PRESERVATION AND SHELF LIFE EXTENSION OF ANCHOVY (*Engraulis encrasicolus*) AND HADDOCK (*Gadus merlangus euxinus*) BY HIGH HYDROSTATIC PRESSURE

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SICENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

CEYDA AKHAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

MAY 2012

Approval of the thesis:

PRESERVATION AND SHELF LIFE EXTENSION OF ANCHOVY (*Engraulis encrasicolus*) AND HADDOCK (*Gadus merlangus euxinus*) BY HIGH HYDROSTATIC PRESSURE

submitted by **CEYDA AKHAN** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Alev Bayındırlı _____
Head of Department, **Food Engineering**

Prof. Dr. Hami Alpas _____
Supervisor, **Food Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Kezban Candoğan _____
Food Engineering Dept., Ankara University

Prof. Dr. Hami Alpas _____
Food Engineering Dept., METU

Assoc. Prof. Dr. Behiç Mert _____
Food Engineering Dept., METU

Assist. Prof. Dr. İlkay Şensoy _____
Food Engineering Dept., METU

Assist. Prof. Dr. Yeşim Soyer _____
Food Engineering Dept., METU

Date: 17.05.2012

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

Name, Last Name : Ceyda Akhan

Signature :

ABSTRACT

PRESERVATION AND SHELF LIFE EXTENSION OF ANCHOVY (*Engraulis encrasicolus*) AND HADDOCK (*Gadus merlangus euxinus*) BY HIGH HYDROSTATIC PRESSURE

AKHAN, Ceyda

M.Sc., Department of Food Engineering

Supervisor: Prof. Dr. Hami ALPAS

May 2012, 80 pages

High Hydrostatic Pressure (HHP) application, alone or in combination with refrigeration, ambient or moderate heating temperatures; inactivates pathogenic and spoilage microorganisms and conserves the product ‘‘freshness’’ with fewer changes when compared to conventional food preservation processes. During handling and frozen storage, quality deterioration of seafood brings the risk of contamination, quality loss and a potential threat to food safety and human health. In this respect, the aim of this study was to determine the effect of HHP on quality factors and shelf life of anchovy (*Engraulis encrasicolus*) and haddock (*Gadus merlangus euxinus*) under different pressure, temperature and time combinations. Due to their high perishability and easy availability in Turkey, anchovy (*Engraulis encrasicolus*) and haddock (*Gadus merlangus euxinus*) samples were chosen and pressurized with HHP at 200, 300 and 400 MPa, at 5, 10 and 15 °C for 5 and 15 min.

According to the results of physical and chemical analysis which were performed to HHP treated fish samples; such as color (L*, a*, b* and ΔE), trimethylamine nitrogen (TMA-N), and 2-thiobarbituric acid (TBA); best HHP combinations were selected for shelf life analysis as 200 MPa, 5 °C for 5 min for anchovy; both 200 MPa, 5 °C for 5 min and 400 MPa, 15°C for 5 min for haddock.

In order to determine the effect of HHP on the shelf life, untreated and HHP treated samples were stored at refrigeration temperature (4 °C) for 15 days. Every each day, sensory, color (L*, a*, b* and ΔE), pH, trimethylamine-nitrogen (TMA-N), thiobarbituric acid (TBA), total volatile basic-nitrogen (TVB-N) and total plate count (TPC) analysis including statistical analysis (ANOVA) were monitored for untreated and treated samples during 15 days at refrigeration temperature (4 °C). Considering the rejection limits for consumption, effect of HHP to shelf life was evaluated. When all conclusions were combined, the shelf life extension was determined averagely as 9 days for pressurized anchovies (200 MPa 5 °C for 5 min) when stored at 4 °C. Also for haddock, shelf life was extended to 13 days and 15 days when samples pressurized with 200 MPa at 5 °C for 5 min and 400 MPa at 15 °C for 5 min, respectively; since all control samples of anchovy and haddock became unacceptable within 3 days.

Keywords: anchovy, haddock, quality, high hydrostatic pressure, shelf life

ÖZ

YÜKSEK HİDROSTATİK BASINÇ UYGULAMASI İLE MEZGİT (*Gadus merlangus euxinus*) VE HAMSİNİN (*Engraulis encrasicolus*) MUHAFAZASININ VE RAF ÖMRÜNÜN UZATILMASI

AKHAN, Ceyda
Yüksek Lisans, Gıda Mühendisliği
Tez Yöneticisi: Prof. Dr. Hami ALPAS

Mayıs 2012, 80 sayfa

Yüksek Hidrostatik Basınç (YHB) uygulaması, tek başına ya da buzdolabı sıcaklığında, oda sıcaklığında ya da orta derecede ısıl uygulama ile kombinasyonu ile; diğer alışılagelmiş gıda koruma proseslerine nazaran, gıdanın "tazeliği" koruyarak veya çok az değişikliğe sebep olarak, patojenik ve bozulmaya yol açan mikroorganizmaların inaktivasyonunu sağlayan bir teknolojidir. Dağıtım ve soğuk depolama esnasında deniz ürünlerinde kalite kaybı, kontaminasyon riski, gıda güvenliği ve insan sağlığı için potansiyel bir tehlike getirir. Bu hususta dolayı, bu çalışmanın amacı; farklı basınç, sıcaklık ve zaman kombinasyonları altında seçilen balık türlerinin raf ömürleri ve kalite unsurları üzerinde YHB uygulamasının etkilerini belirlemektir. Yüksek derecede bozulabilirlikleri ve Türkiye'de kolay bulunabilirlikleri göz önünde bulundurularak, hamsi (*Engraulis encrasicolus*) ve mezgit (*Gadus merlangus*

euxinus) balıkları bu çalışma için seçilmiş; 200, 300 ve 400 MPa 5, 10 ve 15 °C'de 5 ile 15 dakika boyunca Yüksek Hidrostatik Basınç uygulamasına tabi tutulmuştur.

YHB uygulamalarına tabi tutulmuş balık örneklerinin, renk (L*, a*, b* ve ΔE), trimetilamin azot (TMA-N), ve 2-tiyobarbitürik asit (TBA) gibi fiziksel ve kimyasal analizlerin sonuçları doğrultusunda; hamsi için 200 MPa, 5 °C'de 5 dk, mezgit için ise hem 200 MPa, 5 °C'de 5 dk hem de 400 MPa, 15°C'de 5 dk, raf ömrü çalışmaları için en iyi YHB kombinasyonları olarak seçilmiştir.

YHB uygulamasının raf ömrü üzerindeki etkisini görmek için, belirlenen kombinasyonlarda yüksek basınç uygulanmış ve uygulanmamış örnekler buzdolabı sıcaklığında (4 °C) 15 gün boyunca muhafaza edilmiştir. İstatistiksel analiz (ANOVA) dahil olmak üzere; duyusal, renk (L*, a*, b* and ΔE), pH, Trimetilamin-azot (TMA-N), Tiyobarbiturik asit (TBA), Toplam uçucu bazik-azot (TVB-N) ve Toplam aerob bakteri sayımı (TPC) analizlerinin sonuçları basınç uygulanmış ve uygulanmamış tüm örnekler için, 15 gün boyunca buzdolabı sıcaklığında (4 °C) izlenmiştir. Tüm analiz sonuçları tüketim için kabul edilebilirlik limitler ile kıyaslanarak, YHB uygulamasının raf ömürlere etkisi değerlendirilmiştir. Tüm sonuçlar biraraya getirildiğinde; 200 MPa-5°C-5 dk koşulunda basınç uygulanan hamsi örnekleri buzdolabı koşullarında (4 °C) muhafaza edildiğinde 9 gün kadar raf ömrü uzatılmıştır. Mezgit örnekleri için ise; örnekler sırasıyla 200 MPa-5°C-5 dk ve 400 MPa-15°C-5 dk basınç uygulandıklarında, raf ömürleri 13 ve 15 gün kadar uzatılırken; her iki balık türü için kontrol örnekleri ise 3 gün içerisinde tüketilemez duruma gelmiştir.

Anahtar kelimeler: hamsi, mezgit, kalite, yüksek hidrostatik basınç, raf ömrü

To My Family

ACKNOWLEDGEMENTS

I would like to express my grateful thanks to my supervisor Prof. Dr Hami ALPAS for his encouragement, patience, understanding and guidance. I felt his greatest support throughout this study.

I wish to express my thankfulness to my family Şenay, Halil, and Gül GENÇEL, and my father Hüseyin AKHAN for their encouragement, love and belief.

Lastly, I would like to thank to my dearest friends Tuğçe Şentürk, Gülçin Kültür, Altuğ Başkoçak and Fuat Gökbel for their supports and their precious ideas.

Finally, I would like to thank to Unit of The Scientific and Technological Research Council of Turkey, TUBITAK for their financial support.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABS: Absorbance

ANOVA : Analysis of variance

AOAC: Association of Analytical Communities

BHT: 2,6-Di-tert-buthyl-4-methyl phenol

CIE: Commission Internationale de l'Eclairage

$(CH_3)_3N.HCl$: Trimethylamine hydrochloride

DMA-N : Dimethylamine nitrogen

EU: European Union

HCHO: Formaldehyde

HCl : Hydrochloric acid

HHP: High Hydrostatic Pressure

HPP: High Pressure Processing

K_2CO_3: Potassium carbonate

MAP: Modified atmosphere packaging

MDA : Malondialdehyde

MPa: Megapascal

NaOH: Sodium hydroxide

PCA : Picric acid

PED: Pressure equipment directive

TBA : Thiobarbituric acid

TCA : Trichloroacetic acid

TEP : 1,1,3,3- tetraethoxy- propane

TMAC: Total Mesophilic Aerobic Count

TMA-N : Trimethylamine nitrogen

TMA-O : Trimethylamine oxygen

TVB-N : Total Volatile Basic Nitrogen

UHP: Ultra High Pressure

CHAPTER 1

INTRODUCTION

1.1 Sea foods in Turkey

Natural resources became exhausted all over the world and humankind searches new sources continuously to keep on living. Nowadays eating a well-balanced and healthy diet is one of the major problems. Animal sourced proteins are one of the most important foods of diatery nutritions.Essential amino acids should be taken as feed from animal based foods.

Highly rich protein, mineral, vitamin and unsaturated lipid content increases the importance of sea foods especially fish in well-balanced and healthy diet. However meat efficiency and chemical composition vary within fish species. Common knowledge of these varieties features the selection of fish species according to their dietary and economic values (Erkoyuncu, Erdem, Samsun, & Özdamar, 1994).

Annual sea foods production of Turkey was reported as 627,847 tons according to data of 2002. 90.3 % was produced from hunting and 9.7 % was produced from cultivation. 77.4 % of production via hunting, was gained from Black Sea Region of Turkey. Thundering part of total production was stated as anchovy with 295,000 tons. Anchovy brackets together with 28,000 tons of horse mackerel, 8000 tons of haddock, 1400 tons of red mullet, 300 tons of turbot,

22,000 tons of bluefish at the top of the list of 2002 (Çaklı, Kılınç, Cadun, Dinçer, & Tolasa, 2006).

1.2 Anchovy (*Engraulis encrasicolus*) and Haddock (*Gadus merlangus euxinus*)

Anchovy (*Engraulis encrasicolus*) with the average length of 12 cm, is classified as a small fish which can be hunted either in shores of Black Sea or both in shores of the Marmara and East Atlantic. All over the world, Turkey is a high-productive country in anchovy production with the quantity of 24,000 tons (Turhan, Evren, & Yazıcı, 2001). Anchovy production in Turkey was determined as 373,000 tons in proportion to the overall fishery production which was 627,847 tons in 2002. This fishing gear of anchovy is progressed commercially within the period of November to January with purse-seine, which is crucial for Turkey economy and for both fish and fish oil industry (Turhan, Kaya, & Erkoyuncu, 2007).

About 25 % of anchovy goes to fish meal factories while seafood consumption is low, 8 kg per person/year, in Turkey, according to 1999 data. Although anchovy is highly perishable, it is commonly marketed throughout Turkey as fresh, without being chilled, frozen, or preserved in any other way only a small proportion is marketed during the off-season in frozen, canned and salted forms (Köse, 2004).

Anchovy (*Engraulis encrasicolus*) which belongs to the Engraulidae family is a pelagic species with a very thin body and colored with typical pelagic fish color including a dark back in order to avoid detection by birds, and also with a silver belly, to confuse when observed below the surface of the sea and to protect itself from extrinsic factors and hazards; as shown in Figure 1.1.

The chemical composition of anchovy includes high protein content between 74.40 % and 76.17 % during fishing season which is slightly higher in December rather than other months. Lipid content varies within 8.57 % and 9.14 % in January and December, respectively. Fatty fish such as herring and anchovy contains the lipid content of fish meal in the range of 7 % - 13 %, depending upon the spawning season. Generally anchovy contains averagely 33.46 % of total saturated fatty acid within the whole year. In reference to some studies, anchovy has ash content between 0.90 % and 1.93 % in addition to moisture range between 65.34 % and 75 % (İnanlı, Karatan, & Çoban, 2011).

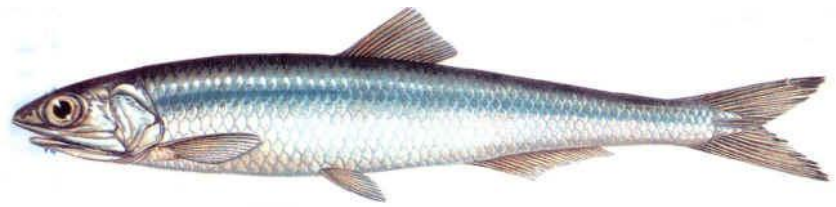

Figure 1.1 Anchovy (*Engraulis encrasicolus*) (luontoportti.com)

Haddock (*Gadus merlangus euxinus*), as shown in Figure 1.2., is sufficiently common all year throughout Turkish water even though there has been a decreasing trend in production since 1996. The production was increased from 16,615 tons in 1994 to 21,450 tons in 1996; then gradually decreased to 13,150 tons in 1998. However, it still covers an important part of Turkish fishing industry. Although it is a perishable fish, it is commonly released to the market as fresh throughout Turkey without chilling, freezing or without applying any other preserving techniques (Köse & Erdem, 2001).

Haddock can be hunted through in shores of generally Black Sea Region within the range of October to July. It is also determined that haddock has 16 % hunting proportion per total hunting quantity. However, in the depths of 60 m, haddock hunting constitutes 65.7 % (1,315 kg) of total fishing gear (Çiloğlu, Şahin, Gözler, & Verep, 2002). It comes from Black Sea which has high level of fish stock and intense study of hunting to Marmara Sea that is served as a bridge strategically in Turkey. Pelagic and demersal fish stocks could not be specified exactly up to now in Sea of Marmara where underlies 15 % of Turkey's fishery (Atasoy, Erdem, Cebeci, & Yerli, 2006). Besides anchovy, haddock which could have the length of 50 cm, is available for hunting from coastal regions of Black Sea throughout the whole year (Akşiray, 1987). Also haddock which lives in the deepest waters of Marmara Sea and has a high hunting quantity, carries weight after some pelagic fish species (Atasoy, Erdem, Cebeci, & Yerli, 2006). Spawning of this species could fluctuate within the whole year, however generally starts in October and progresses till July and August (Bowers, 1957).

In the scope of chemical composition, haddock has 14.91 % protein, 1.09 % fat, 17.92 % dry matter, 82.08 % moisture, 1.1 % ash content proportions, at the same time these contributions vary with respect to sexuality, age, spawning season, feeding conditions and habitat of haddock species (Samsun, Erdem, & Samsun, 2006).

Figure 1.2 Haddock (*Gadus merlangus euxinus*) (pubblicitaitalia.com)

1.3 Traditional Seafood Processing

Processed fish are more convenient to handle, store and prepare rather than unprocessed foods, which is why modern consumers prefer these products. On the other hand, consumers request these foods which are produced in order to maintain their high quality, freshness, and nutrition values. Consumers prefer flavorsome, environmentally friendly processed and economically acceptable food items but also they prefer foods which are produced with more ethical methods. Two distinct types of seafood products occur because of increasing demands, that these demands are the results of increasing expectations on quality. The first type is fresh, chilled products which are packaged, processed and ready-to-cook foods such as salmon steaks or fish fillets. In this type fish has limited time for transportation and for shelf life to consume. It has another disadvantage which is microbiological contamination risk which can be occurred by ice and/or melted water.

The second type is processed, chilled sea foods and ready-to eat products (cold smoked salmon, etc.). A gap for convenience and easy handling is the main subject to classify sea foods into these two types (Venugopal, 2006). Because anchovy and haddock have high perishable characteristics, marketing ways currently used in Turkey are chilling, freezing otherwise preserving in brine. Frozen, canned and marinated types of fish take places in the markets, but only in a small percent of stores rather than fresh sales (Yerlikaya, Gökoğlu, & Uran, 2005). Nowadays, in order to achieve shelf life extension, microbiological inactivation and food safety; thermal processing is the prevailing method. Besides effectiveness, economical benefits and easy availability; thermal technologies have disadvantages such as undesirable effects on food quality, appearance, degradation of nutrition, etc. which should be minimized by food processors (Torres & Velazquez, 2005). Even if thermal treatment inhibits microorganisms like spoilage bacteria; it does not remain the same natural taste and flavor of original food sample, and does destroy minerals and vitamins also.

Minimal processing technologies are developed at this stage to preserve food material with minimum undesired features such as preserving their freshness in taste and other sensorial properties as much as possible. Many different food processing methods which are commonly used in the food industry, could be used in minimal processing potentially either by itself or by combining with other methods. Some novel technologies do not have the detrimental effects of heat treatment as mentioned before (Ohlsson & Bengtsson, 2000).

1.4 High Hydrostatic Pressure (HHP)

High hydrostatic pressure (HHP) application, is alternative to these conventional technologies which provides an extension in fish products shelf life whenever combined with good refrigeration temperatures and good-handling

practices. The researchers demonstrated that HHP is a well-known method in microbial count reduction and inactivation of pathogens in foods (Amanatidou, Schluter, Lamkau, Gorris, Smid, & Knorr, 2000). HHP has lots of advantages such as inactivation of microbiological spoilage and pathogenic bacteria. Moreover vitamins, color and flavor remain largely unaffected in contradiction to thermal processes. So that wholesome foods can be produced with little or no change in nutritional and sensory qualities (Linton, McClements, & Patterson, 2004). The main difference between HHP and other preservation technologies like thermal processing is that HHP is a uniform and instantaneous application throughout the food material being conditional upon food geometry and equipment size. This information gives direction to laboratory scale researches and full-scale production.

High pressure processing (HPP), could be named as high hydrostatic pressure (HHP), or ultra high pressure (UHP) processing also. And this application is specified for either packaged or not liquid or solid phased foods. These kinds of foods can be pressurized between 50 to 1000 MPa. Many researches revealed that HHP technology has potential benefits which alternative to thermal treatment. The inactivation of microorganisms and enzymes, denaturation and alteration of the functionality of proteins and structural changes of food materials are such several examples for the benefits of this technology (Sun, 2005).

Microorganisms are sensitive to various attributes of high pressure induced. Sensitivity of vegetative cells is above pressures of 100 MPa generally and they could be killed rapidly when pressure application is in excess of 500 MPa. The path rate of death for vegetative cells is basically first order kinetics and pronounced survivor tails are substantiated in log survivor versus time graphs in contravention of exceptions. High pressure inactivation affectivity depends on

both chemical and physical circumstances. Because at least 40 percent free water is mandatory in order to inactivate vegetative microorganisms, the large amount of free water featured in fish looms becomes significant. Since bacterial spores have high resistance to pressure; in order to decrease the amount of spores, HHP should be applied in excess of 1000 MPa for several hours. Additionally different species should also be considered. When the temperature increases above 40 °C, bacterial spores appear to be more susceptible to pressure treatment (Earnshaw, 1996).

