

Accepted Manuscript

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PII: S1466-8564(18)31431-0
DOI: <https://doi.org/10.1016/j.ifset.2019.05.010>
Reference: INNFOO 2171

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 20 November 2018
Revised date: 8 March 2019
Accepted date: 17 May 2019

Please cite this article as: M. de Alba, J. Pérez-Andrés, S.M. Harrison, et al., High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets, *Innovative Food Science and Emerging Technologies*, <https://doi.org/10.1016/j.ifset.2019.05.010>

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High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets

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Running title: High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel

Abstract

The objective of this study was to investigate the effect of high pressure processing (HPP) (100, 300 or 500 MPa for 2 or 5 min) on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets. A significant reduction in TVC and H₂S-producing bacteria was detected at 300 MPa for 5 min and 500 MPa for 2 or 5 min. Lightness (L^*) increased and redness (a^*) decreased at the highest treatment intensities. Hardness, chewiness and springiness increased with the most intense treatments but neither cohesiveness nor TBARS values were affected by pressurization. HPP significantly decreased levels of EPA, PUFAs, HUFAs, DHA, CLAs and increased MUFAs and SFAs. TI significantly increased at the highest pressurization intensities and AI was affected when HPP was held for 5 min. However, the ratio PUFA/SFA above 0.45 in pressurized mackerel fillets indicated that HPP did not compromise the nutritional value of this pelagic fish.

Industrial relevance: The potential of HPP to inhibit spoilage and increase the shelf-life of mackerel fillets, while maintaining its quality and healthy attributes, could help the fish processing industry to ensure better quality raw material for further processing, thereby enabling the development of new, value-added products with extended shelf-life. The reduction in the processing time with the subsequent saving of energy compared to conventional thermal methods makes HPP a relatively energy efficient and suitable preservation treatment for the fish industry.

Keywords: Mackerel fillets; High Pressure Processing; microbial inactivation; colour; texture; nutritional quality

1. Introduction

There is a growing demand among consumers for healthier and safer products with a longer shelf-life. Pelagic fish species, such as mackerel (*Scomber* spp.) with a high nutritional value due to their high omega-3 polyunsaturated fatty acids (PUFAs) levels, constitute a valuable food resource in a healthy diet and are often perceived by consumers as beneficial for human health (Ruxton, Calder, Reed, & Simpson, 2005). Raw fish is highly perishable due to its pH close to neutral, high water activity (a_w), as well as high amounts of unsaturated fatty acids and free amino acids. In addition, a high content of autolytic enzymes makes it an ideal substrate for microbial and oxidative degradation (Lougovois & Kyra, 2005), with subsequent detrimental effects on sensory quality and shelf-life. The microbiota present on fish and shellfish depends on different factors, such as the environment, the species of fish, their eating habits and the mode of capture, and the conditions during storage which will determine which bacteria are responsible for spoilage (Chen, 1995). According to Svanevik and Lunestad (2011), the bacterial profile of Atlantic mackerel is dominated by Gram-negative bacteria i.e. *Psychrobacter* sp., *P. immobilis*, *P. marincola*, *P. cibarius*, *P. faecalis*, *Proteus* sp., *P. vulgaris*, *Photobacterium* sp., *P. phosphoreum*, *Vibrio* sp., *V. kanaloae*, *V. splendidus*, *V. pomeroyi*, *Shewanella* sp., *S. putrefaciens*, *Oceanisphaera* sp., *Flavobacteriaceae*, *Bizonia* sp., *B. paragorgiae*, *Pseudoalteromonas* sp., *P. tetradonis*, *Synechococcus* sp. and γ -proteobacteria. Among them, *P. phosphoreum*, *S. putrefaciens* or *Proteus* sp. are H_2S producing Specific Spoilage Bacteria (SSB) which give rise to off-flavours

associated with spoilage and can be used to predict remaining product shelf-life because their numbers correlate closely with sensory rejection (Gram & Dalgaard, 2002). Due to the fact that fish is devoid of carbohydrates (<0.5%) and only a small amount of lactic acid is produced post-mortem, the high final pH (usually >6) allows low pH sensitive spoilage bacteria such as *S. putrefaciens* to grow (Gram & Huss, 1996).

Although conventional food processing methods may be very efficient in generating safe products with an acceptable shelf-life, some changes in nutritional, chemical/biochemical and sensorial properties of food that compromise their acceptance by consumers may occur (Barba, Koubaa, do Prado-Silva, Orlie, & de Souza Sant'Ana, 2017). To address these disadvantages, non-thermal or mild processing technologies (<40°C) constitute useful alternatives to thermal inactivation methods for the food industry in order to preserve thermolabile nutrients, sensory attributes and functional properties of foods, while ensuring their safety and extending their shelf-lives. Reductions in processing times, with subsequent saving in energy consumption make non-thermal technologies beneficial for the industry (Misra et al., 2017). High pressure processing (HPP), applied at refrigeration, ambient or moderate heating offers many advantages to food processors compared to traditional thermal methods. It allows inactivation of pathogenic and spoilage bacteria with fewer changes in colour, texture and flavour (Barba, Esteve, & Frigola, 2012; Knorr, 1993). The principles, historical developments and HPP market in the 21st century have been reviewed by Misra et al. (2017). Basically, the energy consumption is lower (Farr, 1990), the process is not dependent on the shape or size of the food (Knorr, 1999) unlike thermal treatment and as the food is already in its final packaging when treated, post-processing contamination after pressurization is prevented.

