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Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon

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ABSTRACT

The effect of high pressure treatment (HPP) (150 MPa and 300 MPa for 15 min) and cooking on quality of Atlantic salmon based on microbial activity, lipid oxidation, fatty acid profile, colour and texture during 6 days of storage was studied. High pressure and cooking significantly ($p < 0.05$) reduced microbial growth. The 300 MPa treatment and cooking showed higher L^* and b^* values but lower a^* values for dark muscle compared to control and samples treated at 150 MPa. An increase in pressure resulted in an increase in hardness, gumminess and chewiness parameters, and a decrease in adhesiveness compared to control and cooked samples. Whereas cooking and 150 MPa led to similar oxidation development as control in dark muscle, the 300 MPa treatment effectively reduced the samples susceptibility to oxidation. Fatty acid profile of cooked Atlantic salmon dark muscle showed significantly ($p < 0.05$) lower amounts of total saturated, $n-3$ Polyunsaturated fatty acids (PUFA) and $n-6$ PUFA and significantly ($p < 0.05$) higher amounts of monoenes than HPP treated samples during the entire storage period. However, the most important finding of this study was that there was no significant ($p > 0.05$) difference between control and HPP treated samples in terms of total saturated, monoenes, $n-3$ PUFA and $n-6$ PUFA fatty acid profile. This demonstrates that HPP is a very mild process in terms of its effect on fatty acids.

1. Introduction

Recognition of health benefits associated with consumption of $n-3$ fatty acids from seafood is one of the most promising developments in nutrition research in the past 20 years. Research has provided a wide array of explanations for the positive influence of $n-3$ fatty acids. The $n-3$ fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have direct effects on heart muscle itself, increasing blood flow, decreasing arrhythmias, improving arterial compliance, decreasing the size of the infarct, and reducing several chemical and cellular processes that compromise heart function (Nestel, 1990). As a result, seafood remains a healthy, attractive choice to consumers. NOAA Fisheries Service reported that the consumption of seafood in the US has reached 16.3 pounds per person in 2007 (NOAA, 2007). In general, seafood is highly perishable with a 14 day shelf-life for a fresh or thawed product. Usually beyond 7 days of cold storage, the product is considered being of a lower grade and frequently sold at reduced cost or discarded. Moreover, seafood is more susceptible to post-mortem texture deterioration than meats from land animals (Ashie, Smith, & Simpson, 1996).

Processing techniques that can extend the shelf-life of seafood past 14 days while maintaining sensory attributes and characteristics of a fresh quality product are demanded by consumers. Cooking can lead to inactivation of microbial growth and lipolytic enzymes. However, it may damage vitamins, flavour compounds and polyunsaturated fatty acids. High pressure processing (HPP) has been applied to food as a preservation method with its major advantage being the maintenance of fresh quality attributes. High pressure processing is a promising seafood preservation method. This novel technology reportedly provides long shelf-life and minimum quality loss since it does not have many of the undesirable changes that are associated with thermal processing. There is limited information on the influence of high pressure on the oxidative stability and quality changes of fish muscle. The major purpose of HPP is to enhance the safety of most of the seafood by inactivating microorganisms and parasites without changing sensorial quality attributes (Cheftel & Culioli, 1997).

Muscle colour plays an important role in consumer perception of meat quality (Jeremiah, Smith, & Carpente, 1972). Studies on seafood products have shown that consumers associate colour with freshness of a product having better flavour and higher

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quality (Gormley, 1992). Many authors (Hayashi, Kawamura, Nakasa, & Okinaka, 1989; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Ohshima, Ushio, & Koizumi, 1993) claim that HPP has less impact on colour compared to thermal processing treatments.

Applications of HPP in food processing and preservation follow the premise that it can destroy microorganisms while heat-labile compounds undergo limited degradation compared with cooking (Hayashi, Kawamura, Nakasa, & Okinaka, 1989). High pressure processing can diminish the microbial load without any additives, while flavour, vitamins, colour and other properties of food are presumably unchanged, or changed only to a small extent (Hendrickx et al., 1998; Ohshima et al., 1993).

One major problem associated with seafood is lipid oxidation. Various compounds can initiate and mediate oxidation in seafood, including heme proteins, which on pressurisation can become denatured and more pro-oxidative. Limited information is available on HPP of seafood, and more specifically, its effect on lipid oxidation and fatty acid profile. Dark muscle has not only a higher amount of unsaturated lipids than white muscle but also a higher amount of pro-oxidants such as iron and heme proteins (Hultin, 1992). The lipids in dark muscle are more vulnerable to lipid oxidation than white muscle because dark muscle has more unsaturated membrane lipids. Since fish has a high concentration of polyunsaturated lipids, oxidative changes induced by HPP could be very significant. The oxidative quality changes during processing and following storage can directly affect quality of seafood products.

The aim of this study was to investigate the effect of different pressures and cooking on quality changes (colour, lipid oxidation, fatty acid profile, texture and total plate count) of Atlantic salmon fillets during cold storage.

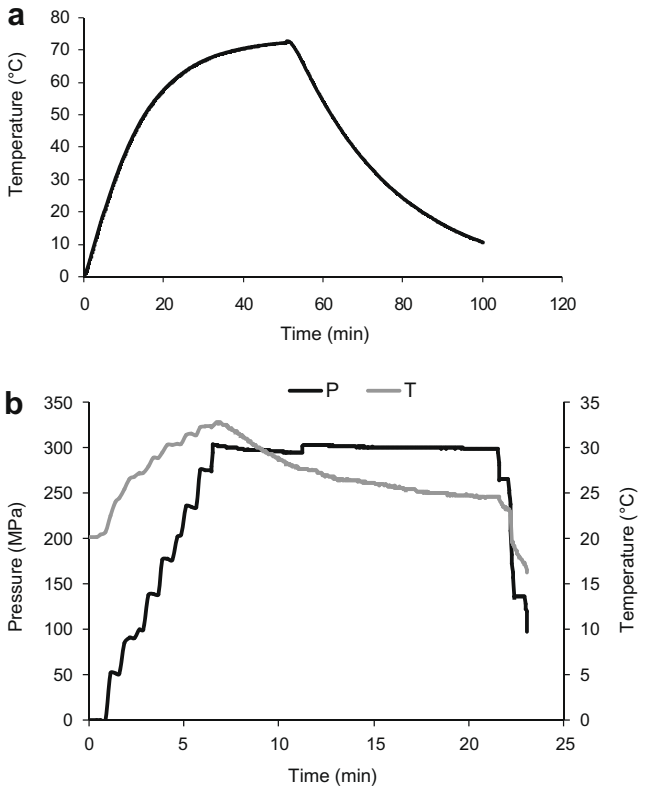

Fig. 1. (a)Temperature profile of Atlantic salmon during cooking, followed by cooling. (b) Pressure and temperature profiles during high pressure processing at 300 MPa.

