

Effect of High Hydrostatic Pressure Treatment (HHPT) on Quality and Shelf Life of Atlantic Mackerel (*Scomber scombrus*)

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Abstract The ability of high hydrostatic pressure treatment (HHPT) to extend the shelf life of Atlantic mackerel (*Scomber scombrus*) was assessed in this study. For that purpose, fillets were subjected to pressure treatments at 200, 300, 400 MPa at 5, 10, 15 °C for 5 and 15 min. The influence of pressure treatments on the levels of trimethylamine nitrogen (TMA-N) and thiobarbituric acid (TBA) as well as color changes was investigated. The suitable combinations were determined as 200 MPa, 15 °C for 5 min. and 400 MPa, 5 °C for 5 min. In the second stage, the shelf life of samples, which were treated at these conditions, stored at 4 ± 0.5 °C were studied by monitoring pH, color, sensorial features (appearance and odor), TMA-N, TBA, total volatile basic nitrogen, histamine, and total mesophilic aerobic count. The unpressurized mackerel samples were acceptable up to only 7 days compared to 17 and 19 days after 200 and 400 MPa treatments; respectively. The results obtained in this study showed that HHPT in combination with chilled storage can improve the shelf life and quality of fish.

Keywords High hydrostatic pressure treatment · Quality · Atlantic mackerel · Shelf life · *Scomber scombrus*

List of abbreviations

HHPT High hydrostatic pressure treatment
TMA Trimethylamine
TMA-N Trimethylamine nitrogen

TMA-O Trimethylamine oxygen
TBA Thiobarbituric acid
TVB-N Total volatile basic nitrogen
MDA Malondialdehyde
TMAC Total mesophilic aerobic count
TEP Tetraethoxy-propane
PCA Plate count agar
CFU Colony-forming unit
TCA Trichloroacetic acid
KOH Potassium hydroxide
BHT Butylated hydroxytoluene

Introduction

Atlantic mackerel, which belongs to the family Scombridae, is a pelagic schooling fatty fish which inhabits in various oceans. It demonstrates an exceptional nutritional value in the human diet being rich in minerals, vitamins, and polyunsaturated fatty acids (Ozden 2010). Although it is declared as a wholesome food, it remains underutilized due to its short frozen shelf life (Aubourg et al. 2005).

Fresh form of Atlantic mackerel is the product most marketed, followed by frozen, smoked, and canned form (<http://www.fishbase.org>, Accessed 30 August 2011). Although freezing is the main commercially used method to slow down enzymatic and bacterial degradation of fish muscle, solidification of water brings about numerous complex physical and physicochemical changes such as gaping, moisture loss, and mechanical damage due to formation of large ice crystals. Occurrence of these degradations has negative economic impact and fish with a lower quality can only be sold as minced or low-priced product, such as canned fish (Jiang and Lee 2006; Love 2006). However, in the canning process, the nutritional and sensorial values of processed fish are reduced (Aubourg 2001).

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High hydrostatic pressure treatment (HHPT) is one of the non-thermal technologies that provides safer, high quality, value-added foods which possess higher vitamin retention and higher consumer acceptance than both conventional (e.g., thermally processed foods) and other non-thermal technologies (Doona and Feeherry 2007). According to different factors such as species nature, chemical composition, and size, different effects of HHPT on fish have been reported (Yağız et al. 2007; 2009). Potential detrimental changes, in appearance, texture, and chemical parameter in HHPT products are also dependent on the processing conditions mainly to the applied pressure. Therefore, the undesirable changes may only be minimized by the judicious selection of treatment parameters namely pressure–temperature and the time (Mol et al. 2004; Gómez-Estace et al. 2007; Erkan et al. 2010a,b).

Fish deterioration occurs due to presence of spoilage microflora and endogenous enzymes. During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Tülsner 1994). HHPT can significantly reduce the microbial load by breaking the non-covalent bonds and piercing or changing the permeabilization of the cell membrane, consequently causing enzyme inactivation. The effects on non-covalent bonds together with changes in the permeabilization of the cell membrane cause the microbial reduction enabling a safer product with prolonged shelf life without affecting flavor and nutritional properties (Alpas et al. 1999; 2000; Bozoglu et al. 2004). HHPT is able to achieve these effects via the two causative agents of food spoilage, namely autolysis as well as microbial growth and metabolism (Heinz and Bukow 2010). On the other hand, HHPT induces damage to membranes, denatures proteins and changes the cell morphology; weak energy bonds like hydrogen and hydrophobic bonds can be irreversibly modified, causing substantial effects on the secondary, tertiary and quaternary structures in proteins. It is also reported that HHPT induces oxidative changes in lipids, therefore an important loss of oxidative stability has been underlined (Ohlsson and Bengtsson 2002; Aubourg et al. 2010). Hence, it is important to monitor the physical (color changes) and chemical parameters (trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA)) as important quality indicators (Erkan et al. 2011a; b).

TMA-N is formed from trimethylamine oxygen (TMA-O) as a result of bacterial enzyme or other endogenous enzymes activities (Erkan and Ozden 2008). Fishy odor takes its source from this compound.

Oxidative rancidity is an important organoleptic characteristic for rejection or approval of fish after prolonged shelf life. Lipid oxidation that can be originated from non-enzymatic and enzymatic reactions is expressed as TBA level. The lipids of fish are more vulnerable to oxidation than other types of meat due to the high

concentration of polyunsaturated fats (Amanatidou et al. 2000).

