EFFECT OF HIGH HYDROSTATIC PRESSURE ON QUALITY FACTORS AND SHELF LIFE OF ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*) AND RED MULLET (*MULLUS BARBATUS*)

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ABSTRACT

EFFECT OF HIGH HYDROSTATIC PRESSURE ON QUALITY FACTORS AND SHELF LIFE OF ATLANTIC MACKEREL (*SCOMBER SCOMBRUS*) AND RED MULLET (*MULLUS BARBATUS*)

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The ability of high hydrostatic pressure (HHP) to extend the shelf life of Atlantic mackerel *(Scomber scombrus*) and red mullet (*Mullus barbatus*) was assessed in this study. For that purpose, fillets of both atlantic mackerel and red mullet were subjected to pressure treatments at 200, 300, 400 MPa at 5, 10, 15°C for 5 and 15 minutes. The influence of the treatments on Trimethylamine Nitrogen (TMA-N) level, lipid oxidation stability (Thiobarbituric Acid, TBA level) was investigated as well as color changes. The suitable combinations for Atlantic mackerel were determined as 200 MPa, 15°C for 5 min and 400 MPa, 5°C for 5 min; and for red mullet 200 MPa, 15°C for 5 min. In the second stage, the shelf life of fish samples, which were treated with these conditions and stored at 4°C, were studied by measurement of pH, color, sensorial features (appearance and odor), TMA-N, TBA, Total Volatile Basic Nitrogen (TVB-N), Histamine and Total Mesophilic Aerobic Count (TMAC) formations. Based on these analyses, the unpressurised

mackerel samples were acceptable up to only 7 days compared to 17 and 19 days after 200 and 400 MPa treatments; respectively. For red mullet samples pressurization at 200 MPa extended the shelf life an additional 3 days (from 1 week to 10 days). HHP treatment in combination with chilled storage can improve the shelf life and quality of fish.

Keywords: high hydrostatic pressure, quality, *Scomber scombrus*, *Mullus barbatus*, shelf-life

ÖZ

YÜKSEK HİDROSTATİK BASINÇ UYGULAMASININ USKUMRU (*SCOMBER SCOMBRUS*) VE BARBUNUN (*MULLUS BARBATUS*) KALİTE FAKTÖRLERİ VE RAF ÖMRÜ ÜZERİNE ETKİSİ

Şentürk, Tuğçe
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Çalışmada Yüksek Hidrostatik Basınç (YSB) uygulamasının uskumru *(Scomber scombrus*) ve barbun (*Mullus barbatus*) balıklarının raf ömrü süreleri üzerine olan etkileri incelenmiştir. Bu amaçla, hem uskumru hem de barbun filetoları 5, 10, 15 °C'de 5 ve 15 dakika boyunca 200, 300, 400 MPa basınçlara tabi tutulmuşlardır. Uygulanan işlemlerin, Trimetilamin Azot (TMA-N), yağ oksidasyon stabilitesi (Tiyobarbiturik Asit, TBA değeri) üzerine olan tesirleri incelendiği gibi renk değişimleri üzerine olan etkileri de incelenmiştir. Uskumru için en uygun kombinasyonlar 200 MPa, 15°C, 5 dak ve 400 MPa, 5°C, 5 dak.; barbun için 200 MPa, 15°C, 5 dak. olarak belirlenmiştir. Deneyin ikinci aşamasında belirtilen koşullarda işlem görmüş balıklar 4°C sıcaklıkta saklanmış ve raf ömrü süreleri pH, renk, duyusal özellikler (görüntü ve koku), TMA-N, TBA, Toplam Uçucu Bazik Azot (TVB-N), Histamin ve Toplam Mezofilik Aerobik Mezofilik Mikroorganizma Sayısı (TMAS) ölçümleri yapılarak çalışılmıştır. Yapılan

analizlere dayanarak, basınçlanmamış uskumru örnekleri 7 güne kadar kabul edilebilir limitleri aşarken, 200 ve 400 MPa uygulanmış örnekler sırasıyla 17 ve 19 gün sonunda bu limitlere ulaşmışlardır. Barbun balığında ise 200 MPa basınç raf ömrünü ilave 3 gün (1 haftadan 10 güne) arttırmıştır. YHB işlemi soğukta depolama ile birlikte kullanıldığında balık kalitesini ve raf ömrünü arttırabilir.

Anahtar Sözcükler: yüksek hidrostatik basınç, kalite, *Scomber scombrus*, *Mullus barbatus*, raf ömrü

To My Beloved Family

and in Memory of My Grandfather

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LIST OF ABBREVIATIONS

ANOVA : Analysis of variance

CD-ELISA : Competitive direct enzyme-linked immunosorbent assay

DMA : Dimethylamine

HHP: High Hydrostatic Pressure

HCl : Hydrochloric acid

ID: Internal Diameter

MDA : Malondialdehyde

MA : Methylamine

PCA : Plate Count Agar

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

TVC : Total Viable Count

CHAPTER 1

INTRODUCTION

1.1 Fishery Industry

Marine foods can supply important ingredients for billions of people worldwide, such as lipid soluble vitamins, nutritional and digestive proteins, microelements (such as I, Fe, Ca, etc.) and high content of ω-3 polyunsaturated fatty acids. Besides the health benefits, fisheries have great economic importance (Aubourg, 2001). Total captured and aquaculture production of fishery of the world over the years is shown in Figure 1.1. In 2008, fish consumption of the world reached 115 million tones which supplies at least 15% of their average animal protein intake of 3 billion people. As well, world exports of fish and fishery products reached a record US$102 billion and the long term forecast is positive with a growing share of fish production entering international markets (FAO, 2011).

World's population is expected to reach 8.5 billion within the next 25 years. The increasing demand of fish does not only underline the necessity of sustainable management of aquatic resources, but also to supply the protein needs of the world's population, fish production must be doubled. However, recent studies showed that such a large increase in demand can not be covered with the existing stocks (FAO, 2011). Bearing in mind that increasing fishing caused the anchovy stocks of Peru to collapse in seventies, the same situation occurred in Turkey, too.

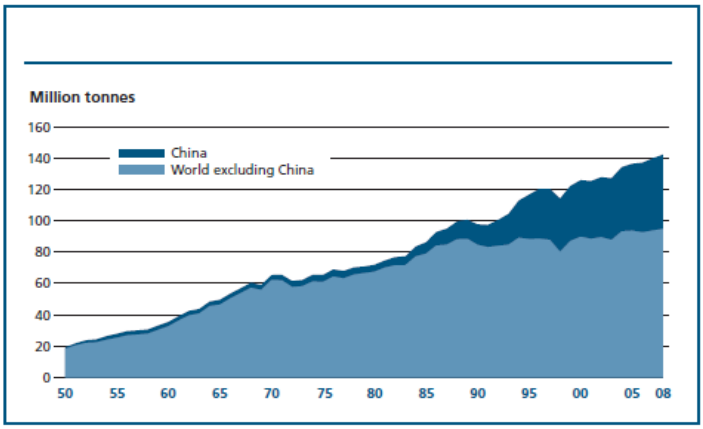

Figure 1.1 *Captured fisheries and aquaculture production of the world. (FAO, 2011)*

Although the production was 700 thousand tones in eighties, this value decreased to 400 thousand tones in nineties. Therefore, overfishing has been the most important problem that threatens the world fishing industry. In substitution for this, extension of shelf life of the fisheries by using the new technologies will make a great contribution to the fishery industry.

1.2 Traditional Processing of Fish

Fisheries are highly perishable products due to complex series of chemical, physical, microbiological changes occurring in muscle and the poorness in terms of connective tissue and its porous structure (Love, 2006; Jiang and Lee, 2006). As a highly perishable commodity, fish has a significant capacity for processing. Fresh form of fish was the most important product (49.1 %), followed by frozen fish (25.4 %), prepared or preserved fish (15.0 %) and cured fish (10.6 %). Human consumption of processed fish increased from 46.7 million tonnes in 1998 to 58.6 million tonnes in 2008 (live weight equivalent) (FAO, 2011).

As can be seen, freezing is the main commercially used method to slow down enzymatic and bacterial degradation of fish muscle. In the frozen state, fish can be stored for several months. However solidification of water initiates complex

physical and physicochemical changes that can cause general deteriorative quality changes not ordinarily occurring in fresh foods. Gaping is the major problem of frozen fish. It has a great economic importance because a gaping fillet can not be smoked, sliced or sold. It has to be minced and sold as a low-priced product which represented financial loss. The elapsed time between freezing and the death of the fish is important in gap formation. Slow freezing rate affect the flesh the same as that of prolonging the period preceding freezing. In addition to all disadvantages of freezing method (moisture loss, mechanical damage due to large ice crystals, drip on thawing etc.), these processes can not eliminate changes in quality. (Love, 2006; Jiang and Lee, 2006)

In canning process, raw fish material is subjected to various industrial applications. Although softening of the bones and providing a calcium source are advantages of canning; detrimental effects on labile and essential nutrients of the raw fish such as proteins, vitamins, minerals, lipids etc. are the most important disadvantages of this technology. Nutritional and the sensorial values of the canned product will also be reduced (Aubourg, 2001).

1.3 Features of Atlantic Mackerel (*Scomber scombrus*) and Red Mullet (*Mullus barbatus*)

Atlantic mackerel, which belong to the family Scombridae, is one of the fatty fish and inhabits various oceans. Native distribution map of atlantic mackerel in the world is shown in Figure 1.2. Its' proximate chemical composition with respect to season is given in Table 1.1. Due to its soft flesh, high lipid content and delicate skin, processing of these fish like handling, has remained a problem (Jhaveri, Shing and Constantinides, 1982). Although it is declared as a wholesome food, it remains underutilized due to its short frozen shelf life (Aubourg, Rodríguez and Gallardo, 2005).

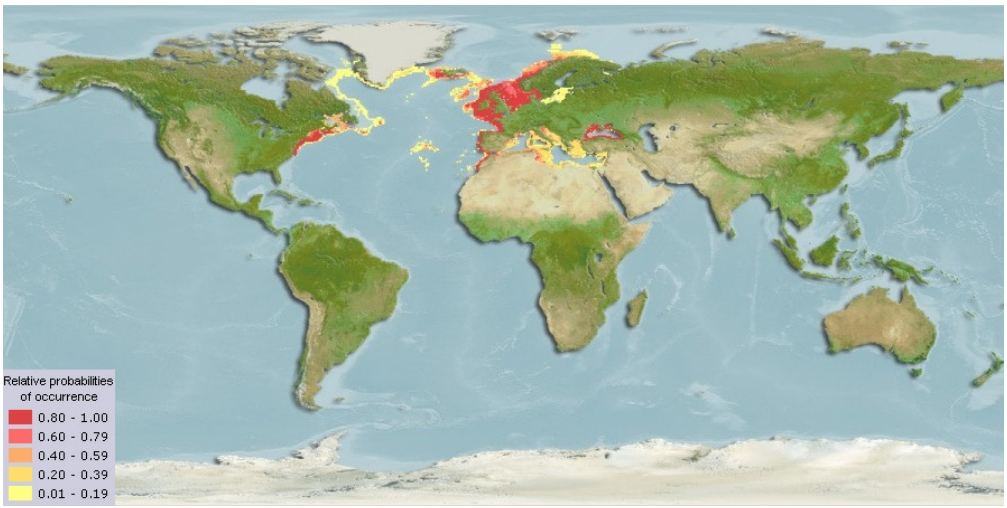

Figure 1.2 *Computer Generated Native Distribution Map of Scomber scombrus (fishbase.org, 2011)*

Table 1.1 Proximate composition (w/w%)[1] of raw atlantic mackerel fillets [1,2,3] (Aminullah Bhuiyan, Ratnayake and Ackman, 1986).

Sample component	Fall[2]	Spring[3]
Water	60.5±0.5	77.7±0.7
Lipid	20.6±0.7 (52.2)	1.8±0.2 (8.2)
Ash	1.6±0.2 (4.5)	1.9±0.2 (8.6)
Protein	17.5±0.5 (44.3)	18.3±0.3 (83.2)

1 Values in parenthesis represent percentage on dry basis.

2 Average of 8 fish

3 Average of 9 fish

Red mullet belong to the family Mullidae. There are two genera *(Mullus* and *Upenus*) and three species (*M. barbatus, M. surmuletus, U. mollucensis*) live in our territorial waters. It is a benthic species. Figure 1.3 shows the native distribution map of red mullet in the world. Its habitat in Turkey is Mediterranean, Anatolia coast of Aegean Sea, Marmara Coasts and the small amount is found in Black Sea coasts. *M. barbatus* has a high economic value in marine fisheries of Turkey. It is also our exportation product (Atar and Mete, 2009). It is a lean fish, composition of which is given in Table 1.2.

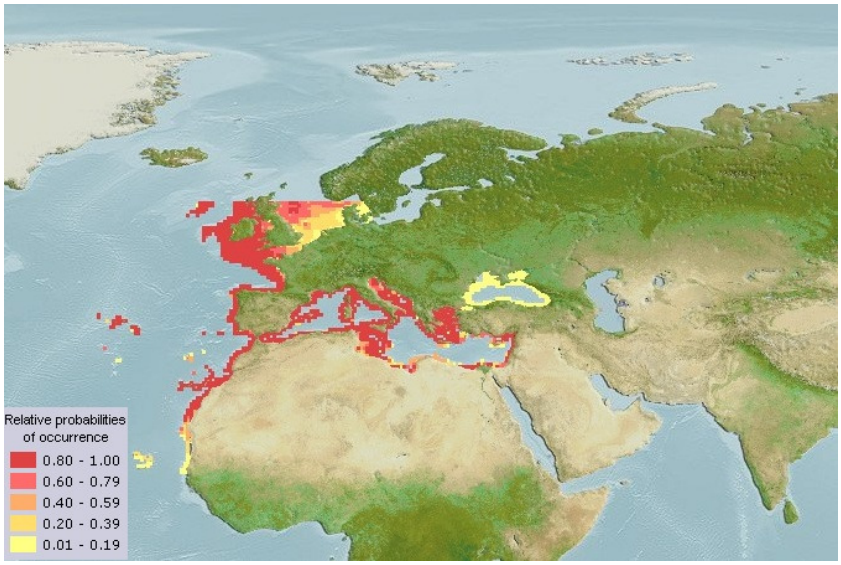

Figure 1.3 Computer generated maps for Mullus barbatus (fishbase.org, 2011)

Table 1.2 Proximate composition (w/w%) of raw red mullet in different seasons [1] (Polat, Kuzu, Özyurt, Tokur, 2007).

Sample component	Fall	Spring
Water	74.13±0.21	73.84±0.25
Lipid	5.76±0.07	3.68±0.34
Ash	1.11±0.01	1.24±0.01
Protein	18.97±0.08	20.43±0.67

1 Means followed by different letters within the same column are significantly different ($P < 0.05$).
The values are expressed as mean SD, n = 3.

These two groups of fish have great commercial importance in both Turkey's and World's fishing industry (Özyurt, Kuley, Özkütük, Özoğul, 2009; fishbase.org, 2011). Both of them are generally consumed fresh, second consumption type is as freezed. They are marketed as bulk of whole fish, packed on ice (fishbase.org, 2011).

1.4 High Hydrostatic Pressure Technology

High Hydrostatic Pressure (HHP), also known as ultra high pressure processing, is the technology that satisfies both scientific and consumer requirements. HHP is one of the non thermal technologies that provides safer, higher qualified, value-added foods which possess higher vitamin retention, enhanced sensory qualifications and higher consumer acceptance than both conventional (e.g. thermally processed foods) and other non thermal technologies (e.g. irradiation) (Doona and Feeherry, 2007). While optimizing the conditions of the process for particular foods, the effects of the technology on the properties of the food product, effects and the kinetics of the food on decelerate the microbial inactivation must be taken into consideration. Due to these reasons, HHP treated products continue to increase in the global market place (Doona and Feeherry, 2007).

