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PII: S0963-9969(19)30626-X
DOI: https://doi.org/10.1016/j.foodres.2019.108740
Reference: FRIN 108740

To appear in: *Food Research International*

Received Date: 13 June 2019
Revised Date: 29 September 2019
Accepted Date: 2 October 2019

Please cite this article as: Fidalgo, L.G., Simões, M.M.Q., Casal, S., Lopes-da-Silva, J.A., Margarida Salgueiro Carta, A., Delgadillo, I., Saraiva, J.A., Physicochemical parameters, lipids stability, and volatiles profile of vacuum-packaged fresh Atlantic salmon (*Salmo salar*) loins preserved by hyperbaric storage at 10 °C, *Food Research International* (2019), doi: https://doi.org/10.1016/j.foodres.2019.108740

Physicochemical parameters, lipids stability, and volatiles profile of vacuum-packaged fresh Atlantic salmon (*Salmo salar*) loins preserved by hyperbaric storage at 10 ºC

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ABSTRACT

Lipid stability, physical properties and volatiles profile of vacuum-packaged fresh Atlantic salmon (*Salmo salar*) loins were evaluated after hyperbaric storage at low temperature (HS/LT: 60 MPa/10 ºC) and compared to atmospheric pressure and conventional refrigeration (AP/5 ºC) after 5, 15 and 30 days, and at low temperature (AP/10 ºC), after 5 and 15 days.

No variations in drip loss and water holding capacity were observed for HS/LT samples. Compared to AP, HS/LT caused lower changes on muscle fibres, visible by scanning electron micrographs, and a decrease of resilience property (only after 30 days). In addition, myofibrillar fragmentation index did not change at HS/LT. Fatty acids were generally not affected by the different storage conditions, while the polyene index at HS/LT was similar to fresh samples during the 30 days of storage, confirmed by the lower lipid oxidation state of these samples, compared to AP. According to the volatile profile (SPME-GC/MS), HS samples showed to be more similar to the fresh ones, retaining fresh-like alcohols and aldehydes, generally not detected in AP samples after 15 days, the latter presenting spoilage-related compounds probably derived from microbial activity.

According to these results, HS/LT represents a promising preservation methodology for fresh salmon loins (and fish in general), retaining better important physicochemical properties for 30 days, when compared to the conventional refrigeration.

Keywords: Hyperbaric storage; refrigeration/low temperature; *Salmo salar*; lipid stability; physical properties; volatiles profile.

1. INTRODUCTION

It is well known that fish is a highly perishable food characterized by short shelf-life, mainly when stored under refrigeration. Deteriorative changes are caused by different damage mechanisms, such as microbial spoilage, autolytic degradation and lipid oxidation (Amit, Uddin, Rahman, Islam, & Khan, 2017). During fish spoilage there is a breakdown of various components and formation of new ones, responsible for changes in odour, flavour and texture. Microbial spoilage produces amines, organic acids, sulphides, alcohols, aldehydes, and ketones, with unpleasant and unacceptable off-flavours (Getu & Misganaw, 2015). Additionally, oxidation decreases fish quality due to taste, texture and consistency losses caused by the formation of odours and lipid peroxides, while reducing the nutritional value (Ghaly, Dave, Budge, & Brooks, 2010). Atlantic salmon (*Salmo salar*) is a good source of polyunsaturated fatty acids (PUFAs), namely two important omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ortiz et al., 2012). However, oxidation susceptibility increases as the number of double bonds in the fatty acid increases, with PUFAs being highly susceptible to oxidation. However, the beneficial effects of ω-PUFAs are increasing, particularly EPA and DHA in the prevention of chronic diseases (Tao, 2015). Otherwise, salmon is characterized by its red/orange coloration as a result of the presence of the carotenoid pigment astaxanthin, which has strong antioxidant activity, providing a higher oxidative stability to salmon muscle (Ambati, Moi, Ravi, & Aswathanarayana, 2014).

Hyperbaric storage (HS) has attracted increasing interest due to the possibility to store food products above atmospheric pressure, increasing their shelf-life and quality comparatively to the conventional method of refrigeration. The effect of HS for fish preservation at LT was already studied by Otero, Pérez-Mateos, & López-Caballero (2017) and Otero, Pérez-Mateos, Holgado, Márquez-Ruiz, & López-Caballero (2019), using Cape hake (*Merluccius* spp.) loins and Atlantic mackerel (*Scomber scombrus*, L.) fillets, respectively, being observed a pronounced extension of shelf-life. In such studies,

a pressure/temperature storage condition of 50 MPa/5 ºC was used in both fish species, resulting in both a stable microbial load and a low total volatile base-nitrogen content after 7 and 12 days, respectively. Nevertheless, drip losses and an increase of the shear resistance and whiteness of the raw fish were verified, but after cooking these changes were imperceptible. These two studies clearly show the potential of HS/LT to increase fresh fish shelf-life, being of great interest further and deeper studies of the HS effect on fresh fish as, for instance, the study for longer storage periods.

In a previous work (Fidalgo et al., 2019) different storage pressure/temperature conditions (40-60 MPa; 5-15°C) were evaluated, finding that a HS/LT condition of 60 MPa/10 ºC allowed maintaining the microbial stability of the product for 50 days, but also increased the formation of secondary lipid oxidation compounds, although to a lesser extent when compared to atmospheric pressure storage (5 and 10 ºC). In this way, Atlantic salmon samples were vacuum-packaged in this work, as an attempt to reduce lipid oxidation, and important physicochemical parameters were evaluated during 30 days of storage at 60 MPa/10 ºC including selected physical properties (drip loss, water holding capacity, texture and scanning electron microscopy), myofibrillar fragmentation index, lipid stability (fatty acids profile and lipid oxidation), and volatiles profile.

2. MATERIALS AND METHODS

2.1. Sample preparation and storage experiments

Atlantic salmon (*Salmo salar*) was acquired from a local market next to the University (5 minutes driving distance) before each experiment storage, being fish samples transported in a closed box with ice. The fish was caught between 24-48 hours before acquisition and each storage experiment was carried out using the same initial fresh salmon to ensure the same fish quality. Salmon loins samples were selected from two individuals with similar weight (*ca.* 9 kg) and length (*ca.* 120 cm), according to the scheme represented in **Figure 1S** (**Supplementary Material)**. The skin from the salmon loins samples was removed in aseptic conditions, and loins with *ca.* 60 g were selected (dimensions: 10 cm × 4 cm × 1.5 cm). Salmon loins were vacuum-packaged in low-oxygen permeable barrier bags (PA/PE-90; Plásticos Macar – Indústria de Plásticos Lda., Palmeira, Portugal) and sealed under vacuum at -1 bar of pressure (Vacuum Packaging Machine Culinary, Albipack, Águeda, Portugal). This level of vacuum was selected according to the results obtained in a preliminary assay (results are shown in the **Figure 2S** in the **Supplementary Material**). In this preliminary assay, salmon samples were

packaged in the presence of air (similarly to a previous work; Fidalgo et al., 2019), with vacuum and with an antioxidant (also with air – to verify if the oxidation value is mainly caused by oxygen presence), revealing better results for vacuum, allowing the reduction of secondary lipid oxidation values, when compared to samples stored with air. Furthermore, no adverse effect on anaerobic bacteria survival was observed on vacuum-packaged salmon samples. Therefore, for the present study all the tested samples were stored under vacuum. To avoid deterioration, all samples were kept on ice, and storage experiments were initiated as soon as all the samples were prepared (within 2 hours maximum).

Salmon samples were stored at 60 MPa/10 ºC for 5, 15 and 30 days. Control samples were always kept at atmospheric pressure (0.1 MPa, AP), under conventional refrigeration (AP/5 ºC) for 5, 15 and 30 days, and at the same temperature (AP/10 ºC) for 5 and 15 days, in the same conditions (in the dark and immersed in the same fluid used for compression). A different maximum storage time was used for AP/10°C, since salmon samples stored under this condition showed clear degradation signs (texture changes and swollen packages), having been decided not to continue to analyse after 30 days. HS was performed using a 200 mL high-pressure equipment (SFP FPG13900, Stansted Fluid Power, Stansted, United Kingdom), with a pressure vessel of 35-mm inner diameter and 250 mm height (maximum of pressure level of 200 MPa). This high-pressure equipment uses a mixture of propylene glycol and water (40:60, v/v) as pressurization fluid. Storage experiments were carried out between March and June 2018.