2. Materials and methods

2.1. Materials

Thirteen Atlantic salmon (*Salmo salar*), with an average total length and weight of 81.6 ± 2.1 cm and 5.5 ± 0.2 kg, were purchased from a local seafood supplier (Save-on-Seafood, St Petersburg, FL) within two days of harvest and fish were transported to the laboratory in ice. The fish were filleted, skinned and divided into three parts: portions close to head, body and tail regions of equal length. The fillets were vacuum packaged using FoodSaver Vacloc vacuum bags (Jarden Co. Rye, NY). After high pressure treatment or cooking, duplicates of equal weight portion were collected from each region. All equipments were sterilized using bleach followed by ethanol before and during the whole study to minimise contamination. Chemicals were purchased from Fisher Scientific (Fair Lawn, NJ). All reagents were ACS grade.

2.2. Methods

2.2.1. High pressure processing

The high-pressure equipment consisted of a Stansted laboratory scale unit (Stansted Fluid Power, Stansted, Essex, UK) with a pressurisation chamber of 114 mm diameter and 243 mm height, providing a usable volume of approximately 2.5 L. Skinned fillets from fresh fish were vacuum packaged and treated at different pressures of either 150 or 300 MPa with 50 MPa/min ramp rate for 15 min at room temperature. Initial, maximum and final vessel temperature was recorded at 19.4, 32.6, and 16.1 °C, respectively. After treatment, samples were stored for 6 days at 4 °C. Samples were removed aseptically from their vacuum bags and placed into oxygen permeable bags for the storage study. Pressure and temperature profiles during high pressure processing at 300 MPa were shown in Fig. 1b.

2.2.2. Cooking treatment

Skinless vacuum packed fillets were placed into a water bath containing boiling water. A USB data logger model DAQ-56 (Omega Engineering, Stamford, CT, USA) was connected to a T-type thermocouple probe placed into the centre of fillets for temperature measurements. The thermocouple probe was calibrated using either boiling water or ice mixed water in the temperature range of 0–100 °C. When the centre temperature of fillets reached 72 °C, all fillets were removed and placed in ice to cool down. The samples' temperature was recorded during both cooking and cooling. After treatment, samples were removed aseptically from the vacuum bags and placed into oxygen permeable bags and stored for 6 days at 4 °C. Temperature profile of Atlantic salmon during heating and cooling was shown in Fig. 1a.

2.2.3. Microbial analysis

Total aerobic microbial growth before and after HPP treatment was determined using Petrifilm™ (3 M Laboratories, St. Paul MN) according to the AOAC Official Method 990.12 (AOAC, 1995a). The 3 M Petrifilm™ aerobic plate is a readymade medium that contains standard nutrients, a cold water soluble gelling agent and a tetrazolium indicator dye, which facilitates colony enumeration. Analysis was done on 10 g fish muscle mixed with 90 ml sterile pre-filled dilution vials of 0.3 mM monopotassium phosphate buffer solution at pH 7.2 (Hardy Diagnostic, Santa Maria CA). The solution was then mixed in a stomacher for 1 min, pH adjusted to 7.2 with 1 N NaOH and then serially diluted (10^{-1}–10^{-7}). For inoculation, 3 M Petrifilm™ was placed on a sterile flat surface and 1.0 ml of the sample was placed at the centre of the film and spread by a

sterile plastic spreader to an area of ~20 cm^2. Duplicate inoculations were conducted for each dilution and no more than 10 plates were stacked at 35.5 °C for an incubation time of 48 ± 3 h.

2.2.4. Colour analysis

The surface colour of treated and control Atlantic salmon muscle was measured during storage by a colour machine vision system, consisting of a light box and a CCD colour camera connected to a computer with a firewire connection. A colour machine vision software program was used to capture images, and to obtain colour results based on L^* (lightness), a^* (redness), and b^* (yellowness) values. Fish fillets were placed in the light box and the digital camera captured a picture of the fillets for each analysis time point. The machine vision system was calibrated using a standard red plate (L^* = 51.13, a^* = 50.00, b^* = 24.03) from Labsphere (North Sutton, NH). Average L^*, a^*, b^* values of whole fillet surface were calculated using a colour analysis program (Luzuriaga, Balaban, & Yeralan, 1997; Yagiz, Kristinsson, Balaban, & Marshall, 2007; Yoruk, Yoruk, Balaban, & Marshall, 2004).

2.2.5. Texture profile analysis

Texture profile analysis (TPA) (Bourne, 1978) was performed as described by Yagiz et al. (2007). Briefly, high pressure treated Atlantic salmon fillets were cut into rectangular shapes with dimensions 2 × 2 × 1.5 cm. Rectangular cuts were taken from each side of the mid-section of fillets. All samples were blotted with filter paper after treatment and stored at 4 °C prior to TPA, which was performed using an Instron Universal Testing Instrument, model 4411 (Canton, MA) at room temperature. Samples were stored at 4 °C for not more than 1 h prior to TPA analysis. Eight replicates for each treatment were compressed twice to 70% of their original height at 100 mm/min speed and 100 N compression load using a cylindrical-shaped probe (38 mm in diameter). Texture analysis parameters (hardness, adhesiveness, chewiness, springiness, cohesiveness and gumminess) were calculated using Blue Hill Software (Norwood, MA).

2.2.6. Lipid oxidation analysis

Lipid oxidation analysis was performed as described by Raghavan and Hultin (2005), measuring secondary products of oxidation in the dark muscle. Dark muscle tissue (5 g) was mixed with 15 ml of TCA extracting solution (7.5% trichloroacetic acid in water, 0.1% propyl gallate and 0.1% EDTA) using a Waring commercial blender (Waring Products Division, Dynamics Corp. of America, CT) for 30 s in a plastic beaker, the suspension filtered using Whatman #1 filter paper and then 2 ml of suspension was mixed with 2 ml of TBA (thiobarbituric acid) in a screw cap tube. The tube was vortexed for 10 s, and placed into boiling water for 40 min. Finally, the tube was placed in ice for 5 min, and absorbance of samples was measured at 530 nm. A standard plot was prepared using tetraethoxypropane (TEP). As each mole of TEP would yield one mole of malonaldehyde, the results were expressed as micromoles of malonaldehyde (MDA) per kg tissue. All analyses were done in six replicates.