The main components of the system are pressure vessel and its closure, pressure generating system, a temperature control device. Depending on the parameters and scale of the operation, expenditure of the application is around US$ 0.05-0.5 per L or kg which is very close to the cost of traditional thermal methods. In the past, the non availability and the costliness of the equipments prevented the applications and distributions of this technology in the market. However, recent developments in the design of the equipments increased the recognition and potential of this application (Rastogi, Raghavarao, Balasubramaniam, Niranjan, Knorr, 2007). Pressure chambers designed for food industry are present up to 500 L volume and 8,000 bars pressure. However, due to the expenditures, there is a practical limitation around 6,000 bars (Ohlsson and Bengtsson, 2002).

HHP can kill microorganisms by breaking the non-covalent bonds and by piercing or changing the permeabilization of the cell membrane. In addition to the microbial inactivation, HHP also inactivates the deteriorative molecules such as hydrolytic and oxidative endogenous enzymes. Therefore, flavor and nutrients are unaffected although microorganisms and enzymes can be inactivated. On the

other hand, HHP gives damage to membranes, denatures proteins and changes the cell morphology; although covalent bonds are not broken, weak energy bonds like hydrogen and hydrophobic bonds can be irreversibly modified, causing substantial effects on the secondary, tertiary and quaternary structures in proteins. Moreover, it is reported in the literature that HHP induces oxidative changes in lipids, therefore an important loss of oxidative stability has been underlined (Ohlsson and Bengtsson, 2002; Aubourg, Munizaga, Reyes, Rodrigues, Won, 2010).

1.4.1 Earlier HHP Studies in Turkey

Although HHP on atlantic mackerel or red mullet is a new topic, there exists several earlier researches on HHP treatments of fish species in our country. One of those is the study of Büyükcan, Bozoğlu and Alpas (2007). They found that pressure treated shrimps (*Parapenaeus longirostris*) and clams (*Venus gallina*) had shelf life of 16 days while untreated ones had 4 days in refrigerated storage. Furthermore, in 2010, effects of HHP on physicochemical properties of horse mackerel (*Trachurus trachurus*), sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) were studied, best treatment combinations were determined (Erkan, Üretener, Alpas, Selçuk, Özden, Buzrul, 2010; Erkan, Üretener, Alpas, 2010; Erkan, Üretener, 2010).

1.5 Freshness Indicators of Fish

Freshness is the most important criterion in determining the quality of fish. The loss of freshness is often caused by a combination of physical, biochemical and microbiological reactions (Chéret, Aránzazu, Delbarre-Ladrat, de Lambarrie, Verrez-Bagnis, 2006). A wide variety of methods (physical, chemical, sensorial, etc.) were found to evaluate freshness and quality changes during storage.

Sensory evaluations are an accurate method for assessing freshness in many fishes. As the storage time increases, characteristic changes of the appearance, odor, taste and texture of the fish occur (Aubourg, 2001; Olafsdottir et al., 1997).

Although lots of instrumental methods have been developed to determine the physical, chemical and biological properties of the foodstuff, they are used for measuring textural and color features and they must be used with sensory evaluation panels to guarantee the accuracy of the results because instrumental methods can not show edibleness of the fish (Dokuzlu, 2004).

One way to perform sensory evaluation is the hedonic scale that Huss developed (Huss, 1988). In this 10-point descriptive scale, a score of 10-9 indicated "very good quality" which meant that it had characteristic odor/appearance of the species. The score of 8.9-8 indicated "good quality" and referred to very fresh/see weedy properties. The score of 7.9-4 "acceptable quality" meant characteristic odor began to disappear and instead of them slight off-odors such as mousy, garlic, bready, sour and rancid odor were observed. The score ≤ 3.9 meant it had strong off-odors/ flavors such as stale cabbage, NH_3, H_2S or sulphides and was denoted as spoiled.

Non-protein nitrogenous compounds constitute 9%-18% of the total nitrogen of osteichthyes (bony fish). The main compounds of this fraction are ammonia, trimethylamine oxide (TMA-O), creatine, free aminoacids, nucleotides, volatile bases such as purine bases and urea in cartilaginous fish. TMA-O takes part in osmoregulation system of marine fish. Therefore, it is not found in fresh water fish. TMA-O acts as a buffer in fish body and it is water soluble (Serdaroğlu and Deniz, 2001). Molecular structure of TMA-O and its degredation products are shown in Figure 1.4. Trimethylamine-nitrogen (TMA-N) is formed from TMA-O as a result of bacterial enzyme (Trimethylamine oxidase) or other endogenous enzymes activities (Erkan, 2005; Erkan and Özden, 2008). Fishy odor takes its source from this compound. Up to 4 mg TMA/100 g, the fish sample was indicated as "very good quality", up to 10 mg TMA/100 g was denoted as marketable, the score of 12 mg TMA/100 g was denoted as spoiled (Kundakçı, 1989; Varlık, Baygar, Özden, Erkan, Metin, 2000; Mol, Özden, Erkan, Baygar, 2004). FAO recommended that 10-15 mg TMA-N/100 g must be regarded as the limit of acceptability for human consumption (Connell, 1990; Huss, 1988). Based

on these literature information 10 mg/100g was used as a threshold TMA-N value in this study.

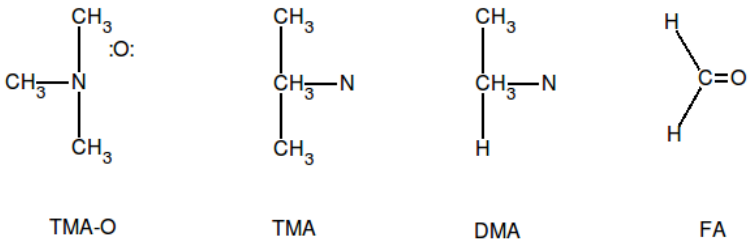

Figure 1.4 *TMA-O and its degredation products (Serdaroğlu and Deniz, 2001).*

Oxidative rancidity is an important organoleptic characteristic feature for rejection or approval of fish after prolonged shelf life (Amanatidou et al., 2000). Lipid oxidation is expressed as TBA level. Due to having high concentration of polyunsaturated fats, the lipids of fish are more vulnerable to oxidation than other types of meat. Lipid oxidation can be originated from non-enzymatic and enzymatic reactions. Phases of lipid oxidation is shown in Figure 1.5. Oxidation level was measured with the help of a reaction which is demonstrated in Figure 1.6. TBA value of 1-2 mg MDA/kg was denoted as the limit of acceptability (Connell, 1975; Goulas and Kontominas, 2005). 1 mg MDA/kg was used as a threshold TBA value for this study.

1. Initiation
   (a) $RH + O_2 \longrightarrow R\cdot + \cdot OOH$
2. Propagation
   (b) $R\cdot + O_2 \longrightarrow ROO\cdot$
   (c) $RH + ROO\cdot \longrightarrow ROOH + R$
   (d) $ROOH \longrightarrow RO\cdot + \cdot OH$
3. Termination
   (e) $R\cdot + R\cdot \longrightarrow R\text{—}R$
   (f) $R\cdot + ROO\cdot \longrightarrow ROOR$
   (g) $ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$

Figure 1.5 *Three phases of lipid auto oxidation which is carried out by autocatalytic mechanism of free radicals. ROOH (hydroperoxides) are the most important initial reaction products of lipid oxidation. Their breakage causes secondary products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) (Fernandez et al., 1997).*

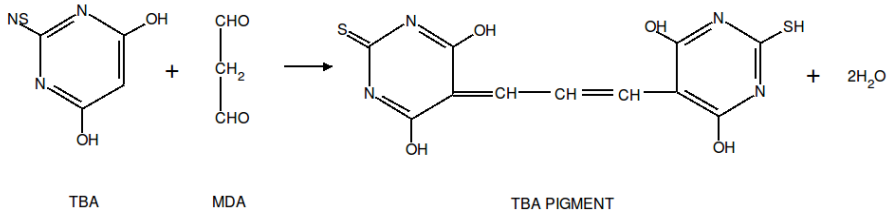

Figure 1.6 *In the analysis of lipid oxidation, MDA reacts with 2 molecules of TBA and produces pink pigment with a maximum absorption at 532 nm (Fernandez et al., 1997).*

TVB-N is interrelated with bacterial growth and an important parameter as a spoilage index (Gomez-Estaca, Gomez- Guille'n, Montero, 2007). During marine fish spoilage, along with TMA-N, ammonia and other basic nitrogenous compounds are also produced and together make up total volatile basic nitrogen (TVB-N) (Baixas-Nogueras, Bover-Cid, Vidal-Carou, Veciana-Nogués, Mariné-Font, 2001). TVB-N value was denoted as "very good quality" up to 25 mg/100g.

Over 35 mg/100 g was indicated as spoiled (Varlık et al., 2000; EEC, 1995; Goulas and Kontominas, 2005).

In literature, pH value of the fresh fish must be between 6.0-6.5. Threshold value for pH was denoted as 6.8-7.0 (Mol et al., 2004). In this study, fish samples which had pH 7.0 were denoted as spoiled.

Among biogenic amines, researchers mostly focused on histamine level. Spoiled fish of the Scombridae and Scomberesocidae families commonly cause the food borne intoxication known as histamine poisoning, in another words "scombroid fish poisoning". Histamine is formed mainly through decarboxylation of histidine by certain bacteria which possess the enzyme, histidine decarboxylase (Figure 1.7) (Taylor, 1989). Histamine concentrations of foods exceeding 50 ppm (5mg/100g fish) were hazardous (FAO, 2011).

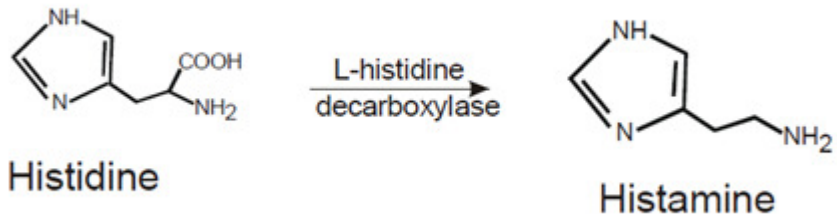

Figure 1.7 *Histamine formation by the enzymatic decarboxylation of the amino acid histidine.*

There is a small amount of pathogenic bacteria in fish. On the other hand, the growth rate of Gram (-) spoilage bacteria are high at low temperatures. Therefore the fish will be spoiled even before dangerous amounts of pathogens or toxins have developed. The metabolites of spoilage organisms cause off-flavors, odors (fishy, ammoniac) and consequently induce consumer rejection (Huss, 1997). The limit of acceptability for mesophilic aerobic bacteria was indicated as 10^6 – 10^7

CFU/g (Gobantes, Choubert, Gomez, 1998; Erkan and Özden, 2008; Gümüş, İkiz, Ünlüsayın, Gülyavuz, 2008). For the present study, 6 log cfu/g was designated as a limit value for consumption.

1.6 Aim of This Study

The primary aim of this study was to investigate the potential of HHP to improve the shelf life of Atlantic mackerel during refrigerated storage. In order to establish the optimum processing conditions (pressure, temperature, time) that would be used in the shelf life study, in the first part, the effects of high hydrostatic pressure (200, 300, 400 MPa), temperature (5, 15°C) and holding time (5, 10, 15 min) treatments on Trimethylamine Nitrogen (TMA-N level), lipid oxidation stability (Thiobarbituric acid (TBA level)) were investigated as well as color changes (Hunter lab color difference). Controls were not pressurized. These treatment ranges were selected due to the necessity of determining the minimum conditions for obtaining desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality. In the second part, unpressurised and pressurized samples with specified conditions (200 MPa, 15°C, 5 min and 400 MPa, 5°C, 5min) were subjected to analysis up to 21 days.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Supplying of Samples

Fresh Atlantic mackerel (average weight and length: 200±20 g and 22.0-31.1 cm, respectively) and red mullet (average weight and length: 150±15 and 20.0±5.0 cm) were purchased in October 2010 from a local market in Ankara and until arrival at the laboratory, the fish was kept on iced containers. Bearing in mind that our study also comprised the effect of HHP on the lipid oxidation, this time of the year was chosen due to high lipid content of fish.

2.1.2 Reagents

All chemicals used were of Analytical grade. The chemicals and biologic materials that were not specified were purchased from Merck (Merck Ltd., Darmstadt, Germany) while picric acid was from Fluka (Fluka Chemie, Steinheim, Switzerland), tashiro indicator was from Ateks (Ateks Kimya Ltd., İstanbul, Turkey).

2.1.3 High Hydrostatic Pressure Treatment

High Pressure treatment was performed with 760.0118 type high pressure equipment supplied by SITEC-Sieber Engineering AG, Zurich, Switzerland. The vessel had a volume of 100 ml with ID 24mm 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 7000 bar at an operating temperature of -10 to 80°C. A built-in heating-cooling system (Huber Circulation Termostat, Offenburg, Germany) was used to maintain and control the required temperature which is measured by a thermocouple type K. It was fitted through the upper plug to measure the inner temperature of the vessel during the pressure treatment. The vessel was filled with a pressure transmitting medium consisting of distilled water. Pressure come-up and release times were less than 20 seconds for each. Pressurization time reported in this study did not include the pressure increase and release times. Temperature increase due to adiabatic heating was reduced to 4-5°C during the time period of pressurization to 400 MPa. Reported temperature is the actual process temperature during hold time at reported pressure levels. Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa.

2.2 Method

2.2.1 Preparation of samples

All the fish were headed, eviscerated, washed thoroughly, filleted and kept in freezer at -25±2°C until they are used. This period did not exceed 1 week. Experiments were carried out on two batches of fish which was obtained at different times. The initial bulk was used in the fist part of the study, the second was used in the shelf life determination part. The samples were prepared by wrapping with stretch film, aluminum foil and once more stretch film in order to avoid the contact of sample with the transmitting medium.

2.2.2 Analyses

Unless specified three independent measurements for all kinds of analysis were performed for each condition. Solely, sensory analysis and color measurements were carried out with six and nine measurements for all conditions, respectively.

2.2.2.1 Sensory Analyses

Sensory analysis was conducted by six panelists, who were laboratory trained graduate students in the Department of Food Engineering at METU, according to the hedonic scale that Huss developed. Sensory assessment of the control and HHP treated samples included the following parameters: appearance and odor. The mean values of these criteria for each fish sample were calculated from the grades given by each panelist.

2.2.2.2 Physical Analyses

2.2.2.2.1 Determination of pH

pH was monitored at room temperature in distilled water (1/10 w/v) by using pH meter (Mettler-Toledo MP220, Schwerzenbach, Switzerland).

2.2.2.2.2 Measurement of Color

Hunter L*, a* and b* parameters which describe the intensity of white, red and yellow color, respectively, (Amanatidou et al., 2000) were measured with a Minolta chromameter model CR-200 (Minolta Camera, Co, Japan). The instrument was standardized each time with a white ceramic plate (L= 73.3; a=8.4; b=23.0). The color difference ΔE was calculated by using the Hunter-Scotfield equation below; subscript '$_0$' indicates initial color. (Hunter, 1975; Lozano & Ibarz, 1997).

$$\Delta E = \sqrt{(a - a_0)^2 + (b - b_0)^2 + (L - \mathrm{L}_0)^2} \quad (1)$$

In order to overcome the heterogeneity problem, measurements were performed at 3 different locations of each 3 different samples. The color was expressed as the mean of nine replicated measurements.

There are two major types of fish skeletal muscles, red and white. Depending on the species red or dark muscle may comprise up to 30% of fish muscle (Walker & Pull, 1975). Therefore, at every turn color analysis based on the measurements on white muscle.