Total volatile basic nitrogen (TVB-N) is interrelated with bacterial growth, during marine fish spoilage along with TMA-N, ammonia and other basic nitrogenous compounds (Baixas-Nogueras et al. 2001; Gómez-Estace et al. 2007). TVB-N analysis is not taken as a criteria to select the optimum conditions in this study as TVB-N level alone is a very unreliable freshness indicator (Castro et al. 2006). Other researchers also underlined that TVB-N was not an index of spoilage; however it could only be evaluated as indicator of advanced spoilage during storage (Ozogul and Ozogul 2000).

The effect of pressure on pH was stated in cod muscle (Angsupanich and Ledward 1998). It was well established that HHPT inactivated 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 could not be evaluated alone, it had to be supported by other analyses. Some studies underscored that pH examination was not useful for quality evaluation (Ledward 1995; Erkan and Ozden 2008; Simeonidou et al. 1998).

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).

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 words “scombroid fish poisoning”. Histamine is formed mainly through decarboxylation of histidine by certain bacteria which possess the enzyme histidine decarboxylase (Taylor 1989).

The aspect and color of the food surface is the first quality parameter which is used as a tool by consumers to accept or reject food. Fish fillet color is correlated with heme-based pigment, physical structure of muscle and amount of unbound water due to the affect of the light scattering. 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 et al. 2005).

Sensory evaluations are an accurate method for assessing freshness in many fish. As the storage time increases, characteristic changes of the appearance, odor, taste and texture of the fish occur (Aubourg, 2001; Olafsdottir et al. 1997). Instrumental methods must be used with sensory evaluation panels to guarantee the accuracy of the results because

instrumental methods can not show edibility of the fish alone (Dokuzlu 2004).

Recent studies with different variety of fish have shown that HHPT may be a useful processing tool. Büyükcan et al. (2009) investigated preservation and shelf life extension of shrimps (250 MPa, 50 °C, 10 min) and clams (220 MPa, 50 °C, 10 min) during storage at 4 °C after HHPT and reported an additional 10 and 16 days of shelflife extension in shrimps and clams; respectively. The effect of HHP treatment on color, TBA and TMA parameters of red mullet was studied by Uretener (2009). The study indicated unchanged TMA-N content for HHPT applied 220 MPa, 15 °C for 10 min, 220 MPa, 25 °C for 5 min, 250 MPa, 7–25 °C for 10 min and 330 MPa, 3 °C for 5 min. Similarly; Erkan and Uretener (2010) determined insignificant TMA-N level than untreated samples in HHPT applied sea bream (220–250–330 MPa, 7 and 15 °C for 5 min; at 330 MPa, 3–7 °C for 10 min). Erkan et al. (2010b) reported 3–4 days of shelf life extension of HHPT red mullet (at 220 MPa for 5 min at 25 °C/at 330 MPa for 5 min at 3 °C) as compared with control samples stored at 4 °C based primarily on microbiological and sensory results. The quality of high-pressure-treated horse mackerel was reported to be best preserved at 250 MPa, 7–15 °C for 5 min, 220 MPa, 15–25 °C for 5 min, 250 MPa, 15 °C for 10 min and 330 MPa, 25 °C for 10 min (Erkan et al. 2011a; b).

Based on these recent literature findings, the effect of different HHPT conditions (200, 300 or 400 MPa at 5, 10, 15 °C for 5 and 15 min) on quality characteristics (color, lipid oxidation, and trimethylamine nitrogen stability) of Atlantic mackerel fillet were investigated to establish the best processing conditions (pressure, temperature, and time) that would be used in the shelf life study. In the second part, the effect of selected HHPT conditions (200 MPa, 15 °C for 5 min and 400 MPa, 5 °C for 5 min) based on chemical, sensory, and microbiological changes, were evaluated during storage of Atlantic mackerel for 21 days at 4 °C. These treatment conditions were selected due to the necessity of determining the minimum HHPT conditions for obtaining desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality. In case the values of the samples exceeded the specified threshold values two times successively, experiment was terminated.

Materials and Methods

Raw Material and Sample Preparation

Fresh Atlantic mackerel (average weight and length, 200 ± 20 g and 26.5 ± 4.5 cm, respectively) were purchased from a local market in Ankara during the fall of 2010; and until arrival at the laboratory, fish was kept in boxes with ice packs. All fish were headed, eviscerated, washed thoroughly, filleted,

and divided in portions of equal weight (15 g). 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 pressure-transmitting medium. They were kept in a freezer at -25 ± 2 °C until they were used. This period did not exceed 1 week. They were thawed in the refrigerator around 4–6 °C overnight and subjected to pressure treatment at 200, 300, or 400 MPa at 5, 10, or 15 °C for 5 or 15 min and TMA-N, TBA, and color analysis were performed to determine the best combination.

For shelf life analysis, samples were prepared as in the first experiment. Two groups of samples were pressurized at 200 MPa at 15 °C for 5 min and at 400 MPa at 5 °C for 5 min and then stored at 4 °C, and the third group samples (control) were stored directly at 4 °C without undergoing high-pressure treatment. After treatment, samples were stored up to 21 days at 4 °C. Samples were placed into oxygen-permeable bags (Seward Medical, England) for the storage study. On each analysis day for each group, samples were taken at random from packaged samples (each packaged sample is approximately 15 g).