Combination of processing technologies with modified-atmosphere storage, salting, heat pasteurization or gamma irradiation with HHP is termed as hurdle technologies concerning enhancement of chilled storage life for several fishery products. This synergism annihilates microbial obstacle for viability of surviving microorganisms (Venugopal, 2006). Advantages and disadvantages of HHP technology were summarized in Table 1. 1 (Venugopal, 2006).

Table 1.1 Advantages and Disadvantages of High Pressure Treatment of Food (Venugopal, 2006)

Advantages	Disadvantages
• Treatment at low or ambient temperature results in minimum change in food components. Unlike heating, no covalent bond breakage and formation of newer compounds occur. Minimum changes in vitamins and other nutrients. Microbial inactivation helps shelf life extension of fresh muscle foods like fish.	• Food enzymes generally require very high pressure for inactivation. At usual range of pressure used, an increase or decrease in enzyme activity may result, affecting the food quality. A blanching process needs to be included to inactivate the enzymes.
• Opportunity to reduce the use of food additives.	• Dissolved oxygen results in oxidative degradation of food components.
• Less energy requirement.	• Most pressure-processed foods need low temperature storage and distribution to retain their sensory properties.
• The treatment is isostatic (uniform throughout the food). The effect on the food is instantaneous.	• Bacterial spores are resistant.
• Minimum damage to food packaging due to isostatic effect.	
• Process is environmentally friendly.	
• Novel texturized foods are possible to produce (e.g. meat, fish, dairy products).	

1.4.1 HHP worldwide

In worldwide, two regulatory attitudes towards commercialization of HHP-manufactured food products become apparent; within the EU or excluding the EU. In countries excluding the EU, no specific legislation applicable to HHP treatment is determined. Despite HHP treated products, such as guacamole and oysters, have already been introduced to the markets; there is still not a specific regulation and the traditional health regulations are still applied to these HHP-products in the USA. In the EU countries, even though national regulations for new products have been replaced, in the application of precautionary principle, "Regulation 258/97/EC" for novel foods and ingredients has been in force by a community regulation since 1997. Commercially, availability of HHP treated sea foods and fish products were shown in Table 1.2.

Table 1.2. HHP treated products in the markets throughout the world (Bhat, Alias & Paliyath, 2012)

Country/Year	Product	HHP treatment	Achievement
USA/1999	Oyster	200-350 MPa for 1-2 min	Opening the shells Inhibition of *Vibrio vulnificus*
USA/2001 Canada/2004	Oyster	240 MPa for 90 s	Selling fresh and frozen oyster with opened shells
Spain/2004	Ready to eat salmon, hake	500 MPa	Producing sanitized fleshes with protected natural color and taste Inhibition of *Listeria* Extend shelf life without additives Ready to eat with 1.5 min at microwave
Italy/2004	Cod	600 MPa	Extend shelf life Microbial inactivation
South Korea/2006	Oyster	Indirect	Opening the shells Inhibition of *Vibrio*

1.4.2 Shelf life Extension Studies by HHP

According to studies, HHP was applied to fish samples in order to improve quality and extend shelf life. For instance, minced albacore muscle was pressurized with 275 and 310 MPa for 2, 4, and 6 min and higher than 22 days at storage temperature of 4 °C and higher than 93 days when stored at −20 °C shelf life were evaluated (Ramirez-Suarez & Morrissey, 2006). Squid muscle samples were extended their shelf life from 7 days (without pressurization) to 28 days with HHP treatment of 400 MPa at 20 °C for 15 min (Paarup, Sanchez, Pelaez & Moral, 2002). Sea bream samples were pressurized at 3 °C/5 min/250 MPa and at 15 °C/5 min/250 MPa and lengthened shelf life to 18 days (Erkan & Üretener, 2010). Similarly carp samples were stored at -8 or -15 ◦C for 50 days when high pressure treated with 170 MPa without any significant changes in texture during storage (Venugopal, 2006). Also pressure treated oyster samples at 400 MPa at 7 ◦C for 10 min, which are subjected to vacuum or aerobic packaging, had shelf life of 21 days under chilling conditions (He, Adams & Morrissey, 2001).

1.5 Quality Attributes of Fish

During storage within several days after harvest, fishery odour and flavor increase rapidly due to reactions occurred by volatile amines which are used as characteristic criteria for determining fish quality. First observation of freshness losses is primarily dehydration ensued by endogenous enzymatic activity, lipid and pigment oxidation reactions. These amines are classified within three molecules of ammonia, dimethylamine nitrogen (DMA-N), trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) (Etienne, 2005). These molecules indicate the spoilage, which help us to measure the quality of many demersal fish objectively for consumption especially during medium-later phases of spoilage.

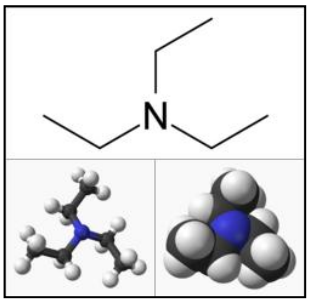

Figure 1.3 Trimethylamine molecule (hmdb.ca)

Firstly DMA-N and TMA-N is degraded from Trimethylamine oxide (TMA-O) which was shown in Figure 1. 4. It is a typical fishery molecule, occurred by a reaction which is named as osmoregulation (Etienne, 2005). Yet TMA-N which is reduced from TMA-O cannot be used by itself as a quality indicator of freshness because of its constant amount within first days of storage in ice (Etienne, 2005). According to literature, after the first days of storage TMA-N increases rapidly with the beginning reactions of specific spoilage bacteria of fish with small amounts (Etienne, 2005). Another criterion should be combined with TMA-N in order to detect the quality of fish.

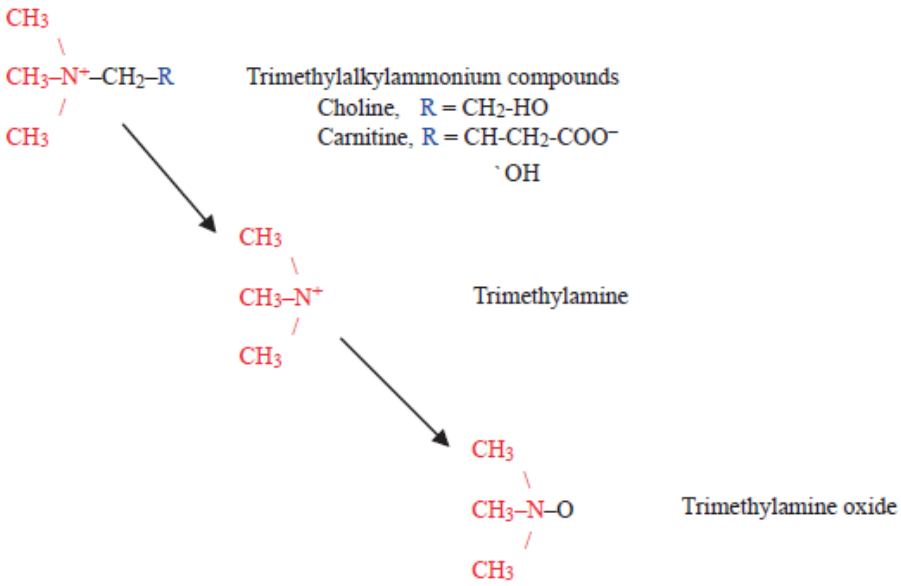

Figure 1.4 Trimethylamine oxide accumulation of fish species (Seibel & Walsh, 2002)

In fish, immediately afterwards the fishing gear TMA-N level is near zero generally, for instance it is near to 2 mg/100 g cod and also near to 2 mg/100g for some species of North-East Atlantic (Etienne, 2005). For fish, the rejection limit was defined as above 5 mg TMA-N/100 g muscle (Sikorski, Kolakowska, & Burt, 1990). For cod and haddock muscle, TMA-N limitations and fish quality specification were identified as; 0–1 TMA-N mg/100 g "1st class-good quality", 1–5 TMA-N mg/100 g is "2nd class – Marketable", and >5 TMA-N mg/100 g is "3rd class – not proper for fresh consumption or manufacturing" (Serdaroğlu & Deniz, 2001).

Besides, total volatile basic nitrogen or a total volatile base which is abbreviated as TVB-N or TVB or TVN is another ammonia involved molecule.

This molecule is derived from reactions occurred in fish fleshes when performed by alkaline and it gives volatile characteristic of TVB-N. It also indicates the spoilage level of some fish species such as such as red fish, flat fish, gadoids, hake and Atlantic salmon, due to the directive of 91/493/EEC. Limits of TVB-N in fish muscle for consumers were determined according to this directive. However, the same situation of being a freshness indicator by itself for TMA-N is present for TVB-N also because of its stable levels during the first days of storage (Etienne, 2005). Due to the autolytic processes which produces these amines, TVB-N content increase slightly during the first days of storage. But after these days with increasing activation of spoilage bacteria, production rate of TVB-N ascent rapidly (Etienne, 2005). In very first hours of harvest, TVB-N content is generally near to 10 mg/100 g of fish and does not exceed 15 mg /100 g except for pelagic fish. But this known fact should not be considered valid for pelagic fish such as anchovy, haddock, sardine, mackerel, albacore tune, with TVB-N levels of 16-18 mg/100 g, 18-20 mg/100 g, and 30mg/100 g respectively According to literature, TVB-N limitations were summarized in Table 1.3 (Erkan & Üretener, 2010; Commission decision 95/ 149/EEC).

Table 1.3 TVB-N Limitation on fish quality (Erkan & Üretener, 2010)

TVB-N content (mg/100 g)	Classification
>25 mg/100 g	Very Good
25 mg/100 g - 30 mg/100 g	Good
30 mg/100 g - 35 mg/100 g	Marketable
35 mg/100 g <	Spoiled

Thiobarbituric acid (TBA) value represents the lipid oxidation and was expressed as milligrams of malonaldehyde (MDA) equivalents per kilogram sample (Raharjo & Sofos, 1993). Malondialdehyde (MDA) is the main responsible compound of degradation of lipid hydro peroxides which decomposed during autoxidation of these highly existing polyunsaturated lipid materials (Botsoglou, Fletouris, Papageorgiou, Vassilopoulos, Mantis, & Trakatellis, 1994). During these reactions, the malonaldehyde-thiobarbituric acid (MDA-TBA) complex is developed. This determination is obtained by means of a pink pigment of this complex which can be quantified spectrophotometrically (Tokur, Korkmaz, & Ayas, 2006).

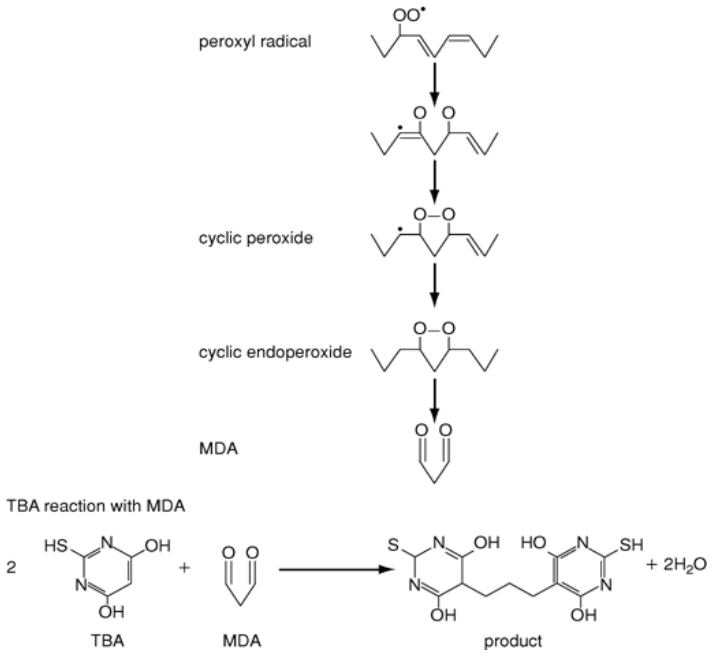

Figure 1.5 MDA and TBA-MDA complex production reactions (Singh, Leuratti, Josyula, Sipowicz, Diwan, Kasprzak, Schut, Marnett, Anderson & Shuker, 2001)

In general, highly purified fats and oils are stated to be insensitive to oxidation reactions when subjected to high pressure according to literature. Meanwhile, water activity is mainly responsible from the relevance of high pressure and sensitivity of fat content to oxidation reactions. During pressurization over 400 MPa lipid oxidation is observed to be occurred in the range of water activies of fish products. With the probable reason of releasing metal ions, oxidation in fat increases due to pressure. Lipid oxidation is induced with convenient amount of antioxidants and metal chelators (Ohlsson & Bengtsson, 2000). TBA value of 3-4 mg MDA/kg was denoted as the limit of acceptability (Huss, 1988; Karaçam & Boran, 1996; Scott, Fletcher, Charles & Wong, 1992).

The combination of these fish quality assessment indicators is utilized to analyze the spoilage determination for most of fish species and they are still the optimum chemical indicators to give opinion for consumption (Etienne, 2005).

1.6 Aim of this study

In Turkey, anchovy and haddock are most widely accessible fish in the local and supermarkets and one of the most known examples for lean and fatty fish categories. These species were chosen for this study according to this reason and their low shelf life because of widely selected fresh consumption. Shelf life extended anchovy and haddock with their remaining fresh attributes could be alternative and reasonable to consumers. In the first part, in order to select optimum pressure treatment application (pressure-temperature-time) for each fish, fish samples were pressurized at 200, 300 and 400 MPa at 5, 10 and 15°C for 5 and 15 min to determine the effect of HHP. Untreated samples were used as control. Color (L*, a*, b* and ΔE), TMA-N and TBA were monitored. In the second part, for shelf life experiments, according to results of first part 200 MPa

at 5 °C for 5 min HHP was selected for anchovy species. In the same way, both 200 MPa at 5 °C for 5 min and 400 MPa at 15 °C for 5 min were chosen for shelf life studies for haddock samples. Then samples were pressurized and stored at 4°C for 15 days. Shelf life extensions of anchovy and haddock were determined according to chemical (TBA, TMA-N, TVB-N, pH), sensory (appearance, odour), color (L*, a*, b*, ΔE), microbiological changes (TMAC) and statistical analysis (ANOVA).

CHAPTER 2

MATERIALS AND METHODS

2.1 Sample Preparation

Anchovies (approximately 12 cm length, 30-35 g weight) and haddocks (up to 50 cm length, 45-50 g weight) were obtained from a local market located in Ankara, in January 2010, transported within 2 h in ice box to the laboratory where they were washed, headed, eviscerated, filleted and cut into small portions for analysis. The fillets were covered with both flexible plastic films and tinfoil in order to protect samples from pressure transmission fluid before HHP application. Samples were treated with high pressure magnitudes of 200, 300 and 400 MPa. High pressure treatments were substantiated with selected temperatures of 5, 10, 15 °C and for compression times of 5 and 15 min. Untreated samples were used as control samples. All untreated and treated fish samples were frozen (-30°C) until physical and chemical analysis were conducted. These analysis were color (L*, a*, b* and ΔE), TMA-N and TBA. Results of untreated and treated anchovy and haddock samples were utilized to determine the optimum pressure-temperature-time combinations of HHP collectively.

Subsequently, 200 MPa-5°C-5 min high pressure combination was selected for anchovy and both 200 MPa-5°C-5 min and 400MPa-15 °C-5 min were selected for haddock, for shelf life analysis. During shelf life studies, both anchovy and haddock samples were pressurized at selected combinations, then stored at refrigeration condition (4 °C) for a period of 15 days. TMA-N, TBA,

TVB-N, color (L*, a*, b* and ΔE), sensory (appearance and odour) and microbiological analysis were evaluated for untreated and treated samples by sampling at every other day. After all the data were obtained, statistical analyses were performed for each analysis results with using SPSS, by regression and ANOVA.

2.2 HHP Equipment

High Hydrostatic Pressure was applied by a 760.0118 type industrial high pressure system (SITEC CH-8124, Zürich, Switzerland) which is shown in Figure 2.1. In order to apply high pressure magnitudes isothermally, temperature should be stable during treatment (Rastogi, Raghavarao, Balasubramaniam, Niranjan & Knorr, 2007). The vessel had a volume of 100 ml with ID 24 mm and length 153 mm. Ethylene glycol was used as a cooling / heating agent that was circulated around the jacketed pressure vessel. The maximum design pressure was 700 MPa at an operating temperature of -10 to 80°C. A built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control the required temperature which was measured by a thermocouple type K. Samples were pressurized at 200, 300 and 400 MPa at 5, 10, 15 °C for 5 and 15 min. Temperature increase due to adiabatic heating was estimated as 4-5°C during the time period of pressurization. Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa.

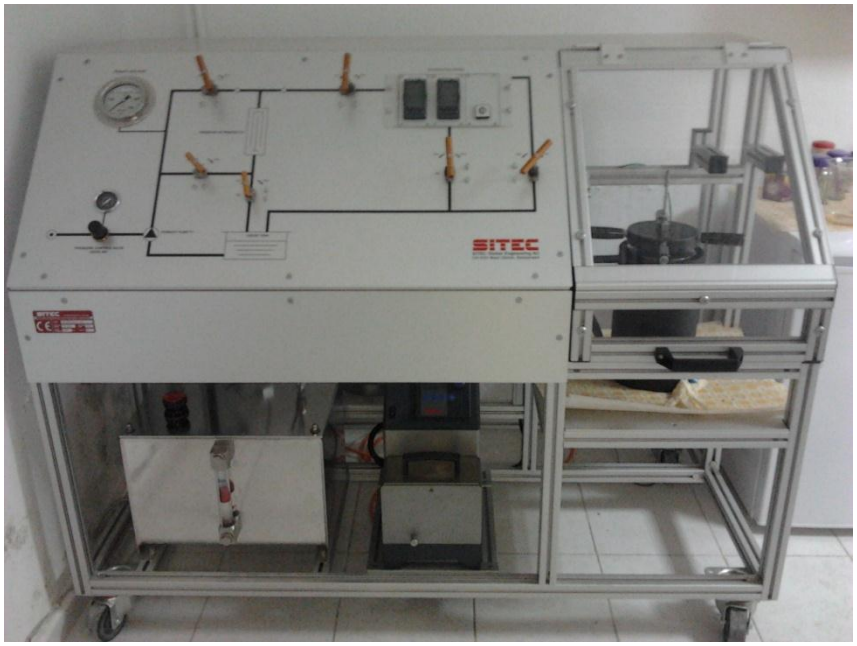

Figure 2.1 Laboratory scale HHP equipment

2.3 Sensory Analysis

Sensory analysis was evaluated according to appearance and odour characteristics of fish samples which were executed by a panel of five experienced judges on each day of sampling. Each panelist was selected to grade this sensorial test impartially and all conditioning parameters like temperature, lightness, etc. were attentive to remain stable. Appearance and odour characteristics of HHP treated and untreated anchovy samples were remarked by five panelists during storage for 15 days according to a nine point scale illustrated in Table 2.1 below, which involves a score card of grades. Each analysis was repeated for 5 times. 5 scores of each days were averaged and panelists were estimated their sensory results with reference to untreated fresh anchovy samples which has the score of 10 equals to “very good quality” (Huss, 1988).

