**Shelf-life extension of fresh Atlantic salmon (*Salmo salar*) under
hyperbaric storage at low temperature by evaluation of microbial and
physicochemical quality indicators**

Liliana G. Fidalgo¹, Rodolfo Castro¹, Marcos Trigo², Santiago P. Aubourg², Ivonne
Delgadillo¹, Jorge A. Saraiva^{1*}

¹ QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro,
3810-193 Aveiro, Portugal.

² Department of Food Technology, Marine Research Institute (CSIC), c/ Eduardo
Cabello 6, Vigo, Spain

** Corresponding author: Tel.: +351 234401513; fax: +351 234370084.*

Email address: jorgesaraiva@ua.pt (J. A. Saraiva)

ABSTRACT

Atlantic salmon (*Salmo salar*) was evaluated by hyperbaric storage at low temperature (HS/LT; 40-60 MPa, 5-15 °C). Results showed HS/LT was efficient to slowdown microbial growth, with additional microbial inactivation at 60 MPa/10 °C after 50 days. Contrarily, atmospheric pressure (AP-5/10 °C) samples exceeded the established limit after 15 days. Furthermore, the established limit of total volatile base-nitrogen was surpassed at 60 MPa/10 °C only after 30 days (contrarily to 6 days at AP/10 °C), but with stable trimethylamine-nitrogen content in the former. Formaldehyde and dimethylamine-nitrogen content increased after 6 days of HS, but only the former progressively increased until the 10th day, indicating possible formation due to enzymatic activity, but also by chemical reactions. Additionally, HS slightly increased lipid oxidation, but to a lower extent compared to AP. HS/LT may represent an interesting methodology for Atlantic salmon shelf-life extension to 15-30 days, compared to 6 days for AP/5 °C.

Industrial relevance: Hyperbaric storage at low temperature (HS/LT: 60 MPa/10 °C) has shown a quality shelf-life extension of fresh fish salmon, as well as possible energy savings when compared to conventional refrigeration at 5 °C. HS only needs energy for compression and decompression phases, and at low temperature of 10 °C, the energy consumption to control temperature is lower than for conventional refrigeration. Besides that, many countries have this kind of room temperature. However, before industrial implementation, more research is needed to clarify the effect of these storage conditions on mechanisms implied in fish quality degradation.

40 **Keywords:** Hyperbaric storage; refrigeration; *Salmo salar*; microbial stability; volatile
41 amines; physicochemical properties.

1. Introduction

Several spoilage mechanisms are responsible for fish deterioration: metabolic activity of microorganisms, endogenous enzymatic activity (autolysis), and chemical lipid oxidation, considering fish products, mainly fresh fish, a highly perishable product with short shelf-life, being this very limiting for commercialization and leading to great losses (Ghaly, Dave, Budge, & Brooks, 2010). The latter causes the formation of odours and lipid peroxides, resulting in taste, texture, and consistency losses, and reduced nutritional value. Transition metals are primary activators of molecular oxygen, which reacts with double bonds of fatty acids, particularly polyunsaturated fatty acids (PUFAs) that are highly susceptible to oxidation (Hultin, 1994).

Microbial spoilage in fish produces amines, organic acids, sulphides, alcohols, aldehydes, and ketones with unpleasant and unacceptable off-flavours (Ólafsdóttir et al., 1997). The occurrence of volatile basic compounds, such as ammonia, trimethylamine-nitrogen (TMA-N), and dimethylamine-nitrogen (DMA-N), is one of the characteristic features attributed to chemical changes occurring in marine fish muscle during spoilage. The analytical parameter, which includes these volatile compounds, is the total volatile basic nitrogen (TVB-N), and this is widely considered to be a useful index of fish freshness (Ozogul, 2009). The main volatile compound accumulated during chilled fish is TMA-N, originating from the bacterial reduction of trimethylamine N-oxide (TMAO), via the enzyme trimethylamine-N-oxide reductase (TMAOase), and this is considered to be the main reason for the off-odours of fish products (Ólafsdóttir et al., 1997). When the bacterial growth is slowed down/inhibited, as is the case of refrigeration/frozen storage, conversion of TMAO to DMA and formaldehyde (FA) by the enzyme TMAO aldolase occurs, also with negative effects on fish quality (Benjakul, Visessanguan, & Tanaka, 2004).

Hyperbaric storage (HS) has attracted great interest lately due to the possibility to preserve food products above atmospheric pressure at room (RT) or low temperatures (LT), increasing shelf-life and quality comparatively to the conventional method of refrigeration (RF). The effect of HS/LT for fish preservation was already studied by Ko, Jao, Hwang, & Hsu (2006) and Fidalgo, Lemos, Delgadillo, & Saraiva (2018) using tilapia fillets and Atlantic salmon (*Salmo salar*), respectively, and at HS/LT by Otero, Pérez-Mateos, & López-Caballero (2017) and Otero, Pérez-Mateos, Holgado, Márquez-Ruiz, & López-Caballero (2019), using Cape hake (*Merluccius capensis*) loins and Atlantic mackerel (*Scomber scombrus*, L.) fillets, respectively, with good results for fresh fish shelf-life extension. In such studies, HS/LT (50 MPa and 5 °C) of both hake loins and Atlantic mackerel fillets resulted in a maintenance of microbial load and TVB-N content after 7 and 12 days, respectively. However, drip losses and an increase of the shear resistance and whiteness of the raw fish were verified, but after cooking, these effects 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 of longer storage periods. For instance, HS/LT was recently studied for watermelon juice, being observed a microbial shelf-life of at least 58 days at 62.5/75 MPa (15 °C), due to a reduction of initial loads, by at least 2.5 log CFU/mL, while pH and colour values did not change (Lemos, Ribeiro, Fidalgo, Delgadillo, & Saraiva, 2017).

Consequently, the aim of this work was to evaluate the endogenous microbial and physicochemical quality of Atlantic salmon stored at HS/LT. An initial screening was carried out, using different pressure/temperature storage conditions (40-60 MPa/5-15 °C) and storage experiments over 10 days. Control samples stored at atmospheric pressure (AP, 0.1 MPa) and at the same temperature (5-15 °C) during the same time

were also performed. These samples were microbiology evaluated (total aerobic psychrophiles, anaerobic bacteria, *Enterobacteriaceae*, lactic acid bacteria, *Pseudomonas* spp. and hydrogen sulphide-reducing bacteria). Afterwards, the results obtained led to carry out a further storage assay using the best condition obtained in the screening assay, 60 MPa/10 °C, during a longer storage time (50 days). The results were compared to control samples stored at AP (5 or 10 °C) during 6 days, by studying several microbial, physicochemical indicators of quality, colour parameters and lipid oxidation.

2. Materials and methods

2.1. Sample preparation and storage experiments

Atlantic salmon was acquired from a local market before each experiment storage and prepared according to the methodology already described in previous work (Fidalgo et al., 2018). Storage experiments were carried out between January and June 2017.

Storage experiments are divided in two parts: (1) A screening assay using different combinations of pressure/low temperatures over 10 days of storage was carried out (40 MPa/5 °C, 50 MPa/5 °C, 50 MPa/10 °C, 50 MPa/15 °C, 60 MPa/10 °C, 60 MPa/15 °C); (2) The best condition, 60 MPa/10 °C, in a microbial stability point of view, was further studied during a longer storage time of 50 days. Control samples were always kept at the same temperature and also at 5 °C under atmospheric pressure conditions, in exactly the same conditions (in the dark and immersed in the same fluid used for compression). Samples from first assay (1) were evaluated through microbial analyses, while in the second assay (2) were also performed pH measurements, TVB-N, TMA-N, DMA-N and FA determinations, colour, and lipid oxidation (primary,

secondary and tertiary lipid oxidation). For each storage condition, a different batch of fresh salmon samples was used.