HHPT

HHPT was performed with 760.0118-type pressure equipment supplied by SITEC-Sieber Engineering AG, Zurich, Switzerland. The vessel had a volume of 100 ml with ID 24 mm and length 153 mm. A built-in heating-cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control required temperature which is measured by a thermocouple type K in the vessel. The vessel was filled with a pressure-transmitting medium consisting of distilled water. Pressurization rates were 400 MPa/min for 200 MPa, 360 MPa/min for 300 MPa and 340 MPa/min for 400 MPa. Pressure increase and release times were less than 20 s. for each. Pressurization time reported in this study did not include the pressure increase and release times. Control group samples were not pressurized.

Analyses

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

Physical Analyses

Measurement of pH

pH was monitored at room temperature in distilled water (1/10 w/v) by using pH meter (Mettler-Toledo MP 220, Schwerzenbach, Switzerland).

Color Analysis

Although there are different color spaces, CIELAB L^* , a^* , b^* color spaces are the most used one due to the uniform distribution of colors. L^* is the luminance or lightness component, parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components. Euclidean distance (ΔE) between two different colors corresponds approximately to the color difference perceived by the human eye. (Leon et al. 2006)

Three different fillet parts were measured in three different locations of each part using CIELAB color system. L^* (lightness, range from 0 to 100), a^* (from green to red, range from -120 to 120) and b^* (from blue to yellow, range from -120 to 120) values were measured with the help of a chromameter (Minolta CR-200, Osaka, Japan). The instrument was standardized before each measurement with a white calibration plate ($L=73.3$; $a=8.4$; $b=23.0$ in CIELAB Color System). Averages and standard deviations of L^* , a^* , and b^* values were calculated as the total color differences.

The total color difference (ΔE), as calculated below, was also used for evaluation where subscript 'o' indicates initial color (Lozano and Ibarz 1997).

$$\Delta E = (\Delta L_o^{*2} + \Delta a_o^{*2} + \Delta b_o^{*2})^{1/2}$$

Microbiological Analyses

For the enumeration of total mesophilic aerobic bacterial count (TMAC), 10 g of fish sample was homogenized in sterile Stomacher bags (Seward Medical, England) with a stomacher (Seward Laboratory Blender Stomacher 400, England) in 90 mL sterile 0.1 % peptone water solution. 0.1 mL samples of serial dilutions (1:10 diluents, 0.1 % peptone water, Merck, Germany) of fish homogenates was spread plated on plate count agar (PCA, Merck, Germany). Total viable counts were determined after incubation for 48 h at 37 °C. Results are expressed as logarithm of colony-forming units per gram of sample ((log CFU)/g fish sample).

Chemical Analyses

Measurement of Trimethylamine Nitrogen Value

TMA-N was determined by the method of Schormüller (1968). Homogenized samples (10 g) were weighed, blended with 90 mL of 10 % trichloroacetic acid (TCA, Merck, Germany) solution and filtrated (Whatman no. 1). 4 ml extract was transferred into test tubes with 1 ml formaldehyde (20 %, Emir Kimya, Turkey), 10 ml toluene (99 %, Emir Kimya, Turkey) and 3 ml potassium hydroxide (KOH) (50 %, Merck, Germany).

The tubes were shaken and 5 mL toluene layer was pipetted. Five milliliters of picric acid working solution (0.02 % with H₂O-free toluene, Fluka Chemie, Germany) was added. Yellow picrates, which were the end product of interaction of picric acid with primary and secondary amines, were analyzed with maximum absorption at 410 nm against the blank in spectrophotometer (Specord50, Analytic Jena AG, Jena, Germany) in quartz cuvettes (Silica, Sigma). The concentration of TMA-N was calculated from a standard (TMA-HCl) curve. Results of TMA-N were expressed as mg per 100 g of muscle.

Measurement of Thiobarbituric Acid Value

TBA was determined according to Weilmeier and Regenstein (2004) and Khan et al. (2006) as detailed in Erkan and Ozden (2008). Ten grams of fish sample was placed in a tube with 500 μ L butylated hydroxytoluene (BHT, Merck, Germany) and 190 mL of 5 % (w/v) TCA solution (Merck, Germany). The mixture was homogenized with Ultra-Turrax (T-18, IKA-Werke GmbH & Co, Staufen, Germany) at high speed for 2 min and filtrated through a Whatman No.1 filter paper. A portion (5.0 mL) of the mixture that was pipetted into a dry stoppered test tube 1 mL of TBA reagent (0.01 M of the solution of 2-thiobarbituric acid in 90 % acetic acid, Merck, Germany) was added. The test tubes were stoppered, vortexed, and placed in a water bath at 75 °C for 40 min, then cooled. Absorbance of the resultant colored solution was read at 532 nm using spectrophotometer (Specord50, Analytic Jena AG, Jena, Germany). The concentration of malondialdehyde (MDA) was calculated from a standard curve using 1,1,3,3-tetraethoxy-propane (TEP, Merck, Germany) as the standard compound. TBA values were expressed as mg of MDA/kg of sample.