2.2. Physical properties

2.2.1. Drip loss and water holding capacity

Drip loss was calculated as the difference in salmon loins weight between day 0 (m_0) and day x (m_x) – **Equation 1**:

$$Drip\ loss\ (\%) = \frac{m_0 - m_x}{m_0} \times 100 \qquad \textbf{Eq. 1}$$

The water holding capacity (WHC) was measured with the method of Szajdek & Borowska (2008). Briefly, a previously weighted (*ca* 1 g) piece of salmon was wrapped in two filter papers (also weighted; Whatman #1) and centrifuged (530×g, 15 min, 4 °C; centrifuge 3K30, Sigma, Osterode, Germany). After centrifugation, the sample was

removed, and the filter papers were weighted. WHC was calculated using the following formula (**Equation 2**):

$$WHC\ (\%) = \frac{W_0 - \Delta W}{W_0} \times 100 \qquad \textbf{Eq. 2}$$

$$\text{where } W_0 = \frac{V_0}{(V_0 + D_0)} \times 100 \qquad \textbf{Eq. 3}$$

$$\text{and } \Delta W = \frac{\Delta V_0}{(V_0 + D_0)} \times 100 \qquad \textbf{Eq. 4}$$

The initial weight of a raw sample m_0 was equal to the sum of the initial water content of raw material, V_0, and dry material, D_0, being estimated by drying previously weighed salmon samples (*ca.* 5 g) at 103 ± 2 ºC during 24 hours (ISO 1442, 1997). The weight of the exudates (the liquid separated from the sample during centrifugation) is named ΔV_0 (**Equations 3 and 4**). The solid contents of the exudates are regarded as negligible in these calculations. All determinations were carried out in triplicate of salmon samples for each condition.

2.2.2. Texture profile analyses (TPA)

Texture profile analyses (TPA) of salmon samples were evaluated perpendicularly to the myotomes. Measurements were taken with a 6 mm diameter probe fitted to a TA.HDi texture analyser (Stable Micro Systems, Surrey, England) equipped with a 5 kg load cell. The crosshead moved at a constant speed of 2 mm/s. The test conditions were two consecutive cycles with a 5-s delay between cycles and 1 cm of penetration. From the resulting force-time curve (as can be seen in **Figure 3S** in **Supplementary Material**), the following parameters were determined (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Veland & Torrissen, 1999): *hardness* (N), maximum force required to compress the sample; *adhesiveness* (N.s), the largest negative force value during the upstroke; *springiness* or *elasticity*, sample ability to recover its original form after the deforming force is removed (time 2/time 1); *resilience*, area of the first upstroke relative to the area of the first downstroke (A1’’/A1’).

2.2.3. Scanning electron microscopy

Longitudinal sections of fibres from freeze-dried salmon (lyophilized at - 70 °C and 0.010 mbar for 72 hours; Telstar Benchtop Freeze Dryer LyoQuest, Barcelona, Spain) were examined by scanning electron microscopy (SEM) on a Tabletop Microscope TM4000Plus from Hitachi (Ibaraki-Ken, Japan), with an accelerating voltage of 15kV.

2.3. Lipid hydrolysis and oxidation

2.3.1. Lipid extraction

Lipid fraction was obtained according to the method of Bligh & Dyer (1959), as already described in a previous work (Fidalgo, Lemos, Delgadillo, & Saraiva, 2018). This lipid fraction was used for fatty acids composition and peroxides values (primary lipid oxidation) determinations, and the upper layers were used for fluorescence ratios determination. Both analyses were performed always after lipid extraction (in the same day) with random sampling. The total extracted lipid content ranged from 5.28 to 10.22% (w/w), values that are within the range reported in the literature for salmon (Blanchet et al., 2005).

2.3.2. Fatty acids determination

For the fatty acid methyl ester (FAME) synthesis (O'Fallon, Busboom, Nelson, & Gaskins, 2007), 1 mL of a surrogate fatty acid (C17:0 – 4 mg/mL in methanol; a surrogate recovery of 93-96% was obtained at the end of FAME synthesis), 5.3 mL of methanol and 0.7 mL of 10 N potassium hydroxide were added into a tube containing 40 µg of oil resulting from the lipid extraction. After vortex-mixing for 10 s, the tube was incubated in a thermostatic bath at 55 ºC with stirring for 1.5 h. The mixture was mixed by inversion and 0.58 mL of 12 M sulphuric acid were added, with the formation of a white precipitate (potassium sulphate). The tube was incubated again at 55 ºC for 1.5 h, thus occurring the FAME synthesis. The tube was cooled in cold water, 3 mL of hexane were added and vortex-mixed again for 5 min. After centrifugation (3000 rpm, 5 min), the hexane layer containing FAMEs was placed into a new tube, which already contained 0.5 g of anhydrous sodium sulphate. After another centrifugation for 5 min, the hexane layer (900 µL) containing FAMEs was placed into a GC vial and 100 µL of the C19:0 internal standard (10 mg/mL of C19:0 methyl ester in hexane) were added.

FAMEs were analysed using a gas chromatograph mass spectrometer (GC-MS Shimadzu QP2010 Ultra) equipped with an AOC-20i autosampler (Shimadzu, Japan), with the electron impact ionization (EI) at 70 eV and high-performance quadrupole mass

filter. The separation of the compounds was carried out on a fused-silica DB-5 MS type capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) using helium as the carrier gas (40 cm/s) and a split ratio = 50. The GC-MS chromatographic conditions were as follows: injection port temperature 300 °C; initial oven temperature 140 °C (0 min), rising to 250 °C (0 min) at 1.0 ºC/min and then rising to 280 °C (for 2 min) at 10.0 °C/min. The mass spectrometer was operated over a range of m/z 50-1000. The ion source was kept at 200 ºC and the interface temperature at 300 ºC. Peaks were automatically integrated (GCMS solution Version 4.20), C19:0 fatty acid methyl ester being used as internal standard for quantitative analysis. The compound/internal standard signal ratio for each peak was used for the calculation of concentrations, using different calibration curves as described in **Table 1S (Supplementary material)**. Peaks were identified by comparison of their retention times with standard FAME mixtures (Supelco 37 FAME Mix, Sigma-Aldrich, Missouri, EUA). Three salmon samples were used for each storage condition with a duplicate GC injection. Results are reported as g of fatty acids (g FA)/100 g of lipids.

The polyene index (PI), as a ratio of polyunsaturated to saturated fatty acids, was calculated as follows in the **Equation 5** (Ortiz, Vivanco, & Aubourg, 2014):

$$\text{Polyene index} = \frac{\text{C20:5} + \text{C22:6}}{\text{C16:0}} \qquad \textbf{Eq. 5}$$

2.3.3. Lipid oxidation

Peroxide value determination (primary lipid oxidation) was carried out by following the ferric thiocyanate method used by Gheisari, Møller, Adamsen, & Skibsted (2010), as already described in a previous work (Fidalgo et al., 2018), using the lipid fraction obtained in the lipid extraction. The peroxide value was expressed as mg Fe III/kg lipids.

Secondary lipid oxidation was evaluated by quantification of secondary lipid oxidation products using the thiobarbituric acid-reactive substances (TBARS) method, which was performed as described by Vyncke (1970) and in a previous work (Fidalgo et al., 2018). TBARS results were expressed as μg malondialdehyde (MDA)/g fish.

Tertiary lipid oxidation compounds resulting from the interaction between oxidized lipids and nucleophilic compounds (namely protein-like molecules) were measured by fluorescence spectroscopy (Hitachi F2000 fluorescence spectrophotometer (Tokyo,

Japan). In agreement with previous research (Aubourg, 1999), fluorescence measurements were carried out at 393/463 nm and 327/415 nm in the aqueous phase (methanol-water layer) resulting from the lipid extraction of fish muscle (Bligh & Dyer, 1959). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission wavelength pair and F_{st} is the fluorescence intensity of a quinine sulfate solution (1 µg/mL in 0.05 M H_2SO_4) at the corresponding wavelength pair. The fluorescence ratio (FR) was calculated as the ratio between the two RF values on **Equation 6**:

$$FR = \frac{RF_{\frac{393\ nm}{463\ nm}}}{RF_{\frac{237\ nm}{415\ nm}}} \qquad \textbf{Eq. 6}$$

2.4. Protein stability

Myofibril fragmentation index (MFI) was determined by the method of Hopkins, Martin, & Gilmour (2004) with slight modifications (Zhang et al., 2013). Muscle tissue was pulverized in liquid nitrogen and 0.5 g of this powdered tissue was homogenized for 1 min (MICCRA D-9 Homogenizer, MICCRA GmbH, Müllheim, Germany) in 30 mL of 25 mM phosphate buffer (0.1 M potassium chloride, 1 mM EDTA acid, pH 7.0). The suspension was filtered to remove connective tissue (Whatman No. 1) and the residue was washed with 10 mL of the same phosphate buffer. Then, filtrate was centrifuged at 1000×g for 15 min at 4 ºC (Heraeus Biofuge Stratos, Thermo, Electron Corporation, Massachusetts, EUA), the precipitate was resuspended in 10 mL of phosphate buffer and centrifuged again. This step was repeated twice and the pellet was suspended in buffer solution (10 mL). Protein concentrations were determined and, after adjustment to a concentration of 0.5 mg/mL using the same buffer, absorbance measurements at 540 nm were done (Multiskan Go microplate spectrophotometer, Thermo Scientific, Waltham, EUA). Protein concentrations were determined by the Bradford assay modified by Zor & Selinger (1996) and using bovine serum albumin (BSA) as the standard (0.1-0.6 mg BSA/mL of phosphate buffer). MFI was calculated by multiplying measurements with 150.