2.2.7. Lipid extraction

Lipids were extracted from the dark muscle using the method of Bligh and Dyer (1959). Dark muscle tissue (1 g) was mixed with 1 ml water using a Waring commercial blender (Waring Products Division, Dynamics Corp. of America, Torrington, CT) for 30 s in a plastic beaker. Samples were transferred to a 10 ml round bottom screw cap centrifuge tube, and 3.75 ml of chloroform:methanol (1:2) was added with vortexing for 10 min. Then 1.25 ml chloroform was added with vortexing for 1 min and 1.25 ml of water was then added with vortexing for another minute before centrifugation for 10 min at 4700*g*. The lower phase was collected with a Pasteur pipette into a pre-weighed glass tube. The chloroform phase (1.88 ml) was added to a centrifuge tube, vortexed, and centrifuged at 4700*g* for 10 min, and the lower phase collected into a pre-weighed glass tube. Chloroform was removed under a nitrogen gas stream using a N-EVAP Nitrogen Evaporator (Organomation Associates Inc., Berlin, MA). The weight of each sample was recorded and percentage of lipids determined gravimetrically. The oil recovered was flushed with nitrogen and stored in amber vials at −80 °C until analysis.

2.2.8. Preparation of fatty acid methyl esters

Determination of fatty acid methyl esters (FAME) was performed as described by Maxwell and Marmer (1983). Oil (20 mg) was placed into a 10 ml round bottom capped centrifuge tube. Oils were dissolved in 1.9 ml *iso*-octane (Sigma grade 99%, St. Louis, MO) with 100 µl of 10 mg/ml tricosanoic acid methyl ester (C23:0) (Supelco®, Bellefonte, PA) used as an internal standard. Then 200 µl of 2 N KOH in methanol (1.12 g/10 ml) was added to the centrifuge tube. After vortexing for 60 s, the solution was centrifuged at 2500*g* for 5 min, and the lower layer discarded. This procedure was repeated twice using 0.5 ml of a saturated solution of ammonium acetate in water, followed by 0.5 ml of deionized water. Fatty acid methyl esters in iso-octane were dried with the addition of approximately 200–300 mg of anhydrous sodium sulphate for 20 min, and centrifuged at 2500*g* for 20 min. Fatty acid methyl esters were transferred into 2 ml screw capped amber GC vials (National Scientific, Rockwood, TN) for chromatographic analysis.

2.2.9. Gas chromatography (GC) analysis

FAMEs were analysed with a HP 6890 gas chromatograph, equipped with a flame ionisation detector and an AT™-Silar-100 cyano silicone capillary column (30 m × 0.25 mm × 0.2 µm) (Alltech Assoc. Inc, Nicholasville, KY). Splitless injection was used. Operation conditions were as follows: injection port temperature, 240 °C; detector temperature, 250 °C; initial oven temperature, 120 °C for 2 min, rising to 200 °C and holding for 10 min, then rising to 210 °C and holding for 4 min, and finally rising to 240 °C and holding for 5 min all at the rate of 4 °C/min. The carrier gas was helium (1 ml/min). Retention times and peak areas were computed automatically by Turbochrom Workstation 6.1.1 (Perkin Elmer, MA, USA). Compounds were tentatively identified by comparison with the retention times of known standards. All standards used in the identification of peaks were purchased from Supelco® (Bellefonte, PA). The standards used were: Supelco 37, Marine Oil PUFA#1, and Menhaden Oil PUFA#3. Tricosanoic acid methyl ester (C23:0) was used as an internal standard. Six fillets were used for each treatment for each day with a duplicate GC injection.

2.2.10. Statistical analysis

Colour data (L^*, a^*, b^* values) was reported as mean and standard deviation for the whole surface of the fillets. Texture, microbial analysis, fatty acid profiles and lipid oxidation data were analysed by analysis of variance (ANOVA) and the mean separations were performed by LS Means Tukey HSD ($p < 0.05$) using the JMP 5 software (SAS Institute Inc., Cary, NC).

3. Results

3.1. Microbial analysis

Microbial evaluation of pressure or cooking treated Atlantic salmon samples during 6 days aerobic storage at 4°C is presented in Fig. 2. The initial microbial load of samples was 3.56 ± 0.17 log cfu/g. The microbial load of control sample reached

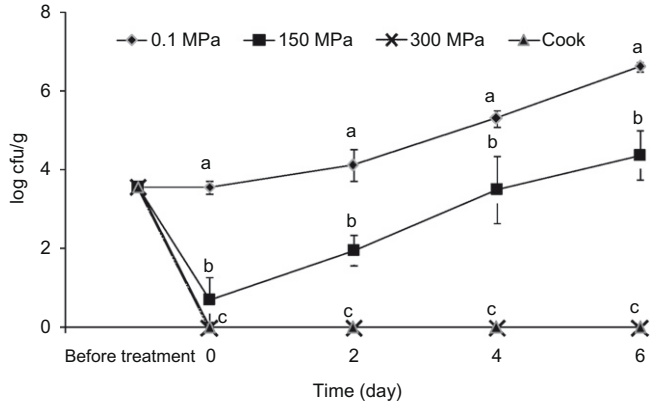

Fig. 2. Total aerobic plate count for Atlantic salmon after 15 min of high pressure processing (150 and 300 MPa) followed by storage at 4 °C for 6 days. Values are means ± standard deviations ($n = 3$). Different letters within each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Table 1
Changes in a^* value (redness), L^* value (lightness) and b^* value (yellowness) for Atlantic salmon dark muscle after HPP treatment followed by storage for 6 days at 4 °C. Control (0.1 MPa) was untreated muscle stored under the same conditions as the treated samples.

Storage day	0.1 MPa	150 MPa	300 MPa	Cook
L^*-Value				
2	46.2 ± 8.2	43.2 ± 9.1	68.4 ± 4.7	75.0 ± 4.9
4	46.7 ± 7.4	44.0 ± 8.8	68.5 ± 4.6	76.9 ± 4.4
6	46.0 ± 7.3	44.1 ± 8.8	67.9 ± 4.0	76.1 ± 3.6
a^*-Value				
2	29.7 ± 5.2	26.2 ± 5.4	24.6 ± 3.9	12.9 ± 3.8
4	30.8 ± 5.1	26.5 ± 5.8	26.2 ± 4.1	10.4 ± 3.5
6	29.0 ± 5.4	25.8 ± 6.0	26.0 ± 3.8	10.1 ± 3.1
b^*-Value				
2	17.0 ± 2.8	14.3 ± 2.6	19.6 ± 2.5	19.8 ± 3.1
4	17.2 ± 2.7	14.2 ± 2.6	19.2 ± 2.5	20.8 ± 3.3
6	16.9 ± 2.4	14.1 ± 2.6	19.2 ± 2.1	21.2 ± 2.6

Values are means ± SD for all pixels of the surface of the fillet.

6.16 ± 0.11 log cfu/g after 6 days of storage. Pressurizing the fillets at 150 MPa for 15 min resulted in a 3 log cycle and 2 log cycle reduction in microbial counts at day 0 and 6, respectively. Samples pressurised at 150 MPa had significantly ($p < 0.05$) lower microbiological counts than control samples during storage at 4 °C. Pressurizing samples at 300 MPa for 15 min led to total microbial reduction, and no bacterial growth was detected for this treatment even after 6 days of storage. Cooking also reduced aerobic microbial load to undetectable levels and no growth was seen during the entire storage period at 4 °C. Among all the samples, the 300 MPa and cooked samples, had significantly ($p < 0.05$) lower microbiological counts than control samples and samples treated at 150 MPa.