Measurement of Total Volatile Basic Nitrogen Value

TVB-N was determined according to the method of Antonacopoulos and Vyncke (1989). 10 g of fish sample was homogenized with 90 ml of 6.5 % perchloric acid (Merck, Germany) for 1–2 min in an Ultra-Turrax (T-18, IKA-Werke GmbH & Co, Staufen, Germany). The homogenates were filtered through a Whatman No.1 filter paper and 50 mL of filtrate was transferred to a Kjeldahl tube with 150 ml distilled water and subsequently alkalized by 20 % NaOH (Merck, Germany). Tubes were distilled in Kjeldahl apparatus (Simsek Laborteknik Co., Ankara, Turkey) until 50 ml distillate was collected. The distillate was titrated with 0.01 N HCl (Merck, Germany). Results of TVB-N were expressed as milligrams per 100 g of muscle.

Measurement of Histamine Value

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

Sensory Analyses

Sensory assessment of the control and HHPT samples (uncooked) included appearance and odor parameters. 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 proposed by Huss (Huss 1988). In this ten-point descriptive scale, a score of 10–9 indicated “very good quality”, 8.9–8 indicated “good quality”, 7.9–4 represents “acceptable quality”, the score below 3.9 means the sample had strong off-odors and was denoted as spoiled.

Statistical Analyses

The results were reported as mean values±standard deviation. They were analyzed by two- and three-way analysis of variance procedure (according to the number of factors) of SPSS 15 for Windows (SPSS Inc., Chicago, IL, USA) to determine the significant differences ($p < 0.05$). Tukey test was used as a post-hoc test if a factor had a significant effect (difference between treatments and days of storage) and if the factor had three or more groups.

Results and discussion

Effect of HHPT Parameters on the Quality of Atlantic Mackerel

The effects of combined application of pressure, temperature, and holding time on TMA-N and TBA levels of Atlantic mackerel are presented in Table 1. The results coincided well with literature TMA-N data of mackerel species, 1.22 ± 0.06 mg/100 g (Goulas and Kontominas 2005). This value was also consistent with the FAO standards which recommended that good quality fish must contain less than 1.5 mg TMA-N/100 g (Huss 1988). Unpressurized control samples had a TMA-N level of 1.241 ± 0.169 mg/100 g and the scores of some HHPTs were higher than control samples insignificantly (Table 1). In literature, this increase was linked to the damage of the

lysosomal membrane by high-pressure treatment (100–200–300–400–500 MPa 10 ± 5 °C for 5 min) and releasing of the enzymes (Chéret et al. 2006). 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 only high pressure as an independent variable. The TMA-N content of horse mackerel samples was not affected also from HHPT application significantly ($p \leq 0.05$) (Erkan et al. 2011a; b).

Goulas and Kontominas (2005) studied 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 be the season of the fish used for experiments. October was the month in which fishing season began in Turkey. TBA number was high due to stocking of fat all summer long. All pressurized samples had higher levels of TBA than the control group. TBA level of the samples increased with increasing pressure. 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. The reason of this increase was due to the denaturation of heme protein by pressure which released metal ions (Fe and Cu) and catalyzed lipid oxidation (Tanaka et al. 1991; Cheah and Ledward 1997; Angsupanich and Ledward 1998). Likewise, during HHPT, metal ions were released from metal salts (Fe-stearate and Cu-stearate) and catalyzed the oxidation reaction (Cheah and Ledward 1997). The oxidative stability decreased (TBA number increased) with the increasing temperature and decreasing with processing time generally. 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 be taken into consideration when determining/selecting the optimum conditions.

The effect of the HHPT on the CIELAB color system (L^* , a^* , b^* and ΔE) of the mackerel was illustrated in Table 2. L^* , a^* , b^* and ΔE values of unpressurized mackerel group were 42.0 ± 0.2 , 12.0 ± 1.3 , 24.9 ± 2.2 , and 31.6 ± 0.4 , respectively. Lightness (L^*) increased, with increasing pressure and although there are few exceptions, generally increased with holding time. However, it decreased with increasing temperature ($p \leq 0.05$); 400 MPa -5 °C, 15 min treatment caused the highest L^* value change (56.5 ± 1.0). Angsupanich and Ledward (1998) indicated that translucency loss was measured with the denaturation of myosins with pressure. Similar results were reported for HHPT-treated sea bass (200–250–330 MPa, 3,7,15 and 25 °C, 5 to 10 min) muscle and dolphinfish (200–300–400 MPa at 20 °C for 15 min) where control samples had the lowest L^* values among all treatment pressures and lightness increased with increasing pressure and holding time (Gómez-Estace et al. 2007; Erkan et al. 2010a). Redness (a^*) of the samples

Table 1 The effect HHPT on TMA-N (mg/100 g sample) and TBA (mg MDA/kg sample) values of Atlantic mackerel flesh

	5 min			15 min		
	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C
TMA-N						
200 MPa	1.516±0.287a	2.329±0.130b	1.213±0.200c	1.674±0.730a	1.985±0.101b	1.761±0.156c
300 MPa	1.251±0.060a	1.968±0.084b	1.856±0.198c	1.169±0.112a	1.713±0.045b	1.786±0.072c
400 MPa	1.059±0.061a	2.042±0.234b	1.892±0.105c	1.370±0.212a	1.600±0.029b	1.613±0.089c
TBA						
200 MPa	0.582±0.052Aa	0.352±0.027Ab	0.410±0.013Ab	0.441±0.052Aa	0.400±0.058Ab	0.367±0.085Ab
300 MPa	0.592±0.139Aa	0.358±0.030Ab	0.446±0.053Ab	0.498±0.047Aa	0.387±0.049Ab	0.386±0.039Ab
400 MPa	0.625±0.219Ba	0.505±0.014Bb	0.592±0.041Bb	0.574±0.045Ba	0.427±0.037Bb	0.523±0.004Bb