The rationale for the use of HPP for fish and fish products is based on its ability to inactivate pathogenic and spoilage microorganisms and autolytic enzymes, resulting in an increased shelf-life (Alves de Oliveira, Neto, Rodrigues dos Santos, Rocha Ferreira, & Rosenthal, 2017) and also, an increased yield of the shucking process of bivalves and crustaceans (Patterson, 2014). However, HPP has been reported to increase discolouration, cooked appearance or lipid oxidation which could limit its use for raw fish products (Truong, Buckow, Stathopoulos, & Nguyen, 2015). Hence, optimisation of treatment conditions such as pressurization intensities, temperature and holding time is required for each specific food matrix. According to Patterson (2014), treatment temperature can affect the microbial inactivation achieved. Due to adiabatic heating during compression of HPP treatments, shifts in temperature that occur seem to be important in terms of microbial inactivation. There is an increase in temperature during processing, which in water-based systems equates to approximately 2 °C per 100 MPa, with the heat quickly dissipated once the pressure is released. The main effects of temperature on microbial inactivation seem to be associated with an increase of permeability and fluidity of microbial membranes which decreases their physical stability (Jayaram, Castle, & Margaritis, 1992).

The aim of this study was to investigate the effect of high pressure treatments of 100, 300 or 500 MPa for 2 and 5 min on mackerel fillets and to evaluate the changes in total viable counts (TVC), H₂S-producing bacteria, colour, texture, lipid oxidation, fatty acid profile and nutritional quality indices of the product.

2. Materials and methods

2.1. Sample origin

Mackerel (*Scomber* spp.) caught in the North Sea off Scotland (East Atlantic Ocean-FAO n° 27) in winter (October-November) were purchased beheaded, eviscerated and opened in butterfly fillets, on three different occasions (three different batches) in a local supermarket in Dublin (Ireland). Prior to and after treatment, samples were kept on ice at 0.5 ± 0.5 °C.

2.2. High pressure processing (HPP)

Butterfly mackerel fillets were 21.3 ± 0.6 cm in length and they weighed 174.8 ± 11.2 g. They were aseptically cut into halves giving rise to fillets which weighed 87.1 ± 8.1 g. They were individually vacuum packaged and pressurized in a high pressure machine (Stansted Fluid Power Ltd., Essex, UK) with 600 MPa as maximum working pressure; a mixture of oil-water (10:90) was used as the pressure transmitting fluid. The HPP unit was cooled to 10°C-12°C before treatments with a cooler unit. Treatments at 100, 300 or 500 MPa for 2 and 5 min were applied. Come up times were approximately 43 s, 84 s and 130 s for 100, 300 and 500 MPa, respectively and the pressure release was 17 s, 22 s and 25 s, respectively. The sample temperature at the starting of compression was 0.5 ± 0.5 °C. The temperature rise in the pressurization fluid during compression was around 2°C per 100 MPa. The temperature of the sample and the medium during the isobaric holding period was around 13.4°C, 17°C, 22.3°C at 100 MPa, 300 MPa and 500 MPa for 2 min, respectively, and 14.5°C, 18°C and 21°C were at 100 MPa, 300 MPa and 500 MPa for 5 min, respectively. The duration of treatment did not include the come up time. Samples were prepared in duplicate and kept on ice prior to and after pressurization treatments. Three independent trials were performed on three different days.

2.3. Microbiological analysis

Samples (10 g) were homogenized in 90 mL of Maximum Recovery Diluent (MRD, Oxoid Ltd., Basingstoke, England) using a IUL Stomacher for 90 s. Decimal dilutions of the homogenate were prepared in MRD. Total Viable Counts (TVC) were determined on duplicate plates of Plate Count Agar (PCA) (Oxoid Ltd., Basingstoke, England) and incubated at 30°C for 48 h. H₂S-producing bacteria, as black colonies, were determined on duplicate plates of Iron Agar supplemented with 0.8% L-cysteine and incubated at 25°C for 72 h. The detection limit was 10 cfu/g.

2.4. Colour and Texture profile analyses (TPA)

L^* (lightness, intensity of white colour), a^* (+a, red; -a, green) and b^* (+b, yellow; -b, blue) values were measured with a HunterLab UltraScan Pro (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were made with the D65 standard illuminant and ten-degree observer angle. Three independent measurements were taken in separate locations in mackerel fillets and the average of these three measurements per sample was expressed as the final value. From these values, the total colour change (ΔE^*) was calculated according to equation (Eq. (1)):

$$(1) \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

Where L_0^* , a_0^* , and b_0^* are the lightness, redness, and yellowness values of the control (non-pressurized) samples.

The texture profile of samples cut into cylinders (13 mm diameter, 18 mm height) was determined at room temperature using an Instron 5543 equipped with a load

cell of 500 N and a 35 mm diameter cylindrical aluminium probe (P/35). Five cylinders were taken from each fillet and compressed to 60% of the original height at a 60 mm/min compression speed to estimate hardness (N), chewiness (N mm), cohesiveness (dimensionless) and springiness (mm), giving five measurements from each fillet.

2.5. Lipid oxidation

Secondary lipid oxidation products were estimated as TBARS (Thiobarbituric acid reactive substances) as described by Botsoglou et al. (1994). Calibration was performed using a standard curve of 1,1,3,3-tetraethoxypropane (TEP) (Fisher Scientific Ireland, Ltd.). Results were calculated from four independent samples and expressed as μmol malondialdehyde (MDA)/g sample.

2.6. Fatty acid profile

2.6.1. Microwave-assisted preparation of fatty acid methyl esters (FAMES)

Microwave assisted FAME preparation was carried out using a MARS 6 Express 40 position Microwave Reaction System (CEM Corporation, Matthews, NC, USA) according to Brunton, Mason, & Collins (2015).