The first set of HS experiments (1) was performed using a 100-mL high-pressure equipment (High-pressure system U33, Institute of High Pressure Physics, Warsaw, Poland) equipped with a pressure vessel of 35-mm inner diameter and 100-mm height. The second set (2) was carried out using a different 200-mL high-pressure equipment (SFP FPG13900, Stansted Fluid Power, Stansted, United Kingdom), which has a pressure vessel of 35-mm inner diameter and 250-mm height. Both high pressure equipment use a mixture of propylene glycol and water (40:60, v/v) as pressurization fluid.

2.2. Microbial analyses and pH determination

For microbial analyses, salmon muscle (5 g) were transferred aseptically into a stomacher bag and Ringer's solution (1:9, w/v) were added. The mixture was homogenized for 60 s (STOMACHER 400, Seward Laboratory Systems Inc., FL, USA) which corresponded to the dilution 10^{-1} . Then, decimal dilutions were made using Ringer's solution. Total aerobic psychrophiles, anaerobic bacteria, *Enterobacteriaceae*, lactic acid bacteria and *Pseudomonas* spp. were determined by the method described previously by Fidalgo et al. (2018). Hydrogen sulphide-producing bacteria (H_2S -producing bacteria including *Shewanella putrefaciens*) were determined by the method of Teixeira et al. (2014). H_2S -producing bacteria were enumerated in pour plate using Lyngby iron agar (Merck), by inoculation of 1 mL of diluted samples and incubation at 20 °C for 4 days, counting the black colonies. In all cases, petri dishes containing 15-300 colony-forming units (CFU) were selected for counting, and microbial loads were calculated as logarithm of colony-forming units per gram (log CFU/g).

The pH of salmon samples was measured with a properly calibrated glass electrode at 25 °C (Crison, Barcelona, Spain).

2.3. Total volatile base-nitrogen assessment

TVB-N values were measured using the method adapted by Vázquez, Fidalgo, Saraiva, & Aubourg (2018). Briefly, 10 g fish muscle were extracted with 60 g/L perchloric acid in 30 mL water and brought up to 50 mL. An aliquot of the acid extracts was rendered alkaline to pH 13 with 200 g/L aqueous NaOH and then steam-distilled. Finally, the TVB-N content was determined by titration of the distillate with 10 mM HCl. Results were expressed as mg TVB-N/100 g muscle.

2.4. Trimethylamine-nitrogen assessment

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described by Tozawa, Erokibara, & Amano (1971). This involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g muscle/25 mL trichloroacetic acid). Results were expressed as mg TMA-N/100 g muscle.

2.5. Dimethylamine-nitrogen and formaldehyde determination

Before DMA-N and FA quantification, extraction using trichloroacetic acid was performed, according to Benjakul et al. (2004). Fish muscle (2 g) and 8 mL of 5% trichloroacetic acid were homogenized using a MICCRA D-9 Homogenizer (MICCRA GmbH, Müllheim, Deutschland). The homogenate was centrifuged at 3000×g for 15 min (Heraeus Biofuge Stratos, Thermo, Electron Corporation, Massachusetts, EUA). To the pellet, 4 mL of 5% trichloroacetic acid were added and homogenized as previously mentioned. The supernatants were combined and neutralized to pH 6.0-6.5 and the final

166 volume was made up to 20 mL using distilled water. The supernatant was then used for
167 DMA-N and FA determination.

168 DMA-N was determined by the copper-dithiocarbamate method, as described by
169 Dyer & Mounsey (1945) with a slight modifications. To 2 mL of neutralized
170 supernatant, 1 mL of copper-ammonia reagent was added and mixed thoroughly,
171 followed by the addition of 4 mL of 5% carbon disulphide-toluene solution. The tubes
172 were then closed tightly, and incubation at 50 °C for 2 min. After mixing for 20 s, 400
173 mL of 30% acetic acid were added, followed by mixing again for 15 s. After leaving for
174 10 min at room temperature, the toluene layer was transferred to a tube containing 0.5-
175 1.0 g of anhydrous sodium sulphate to remove the water. The absorbance was measured
176 at 440 nm (LAMBDA 35 UV/Vis spectrometer, PerkinElmer Instruments, Inc.). Results
177 were expressed as mg DMA-N/100 g muscle.

178 FA was determined using the acetylacetone reagent according to the method of
179 Nash (1953) with slight modifications. To the neutralized supernatant (3 mL), 3 mL of
180 acetylacetone reagent was added and mixed thoroughly. The reaction mixture was
181 incubated at 60 °C for 15 min and cooled in running water. The absorbance was
182 measured at 412 nm (LAMBDA 35 UV/Vis spectrometer, PerkinElmer Instruments,
183 Inc.) and the results expressed as µg FA/100 g muscle.

185 **2.6. Colour analysis**

186 Colour was measured at 25 °C and using a Konica Minolta CM-2300d
187 spectrophotometer (Osaka, Japan). The colour parameters were recorded according to
188 the CIE*lab* system and directly computed through the original SpectraMagic™ NX
189 software (Konica Minolta, Osaka, Japan), obtaining the following parameters: *L**-value

(0, dark; 100, light), a^* -value (+, red; -, green) and b^* -value (+, yellow; -, blue). These colour parameters were used to obtain the total colour difference (ΔE^*):

$$\Delta E^* = \left[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2 \right]^{1/2}$$

2.7. Lipid oxidation assessment

Lipid content was quantified according to the method of Bligh & Dyer (1959) and previously described by Fidalgo et al. (2018), being the extraction done with a chloroform:methanol:distilled water mixture (1/2/1). The total lipid content ranged from 15.4 and 21.9%. For peroxide value determination (primary lipid oxidation) was carried out following the ferric thiocyanate method previously described by Fidalgo et al. (2018). 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 already described in 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: $\text{RF} = F/F_{\text{st}}$, 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 $\mu\text{g}/\text{mL}$ 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: $FR = RF_{393/463\text{ nm}}/RF_{327/415\text{ nm}}$.

2.8. Statistical analysis

For the first set of experiments (10 days of storage assay), microbiology results are shown in relative microbial variation ($\log N - \log N_0$), calculated by difference between the logarithmic load at the end of storage (N) and the initial logarithmic load at day 0 (N_0). This way, positive values denote microbial growth, while negative values indicate microbial reduction compared to initial counts. Fish samples corresponding to each storage condition were tested with a two-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. The level of significance was established at $p < 0.05$.

For the second set of experiment (50 days of storage), microbial, pH, TVB-N, TMA-N, DMA-N, FA, colour and lipid oxidation results are shown in absolute values. Fish samples (initial samples/fresh fish, and samples stored under AP and HS) were tested with a one-way ANOVA, followed by a multiple comparisons test (Tukey's HSD) to identify differences between samples. The level of significance was also established at $p < 0.05$.

3. Results and discussion

3.1. Storage of Atlantic salmon for 10 days: pressure/temperature storage effect

Table 1 (and Table S1 from the Supplementary material) showed the initial microbial counts for raw salmon muscle used in this study. Initial total aerobic psychrophiles counts were between 4.19-5.40 log CFU/g, which were very similar to

those obtained in previous works (Fidalgo et al., 2018). According to the limit of 7.0 log CFU/g of aerobic plate counts established by ICMSF (1986), the salmon samples used in this study were in good conditions for human consumption before the storage experiments.

Evolution of the microbial counts in relative values of Atlantic salmon stored under the different conditions of pressure/temperature is shown in **Figure 1** (statistical analyses are presented in **Table S2** from the **Supplementary material**). Control samples, stored at AP at 5, 10 and 15 °C, showed a progressive increase ($p < 0.05$) of total aerobic psychrophiles counts during the 10 days of storage, with increments of 1.49, 2.05 and 1.93 log units, respectively, at the 3rd day of storage, which correspond to values next or above the acceptable limit of 7.0 log CFU/g (ICMSF, 1986) (**Figure 1a**). Consequently, these samples were already unacceptable for consumption at the 6th day, according to the same acceptability limit, and showed evident visual signs of spoilage, mainly samples stored at AP/15 °C. For the other microorganisms analysed, these samples showed a similar behaviour to total aerobic psychrophiles, with samples stored at AP/15 °C presenting counts above the acceptable limit after 10 days of storage.