2.2.2.3 Microbiological Analyses

10 g of sample was prepared under sterile conditions and homogenized in sterile stomacher bags (Seward Medical, England) with a stomacher (Seward Laboratory Blender Stomacher 400, England) in 90 mL sterile peptone water solution. Subsequently, decimal dilution series of the homogenate were performed in peptone water. For the enumeration of total mesophilic aerobic bacteria, spread plate technique was used on the non-selective Plate count agar (PCA). 100 µl aliquot was poured into plates, spread plated and incubated at 37°C for 48 hour. All counts were expressed as $\log_{10}$ colony forming units (CFU)/g Atlantic mackerel sample. It was calculated by using the formula below (biology.clc.uc.edu, 2011) :

$$CFU/g\ meat = \quad (2)$$

$$CFU/plate \times meat\ suspension\ factor\ (ml/g) \times dilution\ factor \times \frac{1}{aliquot\ factor(ml)}$$

2.2.2.4 Chemical Analyses

2.2.2.4.1 Determination of TMA-N Level

TMA-N was determined by the method of Schormüller (1968). The technique consisted of extracting TMA from 10g of minced and well-mixed fish samples with 10% trichloroacetic acid (TCA) in a ratio of 1:9 (w/v). After filtrating with Whatman No.1 filter paper, 4 ml extract was transferred into test tube with 1ml formaldehyde (20%), 10 ml toluol (99%) and 3 ml KOH (50%). Tubes of blank, standards and samples were stoppered and shaken vigorously by hand ca 80 times. After 15 min. incubation time, 5 mL toluene layer was pipetted and transferred to another tube with 5 mL picric acid solution (0.02%, with H_2O-free toluene) and they were mixed by swirling gently. At this stage, TMA-N which had been extracted in toluene layer reacted with picric acid. By this way picric acid interacted with the primary and secondary amines to produce yellow picrates with maximum absorption at 410 nm in Specord50 spectrophotometer (Analytic Jena AG, Jena, Germany). TMA value can be calculated with the formula below:

$$TMA\ value\ (mg/100\ g\ of\ fish\ sample) = Concentration \times Dilution\ factor \times \frac{1}{Weight\ of\ the\ sample\ (g)} \quad (3)$$

Preparation of Standard TMA Solutions:

170 mg Trimethylamine standard (TMA-HCl) was mixed with 100 mL distilled water. This was the main standard which contained 250µN/mL TMA. 1, 2, 3, 4 ml solutions were taken from main standard and mixed with 100 ml distilled water (Therefore 2.49, 4.98, 7.47, 9.96 µg/ml TMA standards were prepared, respectively). 4 ml of these standards were subjected to the transactions that mentioned above. Thereby, standard TMA curve was drawn. Generated standard curve was located in Appendix A.

2.2.2.4.2 Determination of TBA Level

TBA number was determined according to Erkan and Özden (2008). 10g of homogenized sample were placed in a tube with 500 µL butylated hydroxytoluene (BHT) and 90 mL of %5 TCA solution (w/v). The mixture was homogenized with Ultra-Turrax and filtrated through a Whatman No.1 filter paper. 5 ml of filtrate was mixed with 1 ml of 0.01 M aqueous solution of 2-TBA. Reaction took place in 70-80 °C water bath for 40 min. Tubes were immediately cooled to room temperature. Absorbance of the resultant colored solution was read at 532 nm with using Specord50 spectrophotometer.

$$TBA\ value\ (mg/kg\ of\ fish\ sample) = Concentration \times Dilution\ factor \times \frac{1}{Weight\ of\ the\ sample\ (g)} \quad (4)$$

Preparation of Standard TBA Solutions:

10 µl of 1,1,3,3- tetraethoxy- propane (TEP) was diluted to 10 ml with 0.1 M HCl solution. It was put into the boiling water bath for 5 minutes, then immediately cooled with tap water. 1 ml of this solution was transferred to 100 ml flask; the remaining volume was filled with distilled water. This was the stock solution which had a concentration of 2.92 µg MDA/ml. Serial dilutions from the stock solution were done to prepare 0.002-0.014 µg MDA/ ml standard solutions. Generated standard curve was located in Appendix B (Karataş, Karatepe, Baysar, 2002).

2.2.2.4.3 Determination of TVB-N Level

Total volatile basic nitrogen (TVB-N) was determined according to the method of Antonacopoulos & Vyncke (1989). 10 g of minced fish sample were blended with 90 ml of %6.5 Perchloric acid for 1-2 min in an Ultra-Turrax. The homogenates were filtered through a Whatman No.1 filter paper. 50 mL of filtrates was transferred to Kjeldahl tube with 150 ml distilled water and subsequently

alkalized by %20 NaOH. Tubes were distilled in Kjeldahl apparatus (Şimşek Laborteknik Co., Ankara, Turkey) until 50 ml distillate was collected. The distillate was titrated with 0.01 N HCl. TVB-N level was calculated by using the formula below:

$$TVB - N\ level \frac{mg}{100g} = [Used\ HCl\ for\ sample - Used\ HCl\ for\ blank] \times 0.14 \times 2 \times 100 \times \frac{1}{Weight\ of\ sample} \quad (5)$$

2.2.2.4.4 Determination of Histamine Level

Histamine content of fish flesh during storage was determined with a commercial rapid test kits (Veratox Quantitative Histamine Test Kits, Neogen Co, USA/Canada) by means of the competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method. The tests' results were read in a micro well reader (Stat Fax, Neogen Co, USA) with a 650nm filter. Histamine concentrations were expressed as parts per million (ppm).

2.2.2.5 Statistical Analyses

Means and standard deviations were calculated using Microsoft Excel 2010. The results were analyzed using the analytical procedure of SPSS 15 for Windows (SPSS Inc., Chicago,IL, USA). In the first part of the study, three-way analysis of variance (ANOVA) was used with pressure, application time and temperature as factors; in the second part, two-way ANOVA was used with process and storage time as factors, to determine the significant differences ($p < 0.05$). Tukey Test was used as a post-hoc test if a factor had a significant effect and if the factor had 3 or more groups. An example was given in Appendix C.

CHAPTER 3

RESULTS AND DISCUSSION

All mature fish undergo depletion due to the spoilage microorganisms and enzymes. The spoilage level is determined by complex series of physical, chemical and microbiological changes occurring in fish muscle. These associated processes cause loss or development of undesirable compounds which decrease fish quality (Love, 2006; Jiang and Lee, 2006). The major criteria used in determining the quality of fish were analyzed by observing the effects of high pressure.

3.1 Assessment of Optimum Pressure, Temperature and Time Combination Analyses

Treatment conditions (200, 300, 400 MPa; 5, 10, 15°C; 5, 15 min.) were selected due to the necessity of determining the minimum conditions for obtaining desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality.

3.1.1 Results of TMA-N Analyses

The effects of combined application of pressure, temperature and holding time that studied on TMA levels of Atlantic mackerel could be seen on Table 3.1. Unpressurised control samples had a TMA level of 1.241±0.169 mg /100 g of sample. This result coincided well with literature data of mackerel species, 1.22 ±

0.06 mg /100 g (Goulas and Kontominas, 2005). This value was also convenient with the FAO standards which recommended that good quality fish must contain less than 1.5 mg TMA-N/100 g (Huss, 1988).

As seen in the Table 3.1, TMA-N content of the samples slightly decreased with increasing pressure (T,t = constant), nevertheless difference was not found as significant in Tukey test for pressure as an independent variable. 400 MPa, 5°C, 5 min. treated samples had minimum TMA-N level. Other applications that caused lower TMA-N levels were 300 MPa, 5°C, 15 min and 200 MPa, 15°C, 5 min. Moreover, TMA-N level increased with the increasing temperature. However, this rule was not valid for 10°C generally.

The effects of the combined application on TMA-N values of red mullet were shown in Table 3.2. Unpressurised samples had a TMA-N level of 2.930±0.209 mg/100 g for red mullet. TMA-N value of the fresh sample was specified as 1.50±0.16 mg/100 g in the study of Özyurt et al. (2009). This value was indicated as 2.81±0.19 in the study of Erkan et al. (2010).

Although in some columns (T, t = constant) 300 MPa pressurized samples had higher TMA-N values than 200 MPa treated samples, generally TMA-N level was decreased with pressure. In the same row (P,t = constant), 15°C applied samples had lower values than 5°C treated ones. 10°C applied samples had the highest values. 200 MPa, 15°C, 5 min. and 400 MPa, 15°C, 15 min. combinations resulted with the lowest values.

The TMA-N scores of some treatments were higher than control samples. This increase would be linked to the damage of the lysosomal membrane by high pressure treatment and releasing of the enzymes. (Chéret, Aránzazu, Delbarre-Ladrat, de Lambarrie, Verrez-Bagnis, 2006).

3.1.2 Results of TBA Analyses

TBA values of the mackerel (fatty fish) samples after pressurization can be seen in Table 3.1. TBA level of the control group samples was 0.344±0.011 mg malonaldehide/kg. According to the previous study by Goulas and Kontominas (2005) with unprocessed chub mackerel, TBA level was 0.23 ± 0.05 mg malondialdehyde/kg. The reason of the higher TBA values of control group might take its source from the date of purchasing. October was the month in which fishing season began. TBA number was high due to stocking fat all summer long. All pressurized samples had higher levels of TBA than control group.

TBA level of the sample increased with increasing pressure. These results were supported by literature data. The reason of this increase was due to the denaturation of haem protein by pressure which released metal ions (Fe and Cu) and catalyzed lipid oxidation (Angsupanich and Ledward, 1998; Tanaka, Xueyi, Nagashima,and Taguchi, 1991; Cheah and Ledward, 1997; Wada, 1992). Likewise, during high pressure treatment, metal ions where released from metal salts (Fe-stearate and Cu-stearate) and catalyzed the oxidation reaction (Ledward and Cheah, 1997).

According to the Table 3.1, oxidative stability was decreased (TBA number increased) with the increasing temperature generally. Similarly, Angsupanich and Ledward (1998) indicated that when compared with the fresh sample, 200 MPa treated sample changed the TBA number a little although 400 MPa or higher pressure treatments increased its value considerably. TBA number decreased with increasing time (P,T = constant). Despite the lower TBA results of 200 MPa, 10°C, 5 min and 300 MPa, 10 °C, 5 min treated samples, other chemical parameters had to taken into consideration when determining/selecting the optimum conditions.

TBA values of the unpressurised mullet (lean fish) samples had 0.234±0.016 mg MDA/kg (Table 3.2). In literature it was specified approximately as 0.51±0.07 mg

MDA/kg (Gümüş et al., 2008; Özyurt et al., 2009) for red mullet. Just like the TBA analysis of mackerel, all pressurized samples had higher scores than control group, even so any significant difference could be found in Tukey test, although results confirmed the assumptions of ANOVA (Normality plot and Levene's test) and there were significance in time and other interactions of the independent variables of ANOVA test. Generally, TBA scores increased with increasing application time (P,T = constant). The reason of the closeness of the scores could be due to containing the polyunsaturated fatty acids in small quantities.

Table 3.1 The effect of heat, pressure and time on TMA-N (mg/100 g sample) and TBA (mg MDA/kg sample) values of atlantic mackerel flesh immediately after processing (<4 h at 4±0.5°C) [1,2,3,4].

	5 minutes			15 minutes		
	5°C	10°C	15°C	5°C	10°C	15°C
TMA-N						
200 MPa	$1.516\pm0.287_{a}$	$2.329\pm0.130_{b}$	$1.213\pm0.200_{c}$	$1.674\pm0.73_{a}$	$1.985\pm0.101_{b}$	$1.761\pm0.156_{c}$
300 MPa	$1.251\pm0.060_{a}$	$1.968\pm0.084_{b}$	$1.856\pm0.198_{c}$	$1.169\pm0.112_{a}$	$1.713\pm0.045_{b}$	$1.786\pm0.072_{c}$
400 MPa	$1.059\pm0.061_{a}$	$2.042\pm0.234_{b}$	$1.892\pm0.105_{c}$	$1.370\pm0.212_{a}$	$1.600\pm0.029_{b}$	$1.613\pm0.089_{c}$
TBA						
200 MPa	$0.582\pm0.052^{A}{}_{a}$	$0.352\pm0.027^{A}{}_{b}$	$0.410\pm0.013^{A}{}_{b}$	$0.441\pm0.052^{A}{}_{a}$	$0.400\pm0.058^{A}{}_{b}$	$0.367\pm0.085^{A}{}_{b}$
300 MPa	$0.592\pm0.139^{A}{}_{a}$	$0.358\pm0.030^{A}{}_{b}$	$0.446\pm0.053^{A}{}_{b}$	$0.498\pm0.047^{A}{}_{a}$	$0.387\pm0.049^{A}{}_{b}$	$0.386\pm0.039^{A}{}_{b}$
400 MPa	$0.625\pm0.219^{B}{}_{a}$	$0.505\pm0.014^{B}{}_{b}$	$0.592\pm0.041^{B}{}_{b}$	$0.574\pm0.045^{B}{}_{a}$	$0.427\pm0.037^{B}{}_{b}$	$0.523\pm0.004^{B}{}_{b}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All TMA-N and TBA values are the mean ± standard deviation of three replicates (n=3).

3 Unpressurised control samples had a TMA level of 1.241±0.169 mg /100 g.

4 Unpressurised control samples had a TBA level of 0.344±0.011 mg MDA/kg.

Table 3.2 The effect of heat, pressure and time processing on TMA-N (mg/100g sample) and TBA (mg MDA/kg sample) values of red mullet flesh immediately after processing (<4 h at 4±0.5°C) [1,2,3,4].

	5 minutes			15 minutes		
	5°C	10°C	15°C	5°C	10°C	15°C
TMA-N						
200 MPa	$1.759±0.116_a$	$3.672±0.042_b$	$1.214±0.041_c$	$4.017±0.214_a$	$4.352±1.358_b$	$2.282±0.301_c$
300 MPa	$1.632±0.099_a$	$3.254±0.246_b$	$2.127±0.183_c$	$3.335±0.482_a$	$3.017±0.554_b$	$2.803±0.705_c$
400 MPa	$2.933±0.219_a$	$3.185±0.345_b$	$1.704±0.382_c$	$3.128±0.460_a$	$2.306±0.158_b$	$1.395±0.358_c$
TBA						
200 MPa	0.255±0.012	0.26±0.046	0.254±0.007	0.267±0.014	0.264±0.023	0.29±0.014
300 MPa	0.269±0.02	0.242±0.004	0.249±0.007	0.254±0.013	0.278±0.011	0.285±0.008
400 MPa	0.236±0.005	0.232±0.009	0.273±0.011	0.324±0.009	0.297±0.033	0.287±0.006

1 For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All TMA-N and TBA values are the mean ± standard deviation of three replicates (n=3).

3 Unpressurised samples had a TMA-N level of 2.930±0.209 mg/100 g.

4 Unpressurised samples had a TBA level of 0.234±0.016 mg MDA/kg.

3.1.3 Results of Color Analyses

The effects of the high pressure applications on the Hunter Color system (L*, a*, b* and ΔE) of the mackerel and mullet samples were illustrated in Table 3.3 and Table 3.4, respectively. L*, a*, b* and ΔE values of unpressurised mackerel group were 42.0±0.2, 12.0±1.3, 24.9±2.2 and 31.6±0.4, respectively.

As given at the Table 3.3, lightness increased, redness of the samples decreased with increasing pressure (T, t=constant). No exception was observed. Generally, L* increased with increasing holding time and decreased with increasing temperature. Translucency loss was measured since the denaturation of myosins with pressure. (Angsupanich and Ledward, 1998)

L*, a*, b* and ΔE values of mullet control samples were 34.2±1.3, 8.9±1.5, 17.6±0.6 and 39.1±1.4, respectively. Applied treatments caused an increase in lightness and yellowness and decrease in ΔE values of the samples when compared with the control samples. No contrary situation was observed. Increase in pressure and application time caused an increase of the L* and b* and decrease of ΔE scores.

3.1.4 Selection Criteria

TVB-N analysis was not performed while establishing the optimum conditions although it was done in shelf life determination part of the study. The reason was given in the previous study of Castro et al. (2006) in which the same TVB-N analysis were performed with the same method and on the same fish group that were taken at the same time. However the researchers obtained poor results from the first fish group as no change in the TVB-N level was detected throughout the storage period of 21 days. A drastic increase was obtained from the second group. As a consequence it was underscored that TVB-N level alone, should be evaluated as a very unreliable freshness indicator. In another study, researchers underlined that

TVB-N was not an index of spoilage; however it could only be evaluated as indicator of advanced spoilage (Özoğul and Özoğul, 2000).