3.2. Colour analysis

Colour evaluation was performed by using a colour machine vision system where average L^* (lightness), a^* (redness) and b^* (yellowness) values of the surface of dark muscle was measured (Table 1). As the pressure level increased, the sample became opaque and had a cooked appearance for dark muscle. Table 1 shows the changes in L^*, a^* and b^* values for pressure treated, cooked and control samples during 6 days of storage at 4 °C. L^* value for dark muscle increased slightly as a function of increased pressure or cooking treatment; however, storage time did not show any major difference in L^* values. The 300 MPa treatment and cooking showed higher L^* and b^* values but lower a^* values for dark muscle compared to control samples and the samples treated at 150 MPa.

3.3. Texture profile analysis

Texture profile analysis (TPA) results (hardness, cohesiveness, adhesiveness, springiness, and gumminess) for HPP treated, cooked and control samples are shown in Table 2. Here, springiness indicates the elasticity of the fillets, chewiness indicates the tenderness, gumminess indicates the pastiness, and adhesiveness indicates the stickiness of the fillets. It was observed that as the pressure level increased, hardness, gumminess and chewiness parameters increased whereas adhesiveness decreased compared to control and cooked samples. The highest pressure gave the samples with the highest hardness, gumminess and chewiness values. The 300 MPa treatment led to significantly ($p < 0.05$) higher hardness values than other treatments. There was no significant ($p > 0.05$) difference between samples treated with 150 MPa pressure and control samples in terms of hardness, adhesiveness, gumminess and chewiness. However, it was found that control samples were significantly ($p < 0.05$) different in terms of springiness and cohesiveness than samples treated with 150 MPa pressure. Cooked samples had lower hardness, cohesiveness, gumminess and chewiness values compared to pressure treated and control samples (Table 2).

3.4. Lipid oxidation analysis

Lipid oxidation was measured in the dark muscle section of the salmon fillets during storage at 4 °C using the TBARS method to monitor levels of secondary oxidation products formed, mainly malondialdehyde (MDA) (Fig. 3). TBARS values for all treated and control samples significantly increased during the 6 day storage period at 4 °C. Lipid oxidation of all samples increased from 3.78 μmol MDA/kg muscle to almost 116 μmol MDA/kg after 6 days of storage. The increase in standard deviation with increasing storage time reflected the biological variation between the individual fish samples and the analysis of whole fish. At day 0, there was only a significant ($p < 0.05$) difference in TBARS between samples treated at 150 MPa, and control and cooked samples. On the second day of storage, cooked samples had significantly ($p < 0.05$) higher levels of TBARS compared to pressure treated and control samples. At this time point no significant difference

Table 2
Texture profile analysis (TPA) in terms of hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness for Atlantic salmon muscle after HPP treatment or cooking. Control (0.1 MPa) was untreated muscle.

Treatments	Hardness (N)	Cohesiveness	Adhesiveness (J)	Springiness (mm)	Gumminess(N)	Chewiness(N*mm)
0.1 MPa	19.4 ± 5.9^{bc}	0.14 ± 0.05^{b}	0.00083 ± 0.00042^{ab}	3.3 ± 0.6^{b}	2.7 ± 1.4^{bc}	9.3 ± 5.9^{b}
150 MPa	20.5 ± 1.5^{b}	0.2 ± 0.04^{a}	0.00023 ± 0.00016^{b}	4.5 ± 0.2^{a}	4.1 ± 0.9^{ab}	18.4 ± 3.5^{ab}
300 MPa	25.5 ± 4.4^{a}	0.18 ± 0.05^{ab}	0.00125 ± 0.00116^{a}	3.9 ± 1.1^{ab}	4.6 ± 1.6^{a}	19.1 ± 11.8^{a}
Cook	13.0 ± 4.3^{c}	0.13 ± 0.03^{ab}	0.0015 ± 0.0007^{a}	3.9 ± 1.2^{ab}	1.6 ± 0.7^{c}	6.4 ± 3.9^{ab}

Values are means ± standard deviations, $n = 8$, different letters within a column indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

($p > 0.05$) was observed between 150 and 300 MPa treatments. In addition, control samples showed no significant difference ($p > 0.05$) in TBARS values with samples treated at 150 MPa or cooked samples. At day 4 and day 6, no significant ($p > 0.05$) difference in TBARS was found among control, 150 MPa treated and cooked samples. The 300 MPa treatment did however have significantly ($p < 0.05$) lower oxidation values than the other treatments at day 4 and 6 (Fig. 3).

3.5. Fatty acid analysis

Fatty acid profile of pressure treated, cooked and control Atlantic salmon dark muscle during 6 days of storage at 4 °C is shown in Tables 3–6 and in Figs. 4a–c. The source of total saturated fatty acids mainly came from 14:0, 16:0 and 18:0. There was no significant ($p > 0.05$) difference in total saturated fatty acid composition (g fatty acids/100 g total fatty acids) between control and pressure treated (150 MPa, 300 MPa) samples. The saturated fatty acid composition was also not significantly ($p > 0.05$) affected by storage at 4 °C for 6 days (Fig. 4c). However, cooked samples were significantly ($p < 0.05$) lower in total saturated fatty acids than pressure treated and control samples. All samples had major monoenes, including 16:1*n*−7, 18:1*n*−7, 18:1*n*−9, 20:1*n*−9 and 22:1*n*−11. No significant ($p > 0.05$) difference in 18:1*n*−7 or 18:1*n*−9 at day 0, 2, and 6 were found among the samples, regardless of treatment and storage time. The pressure levels 150 MPa and 300 MPa did not lead to significant differences in 20:1*n*−7 and 22:1*n*−11 at day 0, 2, and 4 compared to control samples. However, cooked samples had significantly ($p < 0.05$) higher levels of these monounsaturated fatty acids than both pressure treated and control samples during the entire storage period.