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

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

Unpressurized control samples had a TMA-N level of 1.241±0.169 mg/100 g

Unpressurized control samples had a TBA level of 0.344±0.011 mg MDA/kg

decreased with increasing pressure and temperature and holding time ($p \leq 0.05$). Yellowness (b^* values) of HHPT-treated Atlantic mackerel samples were not significantly affected ($p \leq 0.05$) with pressure. The significant effect on b^* values of

HHPT-treated sea bass, cold smoked salmon, sliced dolphinfish were also not observed ($p \leq 0.05$) (Gómez-Estace et al. 2007; Erkan et al. 2010a; 2010b). Increase in pressure and application time caused a decrease of ΔE scores.

Table 2 The effect of HHPT on L^* , a^* , b^* , ΔE color values of Atlantic mackerel flesh

	5 min			15 min		
	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C
L^*						
200 MPa	49.8±1.2Aa	49.5±0.8Aa	49.9±1.5Ab	50.9±0.8Aa	51.6±0.8Aa	46.7±2.8Ab
300 MPa	52.1±0.1Ba	51.9±0.3Ba	50.7±1.9Bb	53.6±1.4Ba	52.8±2.5Ba	49.3±4.3Bb
400 MPa	54.9±1.7Ca	53.9±0.3Ca	51.3±2.6Cb	56.5±1.0Ca	55.7±3.3Ca	54.2±0.8Cb
a^*						
200 MPa	12.6±0.2Aa	12.4±0.9Ab	11.4±2.0Ab	11.5±0.3Aa	12.3±0.1Ab	11.2±1.2Ab
300 MPa	11.9±0.9Ba	10.6±1.0Bb	10.0±1.4Bb	11.5±0.6Ba	10.0±0.4Bb	10.2±0.6Bb
400 MPa	10.6±0.7Ca	9.3±0.9Cb	9.2±0.8Cb	10.0±0.5Ca	8.8±0.9Cb	9.4±0.3Cb
b^*						
200 MPa	29.3±0.4a	29.2±1.3b	27.6±2.7c	28.4±0.4a	27.8±0.6b	26.4±0.6c
300 MPa	28.9±1.3a	27.5±1.0b	25.5±1.5c	29.2±0.2a	26.9±0.7b	26.9±1.2b
400 MPa	28.4±0.3a	27.1±0.2b	26.8±1.3c	27.8±0.1a	27.2±1.3b	26.5±0.8c
ΔE						
200 MPa	24.5±1.2Aa	24.7±1.0Aa	24.0±1.8Ab	23.1±0.8Aa	22.4±0.7Aa	26.9±2.6Ab
300 MPa	22.1±0.5Ba	21.8±0.0Ba	22.8±1.7Bb	20.7±1.5Ba	20.8±2.4Ba	24.3±4.1Bb
400 MPa	19.1±1.7Ca	19.7±0.3Ca	22.2±2.4Cb	17.3±1.0Ca	18.0±3.1Ca	19.4±0.8Cb

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

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

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

Table 3 The effect of HHP on TMA (mg/100 g), TBA (mg MDA/kg), TVB-N (mg/100 g), TMAC (log cfu/g) and pH of Atlantic mackerel flesh during refrigerated storage

