0,05
220 MPa 30°C 10 min	counts	30	30	60	40		
	exponents	30000	30000	60000	40000		
	log counts	4,48	4,48	4,78	4,60	4,58	0,14
	log reduction	1,24	1,24	0,94	1,12	1,14	0,14
220 MPa 30°C 20 min	counts	30	40	30	30		
	exponents	30000	40000	30000	30000		
	log counts	4,48	4,60	4,48	4,48	4,51	0,06
	log reduction	1,24	1,12	1,24	1,24	1,21	0,06
220 MPa 40°C 10 min	Counts	103	118	152	133		
	Exponents	1030	1180	1520	1330		
	log counts	3,01	3,07	3,18	3,12	3,10	0,07
	log reduction	2,71	2,65	2,54	2,60	2,62	0,07
220 MPa 40°C 20 min	Counts	67	89	65	66		
	Exponents	670	890	650	660		
	log counts	2,83	2,95	2,81	2,82	2,85	0,07
	log reduction	2,89	2,77	2,91	2,90	2,87	0,07
220 MPa 50°C 10 min	Counts	0	0	0	0		
	Exponents	0	0	0	0		
	log counts	0,00	0,00	0,00	0,00	0,00	0,00
	log reduction	5,72	5,72	5,72	5,72	5,72	0,00
220 MPa 50°C 20 min	Counts	0	0	0	0		
	Exponents	0	0	0	0		
	log counts	0,00	0,00	0,00	0,00	0,00	0,00
	log reduction	5,72	5,72	5,72	5,72	5,72	0,00

Table A.6 HHP treatment for mussels at 250 MPa.

250 MPa for Mussels		Measurements				mean	st dev
		1	2	3	4		
250 MPa 25°C 10 min	Counts	90	60	60	70		
	exponents	90000	60000	60000	70000		
	log counts	4,95	4,78	4,78	4,85	4,84	0,08
	log reduction	0,77	0,94	0,94	0,88	0,88	0,08
250 MPa 25°C 20 min	counts	80	70	50	70		
	exponents	80000	70000	50000	70000		
	log counts	4,90	4,85	4,70	4,85	4,82	0,09
	log reduction	0,82	0,87	1,02	0,87	0,90	0,09
250 MPa 30°C 10 min	counts	60	70	50	60		
	exponents	6000	7000	5000	6000		
	log counts	3,78	3,85	3,70	3,78	3,78	0,06
	log reduction	1,94	1,87	2,02	1,94	1,94	0,06
250 MPa 30°C 20 min	counts	30	40	40	30		
	exponents	3000	4000	4000	3000		
	log counts	3,48	3,60	3,60	3,48	3,54	0,07
	log reduction	2,24	2,12	2,12	2,24	2,18	0,07
250 MPa 40°C 10 min	counts	35	47	48	54		
	exponents	350	470	480	540		
	log counts	2,54	2,67	2,68	2,73	2,66	0,08
	log reduction	3,18	3,05	3,04	2,99	3,06	0,08
250 MPa 40°C 20 min	counts	27	21	46	47		
	exponents	270	210	460	470		
	log counts	2,43	2,32	2,66	2,67	2,52	0,17
	log reduction	3,29	3,40	3,06	3,05	3,20	0,17
250 MPa 50°C 10 min	counts	0	0	0	0		
	exponents	0	0	0	0		
	log counts	0,00	0,00	0,00	0,00	0,00	0,00
	log reduction	5,72	5,72	5,72	5,72	5,72	0,00
250 MPa 50°C 20 min	counts	0	0	0	0		
	exponents	0	0	0	0		
	log counts	0,00	0,00	0,00	0,00	0,00	0,00
	log reduction	5,72	5,72	5,72	5,72	5,72	0,00

Table A. 7 ANOVA table for the effect of pressure on the total microbial reduction in shrimps.

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	16,584	2	8,292	2,248	,111
Within Groups	343,079	93	3,689		
Total	359,663	95			

Table A. 8 Duncan's Multiple Range test for the effect of pressure on the total microbial reduction in shrimps.

Duncan

		Subset for alpha = .05	
Pressure	N	1	2
200	32	2,1050	
220	32	2,4722	2,4722
250	32		3,1109
Sig.		,446	,187

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 32,000.

Table A. 9 ANOVA table for the effect of temperature on the total microbial reduction in shrimps.

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	338,379	3	112,793	487,544	,000
Within Groups	21,284	92	,231		
Total	359,663	95			

Table A. 10 Duncan's Multiple Range test for the effect of temperature on the total microbial reduction in shrimps.