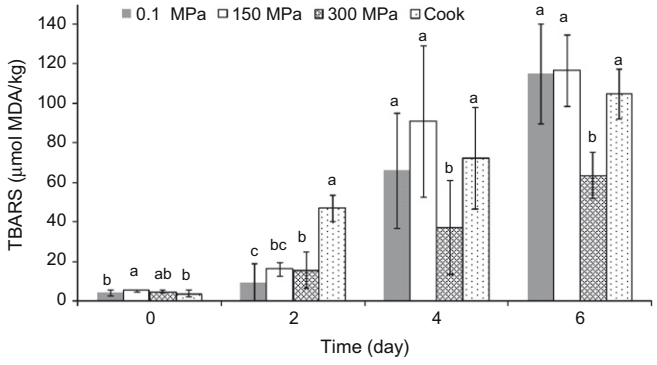

Fig. 3. Changes in lipid oxidation as measured by the formation of thiobarbituric acid reactive substances (TBARS) for dark muscle of Atlantic salmon after high pressure processing (150 and 300 MPa) followed by storage for 6 days at 4 °C. Values are means ± standard deviations ($n = 6$). Different letters within each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Table 4
Fatty acid compositions (g fatty acids/100 g total fatty acids) of Atlantic salmon dark muscle after HPP treatment or cooking followed by storage for 2 days at 4 °C. Control (0.1 MPa) was untreated muscle stored under the same conditions as treated samples.

	0.1 MPa	150 MPa	300 MPa	Cook
14:00	3.4 ± 0.4^{a}	3.2 ± 0.3^{ab}	3.2 ± 0.3^{c}	3.4 ± 0.3^{bc}
16:00	14.8 ± 0.7^{a}	14.6 ± 0.7^{a}	13.4 ± 1.3^{b}	11.5 ± 1.1^{c}
18:00	4.8 ± 0.1^{a}	4.8 ± 0.1^{a}	4.4 ± 0.9^{a}	2.8 ± 0.9^{b}
Total saturated	23.7 ± 1^{a}	23.1 ± 1^{ab}	21.6 ± 2.2^{b}	18.1 ± 1.9^{c}
16:1*n*−7	5.2 ± 0.4^{a}	4.8 ± 0.3^{ab}	4.7 ± 0.4^{b}	5.3 ± 0.6^{a}
18:1*n*−9	15.5 ± 0.7^{a}	15.4 ± 0.4^{a}	15.5 ± 0.6^{a}	14.9 ± 0.8^{a}
18:1*n*−7	4.2 ± 0.1^{a}	4.3 ± 0.1^{a}	4.3 ± 0.2^{a}	4.1 ± 0.2^{a}
20:1*n*−9	2.6 ± 0.1^{b}	3.1 ± 0.5^{b}	5.1 ± 4.1^{b}	11.3 ± 3.7^{a}
22:1*n*−11	1.5 ± 0.3^{b}	1.8 ± 0.4^{b}	3.2 ± 3.5^{b}	10.2 ± 3.8^{a}
Total monoenes	30.6 ± 1.2^{b}	31 ± 1^{b}	34.6 ± 7.9^{b}	47.4 ± 6.7^{a}
18:2*n*−6	6.8 ± 0.1^{a}	6.4 ± 0.5^{a}	5.2 ± 0.9^{b}	3.8 ± 0.9^{c}
20:2*n*−6	0.6 ± 0^{ab}	0.6 ± 0^{a}	0.5 ± 0^{bc}	0.5 ± 0^{c}
Total *n*−6 PUFA	8.6 ± 0.2^{a}	8.3 ± 0.5^{a}	6.9 ± 0.9^{b}	6.8 ± 1.1^{b}
18:3*n*−3	1.2 ± 0^{a}	1.2 ± 0.1^{ab}	1 ± 0.2^{b}	0.7 ± 0.2^{c}
18:4*n*−3	1.4 ± 0.1^{b}	1.3 ± 0^{b}	1.4 ± 0.1^{b}	1.6 ± 0.2^{a}
20:5*n*−3 (EPA)	10.3 ± 0.4^{a}	10.1 ± 0.2^{a}	9.8 ± 1.1^{a}	7.6 ± 0.9^{b}
22:5*n*−3	5.1 ± 0.3^{a}	5 ± 0.2^{a}	4.9 ± 0.8^{a}	3.5 ± 0.7^{b}
22:6*n*−3 (DHA)	17.5 ± 2^{a}	18.3 ± 0.9^{a}	18.5 ± 3.2^{a}	13.3 ± 2.8^{b}
Total *n*−3 PUFA	35.6 ± 1.9^{a}	36 ± 0.9^{a}	35.5 ± 5.1^{a}	26.8 ± 4.3^{b}
n−3/*n*−6	4.2 ± 0.3^{b}	4.4 ± 0.3^{b}	5.2 ± 0.5^{a}	4 ± 0.7^{b}

Results are means ± SD, $n = 12$, different letters within a row for each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Table 3
Fatty acid compositions (g fatty acids/100 g total fatty acids) of Atlantic salmon dark muscle directly (no storage) after HPP treatment or cooking. Control (0.1 MPa) was untreated muscle stored under the same conditions as treated samples.

	0.1 MPa	150 MPa	300 MPa	Cook
14:00	4.6 ± 0.3^{a}	4.6 ± 0.2^{a}	4.5 ± 0.4^{a}	4.4 ± 0.4^{a}
16:00	16.8 ± 0.5^{a}	16.9 ± 0.4^{a}	16.6 ± 1.1^{a}	13.1 ± 0.9^{b}
18:00	4.8 ± 0.1^{a}	4.7 ± 0.1^{a}	4.8 ± 0.2^{a}	2.7 ± 0.6^{b}
Total saturated	27 ± 0.7^{a}	27 ± 0.5^{a}	26.7 ± 1.6^{a}	20.8 ± 1.5^{b}
16:1*n*−7	5.9 ± 0.2^{b}	5.6 ± 0.2^{bc}	5.5 ± 0.4^{c}	6.4 ± 0.5^{a}
18:1*n*−9	14.4 ± 0.3^{a}	14.6 ± 0.9^{a}	14.9 ± 0.6^{a}	14.8 ± 0.5^{a}
18:1*n*−7	5.3 ± 0.3^{a}	5.3 ± 0.3^{a}	5.1 ± 0.6^{a}	5.1 ± 0.3^{a}
20:1*n*−9	1.1 ± 0.1^{b}	1.3 ± 0.4^{b}	1.4 ± 0.2^{b}	7.1 ± 1.7^{a}
22:1*n*−11	1.9 ± 0.1^{b}	2 ± 0.7^{b}	2.2 ± 0.3^{b}	13.9 ± 3.6^{a}
Total monoenes	30.1 ± 0.6^{b}	30.4 ± 1.5^{b}	30.7 ± 1.3^{b}	48.9 ± 5.2^{a}
18:2*n*−6	6.8 ± 0.3^{a}	6.3 ± 0.3^{a}	5.6 ± 0.6^{b}	3.7 ± 0.8^{c}
20:2*n*−6	0.5 ± 0a	0.5 ± 0.1^{a}	0.5 ± 0^{a}	0.4 ± 0^{b}
Total *n*−6 PUFA	8.3 ± 0.4^{a}	7.8 ± 0.4^{a}	7 ± 0.6^{b}	4.7 ± 0.9^{c}
18:3*n*−3	0.4 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0^{b}	0 ± 0^{c}
18:4*n*−3	1.2 ± 0.1^{b}	1.2 ± 0^{b}	1.2 ± 0.1^{b}	1.5 ± 0.1^{a}
20:5*n*−3 (EPA)	9.5 ± 0.3^{a}	9.2 ± 0.3^{a}	9.4 ± 0.6^{a}	7 ± 0.5^{b}
22:5*n*−3	4.6 ± 0.2^{a}	4.5 ± 0.2^{a}	4.6 ± 0.1^{a}	3.2 ± 0.4^{b}
22:6*n*−3 (DHA)	16.8 ± 0.8^{a}	17.7 ± 1.2^{a}	18.2 ± 1.2^{a}	12.7 ± 2.1^{b}
Total *n*−3 PUFA	32.5 ± 1^{a}	32.8 ± 1.3^{a}	33.7 ± 1.2^{a}	24.4 ± 2.9^{b}
n−3/*n*−6	3.9 ± 0.2^{b}	4.2 ± 0.2^{b}	4.9 ± 0.5^{a}	5.2 ± 0.5^{a}