2.6.2. Gas chromatography-flame ionisation detector (GC-FID) analysis

Gas chromatography was carried out using a Clarus 580 Gas Chromatograph fitted with a flame ionisation detector. A CP-Sil 88 capillary column (Agilent, Santa

Clara, California, USA) with a length of 100 m x 0.25 mm ID and 0.2 µm film was used for the separation. The injection volume was 0.5 µL, at a temperature of 250 °C. The oven was set to 80 °C with an initial temperature ramp of 6.2 °C/min to 220 °C which was held for 3.2 min. A second temperature ramp of 6.3 °C/min to 240 °C followed and was held for 6.5 min (runtime 35 min). The carrier gas was hydrogen at a constant flow of 1.25 mL/min, and the split ratio was set at 10:1. The FID was set at 270 °C. Compounds were identified by comparing their retention times with those of authentic standards FAMES from the Supelco 37 FAME mix. The content of each fatty acid was calculated using following equation (Eq. (2)):

$$(2) \text{ FA content} = \frac{\text{Peak Area(FAME)}}{\text{Peak Area(ISTD)}} \times \frac{\text{Weight(ISTD)}}{\text{Weight(Sample)}} \times \text{ISTD purity} \times 10 \times 0.96$$

Where, FA content is the amount of a given fatty acid in the sample (mg/g), 10 is the dilution factor and 0.96 is the conversion factor for the internal standard.

2.7. Nutritional quality indices

Nutritional quality indices of mackerel samples were analyzed from fatty acids composition data. The indices of thrombogenicity (TI) and atherogenicity (AI) were calculated as proposed by Ulbricht and Southgate (1991). TI and AI indices were calculated using Eqs. (3) and (4), respectively. Other nutritional quality indices namely PUFA/SFA and Saturation Index (SI) (Eq. 5) were also determined.

$$(3) \quad TI = \frac{[C14:0+C16:0+C18:0]}{\left[0.5 \times (\sum MUFA + \sum n6) + 3 \times \sum n3 + \frac{\sum n3}{\sum n6}\right]}$$

$$(4) \quad AI = \frac{[C12:0+4 \times (C14:0)+C16:0]}{[\sum MUFA + \sum PUFA]}$$

$$(5) \quad SI = \frac{[C14:0+C16:0+C18:0]}{[\sum MUFA + \sum PUFA]}$$

2.8. Statistical analyses

Data were subjected to an analysis of variance (ANOVA) using SAS proc ANOVA procedure (SAS Version 9.1.3, statistical Analysis Systems) with treatment as the main effect. Tukey's multiple comparison was used to compare treatment means with a confidence interval of 95%.

3. Results and discussion

3.1. Effect of HPP on microbial counts

Initial mean total viable counts (TVC) and H₂S-producing bacteria counts are shown in Table 1. The initial TVC in the non-treated mackerel fillets were 4.17 logcfu/g, in agreement with Albertos et al. (2017), who reported counts of 4.1 log cfu/g in initial total aerobic mesophilic bacteria in Atlantic mackerel. Neither treatment at 100

MPa or 300 MPa for 2 min nor 100 MPa for 5 min significantly affected TVC levels immediately after HPP. However, the treatment at 300 MPa for 5 min significantly ($P<0.05$) reduced the TVC by 0.80 log cfu/g to 3.37 log cfu/g. In addition, HPP at 500 MPa for 2 or 5 min reduced significantly ($P<0.05$) TVC levels and reductions of 1.66 and 2.48 log cfu/g, respectively were achieved. This is in contrast to the results reported by Rode and Hovda (2016), who found undetectable levels of aerobic bacteria in mackerel and cod treated at 500 MPa for 2 min at 8-9 °C, immediately after treatment. Karim et al. (2011) reported a reduction of initial aerobic counts in herring treated at 200, 250 or 300 MPa for 1 or 3 min and significantly ($P<0.001$) lower levels were observed during the first 6 days on ice at 2 °C, as compared to the controls. According to these authors, the initial level of psychrotrophic microorganisms ($P<0.001$) was also reduced resulting in the highest decrease at the higher pressures and longer holding times. As the predominant microbiota at the early stages of conservation of mackerel fillets has been reported as mesophilic (Otero, Pérez-Mateos, Holgado, Márquez-Ruiz, & López-Caballero, 2019), these were focused on in this study. Nonetheless may be considered in future studies.

The mean initial population of H₂S-producing bacteria was 2.53 log cfu/g. The lowest pressurization level, 100 MPa applied for 2 or 5 min, did not significantly affect ($P<0.05$) H₂S-producing bacteria counts. However, HPP at 300 MPa or 500 MPa for 2 or 5 min reduced H₂S-producing bacteria counts to undetectable levels (<1 log cfu/g). Similarly, according to Rode and Hovda (2016), H₂S-producing bacteria levels were not detected on mackerel, cod or salmon treated at 500 MPa for 2 min at 8-9 °C and Cruz-Romero, Kerry and Kelly (2008) found H₂S-producing bacteria levels below the detection limit in oysters treated at 400 or 600 MPa for 5 min at 20 °C, immediately after pressurization.

The interaction of pressure level, process temperature and holding time affect the response of microorganisms to HPP, with all of them key factors for microbial inactivation. Factors such as food composition, bacterial growth phase and bacterial strain (variation between different strains of the same species has been noted) can also have an influence on the microbial inactivation achieved (Patterson, 2014), with Gram-negative bacteria reported, in general, to be relatively pressure sensitive due to the complexity of cell membranes (Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991; Styles, Hoover & Farkas, 1991). In this regard, it is important to highlight that the microbiota of mackerel (*Scomber scombrus*) is dominated by Gram-negative bacteria, such as *Vibrio* sp., *Shewanella* sp., *Proteus* sp. or *Photobacterium* sp., as well as by members of Gram-positive bacteria, such as *Bacillus* sp., *Staphylococcus* sp. or *Clostridia* spp. (Svanevik & Lunestad, 2011) which would influence the impact of the combination of pressure, temperature and holding time applied.