In consequence of the analyses, the mean values were taken into account while determining the best combinations. The condition which caused lower TMA-N level, comparatively high oxidative stability (low TBA level) and in terms of consumer acceptance, the closest color values to the control samples was determined as best combination which was 200 MPa, 15°C, 5 min. for atlantic mackerel. The second combination (400 MPa, 5°C, 5 min.) for the same fish was chosen with respect to the lowest TMA-N level among treatments. 200 MPa, 15°C, 5 min. treatment was chosen as a best combination for red mullet.

Table 3.3 The effect of heat, pressure and time processing on L*, a*, b*, ΔE color values of atlantic mackerel flesh immediately after processing (<4 h at 4±0.5°C) [1,2,3].

	5 minutes			15 minutes		
	5°C	10°C	15°C	5°C	10°C	15°C
L*						
200 MPa	$49.8\pm1.2^{A}_{a}$	$49.5\pm0.8^{A}_{a}$	$49.9\pm1.5^{A}_{b}$	$50.9\pm0.8^{A}_{a}$	$51.6\pm0.8^{A}_{a}$	$46.7\pm2.8^{A}_{b}$
300 MPa	$52.1\pm0.1^{B}_{a}$	$51.9\pm0.3^{B}_{a}$	$50.7\pm1.9^{B}_{b}$	$53.6\pm1.4^{B}_{a}$	$52.8\pm2.5^{B}_{a}$	$49.3\pm4.3^{B}_{b}$
400 MPa	$54.9\pm1.7^{C}_{a}$	$53.9\pm0.3^{C}_{a}$	$51.3\pm2.6^{C}_{b}$	$56.5\pm1.0^{C}_{a}$	$55.7\pm3.3^{C}_{a}$	$54.2\pm0.8^{C}_{b}$
a*						
200 MPa	$12.6\pm0.2^{A}_{a}$	$12.4\pm0.9^{A}_{b}$	$11.4\pm2.0^{A}_{b}$	$11.5\pm0.3^{A}_{a}$	$12.3\pm0.1^{A}_{b}$	$11.2\pm1.2^{A}_{b}$
300 MPa	$11.9\pm0.9^{B}_{a}$	$10.6\pm1.0^{B}_{b}$	$10.0\pm1.4^{B}_{b}$	$11.5\pm0.6^{B}_{a}$	$10.0\pm0.4^{B}_{b}$	$10.2\pm0.6^{B}_{b}$
400 MPa	$10.6\pm0.7^{C}_{a}$	$9.3\pm0.9^{C}_{b}$	$9.2\pm0.8^{C}_{b}$	$10.0\pm0.5^{C}_{a}$	$8.8\pm0.9^{C}_{b}$	$9.4\pm0.3^{C}_{b}$
b*						
200 MPa	$29.3\pm0.4_{a}$	$29.2\pm1.3_{b}$	$27.6\pm2.7_{c}$	$28.4\pm0.4_{a}$	$27.8\pm0.6_{b}$	$26.4\pm0.6_{c}$
300 MPa	$28.9\pm1.3_{a}$	$27.5\pm1.0_{b}$	$25.5\pm1.5_{c}$	$29.2\pm0.2_{a}$	$26.9\pm0.7_{b}$	$26.9\pm1.2_{c}$
400 MPa	$28.4\pm0.3_{a}$	$27.1\pm0.2_{b}$	$26.8\pm1.3_{c}$	$27.8\pm0.1_{a}$	$27.2\pm1.3_{b}$	$26.5\pm0.8_{c}$

Table 3.3 (cont'd)

ΔE						
200 MPa	$24.5\pm1.2^{A}_{a}$	$24.7\pm1.0^{A}_{a}$	$24.0\pm1.8^{A}_{b}$	$23.1\pm0.8^{A}_{a}$	$22.4\pm0.7^{A}_{a}$	$26.9\pm2.6^{A}_{b}$
300 MPa	$22.1\pm0.5^{B}_{a}$	$21.8\pm0.0^{B}_{a}$	$22.8\pm1.7^{B}_{b}$	$20.7\pm1.5^{B}_{a}$	$20.8\pm2.4^{B}_{a}$	$24.3\pm4.1^{B}_{b}$
400 MPa	$19.1\pm1.7^{C}_{a}$	$19.7\pm0.3^{C}_{a}$	$22.2\pm2.4^{C}_{b}$	$17.3\pm1.0^{C}_{a}$	$18.0\pm3.1^{C}_{a}$	$19.4\pm0.8^{C}_{b}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All L*, a*, b*, ΔE values are the mean ± standard deviation of nine replicates (n=9)

3 L*, a*, b* and ΔE values of unpressurised mackerel group were 42.0±0.2, 12.0±1.3, 24.9±2.2 and 31.6±0.4, respectively.

Table 3.4 The effect of heat, pressure and time processing on L*, a*, b*, ΔE color values of red mullet flesh immediately after processing (<4 h at 4±0.5°C) [1,2,3].

	5 minutes			15 minutes		
	5°C	10°C	15°C	5°C	10°C	15°C
L*						
200 MPa	$41.1\pm0.1^{A}_{a}$	$41.1\pm1.0^{A}_{b}$	$42.3\pm0.5^{A}_{c}$	$44.4\pm0.5^{A}_{a}$	$42.1\pm0.9^{A}_{b}$	$43.1\pm1.7^{A}_{c}$
300 MPa	$49.0\pm1.6^{B}_{a}$	$45.1\pm0.7^{B}_{b}$	$45.2\pm0.8^{B}_{c}$	$49.3\pm0.8^{B}_{a}$	$47.0\pm0.3^{B}_{b}$	$48.7\pm1.3^{B}_{c}$
400 MPa	$50.8\pm1.4^{C}_{a}$	$48.1\pm0.7^{C}_{b}$	$51.0\pm1.3^{C}_{c}$	$53.6\pm0.9^{C}_{a}$	$50.7\pm0.9^{C}_{b}$	$53.0\pm0.2^{C}_{c}$
a*						
200 MPa	9.2±1.1	9.6±0.9	8.4±0.5	7.3±1.0	8.8±0.5	9.0±2.1
300 MPa	7.7±0.6	8.8±1.0	8.2±1.2	8.3±0.4	7.1±1.1	8.3±1.0
400 MPa	8.3±0.6	9.2±1,6	6.7±0.9	7.9±1.5	8.8±1.0	7.0±0.5
b*						
200 MPa	21.4±1.5	23.2±1.4	22.4±0.5	21.3±1.1	24.2±0.6	23.5±2.3
300 MPa	22.9±1.1	21.9±1.2	22.4±1.1	23.5±1.2	24.0±1.3	23.4±0.9
400 MPa	23.7±0.7	22.9±1.1	22.5±1.1	24.2±0.7	25.4±1.7	23.4±0.2
ΔE						
200 MPa	$31.8\pm0.2^{A}_{a}$	$31.7\pm1.0^{A}_{b}$	$30.4\pm0.5^{A}_{a}$	$28.5\pm0.6^{A}_{a}$	$30.6\pm0.9^{A}_{b}$	$29.7\pm1.8^{A}_{a}$
300 MPa	$23.8\pm1.6^{B}_{a}$	$27.7\pm0.7^{B}_{b}$	$27.6\pm0.8^{B}_{a}$	$23.4\pm0.7^{B}_{a}$	$25.7\pm0.2^{B}_{b}$	$24.0\pm1.3^{B}_{a}$
400 MPa	$21.9\pm1.4^{C}_{a}$	$24.7\pm0.7^{C}_{b}$	$21.9\pm1.3^{C}_{a}$	$19.2\pm0.9^{C}_{a}$	$22.1\pm0.8^{C}_{b}$	$19.8\pm0.2^{C}_{a}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All L*, a*, b*, ΔE values are the mean ± standard deviation of nine replicates (n=9)

3 L*, a*, b* and ΔE values of unpressurised mullet group were 34.2±1.3, 8.9±1.5, 17.6±0.6 and 39.1±1.4, respectively.

3.2 Results of Shelf Life Analyses

Atlantic mackerel was treated with 200 MPa, 15°C, 5 min. and 400 MPa, 5°C, 5 min. while red mullet was treated with 200 MPa, 15°C, 5 min. combinations for shelf life analysis and all samples were stored in refrigerator at 4±0.5°C.

The threshold values of each parameter were specified in first chapter, part 1.5. Nevertheless, these limits were repeated at the statements under the tables.

In the case the values of the sample exceeded the specified threshold values twice in the row, signified experiment was terminated due to the aim of determining the shelf life. In contradiction to, even if the amount of matter of the sample did not exceed the specified limits, the analysis was terminated at the end of the 21st day.

3.2.1 Results of Chemical and Microbiological Analyses

3.2.1.1 Results of TMA-N Analyses

TMA-N contents of the pressurized and non-pressurized mackerel samples during 21 day of refrigerated storage were shown in Table 3.5. TMA values were increased in the following order 400 MPa treated<200 MPa treated<Control samples. This rate of increase continued for the duration of the experiment. Although the control group has a drastic increase of TMA-N number, 200 MPa pressurized samples had slower increase; moreover 400 MPa treated samples did never exceed the limit of acceptability. Accordingly, control samples were described as spoiled at 11th day of storage, and 200 MPa samples at 17th day of storage.

TMA-N values of the control and 200 MPa, 15°C, 5 min. treated mullet samples during 15 days of storage were shown in Table 3.6. A drastic increase was observed in both samples; however the acceleration of the control group was

higher. Control group samples exceeded the threshold value on 7th day, nevertheless pressure applied samples reached that level on 10th day of storage.

3.2.1.2 Results of TBA Analyses

TBA values of mackerel and mullet samples throughout the storage were shown in Table 3.5 and 3.6, respectively.

Unpressurised samples of fatty and lean fish showed lower ($p<0.05$) oxidation development than their counterpart of pressurized products at the 1st day. However, at the following days, in contrast to the TBA scores of control samples in which continuous increase was observed, fluctuations were observed in the results of pressurized samples especially after 400 MPa treatment. In literature it was noted that TBA records might not bring out the actual lipid oxidation rate due to the interaction between MA and other fish components such as amines, nucleosides, nucleic acids, proteins, amino acids of phospholipids or other aldehydes (Auburg, 1993; Simeonidou, Govaris, Vareltzis, 1998). The other limitation of the test was instability and impossibility of determining the organic alcohols and acids which were produced by oxidation of MDA and other short chain carbon products of lipid oxidation for a long period of time. (Ferněndez, Alvarez, Lopez, 1997)

Unpressurized mackerel exceeded the threshold values on 13th day, pressurized samples (both 200 and 400 MPa treated ones) did not exceed up to 21st day of analyses.

Both control and pressurized samples of lean fish (red mullet) did not transcend the limit of acceptability throughout the analysis. The reason of terminating the analyses on 15th day instead of extending to 21st day was being already spoiled when other parameters were evaluated.

3.2.1.3 Results of TVB-N Analyses

The changes in TVB-N values of mackerel were shown in Table 3.5. Goulas and Kontominas (2005) determined the initial value of unprocessed mackerel as 10.93±0.36 mg N/100 g. Mol et al. (2004) specified this level as 12.34±1.16 mg N/100 g, however researchers underlined that TVB-N level varied according to the place (such as first depot, second depot and the retail sellers) where the samples were received.

A drastic increase in the TVB-N values of unpressurised samples was detected especially on 9th day of storage. The threshold value was exceeded at the same day. Slow and regular increase in the TVB-N scores of 200MPa treated samples was observed throughout the shelf life analysis. Although fluctuations were noted, an increase in the TVB-N values of 400MPa treated samples was observed. At the 21th day of storage, both pressurized samples denoted as spoiled.

The results of TVB-N analyses of red mullet was illustrated in Table 3.6. TVB-N value of the untreated mullet samples were specified as 12.23mg/ 100g by Özyurt et al. (2009), 15.83±0.07mg/100g by Gümüş et al. (2008). The initial value of the study results coincided well with literature data.

The results of TVB-N analyses matched up with TMA values of the fish. High pressure application induced a significant decrease for these two parameters from the first day of storage. Control group and pressurized samples exceeded the critical limits on 7th and 13th day of storage respectively.

3.2.1.4 Results of pH Analyses

pH of the mackerel samples varied significantly in the literature. Goulas and Kontominas, (2005) and Mol et al. (2004) specified this value as 6.12±0.09, although it was indicated as 6.62±0.17 and 6.8±0.1 in other studies. (Venugopal

and Shahidi, 1994; Simeonidou et al., 1998). Similarly pH values of red mullet was specified in a wide range. Erkan et al. (2010) determined this score as 6.4±0.1, Gümüş et al. (2008) indicated as 6.67± 0.00, Simeonidou et al. (1998) specified as 6.92±0.10 and Özyurt et al. (2009) noted as 7.06. This difference could take its source from two reasons. The fishes were kept in -25°C until the beginning of the experiments. Freezing made the remaining solution more concentrated due to converting a large proportion of water into ice. The increased concentration changed the acid-base equilibrium (pH). Secondarily, increased concentration consist of slightly soluble salts, phosphate and other compounds. This phenomena can result in up to 2 point irreversible pH changes. (Love, 2006; Jiand and Lee, 2006)

The results of pH analyses of mackerel were shown in Table 3.5. pH of the 400 MPa treated atlantic mackerel muscle was higher than 200 MPa treated samples (except 7^{th},9^{th} and 11^{st} day of storage), probably due to the denaturation of some protein fractions. Unpressurised samples exceeded the limit on 7^{th} day, while 400 MPa treated ones reached this level on 21^{st} day of storage. 200 MPa treatment did not caused the samples to reach this critic value throughout the storage.

pH results of red mullet was indicated in Table 3.6. As stated above, the reason of higher initial scores of pressurized samples could arise from the denaturation of protein fractions. However, the excessive increase, that the control group exhibited, was not observed in the pressurized samples. Therefore, control group attained the critical limit on 7^{th} day, while pressurized samples reached that level on 10^{th} day of storage.

The effect of pressure on pH was stated in cod muscle before (Angsupanich and Ledward, 1998). It was well established that high pressure treatment killed microorganisms, some of which could be responsible from formation of volatile substances (amines, bases, acids etc.). Hence, lower and more stable pH could be expected. Nevertheless, it couldn't be evaluated alone, it had to be supported by

other analyses. Some studies underscored that pH examination was not useful for quality evaluation. (Shoji and Saeki, 1989; Spinelli, Eklund, Miyauchi, 1964; Ledward, 1995; Erkan and Ozden, 2008; Simeonidou et al., 1998).

3.2.1.5 Results of Total Mesophilic Aerobic Bacteria (TMAC) Analyses

At the beginning of the shelf life analyses, the microbial load of both control and pressurized samples were at undetectable levels (< 3.00 log cfu/g). However, as the shelf life lengthened out, the application of pressure slowed down the microbial growth as can be seen from the Table 3.5. Moreover, the retardation time was extended as the applied pressure increased. The results that led to this interpretation could be seen in every experiment day.

Hereunder, unpressurised control samples exceeded the limit at 11^{th}, 200 MPa, 15 °C, 5 min. processed ones at 17^{th} and 400 MPa, 5 °C, 5 min. processed samples at 19^{th} day of the analyses.

Total mesophilic aerobic bacteria count results for red mullet were illustrated in Table 3.6. The load was specified as 3.16±0.04 in the study of Gümüş et al. (2008) and 3.77±0.11 in the study of Erkan et al. (2010). Unpressurised samples reached the critical level on 10^{th} day, pressurized samples exceeded the level on 13^{th} day of storage.

These results confirmed the effect of high pressure on the reduction of microbial growth.

Table 3.5 The effect of treatment on TMA (mg/100g), TBA (mg MDA/kg), TVB-N (mg /100g), Total viable count (log cfu/g) and pH of atlantic mackerel flesh during refrigerated storage (at 4 ±0.5°C) [1,2,3,4].