Duncan

Temperature	N	Subset for alpha = .05			
		1	2	3	4
25	24	,6954			
30	24		1,0617		
40	24			3,0879	
50	24				5,4058
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 24,000.

Table A. 11 ANOVA table for the effect of time on the total microbial reduction in shrimps.

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1,224	1	1,224	,321	,572
Within Groups	358,439	94	3,813		
Total	359,663	95			

Table A. 12 ANOVA table and Duncan's Multiple Range test for the effect of HHP treatments applied at 40 and 50 °C on shrimps.

MicrobialReduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	70,285	11	6,390	380,096	,000
Within Groups	,605	36	,017		
Total	70,890	47			

Table A. 12 ANOVA table and Duncan's Multiple Range test for the effect of HHP treatments applied at 40 and 50 °C on shrimps. (Continued)

Homogeneous Subsets

Treatments

1. 200 MPa, 40 C, 10 min
2. 200 MPa, 40 C, 20 min
3. 200 MPa, 50 C, 10 min
4. 200 MPa, 50 C, 20 min
5. 220 MPa, 40 C, 10 min
6. 220 MPa, 40 C, 20 min
7. 220 MPa, 50 C, 10 min
8. 220 MPa, 50 C, 20 min
9. 250 MPa, 40 C, 10 min
10. 250 MPa, 40 C, 20 min
11. 250 MPa, 50 C, 10 min
12. 250 MPa, 50 C, 20 min

MicrobialReduction

Duncan

Treatment	N	Subset for alpha = .05							
		1	2	3	4	5	6	7	8
1	4	2,525							
2	4	2,693							
5	4		3,090						
6	4		3,250	3,250					
9	4			3,360					
10	4				3,610				
3	4					4,968			
4	4					5,135	5,135		
7	4						5,270		
8	4							5,463	
11	4								5,733
12	4								5,868
Sig.		,076	,089	,238	1,000	,076	,150	1,000	,150

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4,000.

Table A. 13 ANOVA table for the effect of pressure on the total microbial reduction in mussels.

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	8,444	2	4,222	1,297	,278
Within Groups	302,706	93	3,255		
Total	311,150	95			

Table A. 14 Duncan's Multiple Range test for the effect of pressure on the total microbial reduction in mussels.

Duncan

Pressure	N	Subset for alpha = .05
		1
200	32	2,2244
220	32	2,6116
250	32	2,9503
Sig.		,132

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 32,000.

Table A. 15 ANOVA table for the effect of temperature on the total microbial reduction in mussels.

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	295,345	3	98,448	573,074	,000
Within Groups	15,805	92	,172		
Total	311,150	95			

Table A. 16 Duncan's Multiple Range test for the effect of temperature on the total microbial reduction in mussels.

Duncan

Temperature	N	Subset for alpha = .05			
		1	2	3	4
25	24	,8042			
30	24		1,4054		
40	24			2,8138	
50	24				5,3583
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 24,000.

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

Microbial Reduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	,476	1	,476	,144	,705
Within Groups	310,674	94	3,305		
Total	311,150	95			

Table A. 18 ANOVA table and Duncan's Multiple Range test for the effect of HHP treatments applied at 40 and 50 °C on mussels.

MicrobialReduction

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	85,735	11	7,794	170,969	,000
Within Groups	1,641	36	,046		
Total	87,376	47			

Table A. 18 ANOVA table and Duncan's Multiple Range test for the effect of HHP treatments applied at 40 and 50 °C on mussels (Continued).

Homogeneous Subsets

Treatments

1. 200 MPa, 40 C, 10 min
2. 200 MPa, 40 C, 20 min
3. 200 MPa, 50 C, 10 min
4. 200 MPa, 50 C, 20 min
5. 220 MPa, 40 C, 10 min
6. 220 MPa, 40 C, 20 min
7. 220 MPa, 50 C, 10 min
8. 220 MPa, 50 C, 20 min
9. 250 MPa, 40 C, 10 min
10. 250 MPa, 40 C, 20 min
11. 250 MPa, 50 C, 10 min
12. 250 MPa, 50 C, 20 min

MicrobialReduction

Duncan

Treatment	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
1	4	2,3975						
5	4	2,6250	2,6250					
2	4		2,7500	2,7500				
6	4		2,8675	2,8675				
9	4			3,0650	3,0650			
10	4				3,2000			
3	4					4,4500		
4	4						4,8200	
7	4							5,7200
8	4							5,7200
11	4							5,7200
12	4							5,7200
Sig.		,141	,138	,055	,377	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4,000.