3.2. Effect of HPP on colour and texture properties

The effect of pressurization treatments on colour attributes (L^* , a^* and b^*) is shown in Table 2. Lightness (L^*) increased significantly ($P<0.05$) in mackerel fillets treated at 300 MPa or 500 MPa for 2 or 5 min causing a brighter and less translucent appearance of the muscle, while redness (a^*) values decreased significantly ($P<0.05$) under the same treatment conditions. These results are in accordance with Christensen, Hovda and Rode (2017), who detected an increase of L^* and a decrease of a^* in mackerel treated at 500 MPa for 2 min. However, in contrast to the results obtained in the present work, the magnitude of the increase in L^* values shown by those authors was found to be lower when non-pressurized samples were compared to mackerel

treated at 500 MPa for 2 min. In general, a very light cooking appearance in fish muscle treated below 300 MPa and up to 30 min holding time has been reported. An increase in L^* values has been attributed to sarcoplasmic and myofibrillar denaturation but depends on pressure level, holding time and fish species (Matser, Stegeman, Kals, & Bartels, 2000). On the other hand, the decrease in redness values may be related to conversion of oxymyoglobin or deoxymyoglobin to metmyoglobin, as a result of pressure (Carlez, Veciana-Nogues, & Cheftel, 1995).

The total colour difference (ΔE^*) indicates the magnitude of colour difference between processed and unprocessed food or before/after storage (Barba et al., 2012). Those values are considered to be no perceptible (0.0-0.2), very small (0.2-0.5), small (0.5-1.5), distinct (1.5-3.0), very distinct (3.0-6.0), great (6.0-12.0) and very great (>12.0) (Drlange, 1994; Silva & Silva, 1999). According to this scale, colour differences detected in the present work between non-pressurized and mackerel fillets treated at 300 MPa for 2 min or 5 min (ΔE^* 15.19 or ΔE^* 14.56, respectively) or between non-pressurized samples and those treated at 500 MPa for 2 or 5 min (ΔE^* 20.16 or ΔE^* 19.49, respectively) would be very great and perceptible by consumers. When non-pressurized mackerel fillets were compared to those treated at 100 MPa for 2 or 5 min, colour differences would be very distinct (ΔE^* 3.79 or ΔE^* 5.85, respectively). Total colour differences higher than 6 and perceptible to the naked eye were detected by Otero et al. (2019) in Atlantic mackerel fillets treated at 50 MPa and stored at 5°C for 12 days. According to them, those samples would be perceived by consumers as lighter and more yellowish than controls before storage.

TPA values for treated and untreated mackerel fillets are illustrated in Table 3. Pressurization treatments at 100 or 300 MPa for 2 or 5 min did not significantly ($P>0.05$) affect hardness and chewiness of mackerel. However, a significant ($P<0.05$)

increase in both parameters was detected at 500 MPa for 2 or 5 min. Pressurization treatments did not significantly ($P>0.05$) affect cohesiveness. Springiness increased significantly ($P<0.05$) in samples treated at 300 or 500 MPa for 2 or 5 min, whereas it was not significantly affected ($P>0.05$) by 100 MPa for 2 or 5 min. The results presented here are in accordance with Christensen et al. (2017), who reported an increase in hardness with increasing pressure, but that increase was only significant ($P<0.05$) in cod treated at 200 or 500 MPa for 2 min but not in mackerel or salmon, immediately after pressurization. According to Aubourg, Torres, Saraiva, Guerra-Rodriguez and Vázquez (2013), hardness of Atlantic mackerel was highly affected by pressure level and pressure holding time, resulting in the highest values of hardness at 450 MPa for 5 min. These authors observed that chewiness increased with HPP when high levels of pressure and long pressure holding times were applied. According to them, cohesiveness of mackerel was highly affected by pressure level and also pressure holding time. However, they also reported that springiness was affected mainly by frozen storage and less by pressure level and pressure holding time.

The effect of HPP on the texture of fish has been reviewed by Alves de Oliveira et al. (2017) who report that changes in texture following HPP may be related to protein denaturation and aggregation, changes in actin-myosin interaction, α -actinin release (Chevalier, Le Bail, & Ghoul, 2001; Guyon, Meynier, & Lamballerie, 2016; Yagiz, Kristinsson, Balaban, & Marshall, 2007) and tissue compression (Jantakoson, Kijroongrojana, & Benjakul, 2012) that may be due to reduction of the sarcomere length, and a possible softening effect related to fragmentation of myofibrils structures can also be observed after HP processing (Ashie & Simpson, 1996). The effect of HPP on springiness, gumminess, chewiness, resilience, fracturability, and adhesiveness using TPA has been reported by several authors (Briones-Labarca, Perez-Won, Zamarca,