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
TMA											
Control	1.78±0.02Aa	1.97±0.23Aa	2.52±0.18Aa	5.03±0.10Ab	7.30±0.33Ac	13.32±0.82Ad	18.32±0.48Ae				
Process 1	1.66±0.06Ba	1.38±0.31Ba	1.37±0.00Ba	1.43±0.05Bb	1.80±0.09Bc	2.82±0.29 Bd	4.11±0.07Be	5.32±0.08Bf	10.38±0.31Bg	11.14±0.29Bg	
Process 2	0.90±0.02Ca	0.97±0.05Ca	0.97±0.03Ca	1.23±0.12Cb	1.58±0.11Cc	1.32±0.15Cd	1.34±0.07Ce	1.36±0.11Cf	2.05±0.10Cg	2.09±0.23Cg	2.21±0.07Ch
TBA											
Control	0.45±0.01Aa	0.51±0.03Aa	0.59±0.07Aa	0.72±0.18Ab	0.83±0.19Ab	0.87±0.36Ab	1.29±0.10Ac	1.90±0.09Ad			
Process 1	0.47±0.06Ba	0.33±0.01Ba	0.38±0.02Ba	0.40±0.01Bb	0.42±0.04Bb	0.43±0.07Bb	0.45±0.00Bc	0.48±0.00 Bd	0.48±0.03 Bd	0.53±0.01 Bd	0.54±0.02 Bd
Process 2	0.50±0.02Ca	0.51±0.14Ca	0.57±0.02Ca	0.93±0.16Cb	0.61±0.13Cb	0.66±0.05Cb	0.69±0.11Cc	0.57±0.02Cd	0.52±0.02Cd	0.54±0.01Cd	0.56±0.02Cd
TVB-N											
Control	10.72±0.38Aa	11.73±0.21Aa	15.19±1.50Aa	24.88±2.51Aa	72.95±7.19Ab	96.70±8.57Ac					
Process 1	10.45±2.64Ba	11.24±1.01Ba	14.23±0.94Ba	14.64±0.27Ba	16.60±0.15Bb	17.95±0.47Bc	20.10±0.70 Bd	24.02±1.73 Bd	22.89±6.73 Bd	32.25±2.15Be	36.44±1.26Be
Process 2	11.88±0.71Ba	15.08±0.86Ba	18.68±1.85Ba	16.34±0.05Ba	16.52±0.17Bb	17.00±0.29Bc	17.47±0.06 Bd	17.71±0.36 Bd	20.36±1.20 Bd	34.83±5.97Be	40.80±0.86Be
TMAC											
Control	< 3.00±0.15Aa	3.71±0.09Ab	4.42±0.01Ac	4.90±0.09Ac	5.69±0.04Ad	6.43±0.03Ae	6.62±0.22Ae				
Process 1	< 3.00±0.02Ba	3.27±0.56Bb	3.40±0.09Bc	3.49±0.23Bc	4.68±0.26 Bd	5.14±0.29Be	5.03±0.10Be	5.43±0.06Bf	6.30±0.07Bg	6.41±0.05Bg	
Process 2	< 3.00±0.19Ca	< 3.00±0.22Cb	3.20±0.06Cc	3.35±0.03Cc	3.88±0.13Cd	4.12±0.09Ce	4.44±0.08Ce	4.63±0.09Cf	5.65±0.20Cg	6.03±0.12Cg	6.42±0.19Cg
pH											
Control	6.85±0.04Aa	6.93±0.01Ab	6.95±0.01Ac	7.05±0.03Ad	7.11±0.04Ae	7.19±0.10Ae					
Process 1	6.31±0.01Ba	6.33±0.03Bb	6.43±0.02Bc	6.78±0.01 Bd	6.79±0.04Be	6.95±0.01Be	6.85±0.05Bf	6.89±0.05Bg	6.89±0.020Bg	6.96±0.02Bg	6.99±0.04Bg
Process 2	6.40±0.02Ca	6.47±0.01Cb	6.65±0.02Cc	6.76±0.03Cd	6.41±0.02Ce	6.20±0.03Ce	6.58±0.07Cf	6.93±0.01Cg	6.97±0.010Cg	6.97±0.02Cg	7.02±0.02Cg

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

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

Control: Unpressurized, Process 1: 200 MPa, 15 °C, 5 min treatment, Process 2: 400 MPa, 5 °C, 5 min treatment

10 mg TMA-N/100 g, 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 4 The effect of HHPT on L^* , a^* , b^* and ΔE of Atlantic mackerel flesh during refrigerated storage

	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±0.5Aa	31.1±0.3Aa	32.6±1.0Aa	36.1±1.7Ab	33.4±1.2Ab	34.0±1.0Ab	40.6±2.6Ac	43.7±0.5Ad			
Process 1	42.5±0.6Ba	36.1±0.3Ba	33.8±0.3Ba	35.0±0.7Bb	35.4±0.9Bb	34.3±0.8Bb	36.4±0.5Bc	39.2±1.5 Bd	41.9±1.9 Bd	41.2±0.4 Bd	39.5±1.1 Bd
Process 2	51.3±0.2Ca	52.4±0.6Ca	51.4±0.5Ca	51.5±0.2Cb	51.8±1.6Cb	49.7±0.5Cb	50.3±0.7Cc	49.1±0.2Cd	48.2±0.5Cd	46.0±0.6Cd	44.5±0.7Cd
a^*											
Control	7.6±1.0A	8.2±1.0A	11.9±1.5A	13.7±1.8A	11.1±0.3A	12.1±0.3A	14.8±2.3A	14.9±1.8A			
Process 1	11.1±1.1A	10.2±0.4A	9.7±2.5A	10.2±1.4A	13.6±2.0A	13.0±0.9A	13.6±0.3A	14.8±0.8A	15.0±4.5A	14.4±0.5A	13.4±0.7A
Process 2	11.0±1.1B	11.7±0.8B	14.3±0.6B	13.3±0.3B	12.7±1.2B	11.6±0.9B	13.8±0.8B	13.9±0.1B	13.9±0.4B	15.6±0.7B	15.8±0.4B
b^*											
Control	19.7±0.7Aa	22.2±1.1Aa	24.3±0.2Aa	28.6±4.2Aa	25.4±1.0Aa	24.5±1.6Aa	30.7±2.6Ab	34.4±0.7Ab			
Process 1	24.1±0.6Ba	21.7±0.7Ba	20.5±1.2Ba	20.9±1.0Ba	21.7±0.8Ba	21.1±0.7Ba	23.8±0.3Bb	24.2±1.2Bb	25.8±1.5Bc	25.2±0.7Bc	22.7±0.5Bc
Process 2	27.3±1.7Aa	26.4±1.8Aa	27.0±0.5Aa	26.0±0.1Aa	26.0±1.3Aa	25.8±0.1Aa	26.3±1.2Ab	26.2±0.3Ab	25.8±0.6Ac	25.4±0.2Ac	25.5±0.2Ac
ΔE											
Control	43.8±0.4Aa	39.9±0.4Aa	38.5±1.1Aa	35.5±1.9Ab	37.6±1.2Ab	37.1±0.9Ab	31.5±2.7Ac	29.5±0.5Ac			
Process 1	29.4±0.7Ba	35.7±0.2Ba	38.1±0.4Ba	36.9±0.6Bb	36.7±0.8Bb	37.8±0.9Bb	35.6±0.4Bc	33.1±1.3Bc	30.8±2.0Bc	31.1±0.3Bc	32.6±1.0Bc
Process 2	20.9±0.3Ca	19.7±0.8Ca	21.3±0.5Ca	20.8±0.1Cb	20.5±1.5Cb	22.3±0.4Cb	22.2±0.4Cc	23.3±0.2Cc	24.1±0.4Cc	26.7±0.6Cc	28.3±0.9Cc