Table A.19 TVB-N values for control measures of storage at 25°C

STORAGE at +25 C						
control measures: ml's						
days	TVB-mussels			TVB-shrimps		
	1. meas	2. meas	Avg	1. meas	2. meas	Avg
0	2,3	2,5	2,4	2,8	3,2	3
2	8,6	9,1	8,85	9,4	9,6	9,5
control measures: calculations ml*300*0,35/25 (2)						
days	TVB-mussels			TVB-shrimps		
	1. meas	2. meas	Avg	1. meas	2. meas	Avg
0	9,66	10,50	10,08	11,76	13,44	12,60
2	36,12	38,22	37,17	39,48	40,32	39,90

Table A.20 TVB-N values (measured ml and calculations) for control measures of storage at 4°C

REFRIGERATED STORAGE at +4 C						
control measures: ml's						
days	TVB-mussels			TVB-shrimps		
	1. meas	2. meas	avg	1. meas	2. meas	Avg
0	2,3	2,5	2,4	2,8	3,2	3
2	5,9	6,2	6,05	6,8	7,1	6,95
4	9,1	9,4	9,25	10	10,4	10,2
control measures: calculations ml*300*0,35/25 (2)						
days	TVB-mussels			TVB-shrimps		
	1. meas	2. meas	avg	1. meas	2. meas	Avg
0	9,66	10,50	10,08	11,76	13,44	12,60
2	24,78	26,04	25,41	28,56	29,82	29,19
4	38,22	39,48	38,85	42,00	43,68	42,84

Table A.21 TVB-N values (measured ml and calculations) for HHP treated mussels during storage at 25°C

TVB values: measured ml's

days	mussels 25 C		
	1. meas	2.meas	Avg
0	2,3	2,5	2,40
2	3,4	3,7	3,55
4	4,2	4,9	4,55
6	5,3	5,4	5,35
8	5,6	5,8	5,70
10	6,1	6,1	6,10
12	7,1	7,3	7,20
14	8,5	8,7	8,60
16			
18			
20			

TVB values: Calculations
ml*300*0,35/25 (2)

days	mussels 25 C		
	1. meas	2.meas	Avg
0	9,66	10,50	10,08
2	14,28	15,54	14,91
4	17,64	20,58	19,11
6	22,26	22,68	22,47
8	23,52	24,36	23,94
10	25,62	25,62	25,62
12	29,82	30,66	30,24
14	35,70	36,54	36,12
16			
18			
20			

Table A.22 TVB-N values (measured ml and calculations) for HHP treated mussels during storage at 4°C

TVB values: measured ml's

mussels 4 C		
1. meas	2.meas	Avg
2,3	2,5	2,40
2,7	2,4	2,55
2,9	3,2	3,05
3,9	4,2	4,05
4,1	4,3	4,20
4,4	4,7	4,55
5,1	5,3	5,20
5,9	6,2	6,05
6,7	7,2	6,95
7,9	8,1	8,00
8,4	8,5	8,45

TVB values: Calculations
ml*300*0,35/25 (2)

mussels 4 C		
1. meas	2.meas	Avg
9,66	10,50	10,08
11,34	10,08	10,71
12,18	13,44	12,81
16,38	17,64	17,01
17,22	18,06	17,64
18,48	19,74	19,11
21,42	22,26	21,84
24,78	26,04	25,41
28,14	30,24	29,19
33,18	34,02	33,60
35,28	35,70	35,49

Table A.23 TVB-N values (measured ml and calculations) for HHP treated shrimps during storage at 25°C

TVB values: measured ml's

shrimps 25 C		
1. meas	2.meas	Avg
2,8	3,2	3,00
4,3	4,6	4,45
4,6	5,6	5,10
5,9	6,2	6,05
6,8	7,3	7,05
7,9	8,2	8,05
8,5	8,5	8,50
8,9	9,2	9,05

TVB values: Calculations
ml*300*0,35/25 (2)

shrimps 25 C		
1. meas	2.meas	Avg
11,76	13,44	12,60
18,06	19,32	18,69
19,32	23,52	21,42
24,78	26,04	25,41
28,56	30,66	29,61
33,18	34,44	33,81
35,70	35,70	35,70
37,38	38,64	38,01

Table A.24 TVB-N values (measured ml and calculations) for HHP treated shrimps during storage at 4°C