Aguilera-Radic, & Tabilo-Munizaga, 2012; Chéret, Chapleau, Delbarre-Ladrat, & Verrez-Bagnis, 2005; Perez-Won, Tabilo-Munizaga, & Barbosa-Canovas, 2005; Yagiz et al., 2007, 2009; Yi et al., 2013). However, to date, no consistent data to support a clear effect of HPP have been reported. Differences in process parameters, fish species and methodology used in these studies are probably the main cause. Nevertheless, an increase in hardness has been reported in a wide range of fish and seafood such as tuna treated by HPP (Ramirez-Suarez & Morrissey, 2006), salmon, cod and trout (Schubring, Meyer, Schlüter, Boguslawski, & Knorr, 2003) or white prawn (Bindu, Ginson, Kamalakanth, Asha, & Srinivasa Gopal, 2013). However, there seem not to be a clear trend about the effect of HPP on cohesiveness of fish and seafood products. A decrease in this textural property, as well as in adhesiveness and gumminess, reported in pressurized cod samples was suggested to be due to the loss of myosin structure (Angsupanich & Ledward, 1998). However, an increase in cohesiveness has been reported in salmon treated at 150 MPa for 15 min at room temperature (Yagiz, et al., 2009) and in Atlantic mackerel (*Scomber scombrus*) treated by HPP as pre-treatment before freezing and frozen storage (Aubourg et al., 2013). However, similar to the present study, no significant effect on cohesiveness was found in barramundi (*Lates calcarifer*) muscle after pressurisation (Truong, Buckow, Nguyen & Stathopoulos, 2016), or in hake subjected to HPP as pre-treatment and subsequent frozen storage (Pita-Calvo, Guerra-Rodríguez, Saraiva, Aubourg, & Vázquez, 2018).

3.3. Effect of HPP on lipid oxidation

No significant differences ($P>0.05$) in TBARS values were observed in HPP treated mackerel as compared to non-treated samples (Figure 1) immediately after

processing. These results are in accordance with those obtained by Erkan et al. (2011), who observed no changes in TBARS values between HPP-treated mackerel at 220, 250 or 330 MPa for 5 or 10 min, respectively at 7°C and non-pressurized samples. However, significant differences ($P<0.05$) in TBARS values were detected by Yagiz et al. (2009) in salmon pressurized at 150 MPa for 15 min as compared to non-treated samples, but no significant differences ($P>0.05$) were reported at 300 MPa, immediately after pressurization. A significant ($P<0.05$) increase in TBARS values in pressurized carp fillets compared to untreated samples has been reported by Sequeira-Muñoz, Chevalier, Le Bail, Ramaswamy, and Simpson (2006), immediately after pressurization treatments, except at 100 MPa for 15 min. Chevalier, Le Bail, and Ghoul (2001) detected a significant ($P<0.05$) increase in TBARS values in turbot pressurized at 100, 140, 180 or 200 MPa for 15 or 30 min compared to non-pressurized samples. Fish species, type of muscle, pressure level and holding time seem to exert a big influence on the effect of high pressure on lipid stability (Medina-Meza et al., 2014; Truong et al., 2015).

In the case of foods of marine origin, the high levels of PUFAs promote the initiation of radical mechanisms which accelerate oxidation in subsequent storage periods (Medina-Meza et al., 2014), affecting the quality of seafood products. For this reason, to evaluate lipid oxidation of non-pressurized and pressurized mackerel during chilled storage merits investigation.

3.4. Effect of HPP on Fatty acid profiling

Thirty seven fatty acids were identified and quantified in non-pressurized mackerel fillets and mackerel fillets treated at 100, 300 or 500 MPa for 2 or 5 min. Non-pressurized mackerel samples showed a high level of PUFAs (35.34%), followed by

MUFAs (monounsaturated fatty acids) (33.82%), SFAs (saturated fatty acid) (30.84%) and HUFAs (highly unsaturated fatty acids) (26.90%) (Table 4). The high proportion of polyunsaturated fatty acids present in mackerel, makes it a highly recommendable food from a nutritional point of view. Among PUFAs, eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) were the most abundant fatty acids quantified in mackerel samples. Reported health benefits of the n-3 PUFAs EPA and DHA include reduction of the risk of cardiovascular disease, hypertension, or general inflammation (Calder & Yaqoob, 2009). The quantity of fat and the fatty acid profile of fish depend on factors such as season and water temperature. The increasing degree of unsaturation of fish lipids with cold temperatures is a known strategy used by aquatic organisms to adapt the fluidity and permeability of their cell membranes to the temperature fluctuations of water (Henderson & Tocher, 1987). No significant degradation on fatty acid profile could be expected in the present work because the processing temperature was $<25^{\circ}\text{C}$ for all the treatments. Further, no effect of HPP on fatty acid profile has been reported at room temperature in seafood products, such as salmon or oysters (Cruz-Romero et al., 2008, Yagiz et al., 2009).

The effect of HPP on the proportion (% FA) of SFAs, MUFAs, PUFAs, HUFAs, EPA, DHA and CLAs (conjugated linoleic acids) in non-pressurized mackerel fillets and in those pressurized at 100, 300 and 500 MPa for 2 or 5 min is shown in Table 4. In the present work, a significant decrease ($P<0.05$) in PUFAs (%) and HUFAs (%) was detected with all pressurization treatments applied, with the lowest proportions (29.53 and 22.43 %, respectively) being detected at 500 MPa for 5 min. However, non-significant differences ($P>0.05$) in PUFAs (%) and HUFAs (%) were observed between non-pressurized samples and those treated at 100 MPa for 2 min.