Table 2.1 Sensory Scale (Huss, 1988)

Attributes/Quality	7-9 = very good	6-6.9 = good quality	5-5.9 = acceptable quality	4.9-1.0 = spoiled
Appearance	White-grey, translucent	White-grey, moist, translucent	Pale white-grey or yellowish, opaque	Pale white-grey or yellowish, opaque, sticky
Odour	Pungent smoky, salty	Smoky salty	Metallic	Rancid

2.4 Color Measurement

Konica Minolta Chromometer (Konica Minolta, Model CR 10, Japan) was utilized for determination of the color of the fish samples. L* (lightness), a* (+a, red; −a, green) and b* (+b, yellow; −b, blue) values were measured which were indicated to lightness, color of red to green, color of yellow to blue, respectively. This scale was illustrated below in a diagram of CIE color space in Figure 2.2. The colorimeter was calibrated using blank reference of white (Gerdes & Santos Valdez, 1991).

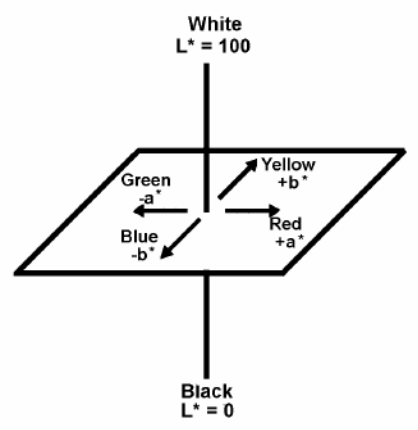

Figure 2.2 CIE L*, a*, b* color scale (hunterlab.com)

Different points of each fillet were measured for each pressure treated and untreated fish samples. The total color difference (ΔE), was calculated with the formula (2.1) of ΔE:

$$\Delta E = \sqrt[2]{(\mathbf{\Delta L^{*2}} + \mathbf{\Delta a^{*2}} + \mathbf{\Delta b^{*2}})} \quad (2.1)$$

where ΔL*, Δa*, and Δb* were calculated with formula (2.2), (2.3), (2.4) given below:

$$\Delta L^* = L^*_{\text{measured value}} - L^*_{\text{blank ref}} \quad (2.2)$$

$$\Delta a^* = a^*_{\text{measured value}} - a^*_{\text{blank ref}} \quad (2.3)$$

$$\Delta b^* = b^*_{\text{measured value}} - b^*_{\text{blank ref}} \quad (2.4)$$

Color determinations of all samples were repeated 9 times (Gerdes & Santos Valdez, 1991).

2.5 Chemical analysis

2.5.1 Determination of Trimethylamine nitrogen (TMA)

Trimethylamine nitrogen (TMA-N) was determined by the method of AOAC Official Methods 971.14 Trimethylamine Nitrogen in Seafood (Hungerford, 1998).

Before determination of TMA-N of anchovy and haddock samples a standard curve should be obtained with TMA-N working solutions which were containing 0.01 to 0.03 mg of TMA-N. These working solutions were prepared from a Trimethylamine (TMA) stock solution by adding 0.682 g of $(CH_3)_3N{\cdot}HCl$ (1 + 3) and diluting with 100 ml of anhydrous toluene. With the help of this stable solution, working solution involving 0.01 mg TMA-N/mL was prepared by adding 1 mL stock solution to 1 mL HCl (1 + 3) and diluting with 100 mL of toluene. For recognized TMA-N contents of standard solutions, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of this working solution was diluted to 4 mL with toluene. For blank solution full of 4 mL of toluene was used. Afterwards, 1 mL of 20% diluted formaldehyde (HCHO), 10 mL of anhydrous toluene, and 3 mL of potassium carbonate (K_2CO_3) solutions were added to both standard and blank solutions. Tubes were shaken vigorously by hand for 40 times. 5 mL of each tube was pipetted into dry colorimeter tubes with adding on 5 mL of picric acid solution and mixing by swirling. Besides picric acid solution was prepared by dissolving 2 grams of picric acid in 100 mL of H_2O-free toluene, and working solution was a dilution of 1 mL of stock solution to 100 mL of H_2O-free toluene. Secondly, 100

g of K_2CO_3 was diluted in 100 mL H_2O so as to prepare potassium carbonate solution. All absorbance of stock solutions were determined at 410 nm against blank solution. This standard curve as shown in Appendix A was utilized to initiate the relationship between absorbance with mg TMA/mL for further TMA analysis which was committed for both haddock and anchovy samples.

After sample preparation both HHP treated or untreated anchovy and haddock samples, ten grams were weighed and blended with 90 mL of 7.5 % aqueous solution of trichloracetic acid (Merck, Cat No: 100807) solution homogeneously then filtrated. In order to procure a blended solution with crumbled fish pieces, dispersing with Ultra Turrax (IKA T18 basic, Staufen, Germany) for a few minutes at 2000-3000 rpm should be more sufficient instead of just blending, mincing and chopping. After filtration, 4 mL of extract was pipetted into test tubes and 4 mL of toluene was used as blank. Then 1 mL of 20% formaldehyde solution, 10 mL anhydrous toluene, and 3 mL of potassium carbonate solution were added to both sample tubes and blank as the same as described for standard curve preparation. The tubes were shaken gently and 5 mL was pipetted off. 5 mL picric acid working solution was added. The contents were mixed and transferred to spectrophotometric tubes. Absorbance at 410 nm against the blank was measured in spectrophotometer (Boeco, S-22/Vis. Spectrophotometer, Germany). Determinations were carried out triplicate for each HHP treated and untreated control samples. In order to calculate TMA-N content per 100 g of anchovy or haddock samples, the formula below was utilized;

$$\text{mg TMA-N/100 g sample} = \frac{(0.013 * ABS_{sample}) * (\text{Dilution factor})}{\text{sample weight}} \qquad (2.5)$$

where 0.013 is the coefficient obtained from TMA-N standard curve in Appendix A. Triplicate results were obtained for each samples in all analysis (Hungerford, 1998).

2.5.2 Determination of Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was measured according to the Antonacopoulos and Vyncke method by using nitrogen content estimation with Kjeldahl method or Kjeldahl digestion (Antonocoupoulos & Vyncke, 1989). After sample preparation stage, 10 grams of fish fillets of both HHP treated and untreated samples were weighted, blended and homogenized with 6.5 % perchloric acid (Merck, Cat No: 100519) (90 mL) for 1 min using a blender and also an Ultra-Turrax (IKA T18 basic, Staufen, Germany). Through a filter paper (Whatman no. 1) solutions were filtered additionally filtrates alkalized by 20% NaOH (Merck, Cat No: 106462) to distillate with a Kjheldahl apparatus. In distillation point of view, 50 mL of solution which was filtered to gain lucent, was transferred into Kjeldahl tubes. 50 mL of distillate water was utilized as blank reference. 150 mL distillated water, and 10 mL of 20 % diluted sodium hydroxide (NaOH) solution and finally a few drop of anti-foaming agent (silicone based) were added upon transferred solutions. Separately, a solution was prepared by adding 5 mL of 3 % diluted boric acid solution and a few drops of tashiro indicator in a 250 mL Erlenmeyer flask. This flask was incorporated into the exit of distillation component of Kjeldahl machinery. In an approximately 7 to 10 minutes, 50 mL of distillates were accumulated within the Erlenmeyer flask which was placed in the exit of distillation component of Kjeldahl. This accumulated 50 mL distillate for each samples and blank were titrated with 0.01 N HCl (Merck, Cat No: 100314). According to the amount of consumptive HCl, mg TVB-N content per 100 g sample was calculated with the formula below;

$$\text{mg TVB-N/100 g sample} = \frac{(\text{HCl sample} - \text{HCl blank}) * 0.14 * (\text{Dilution factor})}{\text{sample weight}} \quad (2.6)$$

where 0.14 was the conversion factor for molecular weight of nitrogen. All analysis was demonstrated triplicate for each sample (Antonocoupoulos & Vyncke, 1989).

2.5.3 Determination of pH

pH was monitored for both HHP treated and untreated triplicate samples with using a pH meter (Mettler Toledo MP220). Before determination, pH meter was calibrated with both pH 4 and pH 7 buffers periodically. During analysis, 10 grams of both anchovy and haddock samples were homogenized for 1 minute at room temperature in an Ultra-Turrax (IKA T18 basic, Staufen, Germany). Homogenization was prepares in 1:10 ratio with 100 ml distilled water. Solutions were filtrated with a filter paper (Whatman no. 1). This clear liquid was utilized for pH meter (Tejada & Huidobro, 2002).

2.5.4 Determination of Thiobarbituric acid (TBA)

Thiobarbituric acid (TBA) determination was carried out with method of Erkan & Özden (2008). Before determination of TBA for anchovy and haddock samples, a standard curve was obtained with TBA working solutions which were prepared from a malondialdehyde standard solution (MDA). In order to prepare MDA standard solution, 1,1,3,3-tetraethoxypropane (TEP) (Merck, Cat No: 805797) with a 10 µL quantity was diluted with 10 mL of 0.1 M HCl solution. This standard solution was boiled in a water bath (Wise Circu WCB-6, Germany) at 70-80 °C approximately for 5 minutes. After cooling of standard, it was diluted

with 100 mL of water; this stock solution contains 2.92 μg/mL of MDA. Working solutions which contain the range of 0.002-0.014 μg/mL of MDA quantities were prepared with dilutions of standard solution for TBA standard curve (Karatepe, 2004). 5 mL of both blank and working solutions were mixed with 1 mL of a 0.01 M of 2-TBA reactive solution and placed into the water bath at 70-80 °C for 40 minutes. For blank, 5 mL of diluted water was used. After cooling of all reactive tubes, absorbances were monitored at 532 nm using a spectrophotometer (Boeco S-22/Vis. Spectrophotometer, Germany). This standard curve which was illustrated in Appendix B was used for relation of absorbance of both HHP treated and untreated haddock-anchovy samples, and was used for all TBA determination analysis.

After sample preparation, for determination of TBA of HHP treated and untreated fish samples, 10 grams of filleted fish was weighted with an assay balance of 0.01 g sensitivity; then blended with the solution including 90 mL of a 5 % of TCA solution and 500 μL BHT (2,6-Di-tert-buthyl-4-methyl phenol, Merck Cat No: 822021). This mixture was homogenized and dispersed more efficiently with both a blender and Ultra Turrax for a few minutes at 2000-3000 rpm (moderate speed). After homogenization and filtration with Whatman No.1 filter paper, 5 mL of filtrated solution was pipetted and mixed with 1 mL of a 0.01 M of 2-TBA reactive solution within stopper tubes. All mixtures were heated in water bath at approximately 75 °C for 40 min similarly implemented for standard curve preparation, and then tubes were cooled to room temperature. The absorbance of all samples which were pink colored was monitored at 532 nm with spectrophotometer. Calculated TBA values with given formula below, were expressed as milligram malondialdehyde (MDA) equivalents per kilogram of fish meat.

$$\text{mg TBA/kg sample} = \frac{(\mathbf{0.0073 * ABS_{sample}}) \mathbf{*} (\textbf{Dilution factor})}{\textbf{sample weight}} \qquad (2.7)$$

where 0.0073 is the coefficient obtained from TBA standard curve in Appendix B. Determinations were carried out triplicate for both treated and untreated samples (Erkan & Özden, 2008).

2.6 Microbiological analysis

Determination of microbiological loads of fish samples was demonstrated via total mesophilic aerobic bacteria count. For that purpose, 10 grams of fish fillet, was transferred aseptically to a Stomacher bag containing 90 mL of 1 % sterile peptone water and homogenized with using a stomacher (Steward stomacher400) for 60 s at moderate speed. Later on, 0.1 mL serial dilutions (1:10, diluents, 1 % peptone water) of fish homogenates were prepared and dilutions were spread on the surface of dry media of plate count agar (PCA) (Merck Cat No: 105463). After incubation for 48 h at 37 °C, results of triplicate repeated for each sample were evaluated as the logarithm of colony forming units (log cfu/g) per gram of sample (Erkan & Özden, 2008).

2.7 Statistical analysis

Results obtained from all analysis (pH, color, TBA, TVB-N, TMA-N and microbiological analysis) for both anchovy and haddock samples were statistically analyzed by using SPSS with Regression and Analysis of Variance (ANOVA). The measurements were repeated for each sample (n=3); except sensory analysis and color analysis were repeated for 5 times and 9 times, respectively. All results were evaluated to find out the significance through

Tukey's honestly significant difference test. The differences between the means were considered significant when $p<0.05$ (Erkan & Özden, 2008).

In this study pressure, temperature and time were chosen as independent variables. Appearance, odour, L*, a*, b*, ΔE, pH, TBA, TVB-N, TMA-N, TMAC (log cfu/g) were the dependent variables involved in first and second parts of this study. In order to check the normality, results were approached with histogram and normality plot from "linear regression". Then "equality of variance/homogenity" was checked with Levene test. When results were procured these two hypotheses; ANOVA was applied to all results. All interactions of variables were controlled for both fish species. Especially, for anchovy, "One Way Anova" was utilized, because of fewer than three groups of pressure variable in shelf life analysis. For haddock, "Two Way Anova" was used in order to evaluate significancy within storage days. Significancy of variables lower than 0.05 ($p<0.05$); was interpreted as a significant change on independent variable. Difference of both capital, superscript letters (A, B, C) in the same column and small, superscript letters (a, b, c) in the same line were indicated significant differences ($p<0.05$) in all tables. As it is shown in Figure 2.3, all experiments were designed for both HHP treated and untreated anchovy and haddock samples.

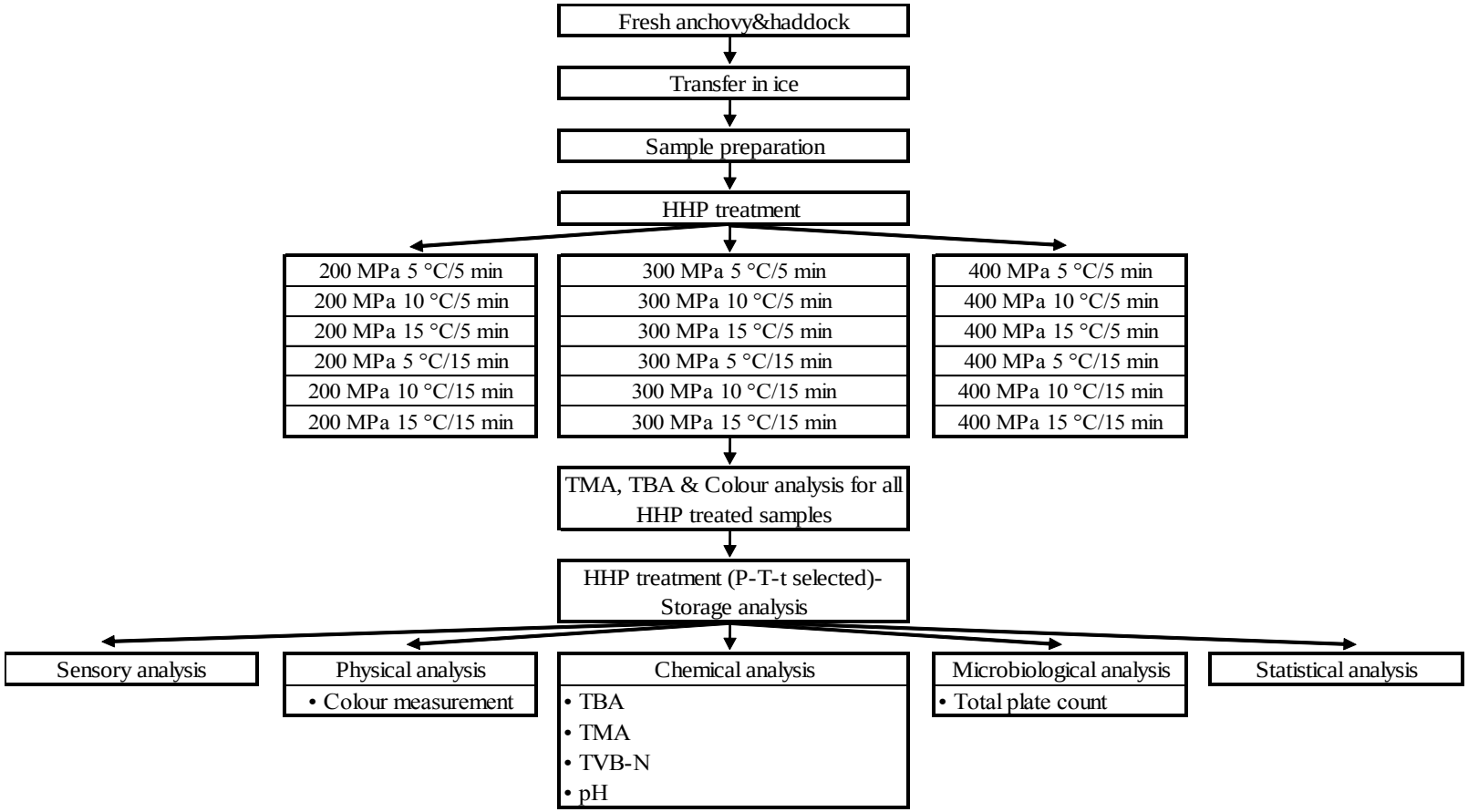

Figure 2.3 Experimental design for HHP treated anchovy and haddock

CHAPTER 3

RESULTS AND DISCUSSION

3.1.Effect of HHP on color

3.1.1. Change in color (L*, a*, b*, ΔE) for anchovy

The effect of HHP on changes in color values demonstrated by varying pressure magnitudes, is shown in Table 3.1 for anchovy fillets. In the CIE Lab system, L* expresses lightness from black to white; a* is red to green; and b* represents yellow to blue. The CIE L*, a*, b* color values and ΔE values of unpressurized control samples for anchovy were 42.12±3.50, 1.67±0.83, 0.12±2.38, 42.62±2.94 respectively.

All L* values decreased as pressure increased compared with untreated anchovy samples L* of anchovy started to decrease around 400 MPa with respect to 200 and 300 MPa significantly ($p<0.05$) (Table 3. 1). Decrease in L* value results were reported for HHP treated horse mackerel (220, 250 and 330 MPa for 5 and 10 min at 7, 15 and 25 °C), carp fillets and mahi mahi samples (100, 140, 180, 200 MPa for 15 and 30 min) (Sequeira-Munoz, Chevalier, LeBail, H, & Simpson, 2006; Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010). Also for cold smoked dolphin fish samples, 200, 300 and 400 MPa treated fish fillets and slices gave higher L* values than control samples significantly ($p<0.05$) (Gomez-Estaca, Gomez-Guillen & Montero, 2007).

When untreated samples are compared with HHP treated anchovy samples; a* values (redness) varied with pressure, temperature and time but change was insignificant ($p>0.05$) as shown in Table 3. 1. In the literature, a* values decreased after pressurization for mackerel and cod fish (Ohshima, Ushio, & Koizumi, 1993) and a* values with respect to horse mackerel control samples were lower insignificantly ($p>0.05$) when treated with 220–250–330 MPa, 7 °C for 5 min, 220–330 MPa, 15 °C for 5 min, 220–250–330 MPa, 25 °C for 5 min (Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010).