TVB values: measured ml's

shrimps 4 C		
1. meas	2.meas	Avg
2,8	3,2	3,00
2,9	3,2	3,05
3,4	3,7	3,55
4,4	4,7	4,55
5,6	5,6	5,60
5,9	6,2	6,05
6,6	6,6	6,60
7,1	7,3	7,20
8,1	8,4	8,25
9,1	9,4	9,25

TVB values: Calculations
ml*300*0,35/25 (2)

shrimps 4 C		
1. meas	2.meas	Avg
11,76	13,44	12,60
12,18	13,44	12,81
14,28	15,54	14,91
18,48	19,74	19,11
23,52	23,52	23,52
24,78	26,04	25,41
27,72	27,72	27,72
29,82	30,66	30,24
34,02	35,28	34,65
38,22	39,48	38,85

Table A. 25 pH values for untreated shrimps during storage at 4 and 25°C.

	Shrimps 25C			Shrimps 4C		
days	1. meas.	2. meas.	average	1. meas.	2. meas.	Average
0	6,94	6,96	6,95	6,94	6,96	6,95
2	8,11	7,96	8,04	7,4	7,27	7,34
4				8,32	8,73	8,53
6						
8						
10						
12						
14						
16						
18						

Table A. 26 pH values for untreated mussels during storage at 4 and 25°C.

	Mussels 25C			Mussels 4C		
Days	1. meas.	2. meas.	average	1. meas.	2. meas.	average
0	6,82	6,78	6,80	6,82	6,78	6,80
2	7,99	8,05	8,02	7,16	7,27	7,22
4				7,42	8,02	7,72
6				8,57	8,89	8,73
8						
10						
12						
14						
16						
18						

Table A. 27 pH values for HHP (250 MPa, 50 °C, 10 min) treated shrimps during storage at 4 and 25°C.

	Shrimps 25C			Shrimps 4C		
days	1. meas.	2. meas.	average	1. meas.	2. meas.	average
0	6,94	6,96	6,95	6,94	6,96	6,95
2	7,1	7,14	7,12	6,97	7,01	6,99
4	7,24	7,32	7,28	7,08	7,09	7,09
6	7,40	7,44	7,42	7,14	7,2	7,17
8	7,58	7,66	7,62	7,32	7,38	7,35
10	7,82	7,88	7,85	7,42	7,45	7,44
12	7,92	7,96	7,94	7,68	7,74	7,71
14	8,20	8,32	8,26	7,78	7,8	7,79
16				7,86	7,89	7,88
18				7,96	8	7,98

Table A. 28 pH values for HHP (220 MPa, 50 °C, 10 min) treated mussels during storage at 4 and 25°C.

	Mussels 25C			**Mussels 4C**		
Days	**1. meas.**	**2. meas.**	**average**	**1. meas.**	**2. meas.**	**average**
0	6,82	6,78	6,80	6,82	6,78	6,80
2	6,95	6,94	6,95	6,88	6,92	6,90
4	7,09	7,12	7,11	6,93	6,95	6,94
6	7,21	7,23	7,22	6,97	6,99	6,98
8	7,28	7,32	7,30	7,12	7,18	7,15
10	7,39	7,33	7,36	7,19	7,23	7,21
12	7,55	7,68	7,62	7,32	7,39	7,36
14	7,78	7,84	7,81	7,42	7,43	7,43
16	8,02	8,63	8,33	7,61	7,69	7,65
18				7,7	7,74	7,72

Table A. 29 Regression analysis for HHP treated shrimps stored at 25 °C.

Regression Statistics	
Multiple R	0,992
R Square	0,984
Arranged R Sqr	0,981
Standard Error	1,222
Observation	8

ANOVA

	df	SS	MS	F	Significance
Regression	1	551,1452625	551,145	368,933579	1,288E-06
Difference	6	8,963325	1,49389		
Total	7	560,1085875			

	Coeff.	Std Error	t Stat	P-value	Low %95	High %95	Low %95	High %95
Int.	14,228	0,813	18,033	0,000	12,297	16,158	12,297	16,158
X Var	1,811	0,097	19,208	0,000	1,581	2,042	1,581	2,042

Table A. 30 Regression analysis for HHP treated shrimps stored at 4 °C.

Regression Statistics	
Multiple R	0,992
R Square	0,983
Arranged R Sqr	0,981
Standard Error	1,257
Observation	10

ANOVA

	df	SS	MS	F	Significance
Regression	1	738,0068182	738,007	466,880839	2,217E-08
Difference	8	12,64574182	1,58072		
Total	9	750,65256			

	Coeff.	Std Error	t Stat	P-value	Low %95	High %95	Low %95	High %95
Int.	10,523	0,739	14,240	0,0000	8,819	12,227	8,819	12,227
X Var	1,496	0,069	21,607	0,0000	1,336	1,655	1,336	1,655

Table A. 31 Regression analysis for HHP treated mussels stored at 25 °C.