Results are means ± SD, $n = 12$, different letters within a row for each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Table 5
Fatty acid compositions (g fatty acids/100 g total fatty acids) of Atlantic salmon dark muscle after HPP treatment or cooking followed by storage for 4 days at 4 °C. Control (0.1 MPa) was untreated muscle stored under the same conditions as treated samples.

	0.1 MPa	150 MPa	300 MPa	Cook
14:00	3.8 ± 0.5^{a}	4 ± 0.4^{a}	4.3 ± 0.4^{a}	4.2 ± 0.5^{a}
16:00	15 ± 1.9^{a}	16.2 ± 0.7^{a}	16.2 ± 0.8^{a}	12.2 ± 0.4^{b}
18:00	4.5 ± 1^{a}	4.7 ± 0.1^{a}	4.6 ± 0.1^{a}	2.4 ± 0.1^{b}
Total saturated	23.9 ± 3.1^{a}	25.5 ± 1.1^{a}	25.8 ± 1.2^{a}	19.1 ± 0.9^{b}
16:1*n*−7	5.4 ± 0.4^{b}	5.4 ± 0.3^{b}	5.4 ± 0.3^{b}	6.2 ± 0.4^{a}
18:1*n*−9	14.9 ± 0.4^{a}	14.8 ± 0.5^{a}	14.9 ± 0.7^{a}	14.1 ± 0.6^{b}
18:1*n*−7	4.1 ± 0.3^{a}	4.4 ± 0.2^{a}	4.3 ± 0.3^{a}	4.3 ± 0.1^{a}
20:1*n*−9	4.3 ± 4.6^{b}	2.7 ± 0.4^{b}	3 ± 0.4^{b}	12.3 ± 0.7^{a}
22:1*n*−11	3.4 ± 3.7^{b}	2.1 ± 0.2^{b}	2.1 ± 0.3^{b}	12.8 ± 0.6^{a}
Total monoenes	33.7 ± 8.6^{b}	31 ± 0.9^{b}	31.4 ± 1.3^{b}	51.2 ± 0.7^{a}
18:2*n*−6	6.3 ± 1.3^{ab}	6.5 ± 0.4^{a}	5.6 ± 0.5^{b}	3.4 ± 0.1^{c}
20:2*n*−6	0.5 ± 0^{ab}	0.6 ± 0.1^{a}	0.5 ± 0^{b}	0.4 ± 0^{c}
Total *n*−6 PUFA	8.1 ± 1.3^{a}	8.2 ± 0.5^{a}	7 ± 0.6^{b}	4.5 ± 0.5^{c}
18:3*n*−3	1.1 ± 0.3^{b}	1.1 ± 0.2^{b}	1 ± 0^{b}	1.7 ± 0.3^{a}
18:4*n*−3	1.4 ± 0.1^{b}	1.3 ± 0^{bc}	1.3 ± 0.1^{c}	1.6 ± 0.1^{a}
20:5*n*−3 (EPA)	9.4 ± 1.2^{a}	9.5 ± 0.4^{a}	9.1 ± 0.4^{a}	6.6 ± 0.3^{b}
22:5*n*−*n*−3	4.5 ± 0.8^{a}	4.6 ± 0.3^{a}	4.5 ± 0.2^{a}	2.9 ± 0.1^{b}
22:6*n*−3 (DHA)	16.2 ± 2.6^{b}	16.9 ± 1^{ab}	18.1 ± 1.6^{a}	11.5 ± 1.3^{c}
Total *n*−3 PUFA	32.7 ± 4.6^{a}	33.5 ± 1.2^{a}	34 ± 1.8^{a}	24.2 ± 1.3^{b}
n−3/*n*−6	4.1 ± 0.4^{b}	4.1 ± 0.3^{b}	4.9 ± 0.6^{a}	5.5 ± 0.7^{a}

Results are means ± SD, $n = 12$, different letters within a row for each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Table 6
Fatty acid compositions (g fatty acids/100 g total fatty acids) of Atlantic salmon dark muscle after HPP treatment or cooking followed by storage for 6 days at 4 °C. Control (0.1 MPa) was untreated muscle stored under the same conditions as treated samples.

	0.1 MPa	150 MPa	300 MPa	Cook
14:00	5.6 ± 0.5^{a}	5.4 ± 0.4^{a}	4.7 ± 0.4^{a}	5.1 ± 0.4^{a}
16:00	16.7 ± 1.6^{b}	18.1 ± 1^{a}	16.8 ± 0.8^{b}	13.4 ± 0.5^{c}
18:00	3.9 ± 1^{b}	4.5 ± 0.1^{a}	4.6 ± 0.1^{a}	2.4 ± 0.1^{c}
Total saturated	26.9 ± 2.4^{b}	28.9 ± 1.4^{a}	27 ± 1.2^{b}	21.5 ± 0.8^{c}
16:1*n*−7	6.8 ± 0.7^{a}	6.2 ± 0.4^{b}	5.6 ± 0.4^{b}	6.9 ± 0.6^{a}
18:1*n*−9	14.4 ± 0.4^{a}	14.5 ± 0.3^{a}	14.3 ± 0.7^{a}	14.3 ± 0.6^{a}
18:1*n*−7	4.6 ± 0.4^{a}	4.7 ± 0.5^{a}	4.9 ± 0.5^{a}	4.6 ± 0.2^{a}
20:1*n*−9	3.7 ± 3.5^{b}	1.6 ± 0.4^{c}	1.5 ± 0.2^{c}	8.1 ± 0.6^{a}
22:1*n*−11	5.5 ± 5.3^{b}	2.1 ± 0.3^{c}	2.1 ± 0.1^{c}	14 ± 1.8^{a}
Total monoenes	36.6 ± 9.5^{b}	30.5 ± 0.6^{c}	30 ± 1.3^{c}	49.6 ± 1^{a}
18:2*n*−6	5.7 ± 1.7^{ab}	6.4 ± 0.6^{a}	5.3 ± 0.4^{b}	3.5 ± 0.1^{c}
20:2*n*−6	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}
Total *n*−6 PUFA	7.1 ± 2.1^{ab}	7.9 ± 0.6^{a}	6.6 ± 0.5^{b}	4.4 ± 0.2^{c}
18:3*n*−3	0.4 ± 0.3^{a}	0.4 ± 0.1^{a}	0.3 ± 0^{a}	0 ± 0^{b}
18:4*n*−3	1.4 ± 0.2^{b}	1.2 ± 0^{c}	1.2 ± 0.1^{c}	1.6 ± 0.1^{a}
20:5*n*−3 (EPA)	8.3 ± 1.3^{b}	8.9 ± 0.3^{ab}	9.5 ± 0.5^{a}	6.7 ± 0.2^{c}
22:5*n*−3	3.9 ± 0.9^{b}	4.2 ± 0.2^{ab}	4.6 ± 0.2^{a}	3 ± 0.1^{c}
22:6*n*−3 (DHA)	13.8 ± 2.6^{c}	15.9 ± 1.5^{b}	18.7 ± 1.8^{a}	12.2 ± 1.1^{c}
Total *n*−3 PUFA	27.8 ± 4.9^{b}	30.8 ± 1.8^{b}	34.3 ± 1.7^{a}	23.5 ± 1.1^{c}
n−3/*n*−6	4.1 ± 0.7^{b}	3.9 ± 0.5^{b}	5.2 ± 0.5^{a}	5.4 ± 0.4^{a}