For anchovy samples, generally most of the b* values (yellowness) increased with increasing pressure magnitudes; however these raises varied with temperature and compressed time of pressure (Table 3.1). For each temperature and time combinations; when pressure increased from 200 MPa to 400 MPa, change in b* values was insignificant ($p>0.05$). For each pressure magnitudes, after pressurization at 5 °C to 10 °C and 15 °C, b* values decreased significantly, otherwise a significant change was not determined by time ($p<0.05$). In the literature, b* values for HHP treated horse mackerel samples affected insignificantly ($p>0.05$), except 220 MPa, 7 °C for 5 min and 220 MPa, 25 °C for 10 min (Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010). Also a* values decreased with pressurization at ≥200 MPa for raw cod muscle samples (Angsupanich, Edde & Ledward, 1999).

All ΔE values decreased compared to control samples; but in each pressure treatment, this reduction varied with temperature and time combinations. These parameters were insignificant on ΔE values ($p>0.05$). For each temperature and time combinations, after 300 MPa pressurization to 400 MPa, ΔE decreased significantly ($p<0.05$). Parallel decreasing results were obtained for hake (*Merluccius capensis*) muscle, when pressurized at 200 MPa (3 and 5 minutes) at 7 °C (Hurtado, Montero, & Borderias, 2000).

3.1.2. Change of color (L*, a*, b*, ΔE) for haddock

The effect of HHP on changes in color values demonstrated by varying pressure magnitudes, are shown in Table 3.2 for haddock. The CIE L*, a*, b* color values and ΔE values of unpressurized control samples for untreated and treated haddock samples were 25.30±3.85, (-1.06) ±1.16, 1.50±2.29 and 25.46±3.91, respectively.

For each time and temperature parameters L* values decreased for haddock significantly ($p<0.05$) as pressure increased as shown in Table 3.2; except 5 ºC/5 min. Similar decrease of L* value results with increasing pressure were reported for HHP treated rainbow trout (220, 250 and 330 MPa, 7, 15 and 25 ºC for 5 and 10 min), sea bream (at 3, 7, 15 and 25 °C, 5–10 min and 220, 250 and 330 MPa) and red mullet (at 3, 7, 15 and 25 °C, 5 to 10 min and 220, 250 and 330 MPa) (Erkan & Üretener, 2010; Erkan, Üretener, & Alpas, 2010; Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010). Denaturation of proteins proposed as a reason for this decrease in L* value for pressurized fish samples (Carlez, Vecianangues, & Cheftel, 1995).

When untreated samples were compared with pressured haddock samples; a* values varied with pressure, temperature and time but was insignificant ($p>0.05$) as shown in Table 3.2. However all a* values increased compared to untreated fillets in haddock samples with the increasing pressure treatment, observed for each temperature. Also a* values at 400 MPa decreased with respect to 300 MPa significantly ($p<0.05$). Same increase in a* value results were reported for smoked salmon fillets when HHP application increased from 200 to 300 and then to 400 MPa with respect to control samples (Gomez-Estaca, Gomez-Guillen & Montero, 2007). Increasing pressure and holding time for HHP treated turbot fillet (at 100, 140, 180 and 200 MPa for 15 and 20 min at 4°C), increased the a* values significantly ($p<0.05$) (Chevalier, Le Bail & Ghoulb, 2001).

For each pressure magnitude, change in b* values varied due to different time and temperature combinations, but these parameters were also significant on b* values of haddock samples ($p<0.05$). In the literature, b* values increased compared to control samples at all pressure treatments (150, 300, 450 and 600 MPa for 15 min) for rainbow trout and mahi mahi (Yağız, Kristinsson, Balaban, & Marshall, 2007). The cooked appearance with increasing pressure treatments was stated as an interrelation for this increase in b* values which represents yellowness (Yağız, Kristinsson, Balaban, & Marshall, 2007). b* values of carp muscle fillet also increased with increasing pressure of 140, 180 and 200 MPa and compression times of 15 and 20 min (Sequeira-Munoz, Chevalier, LeBail, Ramaswamy, & Simpson, 2006).

In case of pressurization of haddock samples, generally, ΔE values were close to or higher than control samples according to pressurization parameters as depicted in Table 3.2. For each temperature and time combinations, all values increased significantly ($p<0.05$) except 300 MPa-15°C-5 min treatment combination. Time was an insignificant parameter in ΔE change for haddock ($p>0.05$). According to the literature, ΔE values increased progressively with increasing pressure and holding times for carp fillet samples at 100, 140, 180 and 200 MPa pressures for 15 and 20 min at 4°C (Sequeira-Munoz, Chevalier, LeBail, Ramaswamy & Simpson, 2006). Same increase in ΔE results were stated for pressurized turbot muscle at different pressure levels of 100 to 200 MPa for 15 and 20 min at 4°C (Chevalier, Le Bail & Ghoulb, 2001).

Table 3.1 The effect of HHP on L*, a*, b*, ΔE values of anchovy flesh [1,2,3]

Temperature/time		5 °C/5min	10 °C/5min	15 °C/5min	5 °C/15min	10 °C/15min	15 °C/15min
L*							
	200 MPa	12.65^{Aa}±6.16	30.05^{Ab}±6.33	32.51^{Ab}±8.46	22.11^{Aa}±7.47	20.47^{Ab}±7.24	23.02^{Ab}±6.98
	300 MPa	30.85^{Aa}±8.00	27.45^{Ab}±7.87	25.43^{Ab}±10.53	23.13^{Aa}±10.36	24.00^{Ab}±10.25	21.98^{Ab}±5.63
	400 MPa	19.43^{Ba}±9.78	19.34^{Bb}±9.55	23.96^{Bb}±6.97	14.20^{Ba}±8.44	24.80^{Bb}±5.40	19.17^{Bb}±9.91
a*							
	200 MPa	-2.43^{Aa}±2.70	0.61^{Aa}±2.58	2.26^{Aa}±4.37	5.13^{Aa}±7.68	-1.83^{Aa}±2.74	0.23^{Aa}±2.52
	300 MPa	2.76^{Aa}±1.83	0.63^{Aa}±4.33	0.73^{Aa}±1.83	-1.47^{Aa}±2.16	0.88^{Aa}±3.67	0.33^{Aa}±2.50
	400 MPa	-0.50^{Aa}±3.65	-0.07^{Aa}±4.03	1.75^{Aa}±3.85	-0.48^{Aa}±3.97	1.12^{Aa}±4.04	0.18^{Aa}±3.93
b*							
	200 MPa	5.37^{Aa}±3.95	1.36^{Ab}±5.32	1.65^{Ab}±4.26	3.00^{Aa}±3.15	2.15^{Ab}±2.35	1.66^{Ab}±4.24
	300 MPa	0.98^{Aa}±4.82	-1.97^{Ab}±4.44	-0.67^{Ab}±3.80	4.82^{Aa}±4.76	1.01^{Ab}±5.20	2.62^{Ab}±2.37
	400 MPa	5.93^{Aa}±8.13	1.55^{Ab}±1.86	-1.28^{Ab}±4.68	5.86^{Aa}±6.81	0.35^{Ab}±3.32	2.03^{Ab}±4.02
ΔE							
	200 MPa	14.99^{Aa}±5.32	30.63^{Aa}±6.05	33.25^{Aa}±8.10	22.88^{Aa}±7.06	21.02^{Aa}±6.96	23.54^{Aa}±6.98
	300 MPa	31.47^{Aa}±7.52	30.20^{Aa}±2.58	25.85^{Aa}±10.33	24.04^{Aa}±9.38	25.13^{Aa}±9.42	22.45^{Aa}±5.39
	400 MPa	22.48^{Ba}±8.63	20.24^{Ba}±8.66	25.73^{Ba}±5.84	17.48^{Ba}±7.60	25.33^{Ba}±5.39	20.81^{Ba}±7.97

1 Different letters (A, B, C) in the same column indicate significant differences (p<0.05). Different letters (a, b, c) in the same line indicate significant differences (p<0.05).

2 All values are the mean ± standard deviation (n=9).

3 L*, a*, b* and ΔE values of control samples were 42.12±3.50, 1.67±0.83, 0.12±2.38, 42.62±2.94 respectively.

Table 3.2 The effect of HHP on L*, a*, b*, ΔE values of haddock flesh [1,2,3]

Temperature/time	5°C/5 min	10°C/5min	15°C/5min	5°C/15min	10°C/15min	15°C/15min
L*						
200 MPa	29.79^{Aa}±3.91	31.41^{Ab}±3.86	31.39^{Ab}±1.71	32.66^{Aa}±3.19	29.67^{Ab}±2.32	31.06^{Ab}±1.66
300 MPa	30.61^{Ba}±2.71	26.02^{Bb}±0.94	24.18^{Bb}±2.49	27.28^{Ba}±2.95	25.17^{Bb}±2.94	26.38^{Bb}±2.90
400 MPa	25.78^{Ca}±3.77	22.44^{Cb}±3.17	23.44^{Cb}±1.77	25.30^{Ca}±3.22	24.87^{Cb}±2.26	24.16^{Cb}±3.20
a*						
200 MPa	1.59^{Aa}±0.86	2.87^{Aa}±1.29	2.66^{Aa}±1.67	3.40^{Aa}±1.06	2.39^{Aa}±1.37	2.83^{Aa}±1.15
300 MPa	2.27^{Aa}±1.21	1.86^{Aa}±1.85	2.87^{Aa}±2.63	2.98^{Aa}±0.79	2.74^{Aa}±1.71	2.52^{Aa}±1.24
400 MPa	1.71^{Ba}±1.28	1.83^{Ba}±1.38	2.06^{Ba}±0.80	1.27^{Ba}±0.92	1.19^{Ba}±2.56	1.12^{Ba}±1.00
b*						
200 MPa	1.96^{Aa}±1.35	3.60^{Aa}±2.60	4.59^{Aa}±1.62	4.23^{Aa}±1.83	2.92^{Aa}±2.19	3.37^{Aa}±1.98
300 MPa	2.40^{Ba}±0.73	1.74^{Ba}±1.52	0.78^{Ba}±1.93	3.41^{Ba}±1.29	2.84^{Ba}±3.01	2.46^{Ba}±1.28
400 MPa	1.42^{Ca}±1.61	2.70^{Ca}±2.10	1.28^{Ca}±1.07	0.81^{Ca}±1.35	0.01^{Ca}±3.11	0.11^{Ca}±1.68
ΔE						
200 MPa	29.92^{Aa}±3.95	31.83^{Ab}±4.00	31.89^{Ab}±1.78	33.17^{Aa}±3.33	30.01^{Ab}±2.46	31.42^{Ab}±1.91
300 MPa	30.80^{Ba}±2.73	26.22^{Bb}±1.07	24.56^{Bb}±2.60	27.67^{Ba}±3.06	25.64^{Bb}±3.27	26.68^{Bb}±2.83
400 MPa	25.96^{Ca}±1.90	22.77^{Cb}±3.42	23.60^{Cb}±1.85	25.39^{Ca}±3.28	25.14^{Cb}±2.63	24.26^{Cb}±3.24

1 Different letters (A, B, C) in the same column indicate significant differences (p<0.05). Different letters (a, b, c) in the same line indicate significant differences (p<0.05).

2 All values are the mean ± standard deviation (n=9).

3 L*, a*, b* and ΔE values of control samples were 25.30±3.85, (-1.06) ±1.16, 1.50±2.29 and 25.46±3.91, respectively.

3.2. Effect of HHP on TMA-N and TBA on anchovy samples

Effect of HHP on TMA-N and TBA values of anchovy samples are given in Table 3.3. Untreated anchovy samples had TMA-N and TBA values of 4.81±1.70 and 1.19±0.17, respectively. 5 min pressurized samples attained lower TMA-N values with respect to control samples for all temperatures. For each temperature and time combination, change in TMA-N with increasing pressure magnitudes varied depending on time and temperature combinations. When pressure applied to anchovy fleshes (constant T and t), all TMA-N values decreased insignificantly ($p>0.05$). For each pressure level, change in TMA-N was significant ($p<0.05$) when samples pressurized at 10 and 15°C with respect to 5°C. In the literature, the rejection limit was defined as above 5 mg TMA-N/100 g muscle (Sikorski, Kolakowska, & Burt, 1990). Only a small part of bacterial load existing naturally in fish is able to produce TMA-N content. (Pérez-Villarreal & Howgate, 1987). The decrease of TMA-N was initiated with the adverse effect of HHP on microbiological load (Raharjo & Sofos, 1993). For pressurized gilted sea bream samples, estimated significantly lower than control samples when pressure applied at 330 MPa, 7°C for 5 min. At 220–250 MPa 7 °C for 5 min; at 220–250–330 MPa 15°C for 5 min and at 330 MPa 3–7°C for 10 min, changes in TMA-N values were insignificant for sea bream samples ($p>0.05$) (Erkan & Üretener, 2010). For 330 MPa, 7 °C for 10 min; 220–250–330 MPa, 25 °C for 10 min pressurized horse mackerel samples, TMA-N content decreased significantly ($p<0.05$) compared to control samples. This decrease was correlated with proteolytic activity inhibition (Erkan, Üretener & Alpas, 2010).

TBA value of unpressurized and pressurized anchovy samples are shown in Table 3.3. For each pressure (P was constant), increase in TBA was significant at 15 °C. For each temperature and time combinations, as pressure levels increased, TBA content increased significantly ($p<0.05$) after 300 MPa. But at 15°C for all holding times, at 300 and 400 MPa pressures, TBA values had a reverse situation

with decreasing values significantly ($p<0.05$) compared with 200 MPa. Even so, all TBA values exceeded untreated anchovy samples. TBA value of 3-4 mg MDA/kg was recommended as the limit of acceptability (Huss, 1988; Karaçam & Boran, 1996; Scott, Fletcher, Charles & Wong, 1992). 3 mg MDA/kg was used as a threshold TBA value in this study. Increase in lipid oxidation due to HHP treatment was stated as free metal ion (Fe and Cu) content increase in minced pork and cod samples (Angsupanich & Ledward, 1998). In the literature, when both mahi mahi and rainbow trout dark muscle samples were pressurized at 300, 450 and 600 MPa for 15 min, TBA values increased significantly with increasing pressure levels when compared with control samples (Yağız, Kristinsson, Balaban, & Marshall, 2007). In addition, TBA values of 220 MPa, 15°C for 10 min pressurized gilthead sea bream samples increased significantly ($p<0.05$) compared with control samples (Erkan & Üretener, 2010). In some studies this acceleration of lipid oxidation associated with protein denaturation of heme protein with high pressure application. During this denaturation, release of metal ions and heme increase auto-oxidation with lipid oxidation (Yağız, Kristinsson, Balaban, & Marshall, 2007).

Table 3.3 The effect of HHP on TMA-N and TBA values of anchovy flesh[1,2,3,4]

Temperature/time		5 °C/5 min	10 °C/5 min	15 °C/5 min	5 °C/15 min	10 °C/15 min	15°C/15 min
TMA-N (mg/100g)	**200 MPa**	2.17^{Aa} ±0.10	2.18^{Ab} ±0.13	4.67^{Ab} ±1.09	7.03^{Aa} ±0.24	3.03^{Ab}±0.43	1.58^{Ab} ±0.97
	300 MPa	3.01^{Aa} ±0.78	1.86^{Ab} ±0.37	1.91^{Ab} ±0.32	6.12^{Aa} ±1.06	5.61^{Ab} ±0.92	3.86^{Ab} ±4.08
	400 MPa	4.18^{Aa} ±2.07	3.92^{Ab} ±0.21	2.40^{Ab} ±0.48	4.95^{Aa} ±1.73	3.89^{Ab} ±0.10	3.31^{Ab} ±1.21
TBA (mg MDA/kg)	**200 MPa**	1.31^{Aa} ±0.01	2.28^{Aa} ±0.28	2.88^{Ab} ±0.73	3.18^{Aa} ±0.54	1.76^{Aa} ±0.14	2.96^{Ab} ±0.70
	300 MPa	1.41^{Ba}±0.28	2.38^{Ba} ±0.45	2.37^{Bb} ±0.18	1.76^{Ba} ±0.59	1.55^{Ba} ±0.07	2.62^{Bb} ±0.16
	400 MPa	1.53^{Ba}±0.31	2.79^{Ba} ±0.31	2.31^{Bb} ±0.20	1.79^{Ba} ±0.58	2.27^{Ba} ±0.41	2.45^{Bb} ±0.10

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All values are the mean ± standard deviation (n=3).

3 Unpressurized samples had TMA-N value of 4.81±1.70 mg/100 g.

4. Unpressurized samples had TBA value of 1.19±0.17 mg MDA/kg.

3.3. Effect of HHP on TMA-N and TBA on haddock samples

Effect of HHP on TMA-N and TBA values of haddock samples are given in Table 3.4. Untreated haddock samples had TMA-N and TBA values of 0.58±0.11 and 0.52±0.07. For each temperature and time parameters, change in pressure was insignificantly ($p>0.05$) in TMA-N content for haddock samples. However, for each pressure application, temperature was a significant factor on TMA-N ($p<0.05$). As temperature increased to 10°C for all pressures, TMA-N increased significantly ($p<0.05$); yet when temperature increased from 10°C to 15°C TMA-N values decreased significantly ($p<0.05$). Although this decrease was established, these values were still higher than untreated samples. In the literature, cold smoked muscle samples pressurized at 220, 250 and 330 MPa, at 3, 7, 15 and 25ºC for 5 and 10 min. Only 220MPa /7°C/ 5 min, 330 MPa /7°C /5 min and 250 MPa /25°C /5 min combinations gave lower TMA-N results than control samples (Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010). Additionally, at 250–330 MPa, 3°C for 5 min; 220–330 MPa, 7°C for 5 min; 220–250–330 MPa, 15°C for 5–10 min; 250 MPa, 25°C for 5 min treated sea bass samples increased in TMA-N content significantly ($p<0.05$) than these values of control samples (Erkan, Üretener & Alpas, 2010).

TBA values of unpressurized and pressurized haddock samples are shown in Table 3.4. Generally, for all HHP combinations TBA values were lower than control samples this decrease substantiated insignificantly with change of temperature and time parameters ($p>0.05$). For each pressure treatment, as temperature increased to 15 ° C, changes in TBA values varied due to treatment combinations. TBA value increased significantly ($p<0.05$) for both 5 and 15 min compression times. Also all TBA values of untreated and treated samples did not reached to rejection limit of 3-4 mg MDA/kg which was stated for fish quality (Huss, 1988; Karaçam & Boran, 1996; Scott, Fletcher, Charles, 1992). In the literature, gilted sea bream samples pressurized at 3, 7, 15 and 25°C, 5–10 min

and 220, 250 and 330 MPa and generally TBA values decreased significantly ($p<0.05$) by comparison with untreated samples (Erkan & Üretener, 2010). In another study, minced albacore muscle treated with 275 and 310 MPa for 2, 4, and 6 min at 4 and -20°C. TBA values of all pressure-temperature-time combinations were lower significantly ($p<0.05$) than control samples, instead of 275 MPa for 2 min at 4°C (Ramirez-Suarez, & Morrissey, 2006). Additionally, horse mackerel fleshes applied to high pressures of 220, 250 and 330 MPa at 7, 15 and 25 °C for 5 and 10 min. HHP treated samples effected insignificantly compared to untreated samples ($p>0.05$). As a result, haddock samples were seemed to be more sensitive than anchovy samples when applied to high pressure application (Erkan, Üretener, Alpas, Selçuk, Özden, & Buzrul, 2010).