Increasing pressure level, microbial evolution was progressively affected during storage time, similarly to what was verified in our previous work using storage at a room temperature of 25 °C at 75 MPa (Fidalgo et al., 2018). However, the present results showed that decreasing the temperature storage under a lower pressure allowed obtaining an improvement of the microbial stability. For instance, for the pressure level of 50 MPa, at 5 °C, a microbial load reduction ($p < 0.05$) was verified, of about 1.45, 1.00, 0.56 and 1.67 log units for total aerobic psychrophiles (**Figure 1a**), *Enterobacteriaceae* (**Figure 1c**), *Pseudomonas* spp. (**Figure 1e**) and H₂S-producing bacteria (**Figure 1f**), respectively. For anaerobic bacteria (**Figure 1b**) and lactic acid

bacteria (**Figure 1d**), the initial microbial load was maintained during the whole storage time. On the other hand, increasing the temperature storage to 10 °C, and maintaining the same pressure storage (50 MPa), only allowed obtaining a microbial growth slowdown. In this latter condition (50 MPa/10 °C), there is only an exception for lactic acid bacteria counts, which showed a reduction at the 3rd day of about 1.61 log units, further increasing to values similar to AP/5 °C, at the 10th day of storage. However, using a higher pressure of 60 MPa (at same 10 °C of storage temperature), this condition was enough to reduce the initial microbial counts for all the microorganisms analysed after 10 days of storage (1.29, 1.22, 1.99, 1.58 and 2.12 log units for total aerobic psychrophiles, *Enterobacteriaceae*, lactic acid bacteria, *Pseudomonas* spp. and H₂S-producing bacteria, respectively), except for anaerobic bacteria, for which a maintenance of the initial microbial load was observed.

A microbial inhibition was obtained using the other pressure/temperature conditions (50 and 60 MPa, at 15 °C), showing total aerobic psychrophiles counts higher than 7 log CFU/g (established limit) at the 10th day, except for 40 MPa/5 °C, which showed a very close value to the acceptable limit, of about 6.47 log CFU/g (an increase of 1.91 log units), at the end of storage. Accordingly, these samples resulted in an increase of microbial shelf-life to at least 6 days, unlike RF that showed microbial load above the acceptable limit at the same sampling day. Anaerobic bacteria presented a similar behaviour to total aerobic psychrophiles counts, while the other microorganisms, as the case of *Enterobacteriaceae*, lactic acid bacteria, *Pseudomonas* spp. and H₂S-producing bacteria, were more pressure sensible.

Recently, for hake loins (Otero et al., 2017) and Atlantic mackerel fillets (Otero et al., 2019) stored at 50 MPa/5 °C during 7 days, it was reported that microbial growth was inhibited at the beginning of the storage period, while microbial inactivation was

verified in the samples stored under pressure during all storage time. In the current work, similar results were obtained for the same storage condition (50 MPa/5 °C), but for a longer storage time.

As a result, the two pressure/temperature conditions of 50 MPa/5 °C and 60 MPa/10 °C were the most efficient to preserve fresh fish, since in addition to microbial growth inhibition, it was observed additionally microbial inactivation during the 10 days of storage. Since 5 °C is a refrigeration temperature, it was decided to pursue this work, studying a longer storage time of 50 days, in order to verify the possible achievable microbial shelf-life increase of fresh salmon fish at 60 MPa/10 °C, since this allows for energetic costs reduction (10 °C versus 5 °C).

3.2. Storage of Atlantic salmon for 50 days

3.2.1. Microbial evaluation

Microbial evolution of the longer time experiment (50 days) using the pressure/temperature condition of 60 MPa/10 °C is shown in **Table 2**. The initial microbial loads (fresh fish) were very close to those obtained in the first experiment (10 days), and a microbial load decrease ($p < 0.05$) was also verified at day 6 in samples stored at 60 MPa/10 °C, as was verified in the 10-day experiment. On the other hand, and similarly to the previously observed results, storage at AP at 5 and 10 °C, caused an increase of the initial microbial load for all the microorganisms, except for *Enterobacteriaceae* on samples stored at AP/5 °C, for which the initial value was maintained after 6 days. Differently, during the 50 days of storage at 60 MPa/10 °C, a progressive reduction ($p < 0.05$) was observed for all the microorganisms analysed, with values below the detection limit for *Enterobacteriaceae*, lactic acid bacteria, *Pseudomonas* spp. and H₂S-producing bacteria being verified. In the case of total

aerobic psychrophiles and anaerobic bacteria, a reduction of 1.25 and 0.41 log units, respectively, was obtained. These results clearly show the potential of HS to extend the microbial shelf-life of fresh fish.

3.2.2. Physicochemical evaluation

a) pH

Samples stored at 60 MPa/10 °C and at AP (5 and 10 °C) were also evaluated by pH measurements (**Table 3**). The initial pH obtained in raw salmon samples was 6.02 ± 0.02 (**Table 2**), very similar to values obtained in previous work (pH 5.95-6.07) (Fidalgo et al., 2018). Storage at AP/10 °C caused a decrease ($p < 0.05$) of pH, from 6.02 ± 0.02 to 5.86 ± 0.03 and 5.80 ± 0.04 after 6 and 15 days, respectively, which is probably caused by the accumulation of lactic acid produced by microbial glycolysis, as it was referred in previous work (Fidalgo et al., 2018), due to the contribution of acid lactic bacteria to the decrease of pH muscle. For storage at AP/5 °C, there was also a similar decrease of the initial pH, being verified a reduction to 5.88 ± 0.04 after 15 days. Differently, at 60 MPa/10 °C no change in pH was observed. These results are similar to those obtained by Otero et al. (2017) and Otero et al. (2019), which reported that after 7 and 12 days of storage at 5 °C, respectively, no variations were detected in the pH values of Cape hake loins and Atlantic mackerel fillets, respectively, both in AP and HS samples.

b) Total volatile basic nitrogen

The level of total volatile basic nitrogen (TVB-N, **Table 3**) was initially 26.13 ± 1.62 mg N/100 g muscle, similarly to values reported by other authors (Calanche et al., 2013) and did not exceed the legal limit (35 mg/100 g muscle) stipulated for

Atlantic salmon (*Salmo salar*) muscle in accordance to the Regulation (EC) No 1022/2008.

Storage at AP/10 °C caused an increase ($p < 0.05$) of TVB-N content in about 1.69-fold, showing salmon deterioration throughout the storage, which could be correlated with the higher microbial load of these samples. However, 6 days of refrigeration (AP/5 °C) did not affect ($p > 0.05$) the formation of TVB-N, showing similar values to the initial one. Baixas-Nogueras, Bover-Cid, Veciana-Nogués, & Vidal-Carou (2002) observed an increase on TVB-N values for Mediterranean hake (*Merluccius merluccius*) only after 10 days at 6-8 °C, and only a slight increase after 20 days in samples stored in ice. Samples stored at AP/10 °C exceeded the legal limit (Regulation (EC) No 1022/2008) after 6 days of storage, showing an average value of 44.24 ± 4.59 mg N/100 g muscle.

HS at 60 MPa/10 °C showed a maintenance ($p > 0.05$) of the TVB-N formation to at least the 15th day of storage, increasing thereafter. These results are in agreement with those obtained by Otero et al. (2017), who verified that HS at 50 MPa/5 °C seemed not to affect TVB-N content of hake loins during 7 days, contrarily to a 3-fold increase obtained at AP/5 °C at the same sampling day. On the other hand, Otero et al. (2019) observed that HS at the same conditions (50 MPa/5 °C), after 12 days, resulted in an increase of TVB-N content on Atlantic mackerel samples. In our work, after 30 and 50 days of storage of salmon samples, TVB-N values increased in about 1.48- and 1.62-fold, respectively, reaching values similar to AP/10 °C after 6 days, higher than the legal limit (Regulation (EC) No 1022/2008). High pressure processing (250 and 400 MPa, 5 min) of sea bass did not cause changes on TVB-N values up to at least 18 days (Teixeira et al., 2014). However, results from high pressure processing (HPP) cannot be easily

extrapolated to HS, since in the latter pressure is much lower, while time under pressure is much longer.