Regression Statistics	
Multiple R	0,986
R Square	0,972
Arranged R Sqr	0,967
Standard Error	1,493
Observation	8

ANOVA

	df	SS	MS	F	Significance
Regression	1	466,4333625	466,433	209,333085	6,832E-06
Difference	6	13,369125	2,22819		
Total	7	479,8024875			

	Coeff.	Std Error	t Stat	P-value	Low %95	High %95	Low %95	High %95
Int.	11,148	0,964	11,569	0,0000	8,790	13,505	8,790	13,505
X Var	1,666	0,115	14,468	0,0000	1,385	1,948	1,385	1,948

Table A. 32 Regression analysis for HHP treated mussels stored at 4 °C.

Regression Statistics	
Multiple R	0,987
R Square	0,973
Arranged R Sqr	0,971
Standard Error	1,515
Observation	11

ANOVA

	df	SS	MS	F	Significance
Regression	1	757,96875	757,969	330,432566	2,105E-08
Difference	9	20,64481364	2,29387		
Total	10	778,6135636			

	Coeff.	Std Error	t Stat	P-value	Low %95	High %95	Low %95	High %95
Int.	8,047	0,854	9,419	0,000	6,114	9,979	6,114	9,979
X Var	1,313	0,072	18,178	0,000	1,149	1,476	1,149	1,476

APPENDIX B

SHRIMP AND MUSSEL PROCESSING

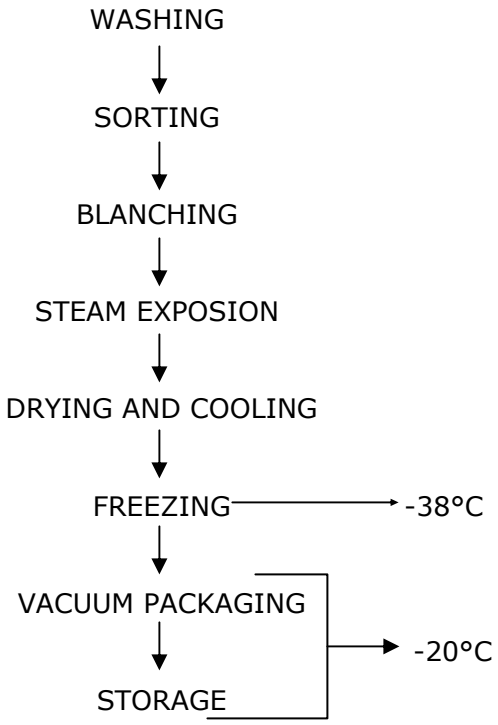

Figure B.1 Frozen shrimp processing.

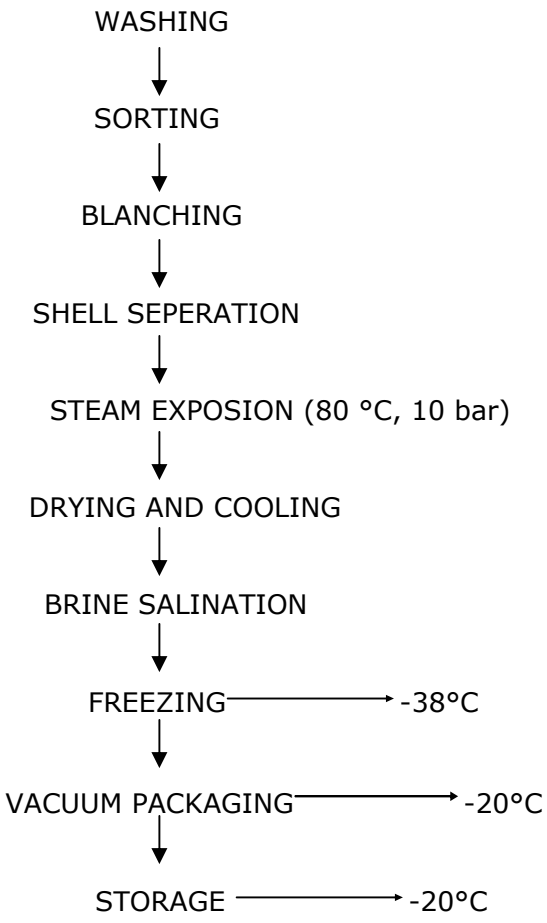

Figure B.2 Frozen mussel processing.