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
TMA											
Control	$1.781\pm0.022^{A}_{a}$	$1.976\pm0.238^{A}_{a}$	$2.521\pm0,186^{A}_{a}$	$5.031\pm0.101^{A}_{b}$	$7.300\pm0.331^{A}_{c}$	$13.325\pm0.822^{A}_{d}$	$18.329\pm0.486^{A}_{e}$				
Process 1	$1.666\pm0.0634^{B}_{a}$	$1.383\pm0.314^{B}_{a}$	$1.378\pm0.006^{B}_{a}$	$1.430\pm0.056^{B}_{b}$	$1.808\pm0.097^{B}_{c}$	$2.825\pm0.290^{B}_{d}$	$4.119\pm0.078^{B}_{e}$	$5.320\pm0.084^{B}_{f}$	$10.383\pm0.318^{B}_{g}$	$11.148\pm0.294^{B}_{g}$	
Process 2	$0.909\pm0.024^{C}_{a}$	$0.972\pm0.052^{C}_{a}$	$0.977\pm0.033^{C}_{a}$	$1.236\pm0.121^{C}_{b}$	$1.582\pm0.113^{C}_{c}$	$1.328\pm0.159^{C}_{d}$	$1.341\pm0.077^{C}_{e}$	$1.364\pm0.113^{C}_{f}$	$2.050\pm0.101^{C}_{g}$	$2.098\pm0.233^{C}_{g}$	$2.219\pm0.071^{C}_{h}$
TBA											
Control	$0.455\pm0.012^{A}_{a}$	$0.510\pm0.03^{A}_{a}$	$0.595\pm0.078^{A}_{a}$	$0.722\pm0.187^{A}_{b}$	$0.835\pm0.190^{A}_{b}$	$0.875\pm0.360^{A}_{b}$	$1.293\pm0.102^{A}_{c}$	$1.906\pm0.093^{A}_{d}$			
Process 1	$0.472\pm0.064^{B}_{a}$	$0.333\pm0.019^{B}_{a}$	$0.388\pm0.020^{B}_{a}$	$0.409\pm0.017^{B}_{b}$	$0.422\pm0.049^{B}_{b}$	$0.439\pm0.073^{B}_{b}$	$0.451\pm0.008^{B}_{c}$	$0.480\pm0.005^{B}_{d}$	$0.489\pm0.034^{B}_{d}$	$0.538\pm0.014^{B}_{d}$	$0.549\pm0.025^{B}_{d}$
Process 2	$0.506\pm0.026^{C}_{a}$	$0.512\pm0.141^{C}_{a}$	$0.576\pm0.025^{C}_{a}$	$0.930\pm0.168^{C}_{b}$	$0.611\pm0.133^{C}_{b}$	$0.668\pm0.058^{C}_{b}$	$0.691\pm0.118^{C}_{c}$	$0.579\pm0.021^{C}_{d}$	$0.521\pm0.026^{C}_{d}$	$0.543\pm0.018^{C}_{d}$	$0.566\pm0.022^{C}_{d}$
TVB-N											
Control	$10.723\pm0.384^{A}_{a}$	$11.739\pm0.216^{A}_{a}$	$15.197\pm1.505^{A}_{a}$	$24.889\pm2.516^{A}_{a}$	$72.952\pm7.191^{A}_{b}$	$96.705\pm8.573^{A}_{c}$					
Process 1	$10.458\pm2.646^{B}_{a}$	$11.243\pm1.016^{B}_{a}$	$14.230\pm0.945^{B}_{a}$	$14.648\pm0.274^{B}_{a}$	$16.604\pm0.157^{B}_{b}$	$17.950\pm0.477^{B}_{c}$	$20.102\pm0.706^{B}_{d}$	$24,023\pm1.739^{B}_{d}$	$22.896\pm6.734^{B}_{d}$	$32.259\pm2.154^{B}_{e}$	$36.440\pm1.262^{B}_{e}$
Process 2	$11.881\pm0.717^{B}_{a}$	$15.085\pm0.864^{B}_{a}$	$18.685\pm1.851^{B}_{a}$	$16.344\pm0.055^{B}_{a}$	$16.520\pm1.715^{B}_{b}$	$17.005\pm0.291^{B}_{c}$	$17.471\pm0.067^{B}_{d}$	$17.718\pm0.365^{B}_{d}$	$20.364\pm1.208^{B}_{d}$	$34.830\pm5.975^{B}_{e}$	$40.801\pm0.867^{B}_{e}$
TMAC											
Control	$< 3.00\pm0.15^{A}_{a}$	$3.71\pm0.09^{A}_{b}$	$4.42\pm0.01^{A}_{c}$	$4.90\pm0.09^{A}_{c}$	$5.69\pm0.04^{A}_{d}$	$6.43\pm0.03^{A}_{e}$	$6.62\pm0.22^{A}_{e}$				
Process 1	$< 3.00\pm0.02^{B}_{a}$	$3.27\pm0.563^{B}_{b}$	$3.40\pm0.092^{B}_{c}$	$3.49\pm0.234^{B}_{c}$	$4.68\pm0.265^{B}_{d}$	$5.14\pm0.295^{B}_{e}$	$5.03\pm0.100^{B}_{e}$	$5.43\pm0.069^{B}_{f}$	$6.30\pm0.072^{B}_{g}$	$6.41\pm0.052^{B}_{g}$	
Process 2	$< 3.00\pm0.19^{C}_{a}$	$< 3.00\pm0.22^{C}_{b}$	$3.20\pm0.06^{C}_{c}$	$3.35\pm0.03^{C}_{c}$	$3.88\pm0.13^{C}_{d}$	$4.12\pm0.09^{C}_{e}$	$4.44\pm0.08^{C}_{e}$	$4.63\pm0.09^{C}_{f}$	$5.65\pm0.2^{C}_{g}$	$6.03\pm0.12^{C}_{g}$	$6.42\pm0.19^{C}_{g}$
pH											
Control	$6.85\pm0.04^{A}_{a}$	$6.93\pm0.01^{A}_{b}$	$6.95\pm0.01^{A}_{c}$	$7.05\pm0.03^{A}_{d}$	$7.11\pm0.04^{A}_{e}$	$7.19\pm0.10^{A}_{e}$					
Process 1	$6.31\pm0.01^{B}_{a}$	$6.33\pm0.03^{B}_{b}$	$6.43\pm0.02^{B}_{c}$	$6.78\pm0.01^{B}_{d}$	$6.79\pm0.04^{B}_{e}$	$6.95\pm0.01^{B}_{e}$	$6.85\pm0.05^{B}_{f}$	$6.89\pm0.05^{B}_{g}$	$6.89\pm0.02^{B}_{g}$	$6.96\pm0.02^{B}_{g}$	$6.99\pm0.04^{B}_{g}$
Process 2	$6.40\pm0.02^{C}_{a}$	$6.47\pm0.01^{C}_{b}$	$6.65\pm0.02^{C}_{c}$	$6.76\pm0.03^{C}_{d}$	$6.41\pm0.02^{C}_{e}$	$6.20\pm0.03^{C}_{e}$	$6.58\pm0.07^{C}_{f}$	$6.93\pm0.01^{C}_{g}$	$6.97\pm0.01^{C}_{g}$	$6.97\pm0.02^{C}_{g}$	$7.02\pm0.02^{C}_{g}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All TMA, TBA, TVB-N, TVC and pH values are the mean ± standard deviation of three replicates (n=3)

3 Control : Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment, Process 2: 400 MPa, 5°C, 5 min. treatment

4 10 mg TMA-N/100g, 1 mg MDA/kg, 35 mg TVB-N/100 g, 6 log cfu/g and pH 7.0 were designated as limit values for consumption.

Table 3.6 The effect of treatment on TMA (mg/100g), TBA (mg MDA/kg), TVB-N (mg /100g), Total viable count (log cfu/g) and pH of red mullet flesh during refrigerated storage (at 4 ±0.5°C) [1,2,3,4].

	Day 1	Day 4	Day 7	Day 10	Day 13	Day 15
TMA						
Control	$3.417\pm0.219_a$	$6.963\pm0.617_b$	$10.126\pm0.450_c$	$18.807\pm2.864_d$		
Process 1	$1.704\pm0.05_a$	$5.434\pm0.282_b$	$7.453\pm1.444_c$	$12.709\pm1.063_d$	$18.339\pm0.445_e$	
TBA						
Control	$0.216\pm0.004_a$	$0.245\pm0.014_b$	$0.287\pm0.004_c$	$0.336\pm0.018_d$	$0.319\pm0.016_d$	$0.388\pm0.007_e$
Process 1	$0.266\pm0.014_a$	$0.294\pm0.019_b$	$0.321\pm0.005_c$	$0.335\pm0.006_d$	$0.349\pm0.006_d$	$0.372\pm0.016_e$
TVB-N						
Control	$13.144\pm0.119_a$	$26.839\pm1.576_b$	$35.383\pm0.610_c$	$42.693\pm1.802_d$		
Process 1	$8.735\pm0.496_a$	$17.528\pm1.955_b$	$29.933\pm2.497_c$	$31.365\pm1.440_d$	$38.884\pm2.981_d$	$45.467\pm1.527_e$
pH						
Control	$6.17\pm0.02_a$	$6.38\pm0.06_b$	$7.13\pm0.03_c$	$7.11\pm0.01_d$		
Process 1	$6.63\pm0.04_a$	$6.76\pm0.02_b$	$6.88\pm0.05_c$	$7.05\pm0.07_d$	$7.14\pm0.02_d$	
TMAC						
Control	$3.14\pm0.11_a$	$3.81\pm0.05_b$	$5.07\pm0.14_c$	$6.15\pm0.10_d$	$7.47\pm0.12_e$	
Process 1	$<3.00\pm0.28_a$	$3.28\pm0.15_b$	$4.16\pm0.11_c$	$5.37\pm0.10_d$	$6.68\pm0.10_e$	$7.31\pm0.06_e$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All TMA, TBA, TVB-N, TVC and pH values are the mean ± standard deviation of three replicates (n=3)

3 Control : Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment

4 10 mg TMA-N/100g, 1 mg MDA/kg, 35 mg TVB-N/100 g, pH 7.0 and 6 log cfu/g were designated as limit values for consumption.

3.2.1.6 Results of Histamine Analyses

Histamine formation was studied up to 20 days of refrigerated storage of atlantic mackerel and was shown in Table 3.7. In literature untreated mackerel samples had a histamine value of 14.40±4.30 (Mol et al., 2004). A great increase in histamine formation of unpressurised samples was observed after in 12th day of storage. Such a large increase was observed on the 20th day for 200 MPa pressurized samples. The histamine concentration of 400 MPa pressurized samples did not exceeded the critical level until the end of the experiment. The reason of lower histamine concentrations than the control group could be reduced histidine decarboxylase activity. Results were consistent with the literature. It had been established that pressures above 300 MPa reduced the enzyme activity to a level of 55% residual activity (Santibanez, 2007).

Table 3.7 The effect of treatment on Histamine values (ppm) of atlantic mackerel flesh during refrigerated storage (at 4 ±0.5°C) [1,2,3,4].

	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	$14.642\pm1.088^{A}_{a}$	$16.810\pm0.888^{A}_{a}$	$21.019\pm3.653^{A}_{a}$	$81.015\pm9.338^{A}_{b}$	$96.332\pm4.944^{A}_{c}$	
Process 1	$6.932\pm0.960^{B}_{a}$	$12.566\pm1.579^{B}_{a}$	$18.087\pm0.360^{B}_{a}$	$39.648\pm2.859^{B}_{b}$	$48.846\pm4.204^{B}_{c}$	$89.269\pm4.662^{B}_{d}$
Process 2	$5.303\pm0.684^{C}_{a}$	$4.638\pm0.320^{C}_{a}$	$10.535\pm0.546^{C}_{a}$	$16.314\pm1.054^{C}_{b}$	$22.132\pm1.012^{C}_{c}$	$37.657\pm1.685^{C}_{d}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All Histamine values are the mean ± standard deviation of three replicates (n=3)

3 Control: Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment, Process 2: 400MPa, 5°C, 5 min. treatment.

4 Histamine concentrations of foods exceeding 50 ppm (5mg/100g fish) are hazardous.

3.2.2 Results of Color Measurements

Due to the affecting light scattering, fish fillet color is correlated with heme based pigment, physical structure of muscle and amount of unbound water. Additionally, modifications of protein matrix, enzymatic and non-enzymatic reactions which occur in consequence of myofibrillar protein degradation and disorganization of myofibrils, can cause color changes during storage (Chéret, Chapleau, Ladrat, Bagnis, Lamballerie, 2005).

Table 3.8 and Table 3.9 indicated change in HUNTER LAB color scale of atlantic mackerel and red mullet respectively, according to the intensity of pressure and to the time of storage.

Lightness, redness and yellowness of the unpressurised mackerel samples increased with increasing storage time. Therefore, ΔE decreased with time as expected. All color values of 200 MPa treated samples showed fluctuations. Redness and ΔE values increased with increasing time, on the other hand lightness decreased with time.

Lightness and the yellowness of the control group of mullet increased, ΔE value decreased with increasing time. Trends of increase and decrease were similar in control group of mackerel. Pressurized samples showed increase in lightness and redness, however ΔE values decreased with increasing shelf life.

Table 3.8 The effect of treatment on L*, a*, b* and ΔE of atlantic mackerel flesh during refrigerated storage (at 4 ±0.5°C) [1,2,3].