An increase ($P<0.05$) of MUFAs (%) was observed at 300 or 500 MPa for 2 min and also, at 500 MPa for 5 min. The proportion of SFAs (%) increased significantly ($P<0.05$) when pressure was held for 5 min, except at 500 MPa where no significant differences ($P>0.05$) among non-pressurized and HPP treated mackerel for 5 min were detected. Non-significant differences ($P>0.05$) in the fatty acid profile have been reported in salmon treated at 150 MPa or 300 MPa for 15 min at 20 °C compared to non-pressurized samples (Yagiz et al., 2009), in turbot treated at 100, 140, 180 or 200 MPa for 15 or 30 min at 4 °C (Chevalier et al., 2001) or in oyster treated at 260 MPa for 3 min, 500 MPa for 5 min or 800 MPa for 5 min at 20 °C (Cruz-Romero et al., 2008). Changes in fatty acid profile of lipid extracted from shrimp cephalothorax treated by HPP have been detected by Gomez-Estaca, Montero, Fernandez-Martin, Calvo, and Gomez-Guillen (2016). Similar to this study, these authors observed an increase of SFAs at 200, 400 or 600 MPa for 15 min or at 200 or 600 MPa for three consecutive cycles of 5 min each without waiting time between cycles. The highest amounts of MUFAs were detected at 200 or 400 MPa for 15 min or at 200 MPa for three consecutive cycles of 5 min each. These authors observed that the amount of PUFAs increased with the least intense treatments whereas decreased at 600 MPa for 15 min or for three consecutive cycles of 5 min each. The reported variations in seafood used for HPP treatments, temperatures at which the treatments were performed, as well as the large variability in pressures and duration, make it difficult to compare results and draw clear conclusions as to which processing conditions are the main factors that will influence the effect of HPP on fatty acid profiles.

As can be seen in Table 4, a high proportion of EPA and DHA was detected in non-pressurized mackerel samples (9.81 and 16.52%, respectively). In the present work, a significant decrease ($P<0.05$) in the proportion of CLAs was detected at 500 MPa for

2 or 5 min and also, at 300 MPa for 2 min. However, a significant increase ($P<0.05$) was observed in samples treated at 100 MPa or 300 MPa for 5 min. On the other hand, EPA decreased significantly ($P<0.05$) with pressurization treatments while significant decreases ($P<0.05$) in DHA were only detected at the highest pressurization levels, at 500 MPa for 2 or 5 min and also at 300 MPa for 2 min. Yagiz et al. (2009) reported no significant differences ($P>0.05$) in pressurized salmon at 150 or 300 MPa for 15 min compared to non-treated samples.

The effect of HPP on fatty acid profile observed in the present work could be due to changes in lipid membranes caused by the possible disruption of the cell membranes at the highest pressure applied on the mackerel fillets.

3.5. Effect of HPP on nutritional quality indices

Nutritional quality indices (PUFA/SFA, SI, AI and TI) are illustrated in Table 5. To the author's knowledge, this is the first time that these indices have been calculated for HPP treated mackerel. According to Ulbricht and Southgate (1991), AI and TI are a measure of influence of diet on coronary heart disease. AI relates the risk of atherosclerosis and is based on fatty acids which can increase (C12:0, C14:0 and C16:0) or decrease (Σ MUFA, Σ PUFA) the level of blood cholesterol. C12:0, C14:0 and C16:0 are considered pro-atherogenic and MUFAs and PUFAs, anti-atherogenic (Ulbricht & Southgate, 1991). TI values relate to the tendency to form clots in the blood vessels, defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, n-6 PUFAs and n-3 PUFAs) (Ulbricht & Southgate, 1991). In the present study, AI was affected significantly ($P<0.05$) when pressurization treatments were held for 5 min, showing an increase of this index at 100 MPa or 300

MPa and a decrease at 500 MPa. The AI values varied from 0.58 to 0.90 in pressurized mackerel, and non-pressurized samples had an AI value of 0.71. The TI values significantly increased ($P < 0.05$) at the highest pressurization intensities, at 300 and 500 MPa for 2 or 5 min. However, non-significant differences ($P > 0.05$) in this nutritional quality index were observed between non-pressurized samples and those treated at 100 MPa for 2 or 5 min. The TI values ranged from 0.25 to 0.29 in mackerel samples treated by HPP and was 0.25 in non-pressurized samples. Very low values for AI and TI are recommended, indicating positive health benefits derived from the product (Ulbricht & Southgate, 1991). According to these authors, AI or TI values would be the highest for the most atherogenic or thrombogenic dietary components, respectively. In this regard, coconut oil was reported to be a highly atherogenic food with an AI value of 13.63. However, raw mackerel, olive and sunflower oil were reported to be low atherogenic foods whose AI values were 0.28, 0.14 and 0.07, respectively. Raw mackerel, as well, was reported to be highly antithrombogenic with a TI value of 0.16, followed by olive and sunflower oil (0.32 and 0.28, respectively). The range of the expected values for AI and TI indices has been reported to be up to 1 and 0.5, respectively (Fernandes et al., 2014). These authors indicated AI values of 0.60 and 0.48 for sardines and mackerel, respectively, and TI values of 0.20 and 0.24 for those fish. According to those authors, AI and TI values detected in the present work would be within the expected range both in non-pressurized and pressurized mackerel fillets. The PUFA/SFA ratio is a good indicator of the nutritional value of dietary fat. According to nutritional recommendations (British Department of Health, 1994), the PUFA/SFA ratio in the human diet should be above 0.45. Lower PUFA/SFA ratios that indicate higher levels of dietary saturated fatty acids have been considered as major risk factors for cardiovascular disease (Dieter & Tuttle, 2017). In the present work, the PUFA/SFA

ratio decreased significantly ($P < 0.05$) in mackerel samples treated by HPP, except at 100 MPa for 2 min, with no significant differences compared to non-pressurized samples. However, the ratio varied from 0.99 to 1.14 in pressurized mackerel and was 1.15 in non-treated samples. According to the nutritional recommendations (British Department of Health, 1994), mackerel would be considered a healthy food because the ratio PUFA/SFA was above 0.45 and pressurization treatments did not decrease the ratio below the recommended value. SI indicates the relationship between the sum of saturated fatty acids (C14:0, C16:0 and C18:0) (pro-thrombogenic) and unsaturated fatty acids (anti-thrombogenic). It has been reported that myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) are associated with an increased risk of coronary heart disease (Zong et al., 2016). Although to author's knowledge, there is no numerical values assigned to SI, a food with lower values of these SFA compared to unsaturated fatty acids would be considered a healthier food. In the present work, SI values ranged from 0.41 to 0.46 in pressurized mackerel and 0.42 in non-pressurized samples. Non-significant ($P > 0.05$) differences were detected between pressurized and non-pressurized mackerel, except for treatments at 100 MPa or 300 MPa for 5 min which significantly increased ($P < 0.05$) SI values compared to non-pressurized samples. A significant increase ($P < 0.05$) in SFA proportion was also detected for the same treatments but among them, only myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) contributed to the calculation of the saturation index.