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

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

Control: Unpressurized, Process 1: 200 MPa, 15 °C, 5 min treatment, Process 2: 400 MPa, 5 °C, 5 min treatment

Shelf Life Analyses

The condition which caused lower TMA-N level, comparatively high oxidative stability (low TBA level), and the closest color values to the control samples was 200 MPa, 15 °C, 5 min for Atlantic mackerel. The second combination (400 MPa, 5 °C, 5 min) was also studied for shelf life analyses with respect to the lowest TMA-N level among the treatments as high-pressure treatment can also promote color and oxidation changes that could modify the sensory characteristics (Erkan et al. 2010b). The selection of two different HHPT combinations also enabled the comparison of the effect of relatively low pressure–high temperature (200 MPa, 15 °C) and high-pressure–low-temperature (400 MPa, 5 °C) treatments on the quality parameters studied within the HHPT conditions (200, 300 or 400 MPa at 5, 10, 15 °C for 5 and 15 min) of the study.

The effect of HHPT on TMA-N (in milligrams per 100 g), TBA (in milligrams MDA per kg), TVB-N (in milligrams per 100 g), TMAC (in log cfu per gram) and pH of Atlantic mackerel flesh during refrigerated storage (at 4 ± 0.5 °C) are given in Table 3.

FAO recommended that 10–15 mg TMA-N/100 g must be regarded as the limit of acceptability for human consumption (Connell 1975; Huss 1988). Up to 4 mg TMA/100 g, the fish sample is indicated as “very good quality”, up to 10 mg TMA/100 g it is denoted as marketable, the score of 12 mg TMA/100 g is denoted as spoiled (Kundakçı 1989; Varlık et al. 2000; Mol et al. 2004). Based on these literature information, 10 mg/100 g was used as a threshold TMA-N value in this study. 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 the 11th day of storage and 200 MPa samples at the 17th day of storage.

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

in this study. Unpressurized samples of fatty fish showed lower ($p<0.05$) oxidation than their counterpart pressurized products at the first day. In literature, it was noted that TBA records might not bring out the actual lipid oxidation rate due to the interaction between malonaldehyde and other fish components such as amines, nucleosides, nucleic acids, proteins, amino acids of phospholipids, or other aldehydes (Aubourg 1993; Simeonidou et al. 1998; Taliadourou et al. 2003). 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 (Fernández et al. 1997). Unpressurized mackerel exceeded the threshold values on the 13th day, pressurized samples (both 200- and 400-MPa-treated ones) did not exceed up to the 21st day of analyses.

TVB-N value was denoted as “very good quality” up to 25 mg/100 g. Over 35 mg/100 g was indicated as spoiled (EEC 1995; Goulas and Kontominas 2005; Varlık et al. 2000). 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 unpressurized samples was detected especially on the ninth day of storage. The threshold value was exceeded at the same day. Slow and regular increase in the TVB-N scores of 200-MPa-treated samples was observed throughout the shelf life analysis.

The limit of acceptability for mesophilic aerobic bacteria was indicated as 10^6 – 10^7 CFU/g (Gobantes et al. 1998; Erkan and Ozden 2008; Gumus et al. 2008). For the present study, 6 log cfu/g was designated as a limit value for consumption. 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 5 The effect of HHPT on Histamine values (ppm) of Atlantic mackerel flesh during refrigerated storage

	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	14.642±1.088Aa	16.810±0.888Aa	21.019±3.653Aa	81.015±9.338Ab	96.332±4.944Ac	
Process 1	6.932±0.960Ba	12.566±1.579Ba	18.087±0.360Ba	39.648±2.859Bb	48.846±4.204Bc	89.269±4.662 Bd
Process 2	5.303±0.684Ca	4.638±0.320Ca	10.535±0.546Ca	16.314±1.054Cb	22.132±1.012Cc	37.657±1.685Cd

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

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

Control: Unpressurized, Process 1: 200 MPa, 15 °C, 5 min treatment, Process 2: 400 MPa, 5 °C, 5 min treatment

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

data given in Table 3. Moreover, the retardation time was extended as the applied pressure increased. Hereunder, unpressurized samples exceeded the acceptability limit at the 11th day; 200 MPa–15 °C–5 min processed ones at 17th day; and 400 MPa–5 °C–5 min processed samples at 19th day of storage. Amanatidou et al. (2000) indicated that commercial shelf life of fish products is usually limited to 1 week of refrigerated (2–8 °C) storage. An inverse relationship between microbiological growth rate and the amount and time of pressure applied was observed in this study as well as the net effect of high pressure on the reduction of microbial growth.

Threshold value for pH was denoted as 6.8–7.0 (Mol et al. 2004). In this study, fish samples which had pH higher than 7.0 were denoted as spoiled. 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). This difference could take its source from two reasons. The fish 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). Secondly, increased concentration consists of slightly soluble salts, phosphate, and other compounds. This phenomenon can result in up to two units of irreversible pH changes (Love 2006; Jiang and Lee 2006). pH of the 400-MPa-treated Atlantic mackerel muscle was higher than 200-MPa-treated samples (except the 7th, 9th, and 11th day of storage), probably due to the denaturation of some protein fractions. Unpressurized samples exceeded the limit on the 7th day, while 400-MPa-treated ones reached this level on the 21st day of storage; 200 MPa treatment did not caused the samples to reach this critic value throughout the storage.