Results are means ± SD, $n = 12$, different letters within a row for each day indicate significant differences at $p < 0.05$ separated by Tukey's HSD.

Total *n*−6 polyunsaturated fatty acids (PUFA) were mainly composed of 18:2*n*−6 and 20:2*n*−6. Cooked samples led to significant ($p < 0.05$) decrease in total *n*−6 PUFA compared to HPP treatment or no treatment. Several *n*−3 PUFAs were detected in the samples: 18:3*n*−3, 18:4*n*−3, 20:5*n*−3 (EPA), 22:6*n*−3 (DHA), 22:5*n*−3. The major individual fatty acids making up the total *n*−3 PUFAs were 20:5*n*−3 (EPA), 22:5*n*−3 and 22:6*n*−3 (DHA). Cooking significantly ($p < 0.05$) reduced the amount of EPA from 9.5 to 7.0 g/100 g at day 0. However, storage at 4 °C for 6 days did not significantly change the level of *n*−3 and *n*−6 polyunsaturated fatty acids in cooked, HPP treated samples or control fillets (Figs. 4a and b). The level of EPA remained significantly low for the cooked samples compared to the other treatments during the entire storage period. On the other hand, HPP treatment did not change the amount of EPA at day 0 (Table 3). Cooking also significantly decreased the amount of DHA from 16.8 to 12.7 g/100 g, unlike HPP (Table 3), which remained lower than before cooking during the entire storage. There were no significant differences in the level of 22:5*n*−3 fatty acids at day 0, 2, and 4 between HPP treated and control samples. However, it was found that cooking significantly reduced levels of 22:5*n*−3 fatty acids, below that of other treatments, and levels remained reduced during the entire storage time. Control and pressure treated samples did not show any significant difference in the levels of 22:5*n*−3 at day 0, 2, 4, and 6 except for 300 MPa, which did show a significant ($p < 0.05$) difference from the control and 150 MPa pressure treated sample at day 6 (Table 3).

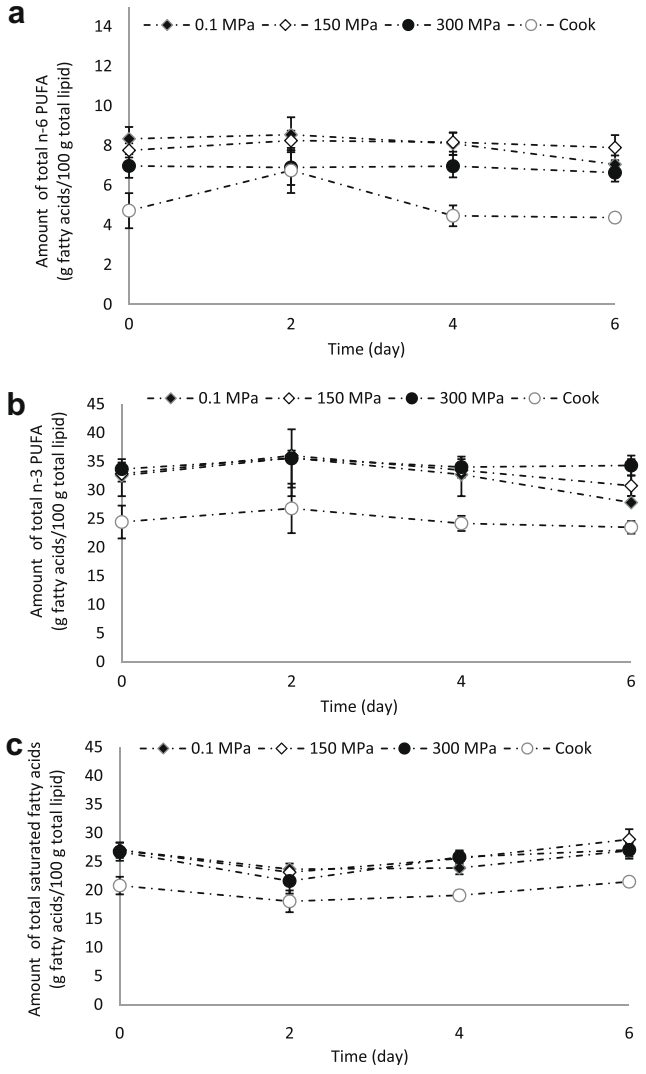

Fig. 4. Fatty acid compositions (g fatty acids/100 g total fatty acids) of Atlantic salmon dark muscle after HPP treatment or cooking followed by storage for 6 days at 4 °C. (a) Total *n*−6 PUFA; (b) total *n*−3 PUFA; (c) total saturated fatty acids.