Table 3.4 The effect of HHP on TMA-N and TBA values of haddock flesh[1,2,3,4]

Temperature/time		5 °C/5 min	10 °C/5 min	15 °C/5 min	5 °C/15 min	10 °C/15 min	15 °C/15 min
TMA-N (mg/100g)	**200 MPa**	0.42^{Aa}±0.05	1.11^{Ab}±0.23	0.44^{Ac}±0.04	1.29^{Aa}±0.12	0.84^{Ab}±0.03	0.38^{Ac}±0.05
	300 MPa	0.66^{Aa}±0.17	1.02^{Ab}±0.12	0.65^{Ac}±0.09	1.45^{Aa}±0.13	0.77^{Ab}±0.14	0.48^{Ac}±0.06
	400 MPa	0.65^{Aa}±0.21	1.49^{Ab}±0.28	0.39^{Ac}±0.03	0.95^{Aa}±0.28	1.27^{Ab}±0.24	0.58^{Ac}±0.14
TBA (mg MDA/kg)	**200 MPa**	0.31^{Aa}±0.08	0.30^{Aa}±0.04	0.53^{Ab}±0.13	0.22^{Aa}±0.01	0.30^{Aa}±0.16	0.45^{Ab}±0.12
	300 MPa	0.28^{Aa}±0.07	0.17^{Aa}±0.13	0.35^{Ab}±0.10	0.25^{Aa}±0.07	0.41^{Aa}±0.08	0.58^{Ab}±0.29
	400 MPa	0.35^{Aa}±0.03	0.15^{Aa}±0.02	0.30^{Ab}±0.13	0.24^{Aa}±0.06	0.30^{Aa}±0.15	0.42^{Ab}±0.14

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All values are the mean ± standard deviation (n=3).

3 Unpressurized samples had TMA-N value of 0.58±0.11.

4 Unpressurized samples had TBA value of 0.52±0.07.

3.4. Decision Criteria

Before shelf life study, all of L*, a*, b*, ΔE color values with TMA, TBA results were compared with control (untreated) anchovy and haddock samples.

For anchovy, lowest TMA-N values were evaluated when samples pressurized at 200 MPa/5°C/5 min insignificantly ($p>0.05$) and 200/MPa/15°C/15 min, 300/MPa/10°C/5 min, 300/MPa/15°C/5 min significantly ($p<0.05$) when compared to control samples. Lowest TBA values were determined for anchovy at 200/MPa/5°C/5 min, 200/MPa/10°C/5 min and 200/MPa/15°C/5 min. Because increase in both TMA-N and TBA demonstrated the protein degradation due to microorganism load and lipid oxidation in time; lowest TMA-N and TBA values were chosen to gain high quality samples with respect to control. For this reason, optimum treatment was selected as 200/MPa/5°C/5 min for anchovy.

For haddock, lowest TMA-N values were determined for 200/MPa/5°C/5 min, 200/MPa/15°C/5 min, 400/MPa/15°C/5 min and 200/MPa/15°C/15 min combinations. Additionally, all TBA values were lower than control samples, except 300/MPa/15°C/15 min. But at 200/MPa/5°C/5 min, 200/MPa/15°C/5 min, 400/MPa/15°C/5 min and 200/MPa/15°C/15 min treated samples had 0.31±0.08, 0.53±0.13, 0.30±0.13 and 0.45±0.12 TBA values. According to these results both 200/MPa/5°C/5 min and 400/MPa/15°C/5 min were selected for shelf life studies for haddock samples.

3.5. Shelf Life Analysis

Anchovy and haddock samples were pressurized and stored at refrigeration temperature (4 °C) for 15 days. Every each day, pressurized and untreated samples of both anchovy and haddock were analyzed for TVB-N, TMA, TBA, pH, color, microbiological and sensory analysis.

3.5.1. Total mesophilic aerobic count (TMAC) results of anchovy

Change in TMAC for untreated control samples and pressurized anchovy samples at 200 MPa, 5°C for 5 min, monitored for 15 storage days at refrigeration temperature (4 °C) as shown in Table 3.5. When untreated and HHP treated samples were compared with each other, in each storage day, pressurized samples were lower in microbial load than control samples. This decrease demonstrated that HHP application caused positive influence on microbiological inactivation. The limit of acceptability was stated as 10^6 cfu/g as upper acceptability limit for marine species and as rejection criterion for fish consumption as was stated in literature (Erkan, 2007). In addition the Total Mesophilic Aerobic Count (TMAC), fish samples which have lower than 3 log cfu/g were classified as "very good fish quality".

Initial bacterial load was lower than 3 log cfu/g for untreated and treated anchovy samples. Statistically, post hoc test was not performed for pressure because there were fewer than three groups. Meanwhile microbial load of both control and HHP treated samples increased after Day 5 significantly ($p<0.05$). After Day 5 control samples lost their good quality and were rejected. However, HHP treated anchovy samples classified as very good quality till Day 9, and samples were still consumable at Day 15 when stored in refrigeration conditions. In the literature, for gilthead sea bream fish samples, unpressurized samples reached to acceptable limit of 6 log cfu/g at Day 19, since 250 MPa, 3°C for 5 min and 250 MPa, 15°C for 5 min pressurized samples reached to this limit at Day 16 (Erkan, & Üretener, 2010).

3.5.2. Sensory results (Appearance and Odour) of anchovy

Sensory analysis results, including appearance and odour notations are given in Table 3.5. In terms of appearance, since scores between 4.9–0 was

denoted as “spoiled”, untreated anchovy samples (4.00±1.37) were classified as spoiled after Day 7. Whereas 200 MPa 5°C for 5 min treated samples (2.10±0.89) were rejected because of spoilage at Day 11 during storage at 4 °C. Appearance values of both control and 200 MPa at 5 °C for 5 min treated samples were decreased significantly ($p<0.05$) with Day 5. For odour attribute, control samples (2.80±0.57) were denoted as spoiled at Day 7, since 200 MPa 5°C for 5 min pressurized samples (3.80±1.15) were spoiled after Day 7, according to odour scores. Odour results of both control and 200 MPa 5°C for 5 min HHP treated anchovy samples started to decrease at Day 3 significantly ($p<0.05$). According to literature, all 4 %, 7 % and 10 % brined, canned anchovy samples were scored for “very good quality” after 85 hr of storage at 0°C (Del Valle, Filsinger, Yeannes & Soule, 1984). In another study, ready-to eat fish patties prepared with minced anchovy, were subjected to sensory analysis and all scores decreased significantly ($p<0.05$) during refrigerated storage (4 °C). The samples had “very good” quality up to 2 days and rejected after Day 6 (Yerlikaya, Gökoğlu & Uran, 2005) .

Table 3.5 The effect of HHP on sensory evaluation of appearance, odour and TMAC of anchovy flesh during refrigerated storage [1,2,3,4]

Storage days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
Appearance	**Control**	$10,00^{Aa}$±0,00	$9,98^{Aa}$±0,05	$8,46^{Aa}$±0,50	$5,70^{Ab}$±1,79	$4,00^{Ab}$±1,37	$2,88^{Ab}$±0,71	$2,00^{Ac}$±0,35	$1,00^{Ac}$±0,00	$0,00^{Ac}$±0,00
	200 MPa 5°C 5 min	$9,66^{Aa}$ ±0,42	$9,00^{Aa}$±0,00	$8,34^{Aa}$±0,42	$7,40^{Ab}$±1,14	$6,96^{Ab}$±0,73	$5,90^{Ab}$±1,43	$2,10^{Ac}$±0,89	$1,10^{Ac}$±0,22	$0,20^{Ac}$±0,45
Odour	**Control**	$10,00^{Aa}$±0,00	$9,90^{Aa}$±0,10	$8,28^{Ab}$±0,47	$6,48^{Ac}$±1,64	$2,80^{Ad}$±0,57	$1,10^{Ad}$±0,74	$0,30^{Ae}$±0,45	$0,30^{Ae}$±0,45	$0,00^{Ae}$±0,00
	200 MPa 5°C 5 min	$9,90^{Aa}$ ±0,22	$9,94^{Aa}$±0,09	$9,04^{Ab}$±1,19	$7,80^{Ac}$±1,96	$3,80^{Ad}$±1,15	$3,18^{Ad}$±0,73	$0,60^{Ae}$±0,65	$0,40^{Ae}$±0,42	$0,00^{Ae}$±0,00
TMAC	**Control**	$1,97^{Aa}$±0,02	$1,97^{Aa}$±0,02	$2,06^{Aa}$±0,03	$2,73^{Ab}$±0,04	$3,94^{Ac}$±0,04	$3,72^{Ad}$±0,05	$4,58^{Ad}$±0,08	$5,00^{Ad}$±0,07	$5,85^{Ae}$±0,07
(log cfu/g)	**200 MPa 5°C 5min**	$1,10^{Ba}$±0,04	$1,12^{Ba}$±0,04	$1,15^{Ba}$±0,00	$1,76^{Bb}$±0,04	$2,41^{Bc}$±0,10	$3,80^{Bd}$±0,03	$3,96^{Bd}$±0,14	$4,09^{Bd}$±0,06	$5,78^{Be}$±0,02

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All appearance and odour values are the mean ± standard deviation of six replicates (n=6). All microbiological analysis are the mean ± standard deviation of triplicate (n=3).

3 Control: Unpressurised

4. For sensory analysis (for both appearance and odour) <4.9 was noted as spoiled by panelists. For marine species < 3 log cfu/g and <6 log cfu/g were selected as “lost good quality” and rejection criteria, respectively.

3.5.3. Color results (L*, a*, b*, ΔE) of anchovy

At the beginning of storage period, L*, a*, b* and ΔE values of control samples were 43.42±1.46, 1.33±0.23, 0.74±2.28 and 43.60±1.46, respectively in Table 3.6. It could be concluded that high pressure gave anchovy muscle a brighter and less transparent appearance. Within ongoing storage days, because a* and b* values were fluctuated; L*, and ΔE values were decreased in color values of anchovy samples. As a result, L* and ΔE values were decreased gradually during shelf life at 4°C for both pressurized and unpressurized samples; this explains the increase in darkness of anchovy samples day by day. All L* and ΔE values were changed significantly with pressure application ($p<0.05$). In the literature, L* values increased significantly when cold smoked dolphin fish samples were pressurized (100–200–300 MPa at 20 °C for 15 min) than untreated samples, during the first 14 days of storage. Also a* and b* values slightly increased with HHP treatment (100–200–300 MPa at 20 °C for 15 min) when stored at 5°C (Gomez-Estaca, Gomez Gulienne & Montero, 2007). In another shelf life study, when HHP treated (3 °C 5 min 250 MPa and 15 °C 5 min 250 MPa) gilted head sea bream samples stored at 4°C, both L* and ΔE values increased significantly after Day 10 during 19 days of storage. But a* and b* values were fluctuated for all pressurized and control samples within 19 days (Erkan & Üretener, 2010). For pressurized albacore tuna (275 MPa for 2-4-6min, 310 MPa for 2-4-6min) L* and ΔE values increased, whereas a* value decreased during 22 days of storage 4 °C. It was also stated that all changes resulted because of increase in lightness and whiteness of albacore samples during shelf life.

In summary, HHP treated and untreated anchovy samples became darker during storage in comparison to sea bream, albacore tuna and dolphin fish. These

decrease in color values were expressed with precipitation and denaturation of the globular heme protein metmyoglobin due to pressurization and storage time (Defaye, Ledward, MacDougall & Tester, 1995). It is widely known that denaturation of this pigment is responsible for color change due to heat treatment of meat. This relation explains the residual cooked appearance (increasing L* and ΔE values) of anchovy samples during 15 days.

Table 3.6 The effect of HHP on L*, a*, b* and ΔE of anchovy flesh during refrigerated storage [1,2,3]

Storage days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
L*	Control	43,42^{Aa}±1,46	41,00^{Aa}±4,71	39,89^{Aa}±6,67	40,04^{Aa}±4,99	37,44^{Aa}±5,76	38,16^{Aa}±5,84	35,84^{Aa}±4,30	37,42^{Aa}±4,10	36,94^{Aa}±2,56
	200 MPa 5°C 5min	35,11^{Ba}±5,84	35,21^{Ba}±6,81	29,49^{Ba}±8,02	32,42^{Ba}±8,39	33,67^{Ba}±7,36	28,37^{Ba}±3,48	29,14^{Ba}±3,42	28,97^{Ba}±1,94	31,10^{Ba}±3,55
a*	Control	1,33^{Aa}±0,23	0,61^{Aa}±2,16	1,58^{Aa}±3,48	0,62^{Aa}±2,59	1,60^{Aa}±3,27	-1,39^{Aa}±3,02	0,19^{Aa}±2,13	2,18^{Aa}±7,01	-0,47^{Aa}±1,16
	200 MPa 5°C 5min	-0,21^{Aa}±1,91	1,28^{Aa}±2,70	2,72^{Aa}±1,71	4,40^{Aa}±5,61	3,28^{Aa}±3,80	3,01^{Aa}±2,00	0,64^{Aa}±2,82	-0,73^{Aa}±1,34	2,69^{Aa}±3,02
b*	Control	0,74^{Aa}±2,28	-1,21^{Aa}±2,80	-0,76^{Aa}±5,73	0,23^{Aa}±1,60	-1,34^{Aa}±3,70	-0,03^{Aa}±3,98	2,07^{Aa}±2,54	1,47^{Aa}±1,71	0,97^{Aa}±1,20
	200 MPa 5°C 5min	-0,62^{Aa}±2,79	0,71^{Aa}±4,11	0,48^{Aa}±2,40	1,21^{Aa}±3,95	-0,62^{Aa}±5,11	0,24^{Aa}±3,00	0,60^{Aa}±2,35	-0,66^{Aa}±1,61	0,93^{Aa}±2,86
ΔE	Control	43,60^{Aa}±1,49	41,17^{Aa}±4,53	40,50^{Aa}±6,21	40,14^{Aa}±5,03	37,83^{Aa}±5,52	38,47^{Aa}±5,91	36,10^{Aa}±4,28	37,71^{Aa}±3,93	37,00^{Aa}±2,54
	200 MPa 5°C 5min	35,27^{Ba}±5,85	35,51^{Ba}±7,06	29,71^{Ba}±8,07	33,38^{Ba}±8,26	34,36^{Ba}±7,51	28,69^{Ba}±3,67	29,33^{Ba}±3,60	29,03^{Ba}±1,89	31,46^{Ba}±3,73

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All color values are the mean ± standard deviation of nine replicates (n=9).

3 Control: Unpressurised

3.5.4. Results of chemical analysis (pH, TVB-N, TMA-N, TBA) for anchovy

Initial pH values of untreated and 200 MPa 5 °C 5 min treated samples were 6.67±0.01 and 6.82±0.05 respectively as shown in Table 3.7. Pressure was a significant factor for anchovy at the beginning of storage ($p<0.05$). For each day of storage pH of control samples increased significantly ($p<0.05$) during 15 days of storage after Day 0. The acceptable limit of pH for fish samples was stated as 7.00 in literature (Cadun, Kılınç, Şen & Çaklı, 2008). According to rejection limit, pH of untreated samples (7.16) exceeded at Day 5. 200 MPa 5 °C 5 min pressurized samples with pH of 7.14, rejected at Day 11. In literature, pH values of anchovy patties were obtained as 6.09 initially when stored in ice (4°C) and reached to rejectable level at day 15 (Yerlikaya, Gökoğlu & Uran, 2005). In another study, when anchovy samples stored at ambient temperature (25°C) pH values of anchovy samples were determined as 6.22 initially and rejected (with average of 7.06) at Day 3. When same samples with initial pH of 6.12 stored in refrigerator (4°C), pH were reached to approximately 7 at the end of 5 days of storage (Köse & Erdem, 2004). As pH value alone could not give direct information about chemical quality about anchovy samples, all attributes were combined during shelf life determination.

The initial TVB-N values of untreated and HHP treated samples were shown in Table 3.7. With 200 MPa 5 °C 5 min HHP application initial TVB-N reduced to 12.61±0.37 from 14.80±1.04 mg/100 g significantly ($p<0.05$); this reduction indicates the effect of HHP on TVB-N. HHP treatment did not just inactivate microorganisms or denaturate proteins, it also inactivated enzymes. This result could be explained with the anticatalyst role of HHP on reactions that produce this volatile molecule by alkaline. For each day of storage rapid increase of TVB-N was monitored significantly ($p<0.05$), especially after the 9^{th} day. Based on rejection criteria, control samples were rejected (74.41±0.50) at 9^{th} day whereas HHP treated samples reached to rejectable limit after 15 days

(36.84±1.41). 200 MPa 5 °C 5 min treated samples also increased significantly ($p<0.05$) after Day 9. According to literature; anchovy patties stored in ice (4 °C) contained TVB-N values of 17.37 mg/100 g initially. Also increase of TVB-N was not determined as significant ($p<0.05$), until samples were reached to 6th day of storage. Then they were rejected at day 15 (38.40 mg/100 g) when stored in ice (4 °C) according to rejection limit of 35 mg/100 g (Yerlikaya, Gökoğlu, & Uran, 2005). In another study of anchovy samples, TVB-N values were 7.3, 26.6 mg/100 g at day 1, 2 respectively and were rejected (39.2 mg/100 g) at Day 3 during storage at ambient temperature (25 °C) (Köse & Erdem, 2004). During refrigeration at 4°C, TVB-N values of anchovy samples were on the average of 5.7 mg/100 g initially and exceeded the rejectable limit at day 5 (36.9 mg/100 g) (Köse & Erdem, 2004). Additionally, according to another study of anchovy surimi, raw anchovy samples were analyzed as 4.2 mg TVB-N/100 g initially. After surimi production samples reached to 6.3 mg /100 g at the end of 150 days frozen storage (–18°C for 9 months) (Kaba, 2005). When this study is compared with literature, effect of HHP on shelf life for only TVB-N criterion could be a successful alternative to preservation techniques like storage at 4 °C.

TMA-N change of untreated and HHP treated samples were shown in Table 3.7. Initially, TMA-N value decreased from 29.34±0.28 to 8.41±0.46 significantly ($p<0.05$) with 200 MPa 5 °C 5 min application. TMA-N values increased significantly ($p<0.05$) after Day 3 for both untreated and 200 MPa 5 °C 5 min treated samples. In literature, fish samples lost their “good quality” when TMA-N reached to 5 mg TMA-N/100 g muscle, and classified as “spoiled” between 5-10 mg TMA-N/100 g (Sikorski, Kolakowska, & Burt, 1990). Through Day 1 to Day 3, untreated samples specified as “spoiled” with 11.28 TMA-N/100 g significantly ($p<0.05$). Likewise 200 MPa 5 °C 5 min lost their “good quality” at Day 3 (7.10 TMA-N/100 g) significantly ($p<0.05$) and rejected because of spoilage (10.70 TMA-N/100g) at Day 13. According to a study done for anchovy, initial TMA-N values was measured as 3.4 mg/100 g when samples stored at ambient temperature (25 °C) and samples were rejected (8.84 mg/100 g) at the

end of 3 days storage (Köse & Erdem, 2004). When same samples were stored at refrigerator (4 °C), TMA-N value was determined as 3.05 mg/100 g on the average of 3 batches, initially. TMA-N value reached to 8.79 mg/100g at the end of 5 days storage. Samples rejected at Day 3 with their TMA-N value of 5.12 mg/100 g at 4 °C (Köse & Erdem, 2004). Effect of HHP on shelf life for TMA-N content of anchovy could be seen in comparison to refrigeration storage.