2.5. Volatile compounds characterization by HS-SPME-GC/MS

The characterization of the volatile fraction from salmon samples was performed by headspace solid-phase microextraction (HS-SPME) gas-chromatography-mass spectrometry (GC/MS).

Salmon samples (2 g) were cut and weighed into a vial containing a micro stirring bar. Then, 1 mL of internal standard (0.473 mg/mL cyclohexanone in water), 3 mL of ultrapure water, and 1.44 g of sodium chloride were added into the vial and sealed with a silicone septum. The sample was equilibrated for 20 min at 50 ºC followed by HS-SPME exposure at the same temperature under stirring (500 ×g). The volatile compounds in the headspace were absorbed onto SPME fibres (DVB/CAR/PDMS 50/30 mm; Supelco, Bellefonte, USA) for 40 min. Salmon samples were analysed in triplicate for each condition. The retained compounds were thermally desorbed from the fibre for 5 min in the injection port (splitless mode; 250 °C). The fibre was maintained for further 5 min in the injector port for cleaning and conditioning for further analyses.

Chromatographic separation was performed on a fused-silica DB-5 MS capillary GC column (30 m ×0.25 mm I.D. × 0.25 μm film thickness, Agilent) with a temperature program from 40 °C to 235 °C, with a total run time of 60 min. The MS transfer line and ion source were at 280°C and 230ºC, respectively, and MS quadrupole temperature at 150 °C, with electron ionization of 70 eV; set in full scan mode (m/z 40 to 650 at 1.2 scan/s). Identification of the volatile compounds detected by GC/MS analysis was based on computer matching with the reference mass spectra of the MS library of NIST 11 and Wiley 7.0, retention times (RTs) and retention index (RIs). Semi-quantitative determinations were carried out by using cyclohexanone as an internal standard. The volatiles' profile results were expressed as the percentage (%) of each compound in total identified compounds for the same condition.

2.6. Statistical analysis

Data were tested with one-way analysis of variance (ANOVA), followed by a multiple comparisons test (Tukey's Honestly Significant Difference, HSD) to identify differences between conditions and during storage period using IBM SPSS Statistics 25 (IBM, Armonk, NY, USA). The level of significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical properties and myofibrillar protein stability during storage

Drip loss and water holding capacity (WHC) were evaluated in salmon samples stored under the different conditions (**Table 1**). Samples stored at AP/10 ºC showed the highest drip loss values, of about 6.1% and 6.8% after 5 and 15 days, respectively, which were not significantly different from samples stored at AP/5 ºC for 30 days (6.6%). For HS at 60 MPa/10 ºC no significant differences on drip loss values were observed during 30 days of storage. However, WHC changed during the 30 days of HS, decreasing from 95.8% to 92.0% after 5 days, but increasing further to 97.4% after 30 days. Generally, high pressure promoted drip loss and reduced WHC, which is normally associated to disruption of electrostatic and hydrophobic interactions, this mean, rupture of non-covalent interactions, which have been reported to affect muscle quality (Marcos & Mullen, 2014). These effects induce changes in protein-protein conformation and denaturation of important myofibrillar and/or sarcoplasmic proteins, leading to exudation from muscles which is collected as drip loss. However, drip loss did not change under HS, with WHC decreasing effect during the 15 days of storage, and slightly increasing after 30 days. Otero et al. (2017) stored Cape hake loins at 50 MPa/5 ºC and observed a similar behaviour; WHC of HS samples increased already after 7 days, being a possible explanation the fact that probably part of the free water in these samples had been previously released as drip loss and the water still present was more strongly retained by the tissue.

Through texture profile analysis test, it was possible to obtain a wide range of food texture properties: hardness, adhesiveness, springiness, and resilience **(Table 2)**. Comparing to fresh salmon, no significant differences were found between the conditions for the texture properties of hardness, adhesiveness and springiness. However, HS at 60 MPa/10 ºC showed lower values for resilience, being statistically different from fresh salmon. After 30 days, samples stored under HS decreased 70% relatively to the initial value, from 1.27±0.29 to 0.38±0.03. Veland & Torrissen, (1999) noted that a resilience value of 1 means that all the work performed by the probe during the downstroke is returned by the muscle during the upstroke. So, according to these authors, resilience is the capacity of the muscle to recover after a deformation, resisting to a subsequent deformation. Cape hake loins (Otero et al., 2017) and Atlantic mackerel (Otero et al., 2019) were stored at 50 MPa/5 ºC during 7 and 12 days, respectively, being verified that storage under pressure increased the shear resistance of Cape hake loins, but no effect on firmness was observed in the case of Atlantic mackerel. The fat content can be a possible reason to this effect, since it is possible to observe a different effect for fatty fish (Atlantic

salmon and Atlantic mackerel) and lean fish (Cape hake). Arnaud, de Lamballerie, & Pottier, (2018) reported that when submitted to a 5-min high pressure processing at 150-300 MPa, salmon (fatty fish) hardness was less effected by pressure than that of a lean fish (cod) as a 150 MPa treatment had no effect on its texture profile and 300 MPa was the threshold pressure value, whereas the threshold pressure seems to be 150 MPa for cod.

The images of muscular fibre sections obtained by SEM are shown in **Figure 1**. In fresh samples (day 0, **Figure 1a**), fibres appear with some extracellular space, being this associated to the dehydration method that could weaken links between fibres and myocommata. Storage of salmon samples at AP/10 ºC (**Figure 1b**) induced numerous cracks inside the fibres, a deterioration of pericellular connective tissue being visible after 15 days of storage. For samples stored at AP/5 ºC, there are no visible differences on muscle fibres after 15 days of storage (**Figure 1c**), while after 30 days of storage (**Figure 1d**) is clearly visible the same deterioration observed on AP/10 ºC samples. This evolution of myofibrils structure is the result of natural degradation by proteases and microorganisms (Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & Lamballerie, 2005), which is in accordance with the high microbial load obtained in a previous work for AP samples (Fidalgo et al., 2019). Additionally, the presence of microorganisms is visible in AP samples, corresponding to the numerous light points observed.

On the other hand, the appearance of the fibres showed minor changes when samples were stored under HS at 60 MPa/10 ºC (**Figure 1e**), when compared to AP samples. From **Figure 1e** it is visible that the extracellular space decreases after 15 days of storage, slightly increasing after 30 days (**Figure 1f**), with some cracks inside de fibres but less pronounced than for AP samples (mainly AP/5 ºC also after 30 days). Hughes, Greenberg, Yang, & Skonberg, (2015) verified that high pressure (300 MPa for 5 min) can cause stretching of the connective tissue with low changes in texture due to the continued presence of collagen in the tissue.

Figure 2 shows the results regarding MFI of salmon samples stored under different conditions. Compared to fresh salmon, no significant differences were verified in HS samples during 30 days of storage, as well as for AP/5 ºC. Differently, for AP/10 ºC there was an increase of the initial values from 11 ± 5 to 25 ± 6 and 28 ± 6 after 5 and 15 days, respectively. MFI could indicate the degradation extension of muscle myofibrillar proteins (Volpelli, Failla, Sepulcri, & Piasentier, 2005). In the present work, there was a pronounced myofibrillar degradation on samples stored at 10 ºC, contrarily to those

observed under pressure and at the same temperature (60 MPa/10 ºC). According to Otero et al. (2019), HS at lower temperature (50 MPa/5 ºC) caused differences on the electrophoretic pattern of the myofibrillar fraction of Atlantic mackerel, what could be due to a direct effect of pressure on myofibrillar proteins but also to a pressure-induced effect on the autolytic capacity of endogenous proteases, maybe due to modifications in its configuration after hyperbaric storage. In the present work, at 60 MPa/10 ºC salmon samples revealed a stability of MFI values, possibly due to an inhibition/inactivation of endogenous enzymes under pressure. According to the literature (Wang, Huang, Hsu, & Yang, 2016), enzyme inactivation could occur by structural rearrangements of proteins under high pressure, such as hydration changes that accompany other intramolecular non-covalent interactions.