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
L*											
Control	$28.1\pm0.5^{A}_{a}$	$31.1\pm0.3^{A}_{a}$	$32.6\pm1.0^{A}_{a}$	$36.1\pm1.7^{A}_{b}$	$33.4\pm1.2^{A}_{b}$	$34.0\pm1.0^{A}_{b}$	$40.6\pm2.6^{A}_{c}$	$43.7\pm0.5^{A}_{d}$			
Process 1	$42.5\pm0.6^{B}_{a}$	$36.1\pm0.3^{B}_{a}$	$33.8\pm0.3^{B}_{a}$	$35.0\pm0.7^{B}_{b}$	$35.4\pm0.9^{B}_{b}$	$34.3\pm0.8^{B}_{b}$	$36.4\pm0.5^{B}_{c}$	$39.2\pm1.5^{B}_{d}$	$41.9\pm1.9^{B}_{d}$	$41.2\pm0.4^{B}_{d}$	$39.5\pm1.1^{B}_{d}$
Process 2	$51.3\pm0.2^{C}_{a}$	$52.4\pm0.6^{C}_{a}$	$51.4\pm0.5^{C}_{a}$	$51.5\pm0.2^{C}_{b}$	$51.8\pm1.6^{C}_{b}$	$49.7\pm0.5^{C}_{b}$	$50.3\pm0.7^{C}_{c}$	$49.1\pm0.2^{C}_{d}$	$48.2\pm0.5^{C}_{d}$	$46.0\pm0.6^{C}_{d}$	$44.5\pm0.7^{C}_{d}$
a*											
Control	7.6 ± 1.0^{A}	8.2 ± 1.0^{A}	11.9 ± 1.5^{A}	13.7 ± 1.8^{A}	11.1 ± 0.3^{A}	12.1 ± 0.3^{A}	14.8 ± 2.3^{A}	14.9 ± 1.8^{A}			
Process 1	11.1 ± 1.1^{A}	10.2 ± 0.4^{A}	9.7 ± 2.5^{A}	10.2 ± 1.4^{A}	13.6 ± 2.0^{A}	13.0 ± 0.9^{A}	13.6 ± 0.3^{A}	14.8 ± 0.8^{A}	15.0 ± 4.5^{A}	14.4 ± 0.5^{A}	13.4 ± 0.7^{A}
Process 2	11.0 ± 1.1^{B}	11.7 ± 0.8^{B}	14.3 ± 0.6^{B}	13.3 ± 0.3^{B}	12.7 ± 1.2^{B}	11.6 ± 0.9^{B}	13.8 ± 0.8^{B}	13.9 ± 0.1^{B}	13.9 ± 0.4^{B}	15.6 ± 0.7^{B}	15.8 ± 0.4^{B}
b*											
Control	$19.7\pm0.7^{A}_{a}$	$22.2\pm1.1^{A}_{a}$	$24.3\pm0.2^{A}_{a}$	$28.6\pm4.2^{A}_{a}$	$25.4\pm1.0^{A}_{a}$	$24.5\pm1.6^{A}_{a}$	$30.7\pm2.6^{A}_{b}$	$34.4\pm0.7^{A}_{b}$			
Process 1	$24.1\pm0.6^{B}_{a}$	$21.7\pm0.7^{B}_{a}$	$20.5\pm1.2^{B}_{a}$	$20.9\pm1.0^{B}_{a}$	$21.7\pm0.8^{B}_{a}$	$21.1\pm0.7^{B}_{a}$	$23.8\pm0.3^{B}_{b}$	$24.2\pm1.2^{B}_{b}$	$25.8\pm1.5^{B}_{c}$	$25.2\pm0.7^{B}_{c}$	$22.7\pm0.5^{B}_{c}$
Process 2	$27.3\pm1.7^{A}_{a}$	$26.4\pm1.8^{A}_{a}$	$27.0\pm0.5^{A}_{a}$	$26.0\pm0.1^{A}_{a}$	$26.0\pm1.3^{A}_{a}$	$25.8\pm0.1^{A}_{a}$	$26.3\pm1.2^{A}_{b}$	$26.2\pm0.3^{A}_{b}$	$25.8\pm0.6^{A}_{c}$	$25.4\pm0.2^{A}_{c}$	$25.5\pm0.2^{A}_{c}$
ΔE											
Control	$43.8\pm0.4^{A}_{a}$	$39.9\pm0.4^{A}_{a}$	$38.5\pm1.1^{A}_{a}$	$35.5\pm1.9^{A}_{b}$	$37.6\pm1.2^{A}_{b}$	$37.1\pm0.9^{A}_{b}$	$31.5\pm2.7^{A}_{c}$	$29.5\pm0.5^{A}_{c}$			
Process 1	$29.4\pm0.7^{B}_{a}$	$35.7\pm0.2^{B}_{a}$	$38.1\pm0.4^{B}_{a}$	$36.9\pm0.6^{B}_{b}$	$36.7\pm0.8^{B}_{b}$	$37.8\pm0.9^{B}_{b}$	$35.6\pm0.4^{B}_{c}$	$33.1\pm1.3^{B}_{c}$	$30.8\pm2.0^{B}_{c}$	$31.1\pm0.3^{B}_{c}$	$32.6\pm1.0^{B}_{c}$
Process 2	$20.9\pm0.3^{C}_{a}$	$19.7\pm0.8^{C}_{a}$	$21.3\pm0.5^{C}_{a}$	$20.8\pm0.1^{C}_{b}$	$20.5\pm1.5^{C}_{b}$	$22.3\pm0.4^{C}_{b}$	$22.2\pm0.4^{C}_{c}$	$23.3\pm0.2^{C}_{c}$	$24.1\pm0.4^{C}_{c}$	$26.7\pm0.6^{C}_{c}$	$28.3\pm0.9^{C}_{c}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$. 2 All L*, a*, b* and ΔE values are the mean ± standard deviation of nine replicates (n=9)3 Control: Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment, Process 2: 400 MPa, 5°C, 5min. treatment

Table 3.9 The effect of treatment on L*, a*, b* and ΔE of red mullet flesh during refrigerated storage (at 4 ±0.5°C) [1,2,3].

	Day 1	Day 4	Day 7	Day 10	Day 13	Day 15
L*						
Control	$36.9\pm0.4_a$	$37.4\pm0.5_a$	$37.9\pm1.4_a$	$40.2\pm1.7_b$	$42.5\pm2.5_b$	$40.9\pm0.9_b$
Process 1	$40.5\pm0.8_a$	$41.5\pm1.5_a$	$42.0\pm0.2_a$	$44.7\pm0.5_b$	$45.0\pm0.7_b$	$45.8\pm0.4_b$
a*						
Control	$6.7\pm0.6_a$	$7.7\pm0.7_b$	$7.5\pm0.6_b$	$8.6\pm0.4_b$	$7.2\pm2.4_b$	$7.3\pm0.4_b$
Process 1	$10.0\pm1.2_a$	$13.6\pm1.8_b$	$13.1\pm1.2_b$	$13.1\pm0.4_b$	$15.5\pm0.8_b$	$15.9\pm0.7_b$
b*						
Control	$19.3\pm0.9_a$	$19.9\pm0.6_a$	$20.9\pm0.6_a$	$24.2\pm0.7_b$	$26.9\pm2.3_b$	$25.6\pm1.1_b$
Process 1	$25.2\pm1.4_a$	$26.8\pm3.1_a$	$24.6\pm0.5_a$	$26.7\pm0.3_b$	$26.4\pm0.5_b$	$26.3\pm0.2_b$
ΔE						
Control	$35.5\pm0.6_a$	$34.8\pm0.5_a$	$34.2\pm1.4_a$	$31.5\pm1.7_b$	$29.3\pm2.4_b$	$30.8\pm0.9_b$
Process 1	$31.1\pm0.9_a$	$30.4\pm1.4_a$	$29.7\pm0.2_a$	$27.1\pm0.5_b$	$27.2\pm0.8_b$	$26.4\pm0.4_b$

1 For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All L*, a*, b* and ΔE values are the mean ± standard deviation of nine replicates (n=9)

3 Control: Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment

3.2.3 Results of Sensory Analyses

For both types of samples, decreasing scores in appearance and odor attributes were obtained with increasing time (Table 3.10). The results corroborated the chemical and microbiological analyses. The pictures of fish on specific days of storage were in Appendix D.

As determined with the numerical results in the color analyses, pressure caused the fish flesh a cooked appearance. These findings were consistent with another study which specified that during 5 minutes process with higher than 150-200 MPa pressure, a cooked appearance of mackerel was obtained (Matser, Stegeman, Kals, Bartels, 2000). According to the panelists of this study, this aspect did not appeal to them, conversely caused them to give lower scores to the pressurized samples at the beginning of the shelf life analysis. Similarly, the reason of lower odor remarks of pressurized samples at the 1st day was due to the chemical smell after pressurization process.

Although the control samples of mackerel had a strong deteriorated fish odor, both of the pressurized samples did not have this kind of intense odor towards the end of the shelf life analysis according to panelist evaluations. Instead, a slight off-odor was reported at the 17th day of storage for 200 MPa pressurized samples. Any fishy smell was not reported for 400 MPa pressurized samples until 21st day.

Control group samples were evaluated as spoiled at 9th day, however pressurized samples exceeded the limits on 17th and 19th days.

The results of red mullet were illustrated in Table 3.11. Although pressurized samples had higher scores for appearance analysis, both pressurized and unpressurised samples exceeded the threshold levels on 10th day. The situation

was different for the results of odor; control samples reached the critical levels on 7^{th} day, however pressurized sample never reached the limit values throughout the storage.

Table 3.10 The effect of treatment on sensory evaluation of appearance and odor of mackerel flesh during refrigerated storage (at 4±0.5°C) [1,2,3,4].

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
Appearance											
Control	$9.9\pm0.08^{A}_{a}$	$8.2\pm0.75^{A}_{b}$	$6.5\pm0.6^{A}_{c}$	$4.1\pm0.62^{A}_{d}$	$2.7\pm0.82^{A}_{e}$	$1.8\pm0.61^{A}_{f}$					
Process 1	$9.5\pm0.38^{B}_{a}$	$9.4\pm0.25^{B}_{b}$	$9.1\pm0.38^{B}_{c}$	$8.6\pm0.35^{B}_{d}$	$7.8\pm0.57^{B}_{e}$	$6.7\pm1.08^{B}_{f}$	$5.7\pm0.69^{B}_{g}$	$4.9\pm0.57^{B}_{h}$	$3.8\pm0.58^{B}_{h}$	$3.2\pm0.39^{B}_{h}$	$2.4\pm0.79^{B}_{h}$
Process 2	$9.2\pm0.28^{C}_{a}$	$9.2\pm0.34^{C}_{b}$	$9.0\pm0.31^{C}_{c}$	$8.7\pm0.22^{C}_{d}$	$8.4\pm0.18^{C}_{e}$	$7.8\pm0.42^{C}_{f}$	$6.1\pm0.66^{C}_{g}$	$5.1\pm0.50^{C}_{h}$	$4.0\pm0.35^{C}_{h}$	$3.7\pm0.47^{C}_{h}$	$3.2\pm0.48^{B}_{h}$
Odor											
Control	$9.9\pm0.10^{A}_{a}$	$8.5\pm0.67^{A}_{b}$	$5.9\pm1.00^{A}_{c}$	$3.7\pm0.43^{A}_{d}$	$2.1\pm0.52^{A}_{e}$						
Process 1	$9.7\pm0.22^{B}_{a}$	$9.3\pm0.18^{B}_{b}$	$9.3\pm0.2^{B}_{c}$	$9.1\pm0.15^{B}_{d}$	$8.9\pm0.13^{B}_{e}$	$8.3\pm0.32^{B}_{f}$	$7.8\pm0.21^{B}_{g}$	$5.9\pm1.1^{B}_{h}$	$4.4\pm1.01^{B}_{i}$	$3.4\pm0.46^{B}_{j}$	$2.3\pm0.67^{B}_{k}$
Process 2	$9.3\pm0.18^{C}_{a}$	$9.3\pm0.15^{C}_{b}$	$9.1\pm0.12^{C}_{c}$	$8.9\pm0.12^{C}_{d}$	$8.5\pm0.28^{C}_{e}$	$8.1\pm0.16^{C}_{f}$	$8.0\pm0.29^{C}_{g}$	$7.4\pm0.38^{C}_{h}$	$6.7\pm0.38^{C}_{i}$	$5.0\pm0.97^{C}_{j}$	$4.6\pm1.0^{C}_{k}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All appearance and odor values are the mean ± standard deviation of six replicates (n=6)

3 Control: Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment, Process 2: 400 MPa, 5°C, 5 min. treatment

4 The score ≤ 3.9 was denoted as spoiled for both analyses.

Table 3.11 The effect of treatment on sensory evaluation of appearance and odor of red mullet flesh during refrigerated storage (at 4±0.5°C)[1,2,3,4].

	Day 1	Day 4	Day 7	Day 10	Day 13	Day 15
Appearance						
Control	$9.8\pm0.2_{a}$	$5.4\pm0.81_{b}$	$4.4\pm0.44_{c}$	$2.9\pm0.5_{d}$	$1.4\pm0.4_{e}$	
Process 1	$9.3\pm0.38_{a}$	$8.5\pm0.48_{b}$	$6.2\pm0.38_{c}$	$3.7\pm0.57_{d}$	$3.3\pm0.45_{e}$	
Odor						
Control	$9.7\pm0.21_{a}$	$3.9\pm0.29_{b}$	$2.8\pm0.51_{c}$	$2.1\pm0.52_{d}$		
Process 1	$9.5\pm0.33_{a}$	$8.6\pm0.43_{b}$	$7.1\pm0.66_{c}$	$6.0\pm0.37_{d}$	$5.4\pm0.43_{e}$	$4.3\pm0.61_{f}$

1 For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$.

2 All appearance and odor values are the mean ± standard deviation of six replicates (n=6)

3 Control: Unpressurised, Process 1: 200 MPa, 15°C, 5 min. treatment.

4 The score ≤ 3.9 was denoted as spoiled for both analyses.

3.3 Summary of Experimental Results

In literature vacuum packaged fresh red mullet had a shelf life of 7 days at refrigerated storage. Salted (20%) and ice stored red mullet had 11 days of shelf life (Gümüş et al., 2008; Özyurt et al., 2009).

TMA-N, TVB-N, pH and odor values of untreated mullet products reached the threshold values on 7th day of storage, although values of TMAC and appearance denoted as spoiled on 10th day and TBA values never reached the critical limit. In accordance with present literature information on red mullet, the present study determined the shelf life of untreated samples as 7 days.

Whereas TMA-N, pH and appearance values of 200 MPa-15°C-5 min. treated mullet samples exceeded the critical limits within 10 days although TVB-N and TMAC levels reached the limits after 13 days. Similar to the control group, TBA values never reached that level. It could be underscored that TBA levels of lean fish were not used as an indicator for deterioration. Until it reached a critical level, fish would already be spoiled due to other parameters.

Overall pressurization treatment extended the shelf life of *Mullus barbatus* for 3 days (from 1 week to 10 days).

Pre-treatment with diluted quince-polyphenolic extract brought the mackerel sample 11 days of shelf life (Fattouch et al., 2008). Mackerel samples preserved in ice had 9 days of shelf life (Jhaveri et al., 1982).

pH and odor values of the control group mackerel samples exceeded the critical limits on 7th day. Appearance and TVB-N values on 9th day, TMA, TMAC values on 11th day, histamine values on 12th day, TBA levels reached that threshold values on 13th day of storage. Consequently, unpressurised samples could be denoted as spoiled after 7 days of storage.

TMA, TMAC and appearance level of the 200 MPa-15°C-5 min. treated sample reached the limits at 17th day, while odor values at 19th day, histamine level on 20th day and TVB-N, pH values exceeded that critical levels on 21st day of storage.

TMAC and appearance values of 400 MPa-5°C-5 min. treated ones reached the limits on 19th day and TVB-N levels on 21st day while the rest did not exceed the threshold values.

When all the parameters were evaluated; the unpressurised *Scomber scombrus* were acceptable up to only 7 days compared to 17 and 19 days at 200 MPa-15°C-5 min and 400 MPa-5°C-5 min treatments; respectively.

CHAPTER 4

CONCLUSION

The aim of the study was to analyze the effects of HHP treatment on quality factors and deterioration period of different fish species (atlantic mackerel and red mullet) and to propose more effective non-thermal technology to extend shelf life. To that end, several pressure-time-temperature combinations were applied to the foodstuff and freshness indicators were measured. In the first part of the study, best combinations, which were 200 MPa, 15°C, 5 min. and 400 MPa, 5°C, 5 min. for atlantic mackerel and 200 MPa, 15 °C, 5 min. for red mullet, were determined. In the second part, shelf life analyses were performed to samples which were treated with specified conditions. Eventually, 10-12 days and 3 days of shelf life extension were achieved for atlantic mackerel and red mullet, respectively; in accordance with the order of the treatment mentioned above. The reason for difference in shelf life extension of these species arose from the moisture and lipid content. Since internal moisture of red mullet was higher and lipid content was lower than atlantic mackerel, microorganisms could grow more rapidly. Therefore, protective effect of HHP was lower.

Ultimately, the whole research demonstrated that HHP treatment extended the shelf life of these two fish species. Especially for fatty fish, HHP can be recommended not only for industrial production but also for safe storage of fresh fish even at refrigeration temperatures.

CHAPTER 5

RECOMMENDATIONS

We underscored that the pressure treatment improved microbiological quality of atlantic mackerel flesh for 12 days although it extended the shelf life of red mullet 3 days. This period can be lengthen out with applying the optimum pressure, time, temperature combination, vacuum packaging technique, applying antioxidants, antimicrobial and non-hazardous coating materials, etc. The risk of microbial contamination and chemical deterioration caused by them were increased with inappropriate handling, processing practices and culinary preparations (Fattouch, Sadok, Raboudi-Fattouch, Slama, 2008). If we hold the fish under hygienic conditions as much as possible from the moment we caught them, the shelf life of the fish would be extended. Minced fish and fillets were more inclined to oxidation development than whole fish due to the more exposure of the fish muscle to oxygen (Aubourg et al., 2005). With the help of larger and economic high pressure chambers the industry can manufacture high quality fish products in addition to existing shell fish products. Tasting panel can also be added to sensory analyses with steam cooked fish.