Özyurt, Kuley, Özkütük, & Özogul (2009) stated that the rise in TVB-N is related to the activity of spoilage bacteria and endogenous enzymes. This parameter includes TMA (produced by spoilage bacteria), ammonia (produced by deamination of amino acids and nucleotides catabolites), and DMA (produced by autolytic enzymes mainly during frozen storage). As in HS samples there was no microbial growth after 30 days of storage, probably the TVB-N formation was caused by enzymatic activity.

c) Trimethylamine-nitrogen

Initial salmon samples showed a value of 0.03 ± 0.01 mg TMA-N/100 g muscle, values close to that obtained by other authors (0.05 ± 0.01 mg TMA-N/100 g) (Rodríguez, Carriles, Cruz, & Aubourg, 2008) also in Atlantic salmon. According to Hansen et al. (2009), salmon contains naturally low levels of TMAO and the TMA formation is low, which is in agreement with the low levels found in this study and in salmon species (Dondero, Cisternas, Carvajal, & Simpson, 2004). After 6 days of storage, TMA-N formation was clearly affected by storage under AP/10 °C, presenting an increase ($p < 0.05$) of the initial values of about 570-fold. In addition, although not as pronounced and without significant statistical differences ($p < 0.05$), there was an increase of TMA-N values on samples stored at AP/5 °C of about 7.80-fold of the initial value, and a progressive increase ($p < 0.05$) on HS samples over storage time, showing an increase of about 57-fold the initial value at the 50th day. The level of TMA-N found in fresh fish rejected by sensory panels varies between species, but it is typically around 10-15 mg TMA-N/100 g in aerobically-stored fish (Dalgaard, Gram, & Huss, 1993).

Consequently, only samples stored at AP/10 °C could be rejected, since these fish samples showed a value of 15.88 ± 1.52 mg TMA-N/100 g.

TMA-N value is one of the most commonly employed quality methods to assess microbial activity in marine species kept under refrigerated conditions. According to Baixas-Nogueras et al. (2002), hake samples stored in ice showed values around 4 mg/100 g only after 20 days, but earlier and higher TMA-N values (> 15 mg/100 g after 10 days) were observed for samples stored at 6-8 °C, which agree to what was obtained in the current study.

On the other hand, according to the microbial inhibition (and inactivation) under HS (60 MPa/10 °C) during the 50 days of storage, an inhibition of the TMA formation was expected to occur. However, the conversion of TMAO into TMA observed in this study may be caused by non-enzymatic processes, endogenous tissue enzymes, or enzymes produced by microorganisms before the storage process. Besides that, Méndez et al. (2017) stated that increase of TMA can be related to protein breakdown caused by HPP (125-200 MPa, 0 min of holding time). Nevertheless, a good correlation between microbial inactivation by HPP and the TMA-N formation was observed in many fish muscles (Karim et al., 2011).

d) Dimethylamine-nitrogen and formaldehyde

DMA-N content on the initial samples (fresh salmon) was 0.134 ± 0.004 mg DMA-N/100 g muscle (**Table 3**), which agrees with the values obtained by other authors using Coho salmon (1.0-1.9 mg DMA-N/kg muscle) (Ortiz et al., 2012) and hake (1.83 mg DMA-N/kg muscle) (Vázquez et al., 2018). After 6 days under refrigeration (AP/5 °C), an increase ($p < 0.05$) of about 1.38-fold was observed. However, for samples stored at AP/10 °C (6 days) there was no effect ($p > 0.05$) on

DMA-N formation. For HS samples, a small increase was verified (to a maximum of ~0.158 mg DMA-N/100 g muscle after 15 and 30 days) and decreasing to 0.151±0.008 mg DMA-N/100 g muscle after 50 days.

Initial salmon sample showed a FA content of about 75.73±17.84 µg/100 g muscle (**Table 3**), which is in agreement with values reported before by Chung & Chan (2009) using Atlantic salmon (<1 mg/kg) and Vázquez et al. (2018) using hake samples (~0.83 mg formaldehyde/kg muscle). All stored samples showed an increase ($p < 0.05$) of the initial FA content. After 6 days of storage, there were no differences ($p > 0.05$) between samples stored at AP/5 °C and HS (60 MPa/10 °C), presenting an increase of FA values to 208.27±17.33 and 206.89±5.57 µg formaldehyde/100 g muscle, respectively, while at AP/10 °C, a less intense increase was verified (163.28±3.39 µg FA/100 g muscle) at the same storage day. For HS samples, FA content increased progressively ($p < 0.05$) until the 50th day of storage to a value of 302.68±13.42 µg formaldehyde/100 g muscle, which corresponds to a rise in about 4-fold of the initial value.

As it was explained previously, TMA formation is mainly caused by microbial activity, while concerning DMA, its formation can be explained based on the TMAO breakdown catalysed by an endogenous enzyme widely present in fish species (i.e. TMAOase) and be produced equimolecularly with FA (Benjakul et al., 2004). A marked inhibitory effect of storage pressure on DMA formation was observed in the current study and this effect was verified in other studies when high pressure treatments (150-450 MPa for 2 min) were applied on hake (*Merluccius merluccius*) before freezing (Vázquez et al., 2018). It is important to mention that, there was a low DMA and FA formation on AP/10 °C-stored samples, which might be due to the high TMA-N content in the same samples, indicating that practically all TMAO was converted to TMA.

However, FA content increased *ca* 4-fold on HS samples along the storage time, which indicates that possibly TMAOase activity is not totally inhibited and occurs enzymatic reduction of TMAO to DMA and formaldehyde. However, DMA-N content increased only between the 6th and 30th days (*ca* 1.2-fold), resulting in similar values to the initial one after 50 days of storage. These results could indicate that, besides natural formation of FA in fish and seafood by enzymatic reaction, other biochemical reactions can also occur (Noordiana, Fatimah, & Farhana, 2011).

e) Colour

The colour parameters values obtained in the initial raw salmon were: 56.54±1.39 for *L** (lightness), 16.61±3.19 for *a** (redness) and 18.06±4.09 for *b** (yellowness) (**Table 3**), similar to values obtained in a previous work (Fidalgo et al., 2018). Nevertheless, according to the literature, there are several reasons for colour differences of salmon muscle, which could be caused by different content in astaxanthin carotenoid, as well as to the haem pigments (Yavuz Yagiz et al., 2010). Accordingly, to minimize these effects, several measurements in different locations of the muscle salmon were carried out in the current study.

Salmon samples stored at atmospheric pressure (RF and 10 °C) after 6 days presented a maintenance ($p > 0.05$) of the colour parameters (lightness, redness and yellowness). Furthermore, compared to fresh fish, colour parameters were not statistically ($p > 0.05$) affected by pressure storage, showing values between 54.71-60.60, 19.11-20.00 and 19.36-21.15 for lightness, redness and yellowness parameters, respectively, as can be seen on **Table 3**. Since these parameters did not change in any sample, this is reflected on the value of ΔE^* , resulting in no differences ($p > 0.05$) between samples. The ΔE^* parameter shows the comparison between samples stored

under the different conditions and the respective initial values obtained in raw salmon samples (fresh fish).

The presence of carotenoids (astaxanthin and canthaxanthin), and somewhat by haem proteins, on the salmon muscle contributes to the characteristic red colour (Yavuz Yagiz et al., 2010), being a very important organoleptic property for the customer acceptance (Alfnes, Guttormsen, Steine, & Kolstad, 2006). Generally, according to the literature, a reduction of salmon redness was obtained by increasing pressure and treatment time, due to a pressure-induced myofibrillar denaturation, including the astaxanthin-binding proteins or structural changes of astaxanthin-actinin (Ojagh, Núñez-Flores, López-Caballero, Montero, & Gómez-Guillén, 2011), as it was observed in salmon muscle treated from 100 to 200 MPa and 5 °C for 10-60 min (Amanatidou, 2000). In the current study, redness of salmon muscle stored under pressure was not affected, as also the other colour parameters, revealing that under these storage conditions astaxanthin and/or myoglobin are probably unaffected. These results are in agreement with those obtained in a previous work (Fidalgo et al., 2018) using higher pressure level/storage temperature (75 MPa/25 °C, during 10 or 25 days). On the other hand, Otero et al. (2017) and Otero et al. (2019) verified that storage under pressure (50 MPa/5 °C, during 7 days) increased the whiteness of the raw hake loins and Atlantic mackerel fillet, respectively, but after cooking the differences disappeared.

f) Lipid oxidation

Salmon muscle is very susceptible to oxidation, due to the high content in unsaturated fatty acids and the oxidation state of salmon muscle samples. In the present study was evaluated by primary, secondary and tertiary lipid oxidation, by the quantification of the peroxide value, TBARS and fluorescent compounds, respectively.