4. Discussion

One of the important criteria for quality evaluation of fresh and frozen seafood products is the initial total microbial count. As stated by the International Commission of Microbiological Standards for Foods (ICMSF, 1978), the maximum acceptable microbial limit in fresh and frozen fish is 7 log cfu/g. The current study indicated that the initial microbial count for control Atlantic salmon sample was about 3.5 which is an acceptable level for ICMSF. The 150 MPa treatment with a 15 min holding time resulted in about a two log reduction in microbial counts. As the pressure increased to 300 MPa, with the same holding time, a total reduction in aerobic microorganisms was observed and no microbial growth was seen during all 6 days of refrigerated storage. Similarly, no microbial activity was found for cooked samples after cooking and during storage (Fig. 2). The 300 MPa treatment was therefore equally as effective as cooking in reducing aerobic microorganisms. The effectiveness of HPP for reducing or inactivating microbial growth of seafood has been previously reported, including sea bass (Cheret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & De Lamballerie, 2005), oyster (He, Adams, Farkas, & Morrissey, 2002), octopus (Hurtado, Montero, Borderias, & Solas, 2001), albacore tuna (Ramirez-Suarez & Morrissey, 2006), mahi mahi and rainbow trout (Yagiz et al., 2007). Various authors have suggested that the mechanisms for the inactivation of microbial growth by HPP could be denaturation/inactivation of key proteins and enzymes and damage to cell membrane, releasing intracellular constituents, which could play a role in microbial cell inactivation (Carlez, Vecianan-ogues, & Cheftel, 1995; Smelt, 1998). The higher the pressure, the more profound these effects, in agreement with the results seen here for Atlantic salmon.

Colour and appearance of seafood are very important in terms of consumer perception of seafood quality, and are dominant factors in consumer purchasing decision. Although seafood texture is a parameter normally not used by many consumers in their

buying decision, it is very important when seafood is consumed. The highest pressure level (300 MPa) resulted in a cooked appearance, increasing 32% in L^* value and decreasing 13% in a^* value for dark muscle at the end of the storage period (Table 1). An increased L^* value and decreased a * value as pressure is increased has been reported for different seafood species such as bluefish (Matser, Stegeman, Kals, & Bartels, 2000), cod (Angsupanich & Ledward, 1998), mackerel (Ohshima et al., 1993), salmon (Amanatidou, Slump, Gorris, & Smid, 2000), sheaphead (Ashie et al., 1996), and turbot (Chevalier, Le Bail, & Ghoul, 2001). The mechanisms of those changes are not entirely clear. However, Carlez et al. (1995) stated that one of the reasons of cooked appearance, decrease in a^* value or increase in L^* value could be denaturation of proteins when pressures of 200–300 MPa are applied for 10 min. In terms of textural changes of the salmon fillet pieces, HPP caused a significant increase in hardness, gumminess, chewiness and springiness over both control and cooked samples (Table 2). These textural parameters increased with an increase in pressure levels. Earlier studies on cold-smoked and high pressure treated salmon showed a decrease in the activity of certain proteolytic enzymes (Lakshmanan, Patterson, & Piggott, 2005) and an increase in hardness with an increase in pressure (Lakshmanan, Miskin, & Piggott, 2005). The reason for these changes could be denaturation of proteins and cellular damage due to HPP. Since HPP led to increases in all textural parameters tested compared to cooking, except for adhesiveness, it can be concluded that pressure treatment led to either more denaturation than cooking or a different type of denaturation, resulting in a unique texture for the pressurised samples.

The dark muscle of Atlantic salmon had a large increase in lipid oxidation for all treated samples and control during 6 day refrigerated storage. It was observed that there was no significant ($p > 0.05$) difference among the control, 150 MPa treated and cooked samples at day 4 and 6, however, the 300 MPa had significantly ($p < 0.05$) lower TBARS values compared to other samples (Fig. 3). It is generally assumed that as the pressure level increases, lipid oxidation increases. The effect of HPP on lipid oxidation could depend on pressure level, holding time, fish species and type of muscle. For example, Ramirez-Suarez and Morrissey (2006) studied the effect of different pressure levels (275–310 MPa) and different holding times (2–6 min) on oxidation of albacore tuna muscle. They stated that control samples had higher TBARS values than all of the HPP treated samples during a 3 month storage period. The pressure levels used in the current study are known to denature proteins, like cooking. Heme proteins are key catalysts of lipid oxidation in fish muscle, and would likely have been denatured at the pressure levels used. Even though TBARS values were higher for the 150 MPa treatment at day 4 and 6 compared to control, they were not significantly higher. Changes in lipid membranes are also to be expected at the pressures used, and one might expect perturbations in membranes could make them more susceptible to oxidation. It is therefore interesting that no difference was seen at 150 MPa in terms of oxidation and those samples were actually less susceptible to oxidation at 300 MPa than all the other treatments. Research on acid treatment of fish muscle has shown that significant changes in cell membrane structure may in fact make them less susceptible to lipid oxidation even though heme proteins are significantly denatured with the same treatment (Hultin, 1994). It is possible that this occurred with the high pressure treatment. In addition, salmon have higher amounts of astaxanthin compared to many seafood products. It has been stated that the antioxidation capacity of astaxanthin is 10 times greater than that of β-carotene, and up to 500 times greater than vitamin E (Shimidzu, Goto, & Miki, 1996). During and after pressure treatment, the changes in the muscle structure might make astaxanthin become more available to protect lipids from lipid oxidation.

Fatty acid profiles of cooked Atlantic salmon dark muscle showed significantly lower amounts of total saturated, $n-3$ PUFA and $n-6$ PUFA and significantly higher amounts of monoenes than HPP treated samples during the entire storage period. However, the most important finding of this study was that there was no significant difference ($p > 0.05$) between control and HPP treated samples in terms of total saturated, monoenes, $n-3$ PUFA and $n-6$ PUFA fatty acid profiles (Tables 3–6). Also, storage of HPP treated samples for 6 days at 4 °C did not significantly change the level of $n-3$, $n-6$ PUFA or saturated fatty acid contents. This demonstrates that HPP is a very mild process in terms of its effect on fatty acids.

Seafood lipids are characterised by a high level of PUFA such as EPA and DHA (Ackman, 1994). It was found that the predominant amount of $n-3$ PUFA came from EPA and DHA in Atlantic salmon dark muscle for all samples. There were no significant changes in amounts of EPA and DHA for control samples and HPP treated samples during storage. However, cooking resulted in a significant loss of EPA and DHA compared to control and HPP treated samples (Tables 3–6). It is well known that HPP does not affect covalent bonds; on the other hand, cooking can break covalent bonds. These findings could be correlated experimentally to HPP processing, which does not affect polyunsaturated fatty acids whereas cooking does.

5. Conclusion

It was found that HPP and cooking significantly reduced microbial growth. The 150 MPa treatment had a smaller effect on colour compared to cooking and 300 MPa. While cooking and 150 MPa led to similar oxidation development as control, 300 MPa treatment effectively reduced the samples' susceptibility to oxidation. Cooking significantly reduced the amount of total $n-6$ PUFA and $n-3$ PUFA, including EPA and DHA fatty acids, however, HPP did not change the level of those fatty acids compared to control. HPP may have some negative effects on muscle structure and functionality. More studies are required to study these changes. Overall, these results prove the usefulness of HPP in seafood processing while providing quantitative parameters in order to help with the application of this process.

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