REFERENCES

Amanatidou, A., Schluter, O., Lemkau, K., Gorris, L.G.M., Smid, E.J., Knorr, D. (2000). Effect of combined application of high pressure treatment and modified atmosphere on the shelf-life of fresh Atlantic salmon. *Innovative Food Science and Emerging Technologies*, *1*, 87–98.

Aminullah Bhuiyan, A. K. M., Ratnayake, W. M. N., Ackman, R. G. (1986). Effect of smoking on the proximate composition of atlantic mackerel (Scomber scombrus). *Journal of Food Science, 51*, 327

Angsupanich, K., Ledward, D. A. (1998). High pressure treatment effects on cod (Gadus morhua) muscle. *Food Chemistry*, *63*, 39–50.

Atar, H. H., Mete, T. (2009). Mersin Körfezi'nde dağılım gösteren barbunya balıklarının (Mullus Sp. Linnaeus, 1758) bazı biyolojik özelliklerinin incelenmesi. *Biyoloji Bilimleri Araştırma Dergisi, 2*, 29-34.

Aubourg, S. P. (1993) Review: interaction of malondialdehyde with biological molecules-new trends about reactivity and significance. ht. *J. Food Sci. Technol. 28,* 323-335.

Aubourg, S. (2001). Review: Loss of quality during the manufacture of canned fish products. *Food Sci Tech Int.*, *7*, 199–215.

Aubourg, S., Rodríguez, A., Gallardo, J. M. (2005). Rancidity development during frozen storage of mackerel (*Scomber scombrus*): Effect of catching season and commercial presentation. *European Journal of Lipid Science & Technology*, *107*, 316-323.

Aubourg, S., Munizaga, G. T., Reyes, J. E., Rodriguez, A., Won, M. P. (2010). Effect of high-pressure treatment on microbial activity and lipid oxidation in chilled coho salmon. *Eur. J. Lipid Sci. Technol.*, *112,* 362–372.

Baixas-Nogueras S., Bover-Cid S., Vidal-Carou M. C., Veciana-Nogués M. T., Mariné- Font A. (2001). Trimethylamine and total volatile basic nitrogen determination by Flow Injection/Gas Diffusion in Mediterranean hake (*Merluccius merluccius*). *J Agric Food Chem 49*(4), 1681-1686.

Barrett, E. L., Kwan, H. S. (1985). Bacterial reduction of trimethylamine oxide. *Ann. Rev. Microbiol., 39,* 131-149.

Büyükcan, M., Bozoğlu, F., Alpas, H. (2007). Preservation and shelf life extension of shrimps and clams by high hydrostatic pressure. *International Journal of Food Science and Technology, 44*, 1495-1502.

Castro, P., Padron, J. C. P., Cansino, M. J. C., Velazguez, E. S., Rafael, M. D. L. (2006). Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control, 17*, 245-248.

Cheah, P. B., Ledward, D. A. (1997). Catalytic mechanism of lipid oxidation following high pressure treatment in pork fat and meat. *Journal of Food Science, 62*, 1135.

Chéret, R., Chapleau, N., Ladrat, C. D., Bagnis, V. V., Lamballerie, M. (2005). Effects of high pressure on texture and microstructure of sea bass (Dicentrarchus labrax L.) fillets. *Journal of Food Science, 70*, 477-483.

Chéret, R., Aránzazu, H. A., Delbarre-Ladrat, C., de Lambarrie, M., Verrez-Bagnis, V. (2006). Proteins and proteolytic activity changes during refrigerated storage in sea bass (Dicentrarchus labrax L.) muscle after high-pressure treatment. *European Food Research and Technology* , *222*, 527–535.

Connell, J.J. (1975). *Control of fish quality*. Farnham, Surrey, UK, Fishing News (Books) Ltd.

Dokuzlu, C. (2004). *Gıda Analizleri* (2nd ed.). Bursa: Marmara Kitapevi Yayınları.

Doona, C. J., Feeherry, F. E. (Eds.). (2007). *High pressure processing of foods*. Iowa: IFT Press.

EEC (1995). Total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. Commission Decision 95/149/EEC of 8 March 1995, *Official Journal of European Communities, L97*, 84-87.

Erkan, N. (2005). Changes in quality characteristics during cold storage of shucked mussels (*Mytilus galloprovincialis*) and selected chemical decomposition indicators. *J Sci Food Agri, 85,* 2625–2630.

Erkan N, Ozden O. (2008). Quality assessment of whole and gutted sardine (Sardine pilchardus) stored in ice. *Int J Food Sci Technol, 43*(9), 1549–1559.

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 Technology, 4,* 1322-1329.

Erkan, N., Üretener, G., Alpas, H. (2010). Effects of high pressure treatment on physicochemical characteristics of fresh sea bass (*Dicentrarchus labrax*). *Journal of Consumer Protection and Food Safety, 5,* 83-89.

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. (2010). The effect of high hydrostatic pressure on microbiological, chemical and sensory quality of fresh gilthead sea bream (*Sparus aurata*). *Eur Food Res Technol, 230*, 533-542.

FAO. (2011). The state of world fisheries and aquaculture 2010. Rome, Italy

Fattouch, S., Sadok, S., Raboudi-Fattouch, F., Slama, B. S. (2008). Damage inhibition during refrigerated storage of mackerel (*Scomber scombrus*) fillets by a presoaking in quince (*Cydonia oblonga*) polyphenolic extract. *Int. J Food Sci Technol, 43*, 2056-2064.

Fernandez, J., Perez-Alvarez, J. A., Fernandez-Lopez, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*, 345-353.

FDA (2011). *Fish and Fishery Products Hazards and Controls Guidance,* 4th Ed. U.S. Dept. of Health and Human Services, Food and Drug Administration Center for Food Safety and Applied Nutrition (240) 402-2300, Washington, DC.

Fishbase a Global Information System on Fishes, www.fishbase.org, last visited on August 2011.

Gobantes I, Choubert G, Gomez R. (1998). Quality of pigmented (astaxanthin and canthaxanthin) rainbow trout (Oncorthnchus mykiss) fillets stored under vacuum packaging during chilled storage. *J Agric Food Chem*, *46*, 4358-4362.

Gomez-Estaca, J., Gomez-Guille´n, M. C., & Montero, P. (2006). High pressure effects on the quality and preservation of cold-smoked dolphinfish (Coryphaena hippurus) fillets. *Food Chemistry*, *102*, 1250-1259.

Goulas, A. E., Kontominas, M. G. (2005). Effect of salting and smoking-method on the keeping quality of chub mackerel (Scomber japonicus): biochemical and sensory attributes. *Food Chemistry*, *93*, 511–520.

Green-Walker, M., Pull, G. (1975). A survey of red and white muscles in marine fish, *J. Fish Biol*., *7*, 295-300.

Gümüş, B., İkiz, R., Ünlüsayın, M., Gülyavuz, H. (2008). Quality changes of salted red mullet (*Mullus barbatus* L., 1758) during vacuum packaged stored at +4°C. *E.U. Journal of Fisheries and Aquatic Sciences, 25*, 101-104.

Hunter, R. S. (1975). Scales for measurements of color differences. In Measurement of Appearance. *Wiley-Interscience*, NY, p. 133.

Huss, H. H. (1997). Control of indigenous pathogenic bacteria in seafood. *Food Control*, *8*, 91-98.

Huss, H. H. (1988). *Fresh fish quality and quality changes*. FAO Fisheries Series, No 29 Rome, Italy: FAO. (Pg. 65)

Jhaveri, S. N., Leu, S., Constantinides, S. M. (1982). Atlantic mackerel (Scomber scombrus, L.): Shelf life in ice. *Journal of Food Science, 47*, 1808–1810.

Jiang, S.T., Lee, T.C. (2006). Freezing seafood and seafood products: Priciples and applications. In Y.H. Hui (Ed.), *Handbook of food science, technology, and engineering* (pp. 39(1)-39(22)). New York: CRC Press.

Karataş, F., Karatepe, M., Baysar, A. (2002). Determination of free malondialdehyde in human serum by high-performance liquid chromatography. *Analytical Biochemistry, 311*, 76-79.

Kundakçı, A. (1989). Kefal ve lüferin ön bekleme koşullarının kaliteye etkileri. *E.Ü. Su Ürünleri Fak. Dergisi, 6*, 23-24.

Ledward, D. A. (1995). High pressure processing-the potential. In *High Pressure Processing of Food,* eds D. A. Ledward, D. E. Johnston, R. G. Earnshaw and A. P. M. Hasting, pp. 1-5. Nottingham University Press, Nottingham.

Love, R.M., (2006). Fish biology and food science. In Y.H. Hui (Ed.), *Handbook of food science, technology, and engineering* (pp. 35(1)-35(20)). New York: CRC Press.

Lozano, J. E., Ibarz, A. (1997). Color changes in concentrated fruit pulp during heating at high temperatures. *Journal of Food Engineering*, *31*, 365-373.

Matser, A. M., Stegeman, D., Kals, J., Bartels, P. V. (2000). Effects of high pressure on colour and texture of fish. *High Pressure Res, 19*, 109–115.

Mol, S., Özden, Ö., Erkan, N., Baygar, T. (2004). İthal uskumruların değişik çözülme koşullarındaki kalite parametrelerinin belirlenmesi. *Turk J Vet Anim Sci, 28*, 1071-1077.

Ohlsson, T., Bengtsson, N. (Eds.). (2002). *Minimal processign technologies in the food industry*. Florida: CRC Press.

Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I., Henehan, G., Nielsen, J., Nilsen, H., (1997). Methods to evaluate fish freshness in research and industry. *Trends Food Sci Technol*, *8*, 258–265.

Özoğul, F., Özoğul, Y. (2000). Comparision of methods used for determination of total volatile basic nitrogen (TVB-N) in rainbow trout (Oncorhynchus mykiss). *Turk J. Zool., 24*, 113-120.

Özyurt, G., Kuley, E., Özkütük, S., Özoğul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry 114*, 505-510.

Polat, A., Kuzu, S., Özyurt, G., Tokur, B. (2007). Fatty acid composition of red mullet (*Mullus barbatus*): a seasonal differentiation. *Journal of Muscle Foods, 20,* 70–78.

Rastogi, N. K., Ragjavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition, 47*, 69-112.

Santibanez, R. (2007). The effect of high hydrostatic pressure on histidine decarboxylase and histamine forming bacteria. *Masters Abstracts International*, *46*, no.03, 89 CSA, Illumina.

Schormüller, J. (1968). Handbuch der Lebensmittel Chemie;Band III/2 Teil.Tiersiche Lebensmittel Eier, Fleisch, Buttermilch. *Spriger* Verlag Berlin - Heidelberg-New York S. 1561-1578-1584.

Serdaroğlu, M., Deniz, E.E. (2001). Balıklarda ve Bazı Su Ürünlerinde Trimetilamin (TMA) ve Dimetilamin (DMA) Oluşumunu Etkileyen Koşullar. *E.U. Journal of Fisheries & Aquatic Sciences, 18,*575-581

Shoji, T., Saeki, H. (1989). Use *of High Pressure in Food,* ed. R. Hayahi, pp. 75-87. San-ei Publications, Kyoto.

Simeonidou, S., Govaris, A., Vareltzis, K. (1998). Quality assessment of seven Mediterranean fish species during storage on ice. *Food Res. Int*., *30*, 479–484.

Spinelli, J., Eklund, M. and Miyauchi, D. (1964). Measurement of hypoxanthine in fish as a method of assessing freshness. *Journal of Food Science 29,* 710-714.

Tanaka, M., Xueyi, Z., Nagashima, Y. and Taguchi, T. (1991). Effect of high pressure on the lipid oxidation in sardine meat. *Nippon Suisan Gakkaishi 57*, 957-963.

Taylor, S. L. (1989). Histamine poisoning (scombroid fish poisoning): An allergy-like intoxication. *Clinical Toxicology*, *27*, 225–240.

University of Cincinnati Clermont College, http://biology.clc.uc.edu, last visited on August 2011.

Varlık, C., Baygar, T., Özden, Ö., Erkan, N., Metin, S. (2000). Soğukta depolanan karideslerin (Parapenaeus longirostris, LUCAS 1846) bazı duyusal, fiziksel ve kimyasal parametrelerinin belirlenmesi. *Turk J Vet Anim Sci, 24*, 181-185.

Venugopal. V, Shahidi, F. (1994). Thermostable Water Dispersion From Myofibrillar Proteins of Atlantic Mackerel (Scomber scombrus). *Journal of Food Science, 59*, 265-268.

Wada, S. (1992). Quality and lipid change of sardine meat by high pressure treatment. In Balny, C., Hayashi, R., Heremans, K., Masson, P. (Eds.), *High Pressure and Biotechnology, 224*, (pp. 235-238). Montrouge, Colloque INSERM/ John Libbies, Eurotext Ltd.

Walker, M. G., Pull, G. A. (1975). A survey of red and white muscle in marine fish. *J. Fish Biol. 7*, 295-300.

APPENDIX A

STANDARD CURVE FOR TMA-N CALCULATION

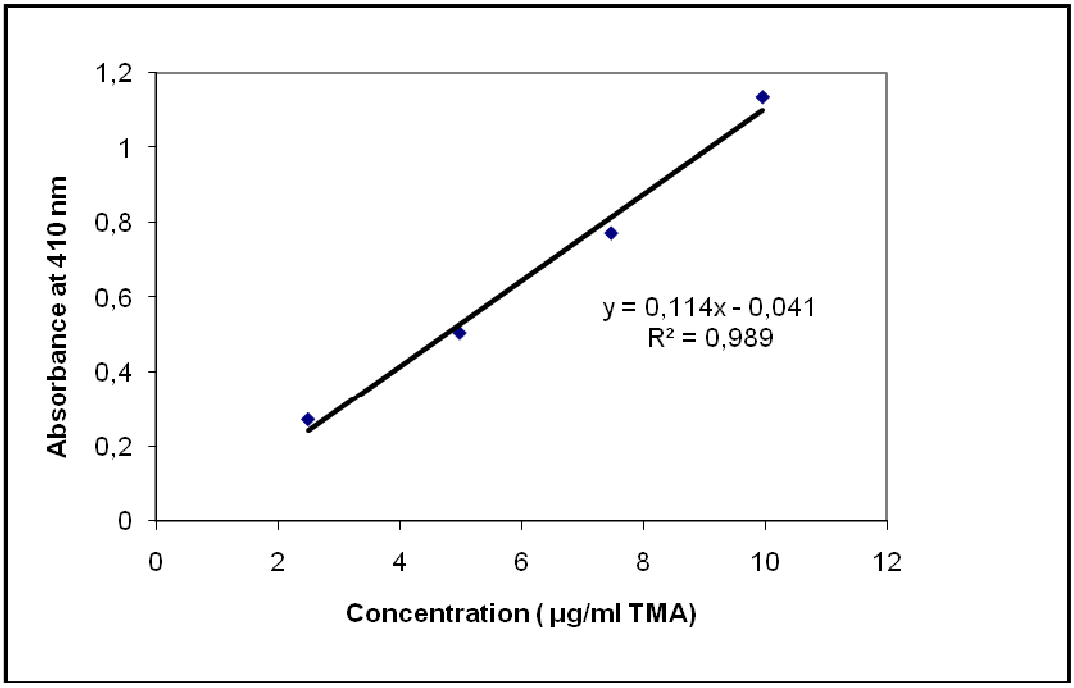

Figure A.1 *The standard curve for Schormüller Method*

$$TMA\ concentration\ (\mu g/ml) = \frac{Absorbance + 0.041}{0.114} \quad (6)$$

APPENDIX B

STANDARD CURVE FOR TBA CALCULATION

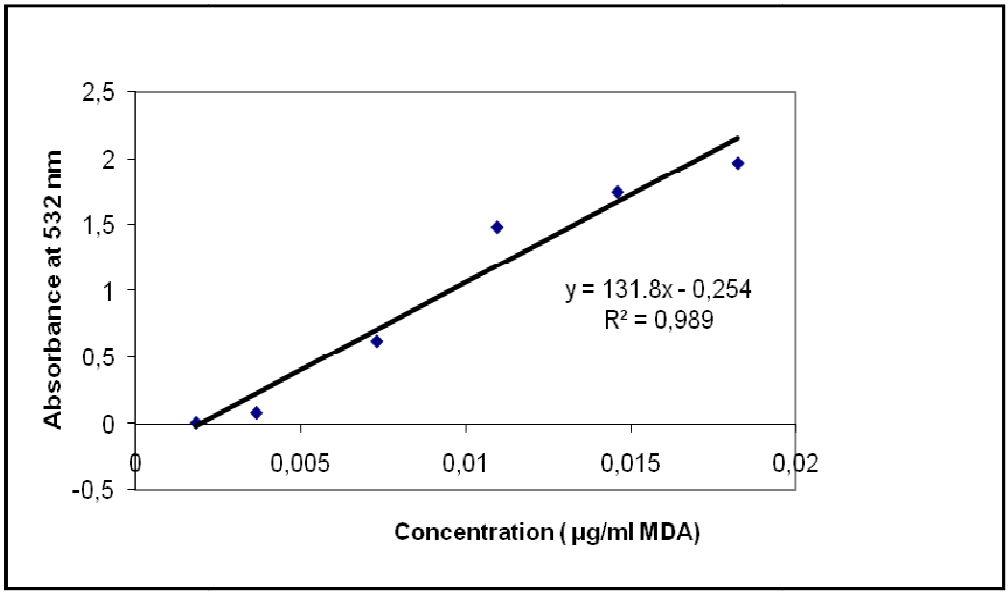

Figure B.1 *Standard curve according to the method of Erkan and Özden (2008)*

$$TBA\ concentration\ (\mu g/ml) = \frac{Absorbance+0.254}{131.8} \quad (7)$$

APPENDIX C

NORMALITY PLOT, LEVENE TEST AND ANOVA RESULTS FOR MEASUREMENT OF TMA-N LEVEL FOR THE DETERMINATION OF OPTIMUM COMBINATIONS PART FOR ATLANTIC MACKEREL