3.2. Lipid stability during storage

The summary of the fatty acids quantified from salmon samples stored under different conditions is shown in **Table 3**. The total saturated fatty acids (SFA, 9.62-13.21 g/100 g lipids), monounsaturated fatty acids (MUFA, 34.03-44.10 g/100 g lipids) and polyunsaturated fatty acids (PUFA, 17.57-22.33 g/100 g lipids) composition did not change between samples, being MUFA predominant in the FA profile of salmon samples. Regarding PUFA, n-6 compounds were identified in higher quantities (9.84-13.28 g/100 g lipids), followed by n-3 compounds in lower amount (6.83-9.66 g/100g lipids), with no significant differences between storage conditions and during time in storage. According to Gonçalves et al. (2017), these results are associated to the feeding of farmed fish caused by the substitution of fish diets with other alternatives with low quantities of n-3 and high levels of n-6 compounds (vegetal source) and SFA (animal source). Otherwise, even though no differences in n-3 and n-6 in different salmon samples were detected, the n-6/n-3 ratio changed under the different conditions studied (**Figure 3a**). HS samples showed a lower n-6/n-3 ratio values and no changes were observed along 30 days of storage (between 1.16 and 1.28 g/100 g lipids), when compared to the values obtained in AP samples after 15 days (1.88 and 1.82 g/100 g lipids for AP/5 ºC and 10 ºC, respectively). According to Simopoulos (2008), a lower ratio of n-6/n-3 ratio is desirable (approximately 1) to reduce the risk of many chronic diseases, such as secondary prevention of cardiovascular diseases.

The polyene index values are shown in **Figure 3b**. HS samples showed a stable polyene index during 30 days of storage. This is in contrast with the values observed for

AP samples; AP/10 ºC polyene index decreased after 5 days from 0.86 ± 0.03 to a value of 0.75 ± 0.05, as samples stored at AP/5 ºC decreased to a similar value after 15 days (0.73 ± 0.03), indicating a loss of docosahexanoic (DHA) and docosapentaenioc acids (EPA), among the most important FA in fish. Polyene index evolution in the salmon loins is a good index to evaluate lipid oxidation (Ortiz et al., 2014), indicating that the decrease of polyene index during storage verified in AP samples showed that oxidation mechanisms are active during storage.

Table 4 shows the primary (peroxides values), secondary (TBARS) and tertiary (fluorescence ratio) lipid oxidations in salmon samples stored under the different conditions. Higher peroxides values were obtained for HS samples (between 5.07 and 5.99 mg Fe III/kg lipids), when compared to AP samples, AP/5 ºC being the condition with the lower value (3.55±0.12 mg Fe III/kg lipids after 15 days). On the other hand, TBARS values did not show significant changes during storage in all the conditions, showing values between 0.12 and 0.28 µg MDA/g fish. However, the fluorescence ratio (tertiary lipid oxidation) increased for the AP/5 ºC and the AP/10 ºC samples after 5 days of storage, going from 0.04±0.01 to 0.33±0.02 and 0.22±0.04, respectively. For HS, 60 MPa/10 ºC, the fluorescence ratio did not change during the 30 days of storage.

Basically, undesirable products formation, resulting from lipid oxidation, could be originated from a complex and sequential mechanism, thus resulting in the formation of primary and secondary products, followed by the reaction of these compounds with amino constituents of the fish muscle (proteins, peptides, free amino acids, and phospholipids), producing interaction compounds, which could be quantified by fluorescence methods (Fidalgo et al., 2018). The results from the present work showed that HS/LT allowed to slowdown lipid oxidation of salmon samples, which was also confirmed by the stable polyene index, when compared to AP samples. Recently, Otero et al., (2019) reported that hyperbaric cold storage (HS at low temperature, 50 MPa/5 ºC) did not reveal significant changes on the lipid oxidation (evaluated by quantification of polymerization compounds through high-performance size-exclusion chromatography), fatty acid composition and polyene index of Atlantic mackerel during 12 days of storage.

3.3. Volatile compounds identification

Table 5 shows the volatile profile identified in the headspace over salmon samples using SPME-GC/MS analysis. In fresh salmon samples, eighteen volatile compounds were identified, including alcohols, aldehydes, alkanes and ethyl acetate. For 60 MPa/10

ºC during 30 days, a similar volatile compounds' profile was identified, differing in the absence of ethyl acetate in HS samples. Otherwise, for salmon samples stored at AP, some volatile compounds disappeared, and new ones appeared. In both AP samples (5 and 10 ºC) new alcohols (phenylethyl alcohol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and butane-2,3-diol), aldehydes (phenylacetaldehyde and 3-methylbutanal) and a ketone (butan-2-one) were detected. Some alcohols, such as phenylethyl alcohol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and butane-2,3-diol, are associated to microbial activity and are considered as possible indicators of fish spoilage or loss of freshness during storage (Parlapani, Mallouchos, Haroutounian, & Boziaris, 2014). The production of 3-methylbutanal and phenylacetaldehyde has been shown to be related to spoilage and could originate from both Strecker degradation and microbial activity on leucine and phenylalanine, respectively, formed by the action between α-dicarbonyl compound and an amino acid (Lee, Suriyaphan, & Cadwallader, 2001; Varlet, Knockaert, Prost, & Serot, 2006). Other ethyl esters, such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate and ethyl 4-methylpentanoate were also detected, although in samples stored at AP/10 ºC after 15 days only. The ethyl esters' formation was associated with esterase activity of lactic acid bacteria (Liu, Holland, & Crow, 2003) and amino acid catabolism of *Pseudomonas* spp. (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015). Additionally, compounds such as pent-1-en-3-ol and oct-1-en-3-ol, hexanal, heptanal, octanal, nonanal, decanal and hepta-2,4-dienal were not detected in AP/10 ºC samples stored after 15 days. Pent-1-en-3-ol and oct-1-en-3-ol are commonly used as markers for salmon freshness, and are originated from n−6 PUFA oxidation (linoleic acid and α-linolenic acid, respectively) by lipoxygenase action and/or by other chemical reactions (Fratini, Lois, Pazos, Parisi, & Medina, 2012; Pan & Kuo, 1994). Furthermore, C6–C9 aldehydes such as hexanal, nonanal, decanal, benzaldehyde, heptanal, octanal, hept-4-enal, and hepta-2,4-dienal are commonly described as the main compounds responsible of typical fresh-fish flavour (Wierda, Fletcher, Xu, & Dufour, 2006), being also produced by lipoxygenase action on n-6 or n-9 PUFAs (Varlet, Prost, & Serot, 2007). Additionally, it has been reported that C8–C19 alkanes such as decane, undecane, dodecane, tridecane, pentadecane, and heptadecane unimportantly contributed to the flavour due to their high odour threshold values (Xu et al., 2014).

In order to simplify the interpretation of the correlation between storage conditions, principal component analysis (PCA) was carried out on the volatile compounds identified in the different salmon samples. **Figure 4** shows the scores plot after PCA of the different

variables by the two first principal components (PC 1 and PC 2), which accounted for 62.9% and 13.2% of total variability, respectively. **Table 6** shows the loadings and the percentage accounted by the two first principal components (PC 1 and PC 2) after PCA. Positive loadings on PC 1 all are related to volatile compounds associated to salmon freshness such as some alcohols (pent-1-en-3-ol, oct-1-en-3-ol), aldehydes (hexanal, heptanal, hepta-2,4-dienal) and alkanes (e.g., dodecane and tridecane). The negative axis of PC 1 is related to compounds associated to spoilage of salmon, such as several alcohols (phenylethyl alcohol and 3-methylbutan-1-ol), aldehydes (phenylacetaldehyde and 3-methylbutanal) and esters. Regarding PC 2, compounds such as 2-methylbutan-1-ol, butane-2,3-diol, phenylacetaldehyde, pentadecane, and ethyl acetate had positive loadings, while dodecane, tridecane, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 4-methylpentanoate and butan-2-one had negative loadings. The distribution of the scores on the first two PCs (**Figure 4**) showed three separate groups of points corresponding to: *(1)* fresh salmon, samples stored at 60 MPa/10 ºC (5, 15 and 30 days) and AP/5 ºC (5 days); *(2)* AP/5 ºC (15 and 30 days) and AP/10 ºC (5 days); and *(3)* AP/10 ºC (15 days). Fresh salmon, samples stored at 60 MPa/10 ºC (5, 15 and 30 days) and AP/5 ºC (5 days) were located in the positive zone of PC 1, while AP/5 ºC (15 and 30 days) and AP/10 ºC (5 and 15 days) were in the negative part. PC 1 could not differentiate salmon samples stored under HS and AP/5 ºC, whereas PC 2 could explain the differences between these samples. Furthermore, samples stored at AP/10 ºC (15 days) are related to the negative PC 2, being characterized by the presence/highest levels in esters compounds and butan-2-one and by the absence/lowest contents in fresh-like compounds. The results clearly show that all HS samples (and also AP/5 ºC (5 days) samples) are more related to fresh salmon samples than the other samples, indicating that HS kept much better the fresh salmon volatiles profiles for up to 30 days.

4. CONCLUSIONS

In conclusion, HS/LT could represent an interesting preservation methodology of fresh salmon loins, since the results obtained revealed that important physicochemical properties were better maintained during the 30 days of storage at 60 MPa/10 ºC, when compared to the conventional refrigeration.