4. Conclusions

Our results show that HPP at 500 MPa for 2 min and 300 MPa or 500 MPa for 5 min were the most effective treatments to increase microbial inactivation in mackerel

without compromising the nutritional value of this pelagic fish. However, the present work only evaluated the effect of HPP in mackerel quality immediately after processing, showing some changes in texture and colour but no effect on lipid oxidation. It is possible that some of the quality indices measured here may be detrimentally affected during the shelf-life of the fillets. Therefore, a study on the effect of HPP on these indices in addition to texture, colour, lipid oxidation and microbiological quality during chilled storage would be warranted.

Acknowledgements

This work has received support from the EU Joint Programming Initiative (JPI) Health Diet for a Healthy Life (HDHL) ProHealth Project (15/HDHL/1 PROHEALTH).

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Table 1

Total viable and H₂S-producing bacteria counts (log cfu/g) in mackerel fillets pressurized at 100, 300 and 500 MPa for 2 or 5 min.

	TVC	H ₂ S-producing bacteria
Non-pressurized	4.17±0.99 ^{ab}	2.53±1.43 ^a
100 MPa/ 2 min	4.10±1.01 ^{ab}	1.98±1.58 ^a
300 MPa/ 2 min	3.64±0.73 ^{bc}	<1.00 ^b
500 MPa/ 2 min	2.51±0.75 ^d	<1.00 ^b
100 MPa/ 5 min	4.27±1.05 ^a	1.96±1.57 ^a
300 MPa/ 5 min	3.37±0.73 ^c	<1.00 ^b
500 MPa/ 5 min	1.69±0.65 ^e	<1.00 ^b

Means within the same column with different superscripts differ significantly at $P<0.05$.

Table 2

Colour parameters in mackerel fillets pressurized at 100, 300 and 500 MPa for 2 or 5 min.

	L^*	a^*	b^*	ΔE^*
Non-pressurized	53.60±1.91 ^b	5.84±3.22 ^a	12.86±1.84 ^a	–
100 MPa / 2 min	54.45±6.44 ^b	4.96±3.10 ^{ab}	13.02±2.80 ^a	3.79±1.30 ^c
300 MPa / 2 min	68.47±1.71 ^a	3.01±1.22 ^{bc}	14.47±1.53 ^a	15.19±1.91 ^b
500 MPa / 2 min	73.61±2.33 ^a	1.90±0.85 ^c	13.61±1.45 ^a	20.16±2.61 ^a
100 MPa / 5 min	55.83±5.42 ^b	5.00±2.29 ^{ab}	14.40±3.68 ^a	5.85±0.45 ^c
300 MPa / 5 min	68.49±2.45 ^a	2.38±0.93 ^c	14.42±1.15 ^a	14.56±1.60 ^b
500 MPa / 5 min	72.73±2.15 ^a	1.68±0.56 ^c	13.18±0.50 ^a	19.49±2.46 ^a

Means within the same column with different superscripts differ significantly at $P<0.05$.

Table 3

Texture properties of mackerel fillets pressurized at 100, 300 and 500 MPa for 2 or 5 min.

	Hardness (N)	Chewiness (N mm)	Cohesiveness (F2/F1) (ratio)	Springiness (mm)
Non-pressurized	7.55±3.48 ^b	46.77±24.46 ^c	1.98±0.45 ^a	4.27±0.25 ^d
100 MPa/ 2 min	6.88±2.24 ^b	42.96±12.13 ^c	2.24±0.45 ^a	4.25±0.33 ^d
300 MPa/ 2 min	6.67±2.21 ^b	46.22±22.50 ^c	2.07±0.36 ^a	4.84±0.92 ^c
500 MPa/ 2 min	17.25±4.57 ^a	154.18±39.60 ^a	2.18±0.46 ^a	7.04±0.96 ^a
100 MPa/ 5 min	7.48±1.52 ^b	50.33±20.20 ^c	2.11±0.77 ^a	4.64±0.27 ^{cd}
300 MPa/ 5 min	8.61±1.16 ^b	62.75±24.75 ^c	2.24±0.47 ^a	5.04±0.32 ^{bc}
500 MPa/ 5 min	14.43±4.51 ^a	115.08±39.50 ^b	2.30±0.30 ^a	5.42±0.48 ^b

Means within the same column with different superscripts differ significantly at $P<0.05$.

Table 4

Proportion (% FA) of SFAs, MUFAs, PUFAs, HUFAs, EPA, DHA and CLAs in mackerel fillets pressurized at 100, 300 and 500 MPa for 2 or 5 min.