Alkaline compounds (ammonium compounds, TMA, etc.) which are mainly derived from microbial action, cause an increase in pH. In this respect, the results of pH, TMA-N, TVB-N, and TMAC of the samples coincided well with each other. Due to the inhibition of bacterial growth with the help of pressure–temperature and time combination applied; it took more time for the pressurized samples to reach the threshold limit.

Throughout the storage period, *L** values of the control group samples increased, although lightness of the 200-MPa-treated samples was quite stable and a trend to decrease was observed for 400-MPa-pressurized samples ($p \leq 0.05$) (Table 4). Erkan et al. (2010b) determined an increase for both pressurized (220 MPa/5 min/25 °C and 330 MPa/5 min/3 °C) and unpressurized red mullet muscle which were covered with flexible plastic films during pressurization process and stored at 4 °C during 17 days of storage. However, Gómez-Estace et

Table 6 The effect of HHPT on sensory evaluation of appearance and odor of Atlantic mackerel flesh during refrigerated storage

	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±0.08Aa	8.2±0.75Ab	6.5±0.6Ac	4.1±0.62Ad	2.7±0.82Ae	1.8±0.61Af					
Process 1	9.5±0.38Ba	9.4±0.25Bb	9.1±0.38Bc	8.6±0.35 Bd	7.8±0.57Be	6.7±1.08Bf	5.7±0.69Bg	4.9±0.57Bh	3.8±0.58Bh	3.2±0.39Bh	2.4±0.79Bh
Process 2	9.2±0.28Ca	9.2±0.34Cb	9.0±0.31Cc	8.7±0.22Cd	8.4±0.18Cc	7.8±0.42Cf	6.1±0.66Cg	5.1±0.50Ch	4.0±0.35Ch	3.7±0.47Ch	3.2±0.48Bh
Odor											
Control	9.9±0.10Aa	8.5±0.67Ab	5.9±1.00Ac	3.7±0.43Ad	2.1±0.52Ae						
Process 1	9.7±0.22Ba	9.3±0.18Bb	9.3±0.2Bc	9.1±0.15 Bd	8.9±0.13Be	8.3±0.32Bf	7.8±0.21Bg	5.9±1.1Bh	4.4±1.01Bi	3.4±0.46Bj	2.3±0.67Bk
Process 2	9.3±0.18Ca	9.3±0.15Cb	9.1±0.12Cc	8.9±0.12Cd	8.5±0.28Cc	8.1±0.16Cf	8.0±0.29Cg	7.4±0.38Ch	6.7±0.38 Ci	5.0±0.97Cj	4.6±1.0Ck

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

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

Control: Unpressurized, Process 1: 200 MPa, 15 °C, 5 min treatment, Process 2: 400 MPa, 5 °C, 5 min treatment

The score ≤ 3.9 was denoted as spoiled for both analyses

al. (2007) and Erkan et al. (2011a; b) indicated that lightness of the pressurized (250 MPa/3 °C/5 min, 250 MPa/25 °C/10 min, and 300 MPa/20 °C/15 min) and control group salmon and dolphinfish was quite stable. L^* value fluctuations were also reported for sea bream for control and pressurized (250 MPa/3 °C/5 min, 250 MPa/15 °C/5 min) groups in literature (Erkan and Uretener 2010). Redness (a^*) of the samples did not significantly changed during storage period ($p \leq 0.05$) in agreement with findings of Gómez-Estace et al. (2007). Yellowness (b^*) of the control group samples increased from 19.7 ± 0.7 to 34.4 ± 0.7 . However, with fluctuations, b^* values of the pressurized samples decreased ($p \leq 0.05$). Total color difference (ΔE) of untreated samples decreased, a slight increase was determined for the pressurized samples.

Histamine concentrations of foods exceeding 50 ppm (5 mg/100 g fish) are hazardous (FAO 2011). Histamine formation was studied up to 20 days of refrigerated storage of Atlantic mackerel (Table 5). 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 unpressurized samples was observed after the 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 exceed 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 in agreement with literature cited. It had been established that pressures above 300 MPa reduced the enzyme activity to a level of 55 % of the residual (Santibanez 2007).

Decreasing scores in appearance and odor attributes were obtained with increasing time (Table 6). The results corroborated the chemical and microbiological analyses. Pressure caused the fish flesh to have a cooked appearance. These findings were consistent with this literature information indicating a 5 min pressure treatment around 150–200 MPa pressure causes a cooked appearance of mackerel (Matser et al. 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 first 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 till 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 the 21st day. Control group samples were evaluated as spoiled on the 9th day; however, pressurized samples exceeded the limits on the 17th and 19th days.

Conclusions

When all the parameters were evaluated; the unpressurized Atlantic mackerel (*Scomber scombrus*) was 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. Accordingly, HHPT in combination with chilled storage can improve the shelf life and quality of fish. For fatty fish, HHPT can be recommended not only for industrial production but also for safe storage even at refrigeration temperatures. With the help of larger and economic high-pressure chambers the industry can manufacture high-quality fish products.

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