Stable drip loss and water holding capacity were observed for HS/LT samples. Longitudinal section images obtained by SEM revealed that HS/LT caused lower changes in muscle fibres than in the samples stored at AP and concerning textural properties, only

resilience was affected by HS/LT after 30 days. No variations of myofibrillar fragmentation index for samples stored at HS/LT were observed. In addition, HS/LT samples showed stable fatty acids profile, polyene index and a lower lipid oxidation state, when compared to AP samples. Furthermore, HS/LT samples exhibited a more similar volatile profile relatively to the fresh ones, since fresh-like alcohols and aldehydes were retained, being not detected in AP samples, mainly the AP/10 ºC samples after 15 days. In these latter samples most of these fresh-like compounds were not detected, presenting mostly spoilage-related compounds from microbial activity.

ACKNOWLEDGEMENTS

Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/QUI/00062/2019) and LAQV-Requimte (UID/QUI/50006/2019) through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement and for supporting the PhD grant of Liliana G. Fidalgo (SFRH/BD/96984/2013).

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Figures

Figure 1 – Scanning electron micrograph of longitudinal sections of: fresh Atlantic salmon muscle (a); stored at AP/10 ºC during 15 days (b); AP/5 ºC during 15 days (c) and 30 days (d); and 60 MPa/10 ºC during 15 days (e) and 30 days (f).

Figure 2 – Myofibrillar fragmentation index of Atlantic salmon stored at 60 MPa/10 ºC and AP/5 ºC during 30 days, and AP/10 ºC during 15 days. Different letters denote significant differences ($p < 0.05$) between salmon samples stored at different conditions and time (a-b).

Figure 3 – Ratio of n-6/n-3 (a) and polyene index (b) of Atlantic salmon stored at 60 MPa/10 ºC during 30 days, and AP/5 ºC and AP/10 ºC during 15 days. Different letters denote significant differences ($p < 0.05$) between salmon samples stored at different conditions and time (a-b).

Figure 4 – Principal component analysis (PCA) score plot of volatile compounds of Atlantic salmon stored at 60 MPa/10 ºC and AP/5 ºC during 30 days, and AP/10 ºC during 15 days.

Figure 1

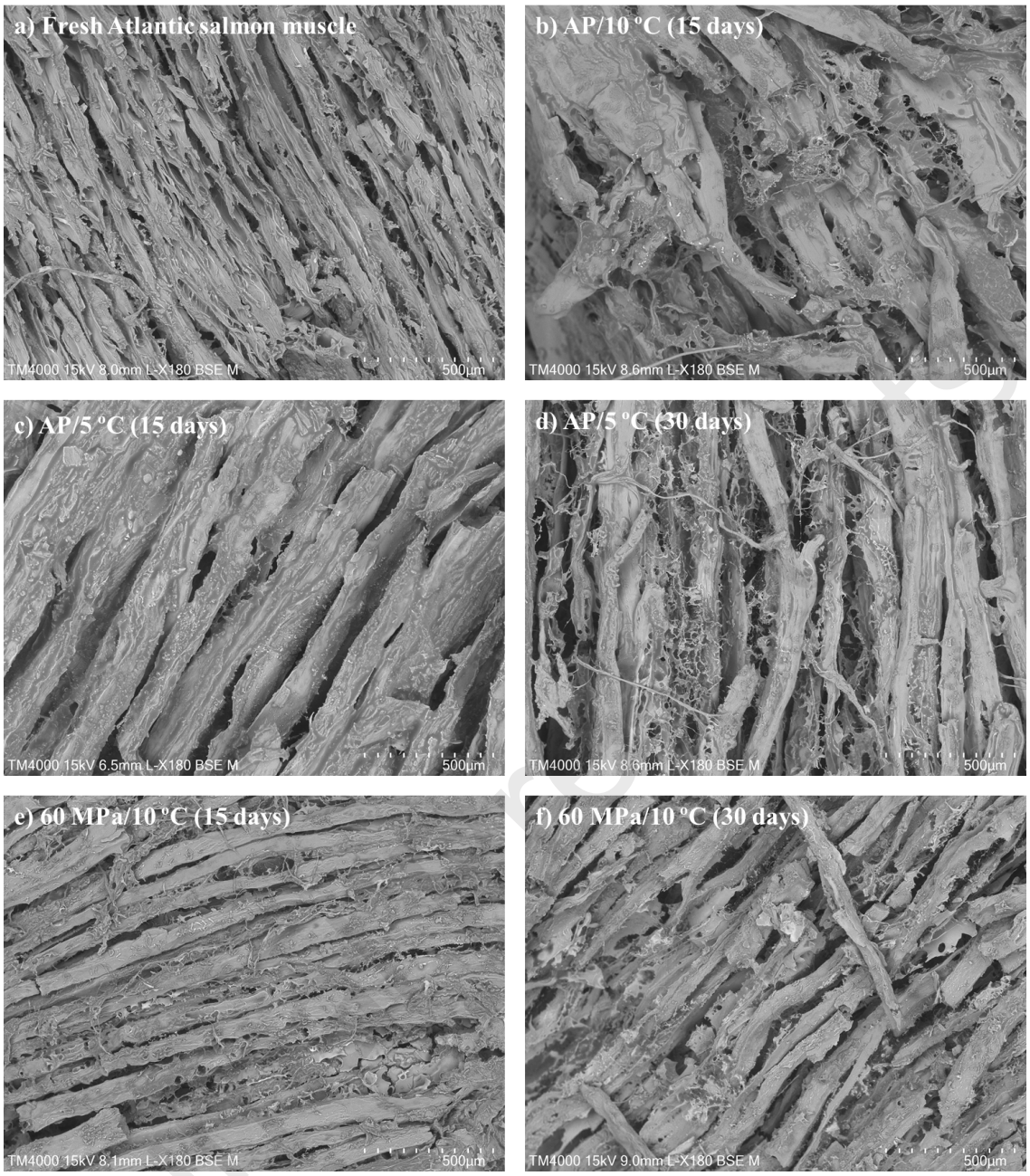

a) Fresh Atlantic salmon muscle
TM4000 15kV 8.0mm L-X180 BSE M
500µm
b) AP/10 ºC (15 days)
TM4000 15kV 8.6mm L-X180 BSE M
500µm
c) AP/5 ºC (15 days)
TM4000 15kV 6.5mm L-X180 BSE M
500µm
d) AP/5 ºC (30 days)
TM4000 15kV 8.6mm L-X180 BSE M
500µm
e) 60 MPa/10 ºC (15 days)
TM4000 15kV 8.1mm L-X180 BSE M
500µm
f) 60 MPa/10 ºC (30 days)
TM4000 15kV 9.0mm L-X180 BSE M
500µm