Initially, the peroxide value and TBARS were 3.80 ± 1.28 mg Fe III/kg lipids and 0.36 ± 0.04 μ g MDA/g fish, which are in agreement to those obtained in previous work (Fidalgo et al., 2018). Concerning fluorescent compounds, the initial value was 0.05 ± 0.01 , being lower to that obtained in a previous work (0.85 ± 0.06) (Fidalgo et al., 2018).

Primary lipid oxidation did not change ($p > 0.05$) on HS and AP samples, with values ranged between 3.30-4.32 mg Fe III/kg lipids (**Table 3**). No variations on values ($p > 0.05$) of TBARS were obtained for both storage conditions at AP (RF and 10 °C). However, HS caused an increment on TBARS values, being observed an accelerated secondary lipid oxidation immediately after 6 days to 1.89 ± 0.15 μ g MDA/g fish, remaining thereafter statistically unchanged ($p > 0.05$) during all storage time (50 days: 2.65 ± 0.68 μ g MDA/g fish). Otherwise, an increase of fluorescent compounds formation ($p < 0.05$) to a value of 0.47 ± 0.01 and 0.21 ± 0.03 for samples stored at AP (10 °C and RF samples, respectively), which correspond to an increase of about 9.5- and 4-fold of the initial value, respectively. On the other hand, no variations ($p > 0.05$) were observed for HS samples during storage time, with the tertiary lipid oxidation products being kept in values similar to fresh salmon samples.

According to Aubourg (1999), the mechanism of lipid oxidation is a process resulted from interactions between hydroperoxides (primary lipid oxidation) and compounds formed on the secondary oxidation (mainly, electrophilic compounds) and protein-type molecules (mainly, nucleophilic compounds) present in the salmon muscle. This mechanism could be the explanation for the obtained results in the AP conditions (lower values of TBARS, and the higher values of fluorescent compounds). Contrarily, an increase of the TBARS values was observed in the HS samples, indicating a possible pro-oxidant effect of pressure, but not as intense as for AP samples. Furthermore, in a

previous work (Fidalgo et al., 2018), higher TBARS values were observed for HS samples stored at a higher storage pressure/temperature (75 MPa/25 °C), with values reaching 7.35 ± 0.30 μg MDA/g fish after 25 days. These results indicate that a lower storage temperature (10 °C) probably retards lipid oxidation, which is in agreement to the results obtained by Otero et al. (2019), verifying that HS, at 50 MPa and 5 °C, did not enhance lipid oxidation in mackerel fillets. Additionally, the higher values of TBARS and lower values of FA observed for HS in the present work, indicates that probably, HS reduced the reactions involved in the mechanism from secondary to tertiary lipid oxidations. Additionally, pressure storage possibly can result in a higher availability of astaxanthin in salmon muscle and exert a protective effect on the fish's lipids (Yagiz, Kristinsson, Balaban, & Marshall, 2007). Overall, HS samples showed a less advanced stage of lipid oxidation.

4. Conclusions

Besides the few published works, HS has shown to be an efficient method to preserve fresh fish. When it is used at low temperatures, as is the case of the current work, under conditions of 50 MPa/5 °C or 60 MPa/10 °C, it allowed to obtain a slowdown of the microbial growth or even a reduction of the initial microbial load, resulting in a microbial shelf-life increase of at least 50 days when stored at 60 MPa/10 °C. Furthermore, these samples did not show colour parameters differences along storage time.

Regarding chemical quality parameters, there was an increase of the TVB-N and TMA-N formation on samples stored during 6 days at AP/10 °C, surpassing the established limit for fresh fish. In addition, samples stored at AP/10 °C showed low DMA-N and FA content, which is correlated to the high TMA-N content in the same

538 samples, since practically the whole TMAO was converted to TMA-N. On the other
539 hand, samples stored at 60 MPa/10 °C only showed an increase on TVB-N formation
540 after 30 days of storage, with values higher than the establish limit. Besides that, TMA-N
541 formation on HS and RF samples was stable and below the established limit. Since the
542 microbial activity was inhibited/reduced under pressure, it was expected that FA content
543 increased on HS samples, but DMA-N content did not change over the storage time in
544 these samples, which indicates that, besides the FA formation by enzymatic activity
545 (TMAO aldolase activity), FA formation by other chemical reactions mechanisms could
546 also occur. Compared to fresh salmon, secondary lipid oxidation (TBARS) was
547 increased by HS. However, tertiary lipid oxidation (fluorescent compounds) increased
548 only for AP samples (RF and 10 °C), revealing an advanced stage of lipid oxidation in
549 these samples.

550 Under the condition of 60 MPa/10 °C, it was possible to increase the shelf-life of
551 Atlantic salmon to minimum of 15 and a maximum of 30 days, by inhibition of
552 microbial load (and additionally initial microbial load) and slowdown chemical
553 indicators formation (volatile amines), as the case of TVB-N, TMA-N and DMA-N.
554 Furthermore, these samples did not show colour parameters differences along storage
555 time. Nevertheless, much more research is required, namely on the oxidation
556 mechanisms (enzymatic and non-enzymatic) that occur during HS of fresh fish and how
557 to avoid or reduce them, or at the level of other quality analyses (for example using
558 inoculated pathogenic microorganisms, enzymatic activity, textural and sensorial
559 parameters, etc).

561 **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) through national funds 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). The authors also acknowledge the financial support provided by the Consejo Superior de Investigaciones Científicas (CSIC) (Spain) through the Research Project PIE 2013-70E001.

References

- Alfnes, F., Guttormsen, A. G., Steine, G., & Kolstad, K. (2006). Consumers' willingness to pay for the color of salmon: a choice experiment with real economic incentives. *American Journal of Agricultural Economics*, 88(4), 1050–1061. <https://doi.org/10.1111/j.1467-8276.2006.00915.x>
- Amanatidou, A. (2000). Effect of combined application of high pressure treatment and modified atmospheres on the shelf life of fresh Atlantic salmon. *Innovative Food Science & Emerging Technologies*, 1(2), 87–98. [https://doi.org/10.1016/S1466-8564\(00\)00007-2](https://doi.org/10.1016/S1466-8564(00)00007-2)
- Aubourg, S. P. (1999). Recent advances in assessment of marine lipid oxidation by using fluorescence. *Journal of the American Oil Chemists' Society*, 76(4), 409–419. <https://doi.org/10.1007/s11746-999-0018-2>
- Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, T., & Vidal-Carou, M. C. (2002). Chemical and sensory changes in Mediterranean hake (*Merluccius merluccius*) under refrigeration (6–8 °C) and stored in ice. *Journal of Agricultural and Food Chemistry*, 50(22), 6504–6510. <https://doi.org/10.1021/jf025615p>
- Benjakul, S., Visessanguan, W., & Tanaka, M. (2004). Induced formation of dimethylamine and formaldehyde by lizardfish (*Saurida micropectoralis*) kidney trimethylamine-N-oxide demethylase. *Food Chemistry*, 84(2), 297–305. [https://doi.org/10.1016/S0308-8146\(03\)00214-0](https://doi.org/10.1016/S0308-8146(03)00214-0)
- Bligh, E., & Dyer, W. (1959). A rapid method of total extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.
- Calanche, J., Samayoa, S., Alonso, V., Provincial, L., Roncalés, P., & Beltrán, J. a. (2013). Assessing the effectiveness of a cold chain for fresh fish salmon (*Salmo salar*) and sardine (*Sardina pilchardus*) in a food processing plant. *Food Control*,