TBA values of untreated samples reduced significantly ($p<0.05$) when treated with 200 MPa 5 °C 5 min, stated in Table 3.7. Oxidation of control samples increased significantly ($p<0.05$) especially after Day 3. When anchovy samples pressurized at 200 MPa 5 °C 5 min, TBA remained stable around approximately 2.88 MDA/kg between 1^{th} and 7^{th} days of storage. Then this value peaked up to 3.83±0.62 MDA/kg at 9^{th} day and rejected at this level at Day 9; according to rejection limit of 3-4 mg MDA/kg which was stated for fish quality (Huss 1988; Karaçam & Boran, 1996; Scott, Fletcher & Charles, 1992); whereas, control samples were rejected at Day1. Statistically, both untreated and HHP treated samples increase significantly ($p<0.05$) after Day 3. According to literature, at ambient temperature (25°C) TBA values of anchovy samples were reported as 1.36, 4.56, 8.43 mg MDA/kg at day 1, day 2, day 3 respectively; that explains samples reached to rejection limit at Day 3 (Köse & Erdem, 2004). At refrigeration temperature, TBA value was measured as 0.81 initially and reached to 8.53 mg MDA/kg at the end of 5 days storage. Similar to our study, anchovy rejected as spoiled with 6.14 MDA/kg at Day 3 at 4°C (Köse & Erdem, 2004). In another study of anchovy patties initial TBA value of 10.61 mg/kg reached to 19.27 mg/kg significantly ($p<0.05$) at day 3 to 4; when patties stored at 4°C (Yerlikaya, Gökoğlu, & Uran, 2005). Additionally, according to Kaba (2006); initial TBA value was determined as 1.09 mg/kg for fresh anchovy, after surimi production TBA value reached to 2.015 mg/kg at the end of 150 days of storage (–18°C for 9 months) (Kaba, 2006). For TBA criterion by itself, effect of HHP could be determined on shelf life (Day 9) when compared with refrigeration (Day 5) or production of patties (Day 3).

Table 3.7 The effect of HHP on pH, TVB-N, TMA-N and TBA of anchovy flesh during refrigerated storage [1,2,3]

Storage days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
pH	**Control**	$6,67^{Aa}$±0,01	$6,62^{Ab}$±0,01	$6,94^{Ac}$±0,02	$7,16^{Ad}$±0,02	$7,47^{Ae}$±0,03	$7,60^{Af}$±0,05	$7,55^{Ag}$±0,02	$7,44^{Ag}$±0,01	$7,45^{Ag}$±0,02
	200 MPa/5C/5min	$6,82^{Ba}$±0,05	$6,69^{Bb}$±0,01	$6,91^{Bc}$±0,01	$7,03^{Bd}$±0,01	$7,00^{Be}$±0,01	$7,00^{Bf}$±0,01	$7,14^{Bg}$±0,01	$7,29^{Bg}$±0,01	$7,34^{Bg}$±0,02
TVB-N (mg/100 g)	**Control**	$14,80^{Aa}$±1,04	$18,48^{Aa}$±0,00	$21,43^{Aa}$±1,52	$21,92^{Aa}$±0,21	$22,51^{Aa}$±0,23	$74,41^{Ab}$±0,50	$86,07^{Ac}$±1,22	$94,17^{Ad}$±1,05	$139,60^{Ae}$±7,01
	200 MPa/5C/5min	$12,61^{Ba}$±0,37	$11,85^{Ba}$±0,75	$13,34^{Ba}$±0,88	$14,42^{Ba}$±0,35	$15,06^{Ba}$±0,21	$16,22^{Bb}$±1,17	$19,98^{Bc}$±1,73	$27,39^{Bd}$±4,88	$36,84^{Be}$±1,41
TMA-N (mg/100 g)	**Control**	$3,65^{Aa}$±0,38	$3,27^{Aa}$±0,03	$11,28^{Ab}$±1,27	$19,79^{Ab}$±1,62	$23,40^{Ab}$±0,55	$29,34^{Ab}$±0,28	$32,47^{Ab}$±0,73	$82,43^{Ac}$±7,49	$96,72^{Ad}$±5,05
	200 MPa/5C/5min	$2,17^{Ba}$±0,10	$4,79^{Ba}$±0,69	$7,10^{Bb}$±0,55	$7,05^{Bb}$±0,49	$6,81^{Bb}$±0,54	$8,41^{Bb}$±0,46	$8,32^{Bb}$±1,15	$10,70^{Bc}$±1,26	$12,66^{Bd}$±1,71
TBA (mg MDA/kg)	**Control**	$1,19^{Aa}$±0,17	$3,40^{Aa}$±0,13	$3,24^{Ab}$±0,08	$5,35^{Ac}$±0,11	$6,51^{Ac}$±0,08	$7,39^{Ac}$±0,32	$8,25^{Ac}$±1,38	$8,11^{Ac}$±0,76	$11,06^{Ad}$±0,67
	200 MPa/5C/5min	$1,31^{Ba}$±0,01	$2,89^{Ba}$±0,30	$2,84^{Bb}$±0,27	$2,94^{Bc}$±0,52	$2,85^{Bc}$±0,46	$3,83^{Bc}$±0,62	$3,96^{Bc}$±0,34	$5,06^{Bc}$±0,35	$5,09^{Bd}$±0,06

1. Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All values are the mean ± standard deviation (n=3).

3. Control: Unpressurized.

4. The acceptable limits were selected as pH 7.00; 35 mg TVB-N/100 g; >5 mg TMA-N; >3 mg MDA/kg for fish samples.

3.5.5. Total mesophilic aerobic count results of haddock

As pressure applied was increased from 200 MPa 5°C 5min to 400 MPa 15°C 5min; significant ($p<0.05$) decrease in initial microbial load was shown in Table 3.8. The limit of acceptability was stated as 10^6 cfu/g for marine species and as a rejection criterion for fish consumption (Lapa-Guimaraes, Aparecida Azavedo Da Silva, Eduardo De Felicio, Contreras Guzman, 2002). Fish samples, which have lower than 3 log cfu/g, TMAC were classified as "very good fish quality" in literature (Erkan, 2007). According to this limitation, control samples were acceptable up to 5 days and 200 MPa 5°C 5 min treated samples were classified as "very good quality" up to 13 and 400 MPa 15°C 5 min treated samples were still marketable for 15 days; respectively. In the literature, when haddock samples were stored at ambient temperature the highest counts changed between 8.78 and 9.46 log (cfu/g) (Köse & Erdem, 2001). At 3th day of storage; whiting fillets were rejected because of their highest results of mesophilic bacterial counts (8.60 log (cfu/g)) for refrigerated (4°C± 1) of samples on the 5th day of storage time. The samples stored at ambient temperature (25°C± 1) were not analyzed for pyschrophilic bacterial counts after the 3rd day of storage because of a highly sensory spoilage rate observed (Köse & Erdem, 2001). HHP extended the rejection day of microbiological criterion up to 13-15 days from 3 days when compared with storage at refrigeration conditions.

3.5.6. Sensory results (Appearance and Odour) of haddock

Sensory results are given in Table 3.8. Untreated haddock samples were at good quality up to day 7 in appearance. Among pressurized samples, haddock was rejected after 13 and 11 days when treated at 200 MPa 5°C 5 min and 400 MPa 15°C 5min, respectively. In odour side of view, control samples were rejected after 7 days; however haddock samples were still at good quality after 9 and 11 days when pressurized with 200 MPa 5°C 5min and 400 MPa 15°C 5min

combinations. When results were examined statistically, for each day, all haddock samples were influenced by pressure parameter significantly ($p<0.05$) after 3 days of storage. After three days and on, all pressurized samples had higher scores from panelists in respect of appearance and odour. For each HHP application and untreated samples, all values decreased in course of time significantly ($p<0.05$). This situation can be explained as natural deterioration of fresh haddock appearance and odour day by day detected by panelists, therefore time also was a significant sensory parameter ($p<0.05$) during shelf life of 15 days. In literature, haddock samples packed with modified atmosphere (60:20:20/$C0_2$:0_2:N_2) stored at 0°C, had 3 days of shelf life extension compared with control samples, based on sensory scores (Dhananjaya & Stroud, 1994). In another study, haddock samples stored at ambient (25°C± 1) and refrigeration (4°C± 1) temperatures were rejected at day 2 and at day 3 on the 5 days of storage time according to their sensory scores which were; very good, 5: good, 4: acceptable, 3: poor, 2: very poor, 1. of 2 (Köse & Erdem, 2001). In another study, three types of fried samples which are plain mince, surimi and pre-cooked whiting balls, gained 9, 10 and 11 days of refrigerated (at 4 ºC) shelf life of 15 days (Boran & Köse, 2007).

Table 3.8 The effect of HHP on sensory evaluation of appearance, odour and TMAC of haddock flesh during refrigerated storage [1,2,3,4]

Storage Days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
Appearance	**Control**	$10,00^{Aa}\pm0,00$	$9,90^{Aa}\pm0,07$	$8,60^{Ab}\pm0,42$	$5,50^{Ac}\pm1,00$	$4,30^{Ad}\pm0,84$	$2,80^{Ae}\pm0,57$	$1,40^{Af}\pm0,55$	$0,20^{Ag}\pm0,45$	$0,00^{Ah}\pm0,00$
	200 MPa 5°C 5min	$9,88^{Ba}\pm0,18$	$9,72^{Ba}\pm0,28$	$8,50^{Bb}\pm0,50$	$7,10^{Bc}\pm0,74$	$6,40^{Bd}\pm0,42$	$5,70^{Be}\pm0,57$	$4,10^{Bf}\pm0,55$	$1,30^{Bg}\pm0,84$	$0,00^{Bh}\pm0,00$
	400 MPa 15°C 5min	$9,38^{Ca}\pm0,41$	$9,12^{Ca}\pm0,18$	$9,00^{Cb}\pm0,35$	$7,72^{Cc}\pm0,26$	$5,00^{Cd}\pm0,94$	$4,20^{Ce}\pm0,91$	$3,60^{Cf}\pm0,42$	$1,80^{Cg}\pm0,84$	$0,00^{Ch}\pm0,00$
Odour	**Control**	$10,00^{Aa}\pm0,00$	$9,84^{Aa}\pm0,05$	$6,70^{Ab}\pm0,45$	$4,90^{Ac}\pm0,74$	$3,20^{Ad}\pm0,76$	$1,90^{Ae}\pm0,55$	$0,50^{Af}\pm0,50$	$0,00^{Ag}\pm0,00$	$0,00^{Ag}\pm0,00$
	200 MPa 5°C 5min	$9,66^{Ba}\pm0,42$	$9,42^{Ba}\pm0,41$	$7,60^{Bb}\pm0,96$	$6,60^{Bc}\pm0,82$	$4,30^{Bd}\pm0,84$	$3,10^{Be}\pm0,74$	$1,20^{Bf}\pm0,84$	$0,70^{Bg}\pm0,67$	$0,00^{Bg}\pm0,00$
	400 MPa 15°C 5min	$9,94^{Ca}\pm0,09$	$9,84^{Ca}\pm0,21$	$8,00^{Cb}\pm0,79$	$7,30^{Cc}\pm1,04$	$6,80^{Cd}\pm0,76$	$5,50^{Ce}\pm0,71$	$4,40^{Cf}\pm0,42$	$0,90^{Cg}\pm0,89$	$0,00^{Cg}\pm0,00$
TMAC (log cfu/g)	**Control**	$2,39^{Aa}\pm0,07$	$2,53^{Aa}\pm0,07$	$2,82^{Aa}\pm0,04$	$2,59^{Ab}\pm0,21$	$3,31^{Ac}\pm0,13$	$3,73^{Ad}\pm0,03$	$3,81^{Ad}\pm0,04$	$3,90^{Ae}\pm0,23$	$4,71^{Af}\pm0,04$
	200 MPa 5°C 5min	$1,64^{Ba}\pm0,05$	$1,34^{Ba}\pm0,04$	$1,41^{Ba}\pm0,03$	$1,67^{Bb}\pm0,10$	$2,22^{Bc}\pm0,04$	$2,55^{Bd}\pm0,10$	$2,79^{Bd}\pm0,20$	$3,18^{Be}\pm0,24$	$4,19^{Bf}\pm0,05$
	400 MPa 15°C 5min	$0,20^{Ca}\pm0,17$	$0,20^{Ca}\pm0,17$	$0,20^{Ca}\pm0,17$	$1,09^{Cb}\pm0,08$	$0,73^{Cc}\pm0,23$	$0,90^{Cd}\pm0,30$	$1,14^{Cd}\pm0,06$	$1,47^{Ce}\pm0,06$	$2,72^{Cf}\pm0,10$

1. Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).
2. All color values are the mean ± standard deviation of nine replicates (n=9).
3. Control: Unpressurised.
4. For sensory analysis (for both appearance and odour) <4.9 was noted as spoiled by panelists. For marine species < 3 log cfu/g and <6 log cfu/g were selected as “lost good quality” and rejection criteria, respectively.

3.5.7. Color results (L*, a*, b*, ΔE) of haddock

L*, a*, b* and ΔE values of haddock samples were shown in Table 3.9. For each day of storage, all color attributes changed significantly ($p<0.05$) with HHP combinations. 200 MPa 5°C 5 min and 400 MPa 15°C 5 min treated samples had L* values of 26.56±0.26 and 25.89±0.22, respectively at Day 9 of storage; since L* value of control samples was 25.30±3.85 at Day 0. In other words, when samples were treated with both of these combination and reached to Day 9, their appearances were still similar to initial fresh haddock samples according to L* values. Change in a* and b* values was remittent within days and within HHP combinations. ΔE values of 200 MPa 5°C 5 min treated samples were higher, since the same values of 400 MPa 15°C 5 min treated haddock were lower than control samples. HHP treated haddock samples reached to rejection limit at day 9 as total color difference approached to fresh haddock appearance. As visual assessment, especially color is very important in order to meet consumer expectations, HHP treatment improved and conserved fresh haddock appearance during the shelf life period studied. Increased L* value, decreased a* value at 150 MPa, 1–5 °C, 60 min and at 200 MPa, 1–5 °C, 10 min have been reported for salmon (Amanatidou, Schuluter, Lamkau, Gorris, Smid & Knorr, 2000). In another study, minimum ΔE values were found in the following HHP condition: 220 MPa, 7–15 °C for 5 min, 220 MPa, 15–25 °C for 10 min, 250 MPa, 25 °C for 10 min, 250 MPa, 7 °C for 5 min for horse mackerel (Erkan, Üretener, Alpas, Selçuk, Özden & Buzrul, 2010).

Table 3.9 The effect of HHP on L*, a*, b* and ΔE of haddock flesh during refrigerated storage [1,2,3]

Storage Days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
L*	**Control**	$25,30^{Aa}\pm3,85$	$30,77^{Ab}\pm0,57$	$24,56^{Ac}\pm0,13$	$28,14^{Ad}\pm0,36$	$25,93^{Ae}\pm0,27$	$29,20^{Ae}\pm0,32$	$30,56^{Af}\pm0,28$	$30,37^{Af}\pm0,55$	$27,29^{Ag}\pm0,15$
	200 MPa 5°C 5min	$32,08^{Ba}\pm0,15$	$32,24^{Bb}\pm0,27$	$27,11^{Bc}\pm0,09$	$28,30^{Bd}\pm0,27$	$36,38^{Be}\pm0,17$	$26,56^{Be}\pm0,26$	$33,57^{Bf}\pm0,15$	$35,01^{Bf}\pm0,27$	$27,36^{Bg}\pm0,25$
	400 MPa 15°C 5min	$19,61^{Ca}\pm2,27$	$20,37^{Cb}\pm0,49$	$24,62^{Cc}\pm0,31$	$32,21^{Cd}\pm0,42$	$21,19^{Ce}\pm0,25$	$25,89^{Ce}\pm0,22$	$26,68^{Cf}\pm0,29$	$24,71^{Cf}\pm0,42$	$27,74^{Cg}\pm0,42$
a*	**Control**	$(-1,06)^{Aa}\pm1,16$	$3,13^{Ab}\pm0,09$	$(-1,82)^{Ac}\pm0,08$	$3,12^{Ad}\pm0,04$	$(-0,2)^{Ae}\pm0,05$	$(-0,63)^{Af}\pm0,05$	$(-5,31)^{Af}\pm0,09$	$2,06^{Ag}\pm0,10$	$2,72^{Ah}\pm0,08$
	200 MPa 5°C 5min	$2,49^{Ba}\pm0,13$	$2,49^{Bb}\pm0,13$	$1,78^{Bc}\pm0,10$	$1,94^{Bd}\pm0,07$	$1,80^{Be}\pm0,05$	$1,03^{Bf}\pm0,11$	$3,81^{Bf}\pm0,08$	$4,73^{Bg}\pm0,05$	$2,67^{Bh}\pm0,09$
	400 MPa 15°C 5min	$0,48^{Ca}\pm2,19$	$(-0,02)^{Cb}\pm0,54$	$2,21^{Cc}\pm0,08$	$2,54^{Cd}\pm0,07$	$0,27^{Ce}\pm0,07$	$(-0,2)^{Cf}\pm0,05$	$0,94^{Cf}\pm0,07$	$0,39^{Cg}\pm0,03$	$3,39^{Ch}\pm0,11$
b*	**Control**	$1,50^{Aa}\pm2,29$	$8,11^{Ab}\pm0,23$	$0,72^{Ac}\pm0,10$	$3,64^{Ad}\pm0,18$	$1,11^{Ae}\pm0,14$	$1,53^{Ae}\pm0,05$	$(-2,57)^{Ae}\pm0,09$	$3,27^{Af}\pm0,09$	$0,90^{Ag}\pm0,09$
	200 MPa 5°C 5min	$6,68^{Ba}\pm0,08$	$6,68^{Bb}\pm0,08$	$5,51^{Bc}\pm0,08$	$5,32^{Bd}\pm0,11$	$6,80^{Be}\pm0,13$	$3,03^{Be}\pm0,11$	$6,58^{Be}\pm0,10$	$10,37^{Bf}\pm0,11$	$0,84^{Bg}\pm0,09$
	400 MPa 15°C 5min	$0,84^{Ca}\pm1,08$	$(-0,69)^{Cb}\pm1,87$	$2,44^{Cc}\pm0,11$	$6,68^{Cd}\pm0,04$	$(-1,02)^{Ce}\pm0,14$	$1,02^{Ce}\pm0,18$	$(-2,92)^{Ce}\pm0,07$	$2,29^{Cf}\pm0,13$	$4,57^{Cg}\pm0,19$
ΔE	**Control**	$25,46^{Aa}\pm3,91$	$31,96^{Ab}\pm0,51$	$24,63^{Ac}\pm0,14$	$28,54^{Ad}\pm0,36$	$25,97^{Ae}\pm0,24$	$29,26^{Ae}\pm0,33$	$31,10^{Af}\pm0,29$	$30,59^{Af}\pm0,54$	$27,42^{Ag}\pm0,17$
	200 MPa 5°C 5min	$32,86^{Ba}\pm0,14$	$33,02^{Bb}\pm0,28$	$27,76^{Bc}\pm0,11$	$28,87^{Bd}\pm0,25$	$37,06^{Be}\pm0,13$	$26,74^{Be}\pm0,26$	$34,41^{Bf}\pm0,15$	$36,82^{Bf}\pm0,22$	$27,56^{Bg}\pm0,19$
	400 MPa 15°C 5min	$19,77^{Ca}\pm2,19$	$20,46^{Cb}\pm0,49$	$24,82^{Cc}\pm0,27$	$33,00^{Cd}\pm0,41$	$21,22^{Ce}\pm0,26$	$25,91^{Ce}\pm0,24$	$26,84^{Cf}\pm0,26$	$24,83^{Cf}\pm0,42$	$28,33^{Cg}\pm0,37$

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All color values are the mean ± standard deviation of nine replicates (n=9).