Figure 2

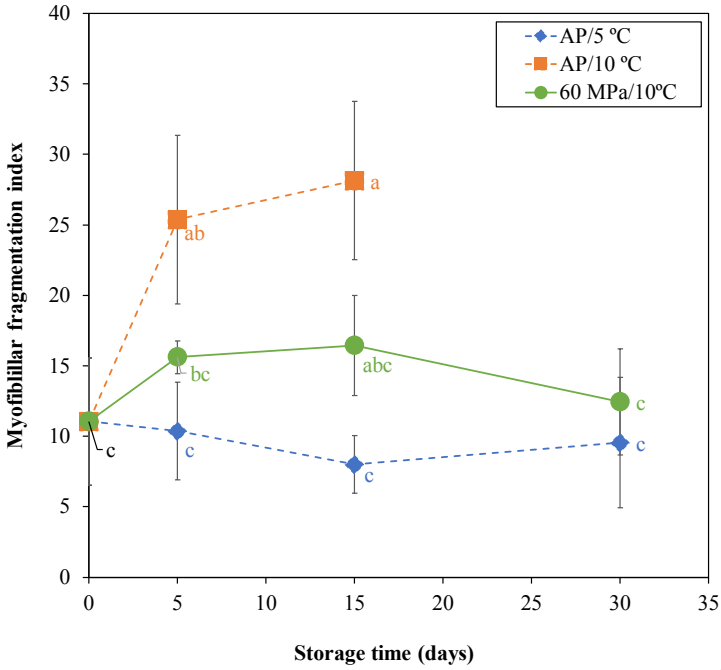


Figure 3

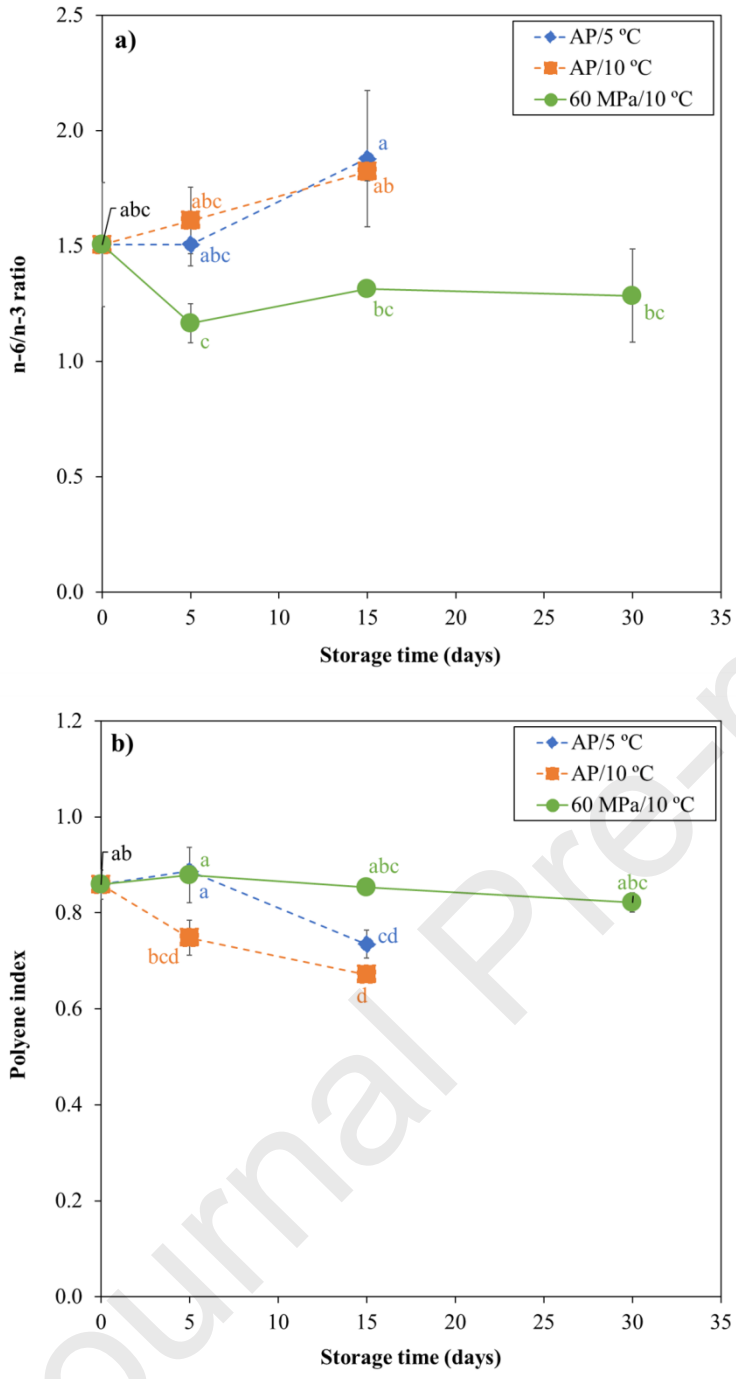
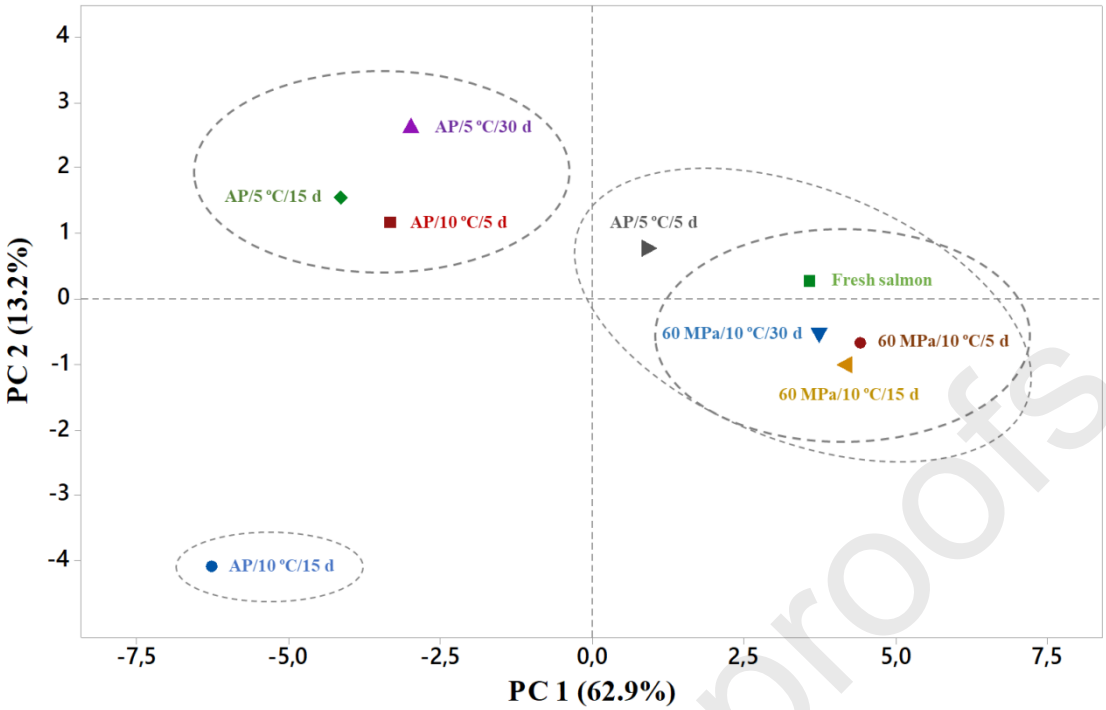


Figure 4



Tables

Table 1 – Physical properties of Atlantic salmon stored at 60 MPa/10 ºC, AP/5 ºC and AP/10 ºC for different storage times. Different letters along each column denote for significant differences ($p < 0.05$); absence of letters means that there were not statistically significant differences.

Storage conditions	Storage time (days)	Water content (%)	Drip loss (%)	Water holding capacity (%)
Fresh fish	***0 days***	71.8 ± 3.2	-	95.8 ± 0.7^{ab}
60 MPa/10 ºC	***5 days***	72.0 ± 0.2	3.6 ± 0.4^{abc}	92.0 ± 1.7^{c}
	15 days	73.7 ± 0.2	3.4 ± 0.8^{bc}	91.9 ± 0.12^{c}
	30 days	73.7 ± 0.3	2.6 ± 0.3^{c}	97.4 ± 0.4^{a}
AP/5 ºC	***5 days***	69.8 ± 1.8	1.4 ± 0.3^{c}	96.5 ± 0.5^{a}
	15 days	69.4 ± 3.0	3.8 ± 1.3^{abc}	93.8 ± 0.9^{bc}
	30 days	73.3 ± 4.3	6.6 ± 1.00^{ab}	97.9 ± 0.7^{a}
AP/10 ºC	***5 days***	71.5 ± 1.3	6.1 ± 1.9^{ab}	95.6 ± 1.0^{ab}
	15 days	68.0 ± 2.6	6.8 ± 1.9^{a}	93.6 ± 0.3^{bc}

Table 2 – Texture properties of Atlantic salmon stored at 60 MPa/10 ºC, AP/5 ºC and AP/10 ºC for different storage times. Different letters along each column denote for significant differences ($p < 0.05$); absence of letters means that there were not statistically significant differences.

Storage conditions	**Storage time (days)**	***Hardness (N)***	***Adhesiveness (N.s)***	***Springiness***	***Resilience***
Fresh fish	***0 days***	3.21 ± 0.51^{ab}	-0.36 ± 0.11^{ab}	1.49 ± 0.12	1.27 ± 0.29^{a}
60 MPa/10 ºC	***5 days***	3.05 ± 0.26^{ab}	-0.17 ± 0.01^{a}	1.13 ± 0.11	0.50 ± 0.34^{ab}
	15 days	3.28 ± 0.05^{ab}	-0.34 ± 0.01^{ab}	1.14 ± 0.04	0.51 ± 0.10^{ab}
	30 days	2.68 ± 0.04^{ab}	-0.28 ± 0.02^{ab}	1.12 ± 0.02	0.38 ± 0.03^{b}
AP/5 ºC	***5 days***	2.74 ± 0.39^{ab}	-0.43 ± 0.12^{ab}	1.37 ± 0.28	0.99 ± 0.65^{ab}
	15 days	3.27 ± 0.28^{ab}	-0.44 ± 0.13^{ab}	1.38 ± 0.10	0.95 ± 0.19^{ab}
	30 days	2.43 ± 0.41^{b}	-0.38 ± 0.18^{ab}	1.42 ± 0.14	0.95 ± 0.21^{ab}
AP/10 ºC	***5 days***	3.47 ± 0.57^{a}	-0.54 ± 0.05^{b}	1.25 ± 0.08	0.67 ± 0.19^{ab}
	15 days	2.67 ± 0.24^{ab}	-0.36 ± 0.16^{ab}	1.25 ± 0.20	0.61 ± 0.40^{ab}

Table 3 – Fatty acids profile [(1)] (wt.%, g FA/100 g of total lipids) of Atlantic salmon stored at 60 MPa/10 ºC, AP/5 ºC and AP/10 ºC for different storage times. For each set of fatty acids no statistically significant differences were observed between all storage conditions.