- 33(1), 126–135. <https://doi.org/10.1016/j.foodcont.2013.02.005>
- Chung, S. W. C., & Chan, B. T. P. (2009). Food Additives and Contaminants : Part B trimethylamine and formaldehyde levels in main traded fish species in Hong Kong, 3210. <https://doi.org/10.1080/02652030902858921>
- Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*, 19(4), 283–294. [https://doi.org/10.1016/0168-1605\(93\)90020-H](https://doi.org/10.1016/0168-1605(93)90020-H)
- Dondero, M., Cisternas, F., Carvajal, L., & Simpson, R. (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food Chemistry*, 87(4), 543–550. <https://doi.org/10.1016/j.foodchem.2004.01.005>
- Dyer, W., & Mounsey. (1945). Amines in fish muscle II. Development of trimethylamine and other amines. *J. Fish. Res. Bd. Can.*, 6(5), 359–367. <https://doi.org/10.1017/CBO9781107415324.004>
- Fidalgo, L. G., Lemos, Á. T., Delgadillo, I., & Saraiva, J. A. (2018). Microbial and physicochemical evolution during hyperbaric storage at room temperature of fresh Atlantic salmon (*Salmo salar*). *Innovative Food Science & Emerging Technologies*, 45, 264–272. <https://doi.org/10.1016/j.ifset.2017.11.003>
- Ghaly, A., Dave, D., Budge, S., & Brooks, M. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences*, 7(7), 859–877. <https://doi.org/10.3844/ajassp.2010.859.877>
- Hansen, A. A., Morkore, T., Rudi, K., Rodbotten, M., Bjerke, F., & Eie, T. (2009). Quality changes of prerigor filleted atlantic salmon (*Salmo salar* L.) packaged in modified atmosphere using CO2 emitter, traditional MAP, and vacuum. *Journal of Food Science*, 74(6), 2–9. <https://doi.org/10.1111/j.1750-3841.2009.01233.x>

- Hultin, H. O. (1994). Oxidation of lipids in seafoods. In F. Shahidi & J. R. Botta (Eds.), *Seafoods Chemistry, Processing Technology and Quality* (1st ed., pp. 49–74). London, UK: Blackie Academic and Professional.
- ICMSF. (1986). Sampling for microbiological analysis: Principles and specific applications. *Blackwell Scientific Publication*, 2, 127–278.
<https://doi.org/10.2307/1268642>
- Karim, N. U., Kennedy, T., Linton, M., Watson, S., Gault, N., & Patterson, M. F. (2011). Effect of high pressure processing on the quality of herring (*Clupea harengus*) and haddock (*Melanogrammus aeglefinus*) stored on ice. *Food Control*, 22(3–4), 476–484. <https://doi.org/10.1016/j.foodcont.2010.09.030>
- Ko, W.-C., Jao, C.-L., Hwang, J.-S., & Hsu, K.-C. (2006). Effect of high-pressure treatment on processing quality of tilapia meat fillets. *Journal of Food Engineering*, 77(4), 1007–1011. <https://doi.org/10.1016/j.jfoodeng.2005.08.029>
- Lemos, Á. T., Ribeiro, A. C., Fidalgo, L. G., Delgadillo, I., & Saraiva, J. A. (2017). Extension of raw watermelon juice shelf-life up to 58 days by hyperbaric storage. *Food Chemistry*, 231, 61–69. <https://doi.org/10.1016/j.foodchem.2017.03.110>
- Méndez, L., Fidalgo, L. G., Pazos, M., Lavilla, M., Torres, J. A., Saraiva, J. A., ... Aubourg, S. P. (2017). Lipid and Protein Changes Related to Quality Loss in Frozen Sardine (*Sardina pilchardus*) Previously Processed Under High-Pressure Conditions. *Food and Bioprocess Technology*, 10(2), 296–306.
<https://doi.org/10.1007/s11947-016-1815-x>
- Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochemical Journal*, 55(3), 416–421. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1269292/>
- Noordiana, N., Fatimah, A. B., & Farhana, Y. C. B. (2011). Formaldehyde content and

- quality characteristics of selected fish and seafood from wet markets. *International Food Research Journal*, 18(1), 125–136.
- Ojagh, S. M., Núñez-Flores, R., López-Caballero, M. E., Montero, M. P., & Gómez-Guillén, M. C. (2011). Lessening of high-pressure-induced changes in Atlantic salmon muscle by the combined use of a fish gelatin-lignin film. *Food Chemistry*, 125(2), 595–606. <https://doi.org/10.1016/j.foodchem.2010.08.072>
- Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., ... Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science & Technology*, 8(August), 258–265.
- Ortiz, J., Vivanco, J. P., Quiral, V., Larraín, M. a., Concha, G., & Aubourg, S. P. (2012). Changes in freshness during frozen storage of farmed coho salmon: effect of replacement of synthetic antioxidants by natural ones in fish feeds. *North American Journal of Aquaculture*, 74(2), 224–229. <https://doi.org/10.1080/15222055.2012.675994>
- Otero, L., Pérez-Mateos, M., Holgado, F., Márquez-Ruiz, G., & López-Caballero, M. E. (2019). Hyperbaric cold storage: Pressure as an effective tool for extending the shelf-life of refrigerated mackerel (*Scomber scombrus*, L.). *Innovative Food Science & Emerging Technologies*, 51(May 2018), 41–50. <https://doi.org/10.1016/j.ifset.2018.05.003>
- Otero, L., Pérez-Mateos, M., & López-Caballero, M. E. (2017). Hyperbaric cold storage versus conventional refrigeration for extending the shelf-life of hake loins. *Innovative Food Science & Emerging Technologies*, 41, 19–25. <https://doi.org/10.1016/j.ifset.2017.01.003>
- Ozogul, Y. (2009). Chapter 13 Methods for Freshness Quality and Deterioration. In L. M. L. Nollet & F. Toldra (Eds.), *Handbook of Seafood and Seafood Products*

- Analysis* (pp. 189–214). CRC Press. Retrieved from <https://books.google.pt/books?id=LfXHWtGvRj8C>
- Özyurt, G., Kuley, E., Özkütük, S., & Özogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry*, 114(2), 505–510. <https://doi.org/10.1016/j.foodchem.2008.09.078>
- Regulation (EC) No 1022/2008. (2008). *Commission Regulation (EC) No 1022/2008 of 17 October 2008 amending Regulation (EC) No 2074/2005 as regards the total volatile basic nitrogen (TVB-N) limits*.
- Rodríguez, A., Carriles, N., Cruz, J. M., & Aubourg, S. P. (2008). Changes in the flesh of cooked farmed salmon (*Oncorhynchus kisutch*) with previous storage in slurry ice (-1.5 °C). *LWT - Food Science and Technology*, 41(9), 1726–1732. <https://doi.org/10.1016/j.lwt.2007.10.002>
- Teixeira, B., Marques, A., Mendes, R., Gonçalves, A., Fidalgo, L., Oliveira, M., ... Nunes, M. L. (2014). Effects of high-pressure processing on the quality of sea bass (*Dicentrarchus labrax*) fillets during refrigerated storage. *Food and Bioprocess Technology*, 7(5), 1333–1343. <https://doi.org/10.1007/s11947-013-1170-0>
- Tozawa, H., Erokibara, K., & Amano, K. (1971). Proposed modification of Dyer's method for trimethylamine determination in codfish. In R. Kreuzer (Ed.), *Fish Inspection and Quality Control* (pp. 187–190). London, UK: Fishing News Books Ltd.
- Vázquez, M., Fidalgo, L. G., Saraiva, J. A., & Aubourg, S. P. (2018). Preservative effect of a previous high-pressure treatment on the chemical changes related to quality loss in frozen hake (*Merluccius merluccius*). *Food and Bioprocess Technology*, 11(2). <https://doi.org/10.1007/s11947-017-2010-4>

- Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette, Seifen, Anstrichmittel*, 72(12), 1084–1087. <https://doi.org/10.1002/lipi.19700721218>
- Yagiz, Y., Kristinsson, H. G., Balaban, M. O., & Marshall, M. R. (2007). Effect of High Pressure Treatment on the Quality of Rainbow Trout (*Oncorhynchus mykiss*) and Mahi Mahi (*Coryphaena hippurus*). *Journal of Food Science*, 72(9), C509–C515. <https://doi.org/10.1111/j.1750-3841.2007.00560.x>
- Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Raghavan, S., & Marshall, M. R. (2010). Correlation between astaxanthin amount and a^* value in fresh Atlantic salmon (*Salmo salar*) muscle during different irradiation doses. *Food Chemistry*, 120(1), 121–127. <https://doi.org/10.1016/j.foodchem.2009.09.086>

FIGURES

Figure 1. Relative values of total aerobic psychrophiles (a), Enterobacteriaceae (b), anaerobic bacteria (c), lactic acid bacteria (d), *Pseudomonas* spp. (e) and H₂S-producing bacteria (f) evolution on Atlantic salmon during 10 days of storage under: hyperbaric storage at low temperature (40 and 50 MPa at 5 °C; 50 and 60 MPa at 10 °C; and 50 and 60 MPa at 15 °C), and under atmospheric pressure (AP, 0.1 MPa) at the same temperatures (5, 10 and 15 °C). Results are shown in relative microbial variation ($\log (N/N_0)$), calculated by difference between the logarithmic load at the end of storage (N) and the initial logarithmic load at day 0 (N₀, values are shown on the **Table S1** of the **Supplementary information**). Statistical analyses are shown in **Table S2** of the **Supplementary information**.

Figure 1

Tables

Table 1. Initial microbial load of the different samples of Atlantic salmon used in the storage experiments.

Microbial parameters (Log CFU/g)	Results	RSD ⁽¹⁾
Total aerobic psychrophiles	4.19 – 5.40	9%
Anaerobic bacteria	1.72 – 4.39	28%
<i>Enterobacteriaceae</i>	1.90 – 2.91	16%
Lactic acid bacteria	1.95 – 3.15	34%
<i>Pseudomonas</i> spp.	4.16 – 6.06	13%
H ₂ S-producing bacteria	3.65 – 5.47	22%

⁽¹⁾ RSD: Relative Standard Deviation.

Table 2 – Microbial evolution of Atlantic salmon stored at 60 MPa/10 °C during 50 days, and AP (5 and 10 °C) during 15 days. Different letters denote significant differences ($p < 0.05$) between storage conditions (a-e).

<i>Microbial analyses</i> ⁽¹⁾	<i>Fresh fish</i>	<i>60 MPa/10 °C</i>				<i>AP/5 °C</i>		<i>AP/10 °C</i>	
	<i>0 days</i>	<i>6 days</i>	<i>15 days</i>	<i>30 days</i>	<i>50 days</i>	<i>6 days</i>	<i>15 days</i>	<i>6 days</i>	<i>15 days</i>
Total aerobic psychrophiles	4.31±0.08 ^c	3.82±0.15 ^{cd}	3.44±0.11 ^{de}	3.31±0.05 ^{de}	3.06±0.13 ^c	5.57±0.51 ^b	≥ 7.00 ^{a (3)}	6.86±0.44 ^a	≥ 7.00 ^{a (3)}
Anaerobic bacteria	2.40±0.25 ^c	1.93±0.17 ^c	≤ 1.00 ^{d (2)}	≤ 1.00 ^{d (2)}	1.99±0.60 ^c	4.12±0.10 ^b	na ⁽⁴⁾	6.66±0.50 ^a	na ⁽⁴⁾
<i>Enterobacteriaceae</i>	2.47±0.06 ^b	≤ 1.00 ^{c (2)}	≤ 1.00 ^{c (2)}	≤ 1.00 ^{d (2)}	≤ 1.00 ^{C (2)}	2.49±0.54 ^b	na ⁽⁴⁾	5.50±0.73 ^a	na ⁽⁴⁾
Lactic acid bacteria	2.00±0.17 ^c	2.25±0.27 ^c	≤ 1.00 ^{d (2)}	≤ 1.00 ^{d (2)}	≤ 1.00 ^{D (2)}	3.23±0.05 ^b	na ⁽⁴⁾	5.93±0.21 ^a	na ⁽⁴⁾
<i>Pseudomonas</i> spp.	4.04±0.23 ^c	2.90±0.82 ^d	≤ 2.00 ^{d (2)}	≤ 2.00 ^{d (2)}	≤ 2.00 ^{D (2)}	6.02±0.41 ^b	na ⁽⁴⁾	7.09±0.18 ^a	na ⁽⁴⁾
H ₂ S-producing bacteria	2.23±0.31 ^c	≤ 1.00 ^{d (2)}	≤ 1.00 ^{d (2)}	≤ 1.00 ^{d (2)}	≤ 1.00 ^{D (2)}	4.65±0.03 ^b	na ⁽⁴⁾	5.75±0.69 ^a	na ⁽⁴⁾

⁽¹⁾ Microbial loads are in Log CFU/g muscle.

⁽²⁾ Counts were below the detection limit (≤ 1 log CFU/g or ≤ 2 log CFU/g in case of *Pseudomonas* spp.).

⁽³⁾ Counts were above the acceptable limit (≥ 7 log CFU/g) according to **ICMSF (1986)**.

⁽⁴⁾ Not analysed.

Table 3 – Physicochemical parameters evolution of Atlantic salmon stored at 60 MPa/10 °C during 50 days, and AP (5 and 10 °C) during 15 days. Different letters denote significant differences ($p < 0.05$) between storage conditions (a-e).

<i>Physicochemical analyses</i>	<i>Fresh fish</i>	<i>60 MPa/10 °C</i>				<i>AP/5 °C</i>		<i>AP/10 °C</i>	
	<i>0 days</i>	<i>6 days</i>	<i>15 days</i>	<i>30 days</i>	<i>50 days</i>	<i>6 days</i>	<i>15 days</i>	<i>6 days</i>	<i>15 days</i>
<i>pH</i>	6.02±0.02 ^b	6.08±0.01 ^a	6.09±0.02 ^a	6.10±0.01 ^a	6.08±0.02 ^a	6.01±0.02 ^b	5.88±0.04 ^c	5.86±0.03 ^c	5.80±0.04 ^c
<i>TVB-N</i> ⁽¹⁾	26.13±1.62 ^b	27.32±1.29 ^b	29.01±1.25 ^b	38.65±3.49 ^a	42.22±6.11 ^a	25.87±1.16 ^b	na ⁽⁶⁾	44.24±4.59 ^a	na ⁽⁶⁾
<i>TMA-N</i> ⁽²⁾	0.03±0.01 ^b	0.16±0.07 ^b	0.58±0.22 ^b	0.71±0.29 ^b	1.58±0.45 ^b	0.22±0.17 ^b	na ⁽⁶⁾	15.88±1.52 ^a	na ⁽⁶⁾
<i>DMA-N</i> ⁽³⁾	0.134±0.004 ^c	0.156±0.005 ^b	0.158±0.005 ^b	0.158±0.012 ^b	0.151±0.008 ^{bc}	0.186±0.003 ^b	na ⁽⁶⁾	0.143±0.007 ^{bc}	na ⁽⁶⁾
<i>FA</i> ⁽⁴⁾	75.73±17.84 ^c	206.89±5.57 ^c	258.19±14.89 ^b	292.43±5.19 ^{ab}	302.68±13.42 ^a	208.27±17.33 ^c	na ⁽⁶⁾	163.28±3.39 ^d	na ⁽⁶⁾
<i>Colour</i>									
<i>L</i> *	56.54±1.39 ^{ab}	54.71±3.05 ^b	59.98±0.57 ^a	59.32±0.53 ^{ab}	60.60±1.72 ^a	56.77±2.17 ^{ab}	na ⁽⁶⁾	57.63±2.26 ^{ab}	na ⁽⁶⁾
<i>a</i> *	16.61±3.19 ^a	19.11±2.88 ^a	20.00±1.16 ^a	18.87±1.38 ^a	19.53±3.62 ^a	16.59±2.32 ^a	na ⁽⁶⁾	19.54±2.23 ^a	na ⁽⁶⁾
<i>b</i> *	18.06±4.09 ^a	19.68±2.70 ^a	21.15±1.05 ^a	19.36±0.57 ^a	20.60±3.24 ^a	18.00±3.55 ^a	na ⁽⁶⁾	20.00±4.04 ^a	na ⁽⁶⁾
ΔE^*		5.12±1.96 ^a	5.80±1.28 ^a	3.93±1.09 ^a	6.96±1.07 ^a	3.75±1.29 ^a	na ⁽⁶⁾	5.17±2.59 ^a	na ⁽⁶⁾
<i>Lipid oxidation</i> ⁽⁵⁾									
<i>Primary</i>	3.80±1.28 ^a	3.35±0.51 ^a	3.67±0.85 ^a	3.74±0.15 ^a	3.91±0.97 ^a	3.30±0.37 ^a	na ⁽⁶⁾	4.32±1.01 ^a	na ⁽⁶⁾
<i>Secondary</i>	0.36±0.04 ^b	1.89±0.15 ^a	2.66±0.36 ^a	2.38±0.12 ^a	2.65±0.68 ^a	0.35±0.03 ^b	na ⁽⁶⁾	0.47±0.01 ^b	na ⁽⁶⁾
<i>Tertiary</i>	0.05±0.01 ^d	0.10±0.01 ^{cd}	0.06±0.01 ^{cd}	0.12±0.01 ^{bcd}	0.15±0.01 ^{bc}	0.21±0.03 ^b	na ⁽⁶⁾	0.42±0.05 ^a	na ⁽⁶⁾