	SFAs	MUFAs	PUFAs	HUFAs	EPA	DHA	CLAs
Non-pressurized	30.84±0.32 ^{cd}	33.82±0.35 ^c	35.34±0.40 ^a	26.90±0.42 ^a	9.81±0.13 ^a	16.52±0.32 ^a	4.43±0.04 ^b
100 MPa / 2 min	30.74±0.74 ^{cd}	34.23±0.34 ^c	35.03±0.55 ^a	26.75±0.57 ^a	9.37±0.08 ^b	16.78±0.52 ^a	4.32±0.06 ^{bc}
300 MPa / 2 min	31.02±0.49 ^c	37.27±0.23 ^b	31.72±0.33 ^{cd}	23.77±0.41 ^{cd}	8.67±0.13 ^c	14.51±0.34 ^b	4.20±0.10 ^{cd}
500 MPa / 2 min	31.10±0.15 ^{bc}	37.81±0.52 ^b	31.10±0.62 ^d	23.36±0.51 ^{de}	8.22±0.21 ^{de}	14.53±0.27 ^b	4.11±0.05 ^d
100 MPa / 5 min	32.03±0.39 ^{ab}	34.57±0.88 ^c	33.40±0.72 ^b	24.96±0.81 ^b	8.39±0.08 ^d	16.00±0.89 ^a	4.80±0.11 ^a
300 MPa / 5 min	32.64±0.41 ^a	34.54±0.39 ^c	32.82±0.24 ^{bc}	24.63±0.21 ^{bc}	8.03±0.03 ^e	15.99±0.20 ^a	4.71±0.02 ^a
500 MPa / 5 min	29.96±0.18 ^d	40.51±0.38 ^a	29.53±0.49 ^e	22.43±0.43 ^e	8.37±0.09 ^d	13.40±0.35 ^c	3.56±0.04 ^e

Means within the same column with different superscripts differ significantly at $P < 0.05$.

SFAs: Saturated fatty acids

MUFAs: Monounsaturated fatty acids

PUFAs: Polyunsaturated fatty acids

HUFAs: Highly unsaturated fatty acids

EPA: Eicosapentaenoic acid

DHA: Docosahexaenoic acid

CLAs: Conjugated linoleic acids

Table 5

Nutritional values of mackerel fillets treated at 100, 300 and 500 MPa for 2 or 5 min.

	PUFA/SFA	SI	AI	TI
Non-pressurized	1.15±0.02 ^a	0.42±0.01 ^{cd}	0.71±0.01 ^b	0.25±0.01 ^{bc}
100 MPa / 2 min	1.14±0.04 ^a	0.42±0.01 ^{cd}	0.69±0.03 ^b	0.25±0.01 ^c
300 MPa / 2 min	1.02±0.03 ^b	0.43±0.01 ^{bc}	0.69±0.02 ^b	0.28±0.01 ^a
500 MPa / 2 min	1.00±0.02 ^b	0.43±0.00 ^{bc}	0.68±0.01 ^b	0.28±0.00 ^a
100 MPa / 5 min	1.04±0.02 ^b	0.44±0.01 ^{ab}	0.86±0.02 ^a	0.27±0.01 ^{ab}
300 MPa / 5 min	1.01±0.02 ^b	0.46±0.01 ^a	0.90±0.02 ^a	0.29±0.01 ^a
500 MPa / 5 min	0.99±0.02 ^b	0.41±0.00 ^d	0.58±0.01 ^c	0.28±0.01 ^a

Means within the same column with different superscripts differ significantly at $P<0.05$.

PUFA/SFA: Polyunsaturated fatty acids/Saturated Fatty acids

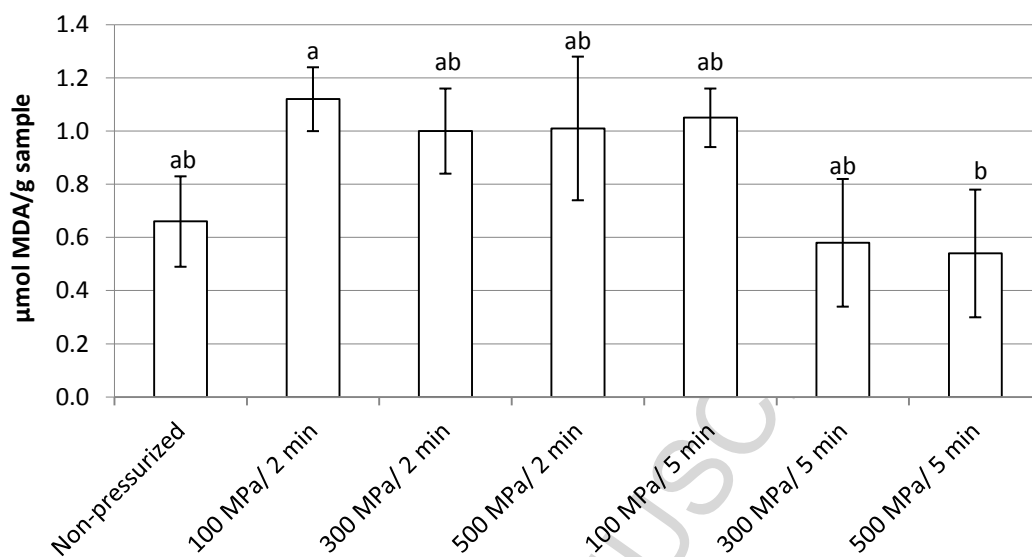
SI: Saturation index

AI: Index of atherogenicity

TI: Index of thrombogenicity

Figure 1

TBARS values ($\mu\text{mol MDA/g}$ sample) of mackerel fillets treated at 100, 300 and 500 MPa for 2 or 5 min.



Different superscripts differ significantly at $P < 0.05$.

Highlights

- High pressure processing did not compromise the nutritional value of mackerel.
- The most effective treatments were 300 MPa for 5 min, 500 MPa for 2 or 5 min.
- Some changes in colour and texture were detected in pressurized mackerel fillets.
- No significant effect on TBARS values were found in pressurized mackerel fillets.