Storage conditions	Storage time (days)	SFA	MUFA	n-3 PUFA	n-6 PUFA
Fresh fish	***0 days***	10.8 ± 1.2	39.9 ± 8.5	8.2 ± 0.9	12.2 ± 2.1
60 MPa/10 ºC	***5 days***	10.9 ± 2.6	34.0 ± 9.3	8.5 ± 2.7	9.8 ± 2.7
	15 days	12.6 ± 1.54	44.1 ± 2.6	9.7 ± 1.2	12.7 ± 1.3
	30 days	13.2 ± 0.6	34.9 ± 10.2	9.5 ± 0.4	12.1 ± 1.5
AP/5 ºC	***5 days***	9.6 ± 1.2	39.4 ± 4.4	7.6 ± 1.0	11.4 ± 1.3
	15 days	10.6 ± 0.4	42.4 ± 6.6	7.1 ± 0.4	13.3 ± 2.0
AP/10 ºC	***5 days***	10.2 ± 1.4	39.9 ± 5.2	6.8 ± 0.6	11.0 ± 1.5
	15 days	11.6 ± 1.2	43.4 ± 3.1	7.2 ± 0.7	13.1 ± 1.2

[(1)] SFA: total saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 4 – Lipid oxidation [(1)] of Atlantic salmon stored at 60 MPa/10 ºC, AP/5 ºC and AP/10 ºC for different storage times. Different letters along each column denote for significant differences ($p < 0.05$); absence of letters means that there were not statistically significant differences.

Storage conditions	Storage time (days)	Lipid oxidation		
		Primary	***Secondary***	***Tertiary***
Fresh fish	***0 days***	4.76 ± 0.50^{abc}	0.21 ± 0.14	0.04 ± 0.01^{d}
60 MPa/10 ºC	***5 days***	5.07 ± 0.99^{abc}	0.28 ± 0.17	0.05 ± 0.01^{d}
	15 days	5.56 ± 0.73^{ab}	0.25 ± 0.09	0.04 ± 0.01^{d}
	30 days	5.99 ± 0.65^{a}	0.22 ± 0.02	0.04 ± 0.01^{d}
AP/5 ºC	***5 days***	4.07 ± 0.68^{bc}	0.17 ± 0.11	0.33 ± 0.02^{a}
	15 days	3.55 ± 0.12^{c}	0.13 ± 0.05	0.25 ± 0.03^{b}
AP/10 ºC	***5 days***	4.34 ± 0.49^{abc}	0.12 ± 0.02	0.22 ± 0.04^{bc}
	15 days	4.37 ± 0.52^{abc}	0.12 ± 0.01	0.18 ± 0.03^{c}

(1) Primary lipid oxidation by peroxides values (mg Fe III/kg lipids); Secondary lipid oxidation by TBARS (μg malondialdehyde (MDA)/g muscle); and tertiary lipid oxidation by fluorescence ratios.

Table 5 – Volatile compounds Atlantic salmon stored at 60 MPa/10 ºC, AP/5 ºC and AP/10 ºC for different storage times. Different letters along each raw denote for significant differences ($p < 0.05$); absence of letters means that there were not statistically significant differences. The volatile profile results are expressed as percentage (%) of each compound in total identified compounds for same condition.

Volatile compounds	*RT (min)*	*KI* [1]	*Fresh fish*	*60 MPa/10 ºC*			*AP/5 ºC*			*AP/10 ºC*	
			0 days	*6 days*	*15 days*	*30 days*	*5 days*	*15 days*	*30 days*	*5 days*	*15 days*
Alcohols											
Pent-1-en-3-ol	2.921	670	4.8 ± 1.1^{a}	4.3 ± 0.7^{a}	4.1 ± 0.2^{a}	4.8 ± 0.5^{a}	3.4 ± 1.4^{ab}	1.1 ± 0.2^{c}	1.8 ± 0.2^{bc}	Traces [3]	ND
3-Methylbutan-1-ol	3.847	717	ND [2]	ND	ND	ND	1.7 ± 0.3^{c}	19.0 ± 1.2^{ab}	13.3 ± 0.6^{b}	17.2 ± 5.0^{ab}	21.9 ± 5.0^{a}
2-Methylbutan-1-ol	3.954	720	ND	ND	ND	ND	0.4 ± 0.2^{c}	3.9 ± 0.1^{b}	6.8 ± 1.8^{a}	4.0 ± 0.6^{b}	2.8 ± 1.9^{bc}
Butane-2,3-diol	5.913	779	ND	ND	ND	ND	2.9 ± 1.2	2.9 ± 0.5	2.4 ± 1.1	3.3 ± 1.0	1.6 ± 1.1
Oct-1-en-3-ol	12.930	973	3.8 ± 3.2^{abc}	5.8 ± 0.7^{a}	4.8 ± 0.7^{ab}	4.5 ± 1.4^{abc}	2.4 ± 0.9^{abc}	1.1 ± 04^{c}	1.5 ± 0.1^{bc}	1.1 ± 0.4^{c}	ND
Phenylethyl alcohol	19.375	1108	ND	ND	ND	ND	0.6 ± 0.2^{c}	4.5 ± 0.7^{ab}	3.5 ± 0.8^{b}	5.4 ± 0.4^{a}	5.7 ± 0.8^{a}
Aldehydes											
3-Methylbutanal	2.569	642	ND	ND	ND	ND	Traces	1.8 ± 0.6^{b}	1.9 ± 0.4^{ab}	2.9 ± 0.8^{a}	2.3 ± 0.3^{ab}
Hexanal	5.533	768	22.9 ± 5.0^{a}	22.2 ± 3.2^{a}	13.8 ± 2.4^{bc}	15.0 ± 2.6^{ab}	15.7 ± 4.4^{ab}	4.9 ± 0.8^{d}	10.6 ± 1.9^{bcd}	5.8 ± 1.7^{cd}	Traces
Hept-4-enal	9.305	893	3.9 ± 1.2^{a}	2.5 ± 1.0^{ab}	1.6 ± 0.2^{b}	1.9 ± 0.4^{b}	2.0 ± 0.3^{b}	Traces	1.1 ± 0.3^{b}	Traces	Traces
Heptanal	9.428	898	4.2 ± 1.6^{a}	4.9 ± 0.6^{a}	3.1 ± 0.7^{abc}	3.6 ± 1.3^{ab}	1.7 ± 0.2^{bc}	1.0 ± 0.3^{c}	1.3 ± 0.4^{c}	1.6 ± 0.2^{bc}	Traces
Benzaldehyde	11.778	951	3.9 ± 2.4	4.9 ± 0.7	4.1 ± 0.9	2.5 ± 1.4	1.8 ± 1.0	2.0 ± 0.5	3.5 ± 0.3	3.5 ± 0.6	2.1 ± 0.5
Octanal	14.104	1004	1.5 ± 0.3^{d}	6.0 ± 0.4^{a}	3.8 ± 0.8^{bc}	4.0 ± 1.3^{b}	2.1 ± 0.7^{cd}	Traces	2.1 ± 0.4^{d}	1.5 ± 0.1^{d}	Traces
Hepta-2,4-dienal	14.420	1005	5.7 ± 1.4^{a}	5.7 ± 0.6^{a}	6.5 ± 1.1^{a}	4.7 ± 1.7^{a}	4.8 ± 0.7^{a}	1.9 ± 0.5^{b}	1.6 ± 0.6^{b}	Traces	ND
Phenylacetaldehyde	15.747	1032	ND	ND	ND	ND	1.1 ± 0.5^{d}	26.4 ± 2.4^{a}	12.7 ± 1.5^{c}	19.4 ± 2.2^{b}	11.7 ± 4.1^{c}
Nonanal	19.044	1101	1.9 ± 0.4^{d}	7.4 ± 0.4^{a}	5.6 ± 0.8^{ab}	4.5 ± 1.3^{bc}	2.9 ± 0.6^{cd}	1.5 ± 0.4^{d}	1.7 ± 0.5^{d}	2.4 ± 0.4^{d}	Traces
Decanal	23.852	1200	1.1 ± 1.0	2.4 ± 0.9	2.3 ± 0.8	1.1 ± 0.1	Traces	Traces	Traces	1.5 ± 0.1	Traces
Alkanes											
Decane	13.961	1000	6.4 ± 2.5^{ab}	3.0 ± 0.7^{bc}	2.7 ± 1.6^{c}	6.5 ± 1.5^{ab}	9.3 ± 1.2^{a}	3.0 ± 0.7^{bc}	2.8 ± 0.5^{c}	2.7 ± 1.0^{c}	1.5 ± 0.4^{c}
Undecane	18.644	1093	8.0 ± 2.8^{ab}	5.5 ± 1.7^{ab}	12.7 ± 5.9^{a}	13.2 ± 4.0^{a}	13.2 ± 2.3^{a}	3.9 ± 0.7^{b}	3.6 ± 0.4^{b}	4.3 ± 1.6^{b}	3.9 ± 0.7^{b}
Dodecane	23.313	1190	4.9 ± 0.4^{cd}	5.5 ± 2.3^{bcd}	11.6 ± 3.2^{a}	10.3 ± 3.3^{ab}	7.2 ± 1.5^{abc}	2.2 ± 0.1^{cd}	2.0 ± 0.3^{d}	2.8 ± 0.9^{cd}	2.3 ± 0.2^{cd}
Tridecane	27.240	1290	2.5 ± 0.2^{bc}	3.4 ± 1.7^{abc}	5.4 ± 1.1^{a}	4.5 ± 1.6^{ab}	3.0 ± 0.6^{abc}	Traces	Traces	1.4 ± 0.4^{c}	1.1 ± 0.1^{c}
Pentadecane	33.403	1496	7.6 ± 1.6^{ab}	5.1 ± 1.7^{bcd}	6.2 ± 0.8^{bcd}	7.0 ± 1.3^{bc}	5.7 ± 1.6^{bcd}	3.4 ± 1.0^{cd}	11.0 ± 1.6^{a}	3.9 ± 0.2^{cd}	3.2 ± 0.6^{d}
Heptadecane	37.830	1680	4.1 ± 0.1^{a}	3.1 ± 0.4^{ab}	3.6 ± 0.9^{ab}	3.5 ± 0.5^{ab}	2.6 ± 0.9^{bc}	1.6 ± 0.4^{c}	2.5 ± 0.2^{bc}	2.2 ± 0.2^{bc}	1.6 ± 0.3^{c}
2,6,10,14-Tetramethylpentadecane	38.335	1700	10.5 ± 0.9^{a}	8.0 ± 0.7^{abc}	8.0 ± 1.9^{abc}	8.3 ± 1.8^{ab}	6.1 ± 2.0^{bcd}	3.6 ± 1.0^{de}	2.1 ± 0.1^{e}	4.7 ± 0.3^{cde}	3.4 ± 0.8^{de}
Esters											
Ethyl acetate	2.256	616	1.7 ± 0.3^{c}	ND	ND	ND	5.8 ± 0.3^{ab}	6.2 ± 1.6^{ab}	8.5 ± 2.5^{a}	3.4 ± 0.7^{bc}	5.3 ± 1.3^{ab}
Ethyl 2-methylpropanoate	4.458	735	ND	ND	ND	ND	ND	ND	ND	ND	2.1 ± 0.3
Ethyl 2-methylbutanoate	7.356	826	ND	ND	ND	ND	ND	ND	ND	ND	1.9 ± 1.4
Ethyl 4-methylpentanoate	12.412	962	ND	ND	ND	ND	ND	ND	ND	ND	2.4 ± 1.1
Ketones											