3 Control: Unpressurised

3.5.8. Results of chemical analysis (pH, TVB-N, TMA-N, TBA) for haddock

pH values of untreated, 200 MPa 5°C 5min and 400 MPa 15°C 5min treated haddock samples were shown in Table 3.10. The acceptable limit of pH for fish samples was stated as 7.00 in literature (Cadun, Kılınç, Şen, & Çaklı, 2008). With respect to this limitation, control samples were rejected by Day 3; unless 200 MPa 5°C 5min and samples were still in the acceptable range up to 11 and 400 MPa 15°C 5 min treated samples exceeded pH 7 limitations at the 15th day. For each day, pH values of HHP treated samples increased slightly and significantly ($p<0.05$) compared with control samples. pH values of 200 MPa 5°C 5 min and 400 MPa 15°C 5 min treated samples were increased due to control samples significantly ($p<0.05$), also storage day was a significant parameter ($p<0.05$) on pH. In literature, with denaturation of protein fractions, pH values of cod muscles increased when pressurized samples with 400 and 800 MPa similar to haddock results (Köse & Erdem, 2001). When haddock samples stored at ambient temperature the lowest and highest pH values were 6.24 and 7.16; and when they stored in the refrigerator, pHs were between 6.14 and 7.22, respectively(Köse & Erdem, 2001). Samples rejected at Day 3 and Day 5, when stored at ambient (25°C± 1) and at refrigeration (4°C± 1) temperatures, respectively (Köse & Erdem, 2001). In 7 days of storage at 4 °C, cod muscles were rejected at Day 7 whereas pressurized samples were still marketable (Angsupanich & Ledward, 1998).

Up to 25 mg/100 g TVB-N content is considered "very good," up to 30 mg/100 g TVB-N content is "good", up to 35 mg/100 g TVB-N "marketable," over 35 mg/100 g TVB-N content is "spoiled (Erkan & Üretener, 2010), (Commission decision 95/ 149/EEC). Based on this criterion, control samples were rejected at day 5, whereas both 200 MPa-5°C-5 min and 400 MPa-15°C-5 min pressure treated samples were still specified as “marketable” at the end of

storage as shown in Table 3.10 . It can be concluded that inhibition of spoilage bacteria of 400 MPa 15°C 5min pressure combination was more effective than 200 MPa 5°C 5 min treatment sustaining drastic difference on TVB-N content development in each storage day. All values were effected significantly ($p<0.05$) depending on storage days and pressure treatments. In literature, haddock samples were "spoiled" at Day 3 when stored at ambient (25°C± 1) temperatures and also rejected at Day 5 when stored at refrigeration conditions (4°C± 1) (Köse & Erdem, 2001). In another study, both control and MAP with CO_2 haddock samples exceeded 35 mg/100g rejection limit within 2- 4 days when stored at 5 and 10 °C. Control and MAP samples rejected at 4 and 6 days, respectively when stored at 0°C (Dhananjaya & Stroud, 1994). Whole sardines stored in ice were rejected at Day 5 since gutted sardines were still marketable at Day 9 (Erkan & Özden, 2008). Otherwise for whiting balls, it was stated that TVB-N contents of the fried balls containing plain mince ranged from 8.13 to 32.21 mg N/100 g, 9.54 to 26.53 mg N/100 g for surimi, and 4.24 to 28.02 mg N/100 g for pre-cooked mince during the 15-day period of storage at 4°C. The values remained within acceptable limits throughout the storage period (Boran & Köse, 2007).

TMA-N values of untreated, 200 MPa 5°C 5 min and 400 MPa 15°C 5 min treated samples were stated in Table 3.10. Pressure was a significant factor on TMA-N content of haddock when compared to untreated samples ($p<0.05$). In literature, it was stated that while selecting the limitation of TMA-N development in fish samples, fish species should also be considered (Serdaroğlu & Deniz, 2001). For cod and haddock muscle, TMA-N limitations and fish quality specification were identified as; 0–1 TMA-N mg/100 g is classified as "1st class-good quality", 1–5 TMA-N mg/100 g is "2nd class – Marketable", and >5 TMA-N mg/100 g is "3rd class – Not proper for fresh consumption or manufacturing" (Serdaroğlu & Deniz, 2001). Control samples were good quality because of their initial freshness at the beginning of storage, marketable up to Day 3 but rejected

at Day 5. When haddock pressurized at 200 MPa 5°C 5min, samples were at good quality as untreated samples initially; they were still marketable during 15 days. 400 MPa 15°C 5min treated samples were first class haddocks at the end of day 5, and were still consumable at Day 15 according to limitations. Each day, all TMA-N values of 200 MPa-5°C-5 min and 400 MPa-15°C-5 min pressurized samples increased slightly and significantly ($p<0.05$) compared with control samples. Also TMA-N values increased significantly ($p<0.05$) depending on storage days. The slow increase in TMA-N during the shelf life of sea bream was stated for 3 °C 5 min 250 MPa and 15 °C 5 min 250 MPa pressurized gilthead sea bream samples when stored at 48 °C for 19 days. In literature, haddock samples were studied as whole fish and fillet for comparison of TMA-N analysis in the findings of Fernandez-Salguero and Mackie (1987) when stored in ice and at 5°C. Whole fish samples rejected up to limitations at Day 10 (6.87 mg/100 g) when stored in ice and rejected at Day 3 (5.45 mg/100 g) when stored at 5°C. While samples analyzed as fillets, samples stored in ice rejected at Day 8 (6.58 mg/100 g) and at Day 3 (8.69 mg/100 g) at 5°C (Fernandez-Salguero & Mackie, 1987). While samples were analyzed as fillets, samples stored in ice were rejected at day 8 (6.58 mg/100 g) and at day 3 (8.69 mg/100 g) at 5°C (Fernandez-Salguero & Mackie, 1987). In another study, when haddock was stored at ambient (25°C± 1) and refrigeration temperatures (4°C± 1), samples reached to unacceptable limit at day 2 and day 3, respectively (Köse & Erdem, 2001). Boran and Köse (2007) determined that TMA level for whiting ball samples containing plain mince and surimi reached 16.23 and 15.25 mg N/100 g, respectively, on the 10th day. These samples selected as “not consumable” by Day 10. (Boran & Köse, 2007).

In the lipid oxidation point of view; initial TBA values of untreated, 200 MPa 5°C 5 min and 400 MPa 15°C 5 min treated samples were shown in Table 3.10. The impact of higher pressure magnitude was shown as inhibition of TBA value in each day of storage. 3-4 mg MDA/kg was defined as souring, spoiled

and quality loss in fish (Huss 1988; Karaçam & Boran, 1996; Scott, Fletcher & Charles, 1992). At day 9, control samples lost their quality classification and exceeded rejection limit, whereas both 200 MPa 5°C 5 min and 400 MPa 15°C 5 min treated haddock samples were still marketable even at day 15. TBA values changed significantly regarding to control samples ($p<0.05$); however, all values were effected insignificantly ($p>0.05$) from storage period. In literature, haddock samples were spoiled at Day 2 when refrigerated at ambient (25°C± 1) and refrigeration (4°C± 1) temperatures in terms of TBA value (Köse & Erdem, 2001). Similarly, for haddock balls, initial level of 0.19 mg TBA/kg was reached to still marketable specification (1.89 mg TBA/kg) at the end of 15 days of refrigeration storage (Boran & Köse, 2007).

Results of shelf life analysis for both anchovy and haddock were summarized in Table 3.11 and Table 3.12, respectively.

Table 3.10 The effect of HHP on pH, TVB-N, TMA-N and TBA of haddock flesh during refrigerated storage [1,2,3]

Storage Days		DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15
pH	**Control**	6,99^{Aa}±0,01	6,97^{Ab}±0,01	7,04^{Ab}±0,01	7,06^{Ac}±0,01	7,05^{Ac}±0,01	7,09^{Ad}±0,01	7,03^{Ae}±0,00	7,15^{Af}±0,01	7,19^{Ag}±0,01
	200 MPa 5°C 5min	7,01^{Ba}±0,01	6,99^{Bb}±0,01	6,99^{Bb}±0,01	6,99^{Bc}±0,01	7,00^{Bc}±0,01	7,00^{Bd}±0,01	7,13^{Be}±0,01	7,09^{Bf}±0,01	7,14^{Bg}±0,02
	400 MPa 15°C 5min	7,08^{Ba}±0,01	7,03^{Bb}±0,01	6,99^{Bb}±0,01	7,02^{Bc}±0,01	7,01^{Bc}±0,00	7,02^{Bd}±0,01	7,03^{Be}±0,01	7,02^{Bf}±0,01	7,13^{Bg}±0,01
TVB-N (mg/100g)	**Control**	10,55^{Aa}±1,23	13,70^{Aa}±1,27	25,48^{Ab}±1,58	38,16^{Ac}±0,77	40,00^{Ac}±1,75	46,70^{Ad}±2,93	56,39^{Ae}±4,30	53,36^{Ae}±1,91	63,26^{Af}±4,61
	200 MPa 5°C 5min	9,88^{Ba}±0,17	9,64^{Ba}±1,28	14,68^{Bb}±0,48	16,65^{Bc}±0,33	16,33^{Bc}±1,12	27,38^{Bd}±0,57	29,26^{Be}±2,21	25,02^{Be}±3,90	29,64^{Bf}±1,21
	400 MPa 15°C 5min	7,70^{Ca}±0,17	8,55^{Ca}±0,61	7,70^{Cb}±0,30	7,20^{Cc}±1,53	8,57^{Cc}±0,60	9,38^{Cd}±1,13	11,33^{Ce}±1,24	11,83^{Ce}±0,32	12,10^{Cf}±1,12
TMA-N (mg/100g)	**Control**	0,74^{Aa}±0,08	3,14^{Ab}±0,03	3,34^{Ab}±0,21	5,65^{Ac}±0,99	6,01^{Ac}±1,11	6,25^{Ac}±0,33	6,37^{Ac}±0,34	6,45^{Ac}±0,45	8,56^{Ad}±0,23
	200 MPa 5°C 5min	1,08^{Ba}±0,17	1,33^{Bb}±0,03	1,67^{Bb}±0,20	1,68^{Bc}±0,16	1,69^{Bc}±0,29	2,32^{Bc}±0,45	2,49^{Bc}±0,33	2,94^{Bc}±0,38	3,37^{Bd}±0,02
	400 MPa 15°C 5min	0,81^{Ca}±0,05	0,83^{Cb}±0,02	0,95^{Cb}±0,06	0,93^{Cc}±0,24	1,10^{Cc}±0,16	1,23^{Cc}±0,10	1,44^{Cc}±0,09	1,68^{Cc}±0,08	3,17^{Cd}±0,11
TBA (mg MDA/kg)	**Control**	0,78^{Aa}±0,25	1,81^{Aa}±0,71	2,42^{Aa}±1,27	2,67^{Aa}±1,00	2,80^{Aa}±0,87	3,31^{Aa}±0,68	3,37^{Aa}±0,78	3,56^{Aa}±0,26	3,85^{Aa}±0,75
	200 MPa 5°C 5min	0,33^{Ba}±0,07	0,32^{Ba}±0,04	0,53^{Ba}±0,15	0,50^{Ba}±0,17	0,64^{Ba}±0,11	0,77^{Ba}±0,05	1,07^{Ba}±0,12	0,89^{Ba}±0,16	1,18^{Ba}±0,18
	400 MPa 15°C 5min	0,36^{Ba}±0,02	0,37^{Ba}±0,17	0,39^{Ba}±0,03	0,44^{Ba}±0,15	0,45^{Ba}±0,04	0,47^{Ba}±0,10	0,53^{Ba}±0,07	0,67^{Ba}±0,06	0,73^{Ba}±0,01

1 Different letters (A, B, C) in the same column indicate significant differences ($p<0.05$). Different letters (a, b, c) in the same line indicate significant differences ($p<0.05$).

2 All values are the mean ± standard deviation (n=3).

3. Control: Unpressurized.

4 The acceptable limits were selected as pH 7.00; 35 mg TVB-N/100 g; >5 mg TMA-N; >3 mg MDA/kg for fish samples.

Table 3.11. Rejection days of shelf life analysis for untreated and HHP treated anchovy[1]

Quality Attributes	Control	200 MPa, 5°C for 5min
Microbiological Analysis	Day 7	Day 9
Appearance	Day 7	Day 11
Odour	Day 7	Day 7
pH	Day 5	Day 11
TVB-N	Day 9	Day 15
TMA-N	Day 3	Day 3
TBA	Day 1	Day 9

1 “Days” are the days of rejected samples during shelf life according to rejection limits mentioned before. “Still Consumable” indicates that samples could still be consumable and did not reach to rejection limit.

Table 3.12. Rejection days of shelf life analysis for untreated and HHP treated haddock[1]

Quality Attributes	Control	200 MPa, 5°C for 5min	400 MPa, 15°C for 5min
Microbiological Analysis	Day 7	Day 13	Still Consumable
Appearance	Day 7	Day 11	Day 9
Odour	Day 7	Day 9	Day11
pH	Day 3	Day 11	Day 15
TVB-N	Day 5	Still Consumable	Still Consumable
TMA-N	Day 5	Still Consumable	Still Consumable
TBA	Day 9	Still Consumable	Still Consumable

1 "Days" are the days of rejected samples during shelf life according to rejection limits mentioned before. "Still Consumable" indicates that samples could still be consumable and did not reach to rejection limit.

CHAPTER 4

CONCLUSION

According to the trials' results (different combinations of 200, 300 and 400 MPa, at 5, 10 and 15 °C for 5 and 15 min) that were performed in the first stage of experiments, color (L*, a*, b* and ΔE), TMA-N and TBA analysis were applied to each samples. For anchovy, L* value decreased, a* value decreased, b* value increased and ΔE decreased when pressure magnitude, temperature and holding time increased. Lowest TMA-N results were obtained with 200 MPa-5°C-5min, 200 MPa-15°C-15min, 300 MPa-10°C-5min, and 300 MPa-15°C-5min treatments. Additionally lowest TBA results were gained with 200, 300 and 400 MPa, 5 °C for 5 min treated samples. Based on these results optimal HHP combination was chosen as 200 MPa at 5 °C for 5 min which is common for all analysis. For haddock, L* value decreased, a*, b* and ΔE values increased with rise of pressure magnitudes, time and temperature. Lowest TMA-N values were measured for 200 MPa, 5°C for 5min, 400 MPa, 15°C for 5min and 200 MPa, 15°C for 15min treated samples. Lowest TBA was determined at 400 MPa 10 ºC for 5 min, 300 MPa 10 ºC for 5 min, and 200 MPa 5ºC for 15 min combinations. Both 200 MPa at 5 °C for 5 min combination with 400 MPa at 15 °C for 5 min treatments were selected for shelf life studies for haddock samples. These selections were substantiated by choosing the lowest color, TMA-N and TBA values in reference to control samples in order to get fresh fish appearance and good quality attributes.

As a summary, according to color values both anchovy and haddock samples changed into light cooked appearance then became darker during storage days with respect to control samples. Considering anchovy microbiological results, control samples lost their good quality at Day 7 significantly ($p<0.05$); but HHP treated samples were very good quality till Day 9 and were still consumable at Day 15. According to panelists' notations, control samples were rejected at Day 7 and Day 7 for odour and appearance attributes, respectively. However; HHP treated samples were acceptable up to Day 7 for odour, Day 11 for appearance. In view of color measurement, L*, and ΔE values decreased for both untreated and treated anchovy, since a* and b* values fluctuated with increase of pressure, time and temperature. For pH values, control samples reached to rejection limit at Day 5, yet 200 MPa, 5°C for 5min pressurized samples were not acceptable at Day 11. According to TVB-N results of control and 200 MPa, 5°C for 5min treated anchovy samples, samples were lost their good quality at Day 9 and Day 15, respectively. Control samples were rejected at Day 3; and 200 MPa, 5°C for 5min treated samples lost their good quality at Day 3, then rejected at Day 3 due to their TMA-N acceleration. In TBA point of view; anchovy was lost their good quality classification at Day 1 when samples were untreated and at Day 9 when samples were applied to 200 MPa, 5°C for 5min. When results of sensory, physical and chemical analysis were blended together; pressurized anchovy samples were rejected within 9 to 11 days while untreated samples were spoiled within 1-3 days. Within the scope of commercialization, the shelf life extension was evaluated as 9 days for pressurized anchovies (200 MPa 5 °C for 5 min) when stored at 4 °C.

As discussed for haddock species, according to color values it was demonstrated that as pressure magnitude and compression time increased to 400 MPa at 15 °C for 5 min (second P-T-t combination) from 200 MPa at 5 °C for 5 min (first P-T-t combination); cooked and brighter appearance occurred in haddock samples. HHP treated haddock samples could be considered as rejected

at day 9 as total color difference approached to fresh haddock appearance at Day 9. But individually, control, 200 MPa at 5 °C for 5 min and 400 MPa at 15 °C for 5 min treated samples were exceeded acceptable level significantly at Day 7, Day 11 and Day 9, respectively with their appearances. Also similarly, at Day 7 control samples, at Day 9 first P-T-t combination, at Day 11 second P-T-t combination treated samples were decreased to rejection limit with their odour, significantly ($p<0.05$). Microbiologically, control samples were acceptable up to Day 7, 200 MPa at 5 °C for 5 min treated samples were lost their quality at Day 13 significantly ($p<0.05$); but 400 MPa at 15 °C for 5 min treated haddock was still tradable at Day 15. pH values of both control and pressurized samples were increased significantly ($p<0.05$) with pressure and storage days. pH of control samples were exceeded good quality limit at Day 3; however at Day 11 first P-T-t combination, at Day 15 second P-T-t combination treated samples were reached to this limitation significantly ($p<0.05$). For TVB-N levels of haddock during storage, all values were increased significantly ($p<0.05$) depending on pressure and storage days. Control samples lost good quality at Day 3 and rejected at Day 5. However, both 200 MPa at 5 °C for 5 min and 400 MPa at 15 °C for 5 treated samples were classified as still marketable and still good quality at Day 15. According to TMA-N contents, control samples were at good quality at Day 3 but rejected at Day 9. When treated with 200 MPa at 5 °C for 5 min, and 400 MPa 15 °C for 5 min; samples were still within good quality limitation significantly ($p<0.05$) at Day 15. TBA values of control samples were exceeded good quality at Day 9. But all values were changed significantly ($p<0.05$) with pressure. At Day 15 first P-T-t combination was also exceeded good quality; yet at Day 15 second P-T-t combination treated samples were still tradable and classified as good quality. When all conclusions were combined, haddock samples were rejected because of their spoilage within few days (3 to 5 days); but 200 MPa at 5 °C for 5 min combination treated samples were rejected at Day 13. However; when samples pressurized with 400 MPa at 15 °C for 5 min combination shelf

life extension of haddock was extended to 15 days of storage. When combined these two treatments, averagely for haddock samples HHP treatment created commercial shelf life extension between 11 to 15 days at refrigeration conditions (4 °C).