A Three-Way ANOVA was conducted to explore the impact pressure, time and temperature on protein. Subjects were divided into groups and labeles for SPSS output as follows:

For pressure	200MPa = 1	For temperature	5°C = 1	For time	5 min. = 1
	300MPa =2		10°C = 2		10 min. = 2
	400MPa= 3		15°C = 3		

The three-way analysis of variance is an extension to the one-way analysis of variance. The three independent variables in a three-way ANOVA are called factors. The idea is that there are three variables, factors, which affect the dependent variable. Each factor will have two or more levels within it, and the degrees of freedom for each factor is one less than the number of levels.

The assumptions are same with the One-Way ANOVA which can be summarized as follows.

- Error terms are normally and independently distributed with mean zero and constant variance.
- Populations (population of each treatment level) have the same variance. This is called equality of variances assumption.

Before conductiong the Three Way ANOVA analysis the model assumptions that we have mentioned above should be satisfied.

One way to check the normality assumption is looking at the normal probability plot of residuals. In SPSS it is under linear regression models, then in order to check the normality assumption we draw the histogram of the residuals that is given below. By looking at the residuals, we can say that residuals come from normal distribution.

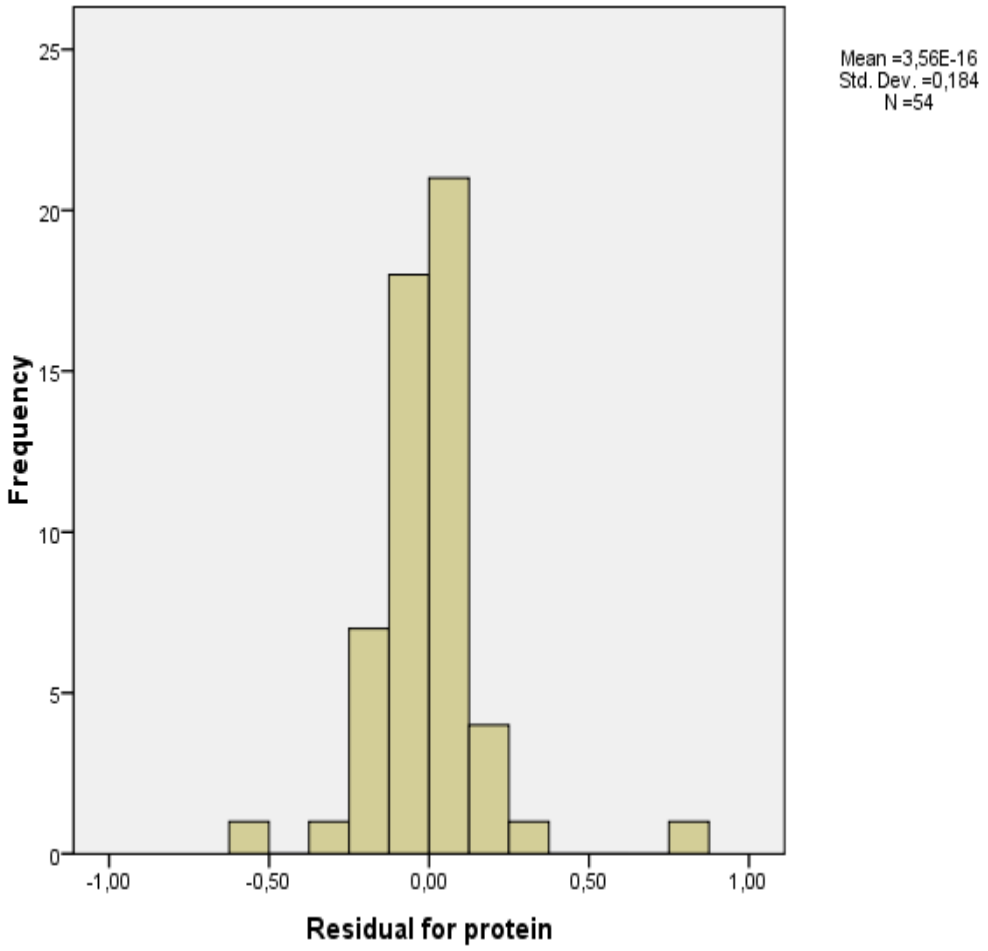

Figure C.1 SPSS output of normal probability plot for residuals for statistical analysis of TMA-N level.

To test the equality of the variances Leven's Test is helpful. Leven's Test indicates that equal variance assumption is not satisfied since corresponding p-value is smaller than 0.05 (0.0) and we can conclude that the variance of our dependent variable across the groups is not equal.

Table C.1 SPSS output of Levene's Test

Levene's Test of Equality of Error Variances[a]

Dependent Variable:protein

F	df1	df2	Sig.
4,016	17	36	,000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + pressure + time + temperature + pressure * time + pressure * temperature + time * temperature + pressure * time * temperature

C.1 Three Way Anova Test Results

We should test for the three factors pressure, time and temperature. We should test for the interaction between pressure, time and temperature and whether there is difference among the mean effects of types of pressure, time and temperature.

H_{01} : The types of time and temperature do not interact to affect the mean response

H_{02} : The types of pressure and temperature do not interact to affect the mean response

H_{03} : The types of pressure and time do not interact to affect the mean response

H_{04} : The types of the pressure, time and temperature do not interact to affect the mean response.

Table C.2 SPSS output of ANOVA Test

Tests of Between-Subjects Effects

Dependent Variable:protein

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5,984[a]	17	,352	7,045	,000
Intercept	147,987	1	147,987	2,962E3	,000
pressure	,231	2	,116	2,312	,114
time	,034	1	,034	,687	,413
temperature	3,263	2	1,631	32,654	,000
pressure * time	,198	2	,099	1,977	,153
pressure * temperature	1,158	4	,289	5,793	,001
time * temperature	,601	2	,300	6,010	,006
pressure * time * temperature	,500	4	,125	2,502	,059
Error	1,799	36	,050		
Total	155,770	54			
Corrected Total	7,783	53			

a. R Squared = ,769 (Adjusted R Squared = ,660)

As you can see from the tests of between subjects effects, the F statistics for the interaction of pressure time and temperature is 2.502 and the corresponding p-value is 0.057. At alpha level 0.05 we reject H_{04} and conclude that there is no three way interaction between pressure, temperature and time.

Since the F statistics for the interaction of pressure and time is 1.977 and the corresponding p-value is 0153. At alpha level 0.05 we reject H_{03} and conclude that there is no interaction between pressure and time.

Since the F statistics for the interaction of pressure and temperature is 5.793 and the corresponding p-value is 0.001. At alpha level 0.05 we reject H_{02} and conclude that there is a significant interaction between time and temperature.

Since the F statistics for the interaction of time and temperature is 6.010 and the corresponding p-value is 0.006. At alpha level 0.05 we reject H_{01} and conclude that there is a significant interaction between pressure and temperature.

C.2 Tukey Test Results

Post-hoc tests are used if a factor has a significant effect. Also they are only used if a factor has 3 or more groups. In our case we can conduct Tukey test which is supported by SPSS and other statistical softwares.

Tukey test;

We can display subsets of groups that have the same means. These calculations use the harmonic mean sample size. Since the only significant independent variable is temperature and besides time has only 2 levels, hence tukey test can be applied only on temperature parameter.

Table C.3 SPSS output of Tukey Test of temperature variable

Multiple Comparisons

Dependent Variable: TMA

Tukey HSD

(I) temperature	(J) temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1,00	2,00	-,59966544*	********	,000	-,78178426	-,41754663
	3,00	-,34685572*	********	,000	-,52897454	-,16473691
2,00	1,00	,59966544*	********	,000	,41754663	,78178426
	3,00	,25280972*	********	,005	,07069091	,43492854
3,00	1,00	,34685572*	********	,000	,16473691	,52897454
	2,00	-,25280972*	********	,005	-,43492854	-,07069091

Based on observed means.

*. The mean difference is significant at the ,05 level.

APPENDIX D

PICTURES OF FISH AT DIFFERENT STORAGE DAYS

Figure D.1 *Picture of unpressurised mackerel at its first day of storage.*

Figure D.2 *Picture of unpressurised mackerel at its 7th day of storage.*

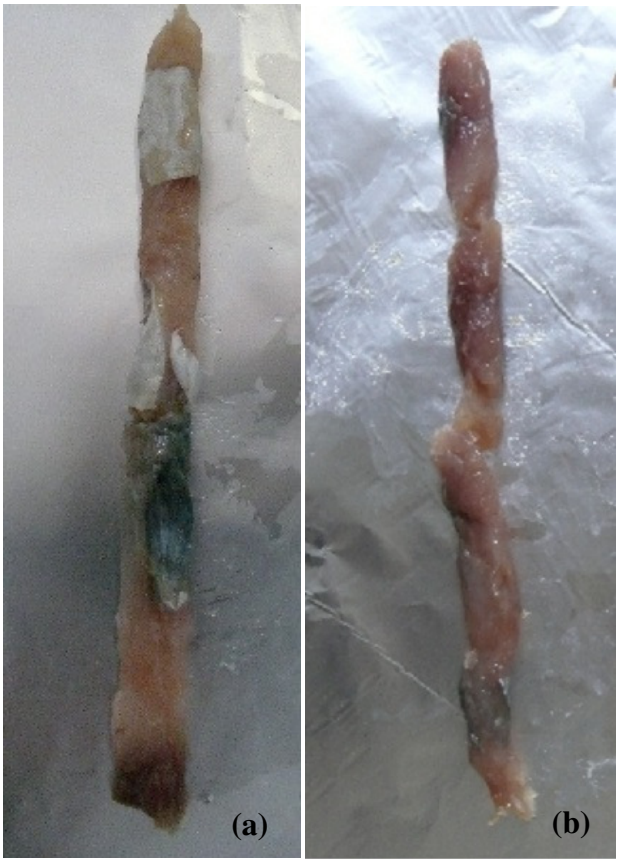

Figure D.3 *(a) Picture of 200 MPa-15°C-5 min. treated atlantic mackerel samples at its 1st day of storage. (b) Picture of 200 MPa-15°C-5 min. treated atlantic mackerel samples at its 17th day of storage.*

Figure D.4 *(a) Picture of 400 MPa-5°C-5 min. treated atlantic mackerel samples at its 1st day of storage. (b) Picture of 400 MPa-5°C-5 min. treated atlantic mackerel samples at its 19th day of storage.*

Figure D.5 Picture of pressurised mullet at its 1st day of storage.

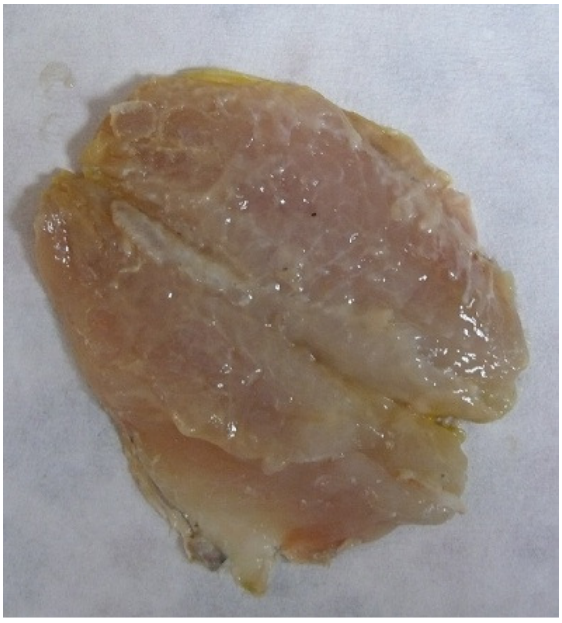

Figure D.6 *Picture of unpressurised mullet at its 7th day of storage.*

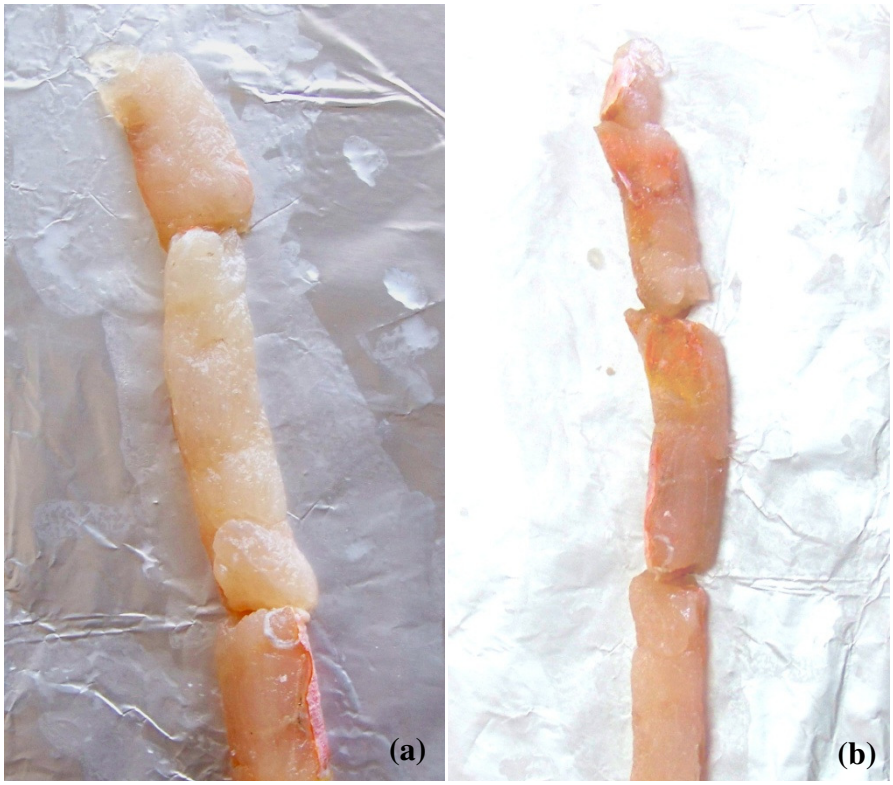

Figure D.7 *(a) Picture of pressurised mullet at its 1st day of storage (b) Picture of pressurised mullet at its 7th day of storage*