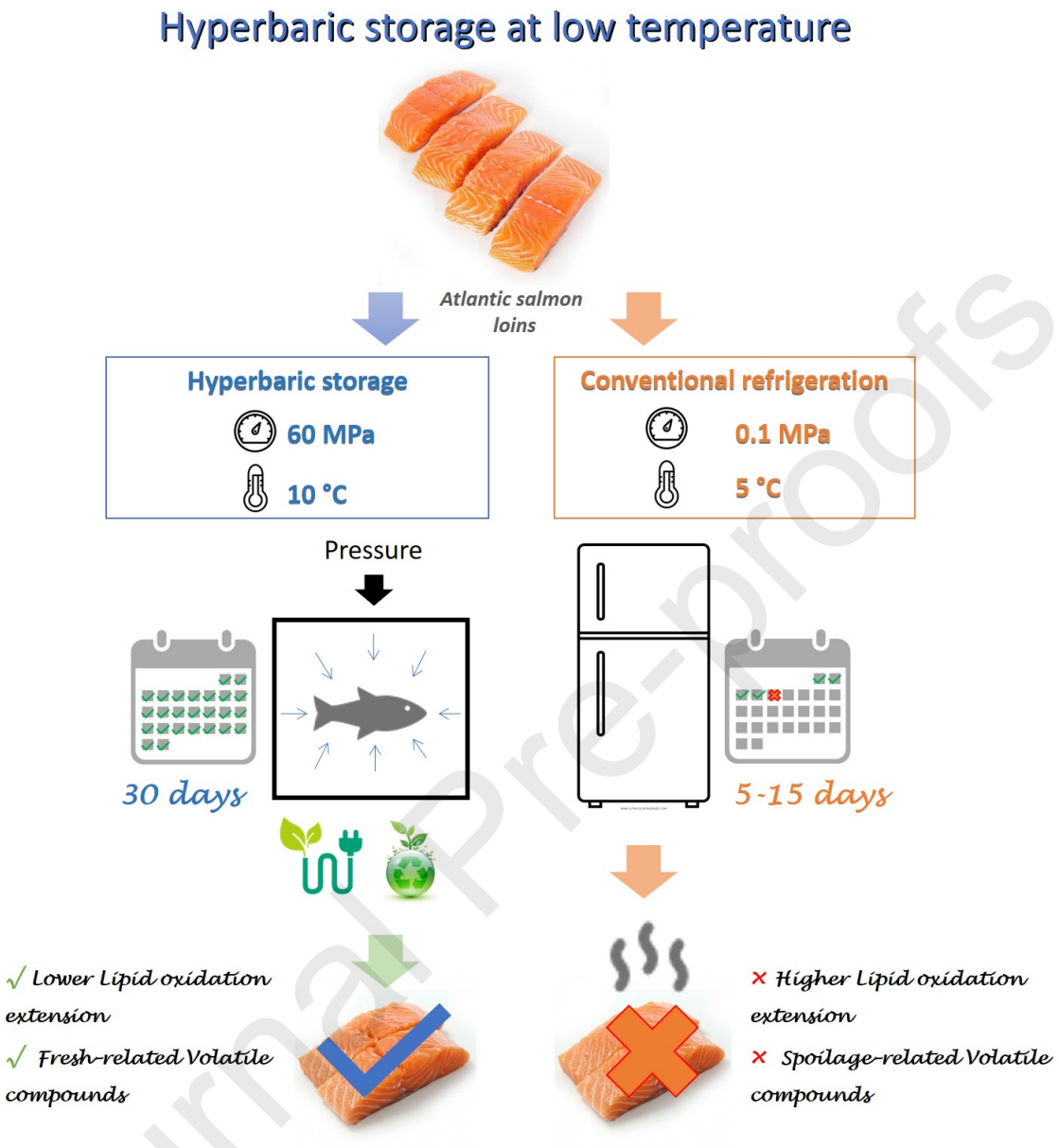
Butan-2-one	2.141	607	ND	ND	ND	ND	1.0 ± 0.4[b]	Traces [(3)]	Traces [(3)]	1.2 ± 0.2[b]	18.7 ± 2.9[a]

[(1)] KI – experiment value of Kovats Index (KI); [(2)] ND – Not detected; [(3)] Traces – Percentage value below 1% of total identified compounds.

Table 6 – Loadings of the variables in the first two principal component analysis of volatile compounds in salmon samples.

Compounds	**Principal components**	
	CP 1	**CP 2**
Phenylethyl alcohol	-0.231	-0.003
2-Methylbutan-1-ol	-0.192	0.230
3-Methylbutan-1-ol	-0.233	-0.016
Butane-2,3-diol	-0.183	0.238
Pent-1-en-3-ol	0.233	0.026
Oct-1-en-3-ol	0.230	-0.028
Hexanal	0.215	0.084
Heptanal	0.219	-0.027
Octanal	0.186	-0.028
Nonanal	0.186	-0.070
Decanal	0.168	-0.080
Benzaldehyde	0.130	0.037
Phenylacetaldehyde	-0.201	0.165
3-Methylbutanal	-0.224	0.065
Hept-4-enal	0.189	0.021
Hepta-2,4-dienal	0.230	0.001
Decane	0.112	0.120
Undecane	0.167	-0.058
Dodecane	0.189	-0.112
Tridecane	0,207	-0.136
Pentadecane	0,075	0.253
Heptadecane	0.218	0.015
2,6,10,14-Tetramethylpentadecan	0.213	-0.096
Ethyl acetate	-0.190	0.198
Ethyl 2-methylpropanoate	-0.134	-0.414
Ethyl 2-methylbutanoate	-0.134	-0.414
Ethyl 4-methylpentanoate	-0.134	-0.414
Butan-2-one	-0.143	-0.399

GA



Highlights

- Hyperbaric storage (HS, 60 MPa/10 ºC retained fresh-like physicochemical quality
- Fatty acid stability and low lipid oxidation extension observed up to 30 days by HS
- No changes on myofibrillar proteins, drip loss and water holding capacity by HS
- Better retention of fresh-like volatile compounds by HS than at refrigeration (RF)
- Overall HS showed promising for better quality than RF for salmon preservation

Conflict of interest

The authors have no conflict of interest to disclose.