CHAPTER 5

RECOMMENDATION

HHP application has distinctive effect on retardation of development of quality attributes such as TMA, TBA and TVB-N which give vent to spoilage of fish products. In order to reinforce the fresh fish, HHP had shown initial reduction on all quality attributes regarding to untreated samples. These effects were adherence to fish species, pressure magnitude, compression time and HHP treatment temperature. With these optimal combinations both anchovy and haddock were remained as fresh; both species were obtained extended shelf life. In order to extend much more shelf life; during sample preparation we could be more careful within the scope of sterile and sanitary working conditions. Consequently, HHP technology should be utilized and adapted to manufacturing processes. Also HHP technology can be combined with other technologies, this hurdle concept could improve quality assessment of fish products; so that HHP technique can be an alternative to any other commonly used processes such as chilling or freezing in the markets.

REFERENCES

Akşiray, F. (1987). *Türkiye deniz balıkları ve tayin anahtarları* (1 b.). İstanbul: İ.Ü. Yayınları.

Amanatidou, A., Schluter, O., Lamkau, K., Gorris, L. G., Smid, E. J., & Knorr, D. (2000). Effect of combined application of high pressure treatment and modified atmosphereson the shelf life of fresh Athlantic salmon. *Innovat Food Sci Emerg Tech , 12*, 87-98.

Angsupanich, K., & Ledward, D. (1998). High pressure treatment effects on cod (Gadus morhua) muscle. *Food Chem , 63*, 39-50.

Angsupanich, K., Edde, M., & Ledward, D, A. (1999). Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. *J Agric. Food Chem. , 47*, 92-99.

Antonocoupoulos, N., & Vyncke, W. (1989). Determination of Volatile Basic Nitrogen in Fish. *Z. Lebensm Unters , 189*, 309-316.

Atasoy, E., Erdem, Ü., Cebeci, M., & Yerli, B. (2006). Marmara Denizi Mezgit (*Merlangius merlangus euxinus* Nordmann, 1840) balığının bazı biyolojik özellikleri. *E.U. Journal of Fisheries & Aquatic Sciences , 23* (1), 33-37.

Bhat, R., Alias, A.K., & Paliyath, G. (2012). *Progress in Food Preservation*, Chichester, West Sussex, UK, John Wiley & Sons Ltd.

Boran, M., & Köse, S. (2007). Storage Properties of Three Types of Fried Whiting Balls at Refrigerated Temperature. *Turkish Journal of Fisheries and Aquatic Sciences , 7*, 65-70.

Botsoglou, N., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff samples. *J. Agric. Food Chem , 42*, 1931-1937.

Bowers, A. B. (1957). Breeding and growth of whiting (*Gagus merlangus L.*) in Isle of Man waters. *J. Mar. Biol. Ass. , 33*, 97-122.

Cadun, A., Kılınç, B., Şen, B., & Çaklı, Ş. (2008). Croquette production from different kinds of frozen and thawed shrimps hunted from different regions and quality changes during freeze storage. *E.U. Journal of Fisheries & Aquatic Sciences , 25*, 191–195.

Carlez, A., Veciananogues, T., & Cheftel, J. (1995). Changes in color and myoglobin of minced beef meat due to high-pressure processing, y, 28 (1995) . *Food Science and Technology , 28*, 528–538.

Chevalier, D., Le Bail, A., & Ghoulb, M. (2001). Effects of high pressure treatment (100–200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. *Food Res. Int. , 34*, 425–429.

Commission decision 95/149/EEC of 8 March 1995 fixing the TVB-N (total volatile basic nitrogen) limit of fishery products and specifying the analytic methods to be used. *Journal of European Communities, L97*, 84–87, 1995.

Çaklı, S., Kılınç, B., Cadun, A., Dinçer, T., & Tolasa, S. (2006). Effects of guttuing and ungutting on microbiological, chemical, and sensory properties of aquacultured seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) stored in ice. *Critical reviews in food science and nutririon , 46,* 519-527.

Çiloğlu, E., Şahin, C., Gözler, A. M., & Verep, B. (2002). Mezgit balığının (*Merlangius merlangus euxinus* Nordmann, 1840) Doğu Karadeniz sahillerinde vertikal dağılımıve toplam av içindeki oranı. *E.U. Journal of Fisheries & Aquatic Sciences , 19* (3-4), 303-309.

Defaye, A.B., Ledward, D.A., MacDougall D.B. & Tester, R.F. (1995). Renaturation of metmyoglobin subjected to high isostatic pressure. *Food Chemistry, 52*, 19-22.

Del Valle, C.E., Filsinger, B.E., Yeannes, M.I. & Soule C.L. (1984). Shelf Life of Brine Refrigerated Anchovies (*Engraulis anchoita*) for Canning. *Journal of Food Science, 49,* 180-182.

Dhananjaya, S., & Stroud, G., D. (1994). Chemical and sensory changes in haddock and herring stored under modified atmosphere. *International Journal of Food Science and Technology, 29,* 575-583.

Earnshaw, R. (1996). High pressure food processing. *Nutrition&Food Science , 2*, 8-11.

Erkan, N. (2007). Freshness and quality of aquacultured sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) stored in ice. *Archives for Food Hygiene , 58*, 98−106.

Erkan, N., & Özden, Ö. (2008). Quality assessment of whole and gutted sardine (*Sardine pilchardus*) stored in ice. *International Journal of Food Science and Technology , 43*, 1549-1559.

Erkan, N., & Üretener, G. (2010). The effect of high hydrostatic pressure on the microbiological, chemical and sensory quality of fresh gilthead sea bream (*Sparus aurata*). *European Food Research and Technology , 230*, 533–542.

Erkan, N., Üretener, G., & Alpas, H. (2010). Effect of high pressure (HP) on the quality and shelf life of red mullet (*Mullus surmelutus*). *Innovative Food Science and Emerging Technologies , 11*, 259-264.

Erkan, N., Üretener, G., Alpas, H., Selçuk, A., Özden, Ö., & Buzrul, S. (2010). Effect of High Hydrostatic Pressure (HHP) Treatment on Physicochemical Properties of Horse Mackerel (*Trachurus trachurus*). *Food Bioprocess Technol , 4* (7), 1322-1329.

Erkan, N., Üretener, G., Alpas, H., Selçuk, A., Özden, Ö., & Buzrul, S. (2010). The effect of Different High Pressure Conditions on the Quality and Shelf life of Cold Smoked Fish. *Innovative Food Science and Emerging Technologies , 12* (2), 104-110.

Erkoyuncu, İ., Erdem, M., Samsun, O., & Özdamar, K. Y. (1994). Karadeniz'de avlanan bazı balık türlerinin et verimi, kimyasal yapısı ve boy-ağırlık ilişkisinin belirlenmesi üzerine bir araştırma. *İstanbul Üniversitesi, Su Ürünleri Dergisi , 8* (1-2), 181-191.

Etienne, M. (2005). Volatile amines as criteria for chemical quality assessment. *Seafood plus-Valid* , 1-22.

Fernandez-Salguero, J., & Mackie, I., M. (1987). Comparative rates of spoilage of fillets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of non-volatile and volatile amines. *International Journal of Food Science and Technology, 22*, 385-390.

Gerdes, D., & Santos Valdez, C. (1991). Modified atmosphere packaging of commercial Pacific red snapper (*Sebastes entomelas, Sebastes flavidus* or *Sebastes godei*). *Food Science and Technology , 24*, 256−258.

Gomez-Estaca, J., Gomez-Guillen, P., & Montero, P. (2007). High pressure effects on the quality and preservation of cold smoked dolphin fish (*Coryphaena hippurus*) fillets. *Food Chem. , 102*, 1250–1259.

He, H., Adams, R.M., Morrissey, M.T. (2001). The Use of High Hydrostatic Pressure to Shuck Oysters and Extend Shelf-life, *J. Food Sci. 67*, 640-45.

Human Metabolome Database, www.hmdb.ca, last visited on May 2012.

Hungerford, J. (1998). AOAC Official Method 971.14 Trimethylamine Nitrogen in Seafood Colorimetric Method. Fish and Other Marine Products. (P. Cunniff, Dü.) *Official Methods of Analysis of AOAC International* , 7.

Hunterlab Application Notes CIE L*a*b* Color Scale, www.hunterlab.com, last visited on May, 2012.

Hurtado, J. L., Montero, P., & Borderias, A. J. (2000). Extension of shelf life of chilled hake (*Merluccius capensis*) by high pressure. *Food Science and Technology International , 6* (3), 243-249.

Huss, H. (1988). Assessment of fish quality, fresh fish quality and quality changes. *FAO Fisheries series , 29*, 61-76.

İnanlı, A. G., Karatan, N., & Çoban, Ö. E. (2011). Sensory, chemical and microbiological quality of anchovy cake. *African Journal of Biotechnology , 10* (48), 9870-9874.

Kaba, N. (2005). The Determination of Technology & Storage Period of Surimi Production from Anchovy (*Engraulis encrasicholus* L., 1758). *Turkish Journal of Fisheries and Aquatic Sciences 6,* 29-35.

Karaçam, H. & Boran, M. (1996). Quality changes in frozen whole and gutted anchovies during storage at –18°C. *International of Journal of Food Science and Technology, 31,* 527-531.

Karatepe, M. (2004). Simulatneous determination os ascorbic acid and free Malondialdehyde in human serum by HPLC-UV. *LCGC Asia Pacific , 7* (2), 1-3.

Köse, S., & Erdem, M. E. (2001). Quality changes of whiting (*Merlangius merlangus euxinus*, N. 1840) stored at ambient and refrigerated temperatures. *Turkish Journal of Fisheries and Aquatic Sciences , 1,* 59-65.

Köse, S., & Erdem, M. E. (2004). An investigation of quality changes in anchovy (*Engraulis encrasicolus*, L. 1758) stored at different temperatures. *Turk. J. Vet. Anim. Sci. , 28,* 575-582.

Lapa-Guimaraes, J., Aparecida Azavedo da Silva, M., Eduardo de Felicio, P., & Contreras Guzman, E. (2002). *Lebensm-Wiss Technol, 35*, 21–29.

Linton, M., McClements, J., & Patterson, M. (2004). Changes in the microbiological quality of vacuum-packaged, minced chicken treated with high hydrostatic pressure. *Innovat Food Sci Emerg Tech. , 5*, 151–159.

Luontoportti Nature Gate, Information System of UNESCO for wild species, www.luontoportti.com, last visited on May 2012.

Ohlsson, T., & Bengtsson, N. (2000). *Minimal processing technologies in the food industry.* Cambrige: Woodhead Publishing Limited.

Ohshima, T., Ushio, H., & Koizumi, C. (1993). High pressure processing of fish and fish products. *Trend Food Science Technology , 4*, 370–375.

Paarup, T., Sanchez, J.A., Pelaez, C & Moral, A. (2002). Sensory, chemical and microbiological changes in vacuum-packaged pressurized squid mantle (*Todaropsis eblanae*) stored at 4 °C, *International Journal of Food Microbiology*, 74, 1–12.

Pérez-Villarreal, B., & Howgate, P. (1987). Spoilage of European hake (*Merluccius merluccius*) in ice. *Journal of the Science of Food and Agriculture , 41*, 335-350.

Pubblicitaitalia Online Data Bank, www.pubblicitaitalia.com, last visited on May 2012

Raharjo, S., & Sofos, J. (1993). Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: a review. *Meat Science , 35*, 145–169.

Ramirez-Suarez, J., & Morrissey, M. (2006). Effect of high pressure processing (HPP) on shelf life of albacore tuna (*Thunnus alalunga*) minced muscle. *Innovative Food Science and Emerging Technologies , 7*, 19-27.

Rastogi, N. K., & Raghavarao K, S. M. (2007). opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition , 47*, 69-112.

Samsun, S., Erdem, M. E., & Samsun, N. (2006). The determination of meal yield and chemical composition of whiting (*Merlangius merlangus euxinus* Nordmann, 1840). *Science and Eng. J. of Fırat Univ. , 18* (2), 165-170.

Scott, D. N., Fletcher, G. C., Charles, J. C. & Wong, R.J. (1992). Spoilage changes in deep water fish, smooth oreo dory during storage in ice. *International Journal of Food Science and Technology*, *27* (5), 577-588.

Seibel, B.A., & Walsh, P.A. (2002). Trimethyleamine oxide accumulation in marine animals: relationship to acylglycerol storage, *The Journal of Experimental Biology, 205*, 297–306.

Sequeira-Munoz, A., Chevalier, D., LeBail, A., H, R., & Simpson, B. (2006). Physicochemical changes induced in carp (*Cyprinus carpio*) fillets by high pressure processing at low temperature. *Innovative Food Science and Emerging Technologies , 7*, 13-18.

Serdaroğlu, M., & Deniz, E.E. (2001). The Factors Affecting Trimetilamine (TMA) and Dimetilamine (DMA) Formation in fish and Some Seafood. *E.U. Journal of Fisheries & Aquatic Sciences , 18* (3-4), 575-581.

Sikorski, Z., Kolakowska, A., & Burt, J. (1990). *Post harvest biochemical and microbial changes seafood.* Boca Raton, USA : CRC Press.

Singh, R., Leuratti, C., Josyula, S., Sipowicz, M., A., Diwan, B., A., Kasprzak, K.S., Schut, H. A. J., Marnett, L.,J., Anderson, L. M., & Shuker, D.E.G. (2001). Lobe-specific increases in malondialdehyde DNA adduct formation in the livers of mice following infection with *Helicobacter hepaticus*, *Carcinogenesis, 22(8)*, 1281-1287.

Sun, D. W. (2005). *Emerging technologies for food processing.* San Diego, California, USA: Elsevier Academic Press.

Tejada, M., & Huidobro, A. (2002). Quality of farmed gilthead seabream (Sparus aurata) during ice storge related to the slaughter method and gutting. *Eur Food Res Technol , 215*, 1-7.

Tokur, B., Korkmaz, K., & Ayas, D. (2006). Comparison of two thiobarbituric acid (TBA) method for monitoring lipid oxidation in fish. *E.U. Journal of Fisheries & Aquatic Sciences , 23* (3-4), 331-334.

Torres, J. A., & Velazquez, G. (2005). Commercial opportunities and research challanges in the high pressure processing of foods. *Journal of Food Engineering, 67*, 95-112.

Turhan, S., Evren, M., & Yazıcı, F. (2001). Shelf-Life of Refrigerated Raw Anchovy (Engraulis encrasicholus) Patties. *E.U. Journal of Fisheries & Aquatic Sciences , 18* (3-4), 391-398.

Turhan, H., Kaya, Y., & Erkoyuncu, İ. (2007). Protein and Lipid content and fatty acid composition of anchovy meal produced in Turkey. *Turk. J. Vet. Anim. Sci. , 31* (2), 113-117.

Venugopal, V. (2006). *Seafood Processing.* Boca Raton, FL, USA: CRC Press, Taylor & Francis Group.

Yağız, Y., Kristinsson, H., Balaban, M., & Marshall, M. (2007). Effect of High Pressure Treatment on the Quality of Rainbow Trout (Oncorhynchus mykiss) and MahiMahi (Coryphaena hippurus). *Journal of Food Science , 72*, 509-515.

Yerlikaya, N., Gökoğlu, N., & Uran, H. (2005). Quality changes of fish patties produced from anchovy during refrigerated storage. *Eur. Food. Res. Technol. , 220,* 287-291.

APPENDIX A

STANDARD CURVE FOR TMA-N CALCULATION

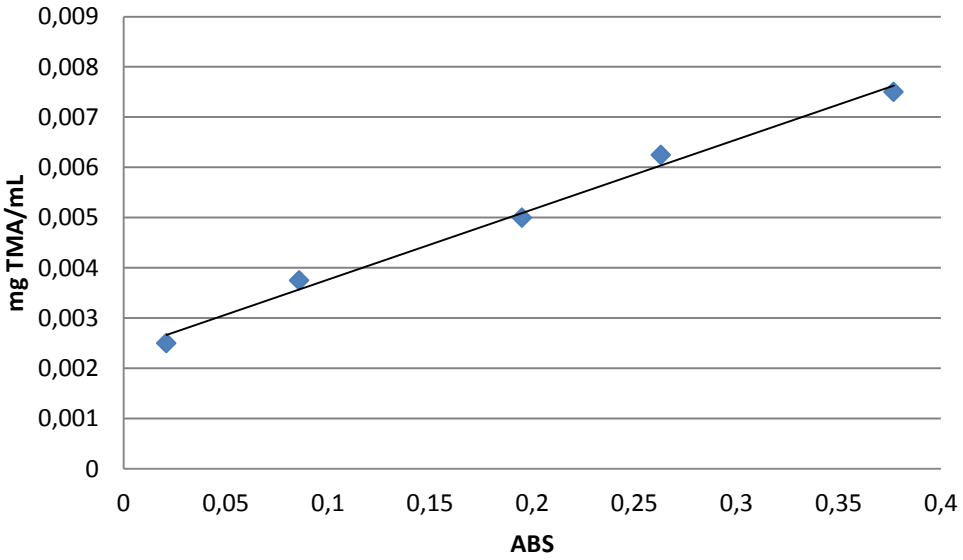

Figure A.1 Trimethylamine nitrogen (TMA-N) standard curve

$$\boldsymbol{TMA\ concentration\ (\mu g/ml)} = \frac{\boldsymbol{Absorbance + 0.154}}{\boldsymbol{76,923}}$$

APPENDIX B

STANDARD CURVE FOR TBA CALCULATION

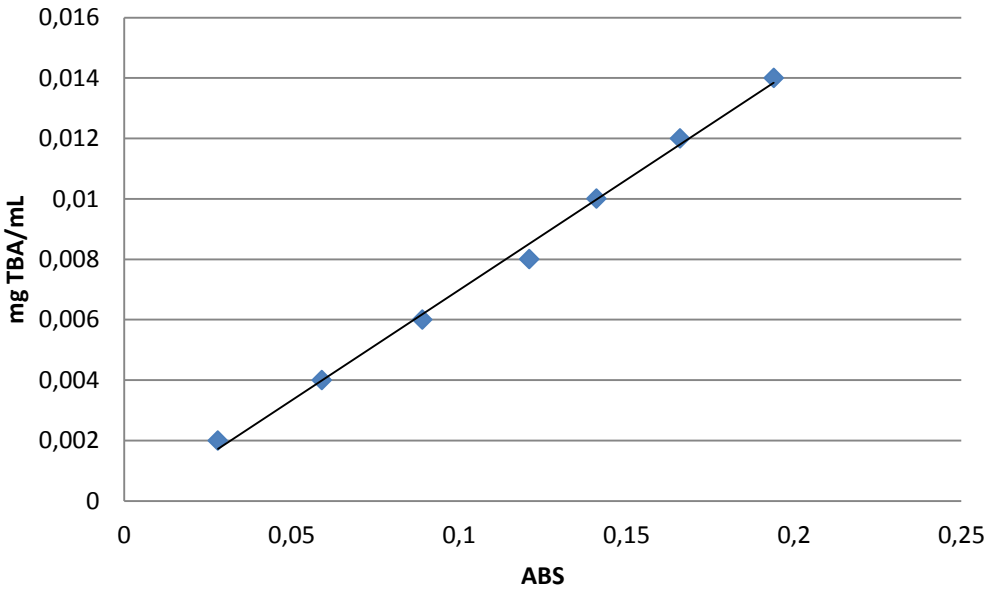

Figure B.1 Thiobarbituric acid (TBA) standard curve

$$\boldsymbol{TBA\ concentration\ (\mu g/ml)} = \frac{\boldsymbol{Absorbance - 0.001}}{\boldsymbol{13.699}}$$