⁽¹⁾ TVB-N: Total volatile basic-nitrogen (mg N/100 g muscle).

⁽²⁾ TMA-N: Trimethylamine-nitrogen (mg TMA-N/100 g muscle).

⁽³⁾ DMA-N: Dimethylamine-nitrogen (mg DMA-N/100 g muscle).

⁽⁴⁾ FA: Formaldehyde (µg FA/100 g muscle).

⁽⁵⁾ 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 compounds.

⁽⁶⁾ Not analysed.

Supplementary information

Table S1 – Corresponding initial counts (N_0 , log CFU/g) for each experiment presented in the Figure 1 of the manuscript.

a) Total aerobic psychrophiles (TAP)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	4.19 ± 0.13
40 MPa/5 °C	4.55 ± 0.07
AP/10 °C, 60 MPa/10 °C	4.64 ± 0.15
50 MPa/10 °C	4.92 ± 0.32
AP/15 °C, 50 MPa/15 °C	5.05 ± 0.15
60 MPa/15 °C	5.40 ± 0.25
b) Anaerobic bacteria (ANA)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	1.72 ± 0.22
40 MPa/5 °C	3.14 ± 0.10
AP/10 °C, 60 MPa/10 °C	2.88 ± 0.07
50 MPa/10 °C	3.29 ± 0.18
AP/15 °C, 50 MPa/15 °C	2.83 ± 0.11
60 MPa/15 °C	4.39 ± 0.41
c) Enterobacteriaceae (ENT)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	1.98 ± 0.18
40 MPa/5 °C	2.29 ± 0.16
AP/10 °C, 60 MPa/10 °C	2.16 ± 0.28
50 MPa/10 °C	1.90 ± 0.10
AP/15 °C, 50 MPa/15 °C	2.91 ± 0.11
60 MPa/15 °C	2.26 ± 0.04
d) Lactic acid bacteria (LAB)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	2.81 ± 0.05
40 MPa/5 °C	1.00
AP/10 °C, 60 MPa/10 °C	2.98 ± 0.03
50 MPa/10 °C	2.79 ± 0.29
AP/15 °C, 50 MPa/15 °C	3.15 ± 0.10
60 MPa/15 °C	1.95 ± 0.23
e) Pseudomonas spp. (PSE)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	4.16 ± 0.29
40 MPa/5 °C	5.05 ± 0.16
AP/10 °C, 60 MPa/10 °C	5.19 ± 0.29
50 MPa/10 °C	4.76 ± 0.24
AP/15 °C, 50 MPa/15 °C	5.41 ± 0.15
60 MPa/15 °C	6.06 ± 0.09
f) Hydrogen sulphide-producing bacteria (HPB)	
<i>Conditions</i>	<i>Initial counts (N_0)</i>
AP/5 °C, 50 MPa/5 °C	3.65 ± 0.16
40 MPa/5 °C	3.61 ± 0.28

AP/10 °C, 60 MPa/10 °C	4.28 ± 0.11
50 MPa/10 °C	5.33 ± 0.09
AP/15 °C, 50 MPa/15 °C	5.47 ± 0.16
60 MPa/15 °C	5.40 ± 0.19

Table S2. Statistical analyses of microbial evolution (in relative values) on Atlantic salmon stored during 10 days under: hyperbaric storage at low temperature (40 and 50 MPa at 5 °C; 50 and 60 MPa at 10 °C; and 50 and 60 MPa at 15 °C), and under atmospheric pressure (AP, 0.1 MPa) at the same temperatures (5, 10 and 15 °C). Different letters denote significant differences ($p < 0.05$) between storage days for each storage condition (a-b) and between storage conditions for each storage day (A-F). * and # symbols mean that microbial counts were ≥ 300 CFU on the 10^{-6} dilution of samples or below the detection limit (≤ 1 CFU/g), respectively.

Storage time	Storage conditions		TAP	ANA	ENT	LAB	PSE	HPB
3 days	0.1 MPa	5 °C	AB b	A b	B b	BC b	A a	A a
		10 °C	A a	A b	BC b	B b	AB b	B a
		15 °C	A b	A b	A c	A b	ABC b	B b
	40 MPa	5 °C	BC b	BC b	E b#	C a#	AB a	C b
	50 MPa	5 °C	D a	D a	E a#	C a#	D ab	CD a
		10 °C	CD c	C c	CD b	DE c	BCD b	C b
		15 °C	CD b	B c	BC a	CD a	CD b	CD b
	60 MPa	10 °C	D a	D a	DE a	E a	D a	D a
		15 °C	D b	D c	C a	C b	CD a	C a
6 days	0.1 MPa	5 °C	A a	AB a	B ab	B a	A a	A a
		10 °C	A a	B a	BC a	A a	AB b	C a
		15 °C	A a	A a	A b	A a	ABC b	A b
	40 MPa	5 °C	B b	C b	E b#	D a#	BCD a	C a
	50 MPa	5 °C	C a	D a	E a#	D a#	E b	D a
		10 °C	B b	C b	D ab	CD b	BCD ab	C a
		15 °C	B b	B b	CD a	CD a	CD ab	CD a
	60 MPa	10 °C	C a	D a	E b#	E a	E a	E ab
		15 °C	B a	C b	E A	BC a	D a	D a
10 days	0.1 MPa	5 °C	A a	AB a	B a	BC b	AB a	A a
		10 °C	AB a	B a	BC ab	A a	A a	B a
		15 °C	A a*	A a*	A a*	A a*	A a*	A a*
	40 MPa	5 °C	B a	C a	DE a	C a#	BC a	B a
	50 MPa	5 °C	C a	D a	F a#	C a#	D a	D b#
		10 °C	A a	B a	BC a	B a	BC a	B a
		15 °C	B a	B a	CD a	C a	C a	B a
	60 MPa	10 °C	C a	D a	F b#	D a	D a	D b

15 °C *B a* *C a* *EF a* *B a* *D a* *A a*

TAP: Total aerobic psychrophiles; ANA: Anaerobic bacteria; ENT: *Enterobacteriaceae*; LAB: Lactic acid bacteria; PSE: *Pseudomonas* spp.; HPB: Hydrogen sulphide-producing bacteria.