Italian Journal of Food Science, 2023; 35 (3): 99–114

Quality and stability of different seafood products treated with high hydrostatic pressure (HPP) intended for raw consumption

Ana Cristina de Aguiar Saldanha Pinheiro[1], Silvia Tappi[1,2]*, Giacomo Braschi[1,2], Jessica Genovese[1], Francesca Patrignani[1,2], Pietro Rocculi[1,2]

[1]Department of Agricultural and Food Science, Alma Mater Studiorum, University of Bologna, Campus of Food Science, Piazza Goidanich 60, Cesena (FC), Italy; [2]Interdepartmental Centre for Agri-Food Industrial Research, Alma Mater Studiorum, University of Bologna, Campus of Food Science, Via Ravennate 933, Cesena (FC), Italy

***Corresponding Author**: Silvia Tappi, Department of Agricultural and Food Science, Alma Mater Studiorum, University of Bologna, Campus of Food Science, Piazza Goidanich 60, Cesena (FC), Italy Email: silvia.tappi2@unibo.it

Received: 15 November 2022; Accepted: 23 June 2023; Published: 23 August 2023

PAPER

Abstract

The consumption of raw fish has rapidly increased in recent years, but being a highly perishable product, it is characterised by a very short microbiological shelf life. High hydrostatic pressure (HPP) processing is a non-thermal technology has emerged recently as a promising alternative to thermal processing for food pasteurization capable of maintaining fresh-like characteristics and nutritional value. However, the induced changes in product quality should be assessed carefully. The present research aimed to investigate the effect of HPP on different seafood products, namely grey mullet, tiger prawn and rose shrimp, intended for raw consumption. Three pressure levels (400, 500 and 600 MPa) were applied for 10 min. During refrigerated storage, microbiological quality, chemical parameters, colour and texture and fat oxidation were analysed. Results showed that the application of lower pressure was able to inactivate *E. coli*, *pseudomonas* and/or positive coagulase *staphylococci*; however, they were able to recover during storage. In addition, the application of 600-MPa pressure extended the microbiological shelf life by up to 30 days. For all samples, general whitening occurred while the texture was affected in a different way for the three considered species. Fat oxidation was only minimally affected and remained quite low during storage.

Keywords: grey mullet, microbiological inactivation, rose shrimp, shelf life, tiger prawn

Introduction

The consumption of raw fish has rapidly increased in recent years, also in the areas where it was not a traditional habit, because of not only changes in food taste but also to the adoption of culinary traditions of other countries. Sushi and sashimi which are typically oriental specialities are becoming increasingly popular in European countries as well. Moreover, the use of low-temperature cooking and processing, such as cold smoking, is spreading fast (Brutti *et al.,* 2010).

These new habits have increased microbiological risks for fish product consumption. Moreover, seafood products are highly perishable, their microbiological shelf life is very short and, in order to sell a fish product to be consumed raw, a strategy to increase its shelf life could increase its marketability. Therefore, a non-thermal technology able to reduce microbial load is highly necessary.

High-pressure processing (HPP) is a non-thermal technology that has recently emerged as a promising alternative to thermal processing for food pasteurization

capable of maintaining fresh-like characteristics and nutritional value. Application of pressure higher than 300 MPa for a few minutes at room temperature has shown to significantly reduce the initial microbial load in many fish species (Truong *et al.,* 2015). The extent of microbial inactivation depends not only on treatment parameters, such as pressure level, holding time and temperature, but also on the characteristics of microflora in the product (Truong *et al.,* 2015). The inactivation of microorganisms by HPP is the result of a combination of factors, including changes in cell membranes, cell walls, proteins and enzyme-mediated cellular functions (Campus, 2010).

Cell membranes are the primary site of pressure-induced damage, with consequent alterations of cell permeability, transport systems, loss of osmotic responsiveness, organelle disruption and inability to maintain intracellular pH.

In general, Gram-negative bacteria and cells in the growth phase are more sensitive than Gram-positive bacteria and cells in the stationary phase. Nevertheless, investigations have shown that cell disruption is more dependent on the geometry of the bacteria, rather than Gram-type. For example, morphological changes in the rod-shaped *Escherichia coli* and *Pseudomonas aeruginosa* were observed whereas *Staphylococcus aureus* (cocci) was more resistant to pressure.

However, in complex matrices such as food, the desired effect on microbial inactivation may also produce physical and biochemical changes, which may affect the product properties negatively.

The denaturation of proteins in fish muscle could cause significant changes of important parameters for consumer acceptability. In particular, the application of high pressure is known to lead to a cooked appearance (Matser *et al.,* 2000), which could be specifically detrimental in products intended for raw consumption. Moreover, the effect of protein structure on enzymatic activity can lead to variations in the textural properties of seafood products not only after the treatment but also during refrigerated storage. The effect of HPP has been studied in a variety of fish and seafood matrices, but results are very variable depending on process parameters as well as specific product characteristics (Truong *et al.,* 2015).

In a product aimed for raw consumption, microbiological quality is of paramount importance throughout the shelf life; however, the effect of HPP on quality could lead to undesirable changes.

The aim of the present research was to investigate the effect of HPP treatment on different types of seafood products, that is, grey mullet, tiger prawn and rose shrimp, intended for raw consumption. Because the European Union (EU) legislation requires seafood products to be frozen for at least 24 h before raw consumption, HPP was applied to frozen-thawed products. Three pressure levels were applied, and microbiological quality, safety, chemical parameters, colour and texture were analysed during the refrigerated storage.

Materials and Methods

Preparation of fish samples

Grey mullet (*Mugil cephalus*) striped prawn (*Melicertus kerathurus*) and deep-water rose shrimp (*Parapenaeus longirostris*) were fished in the Adriatic Sea. Products were fast-frozen in an industrial blast chiller at a temperature of -18°C and kept for 24 h at Economia del Mare (Cesenatico, Italy). Thawing was carried out at 4°C for 16 h; then the seafood samples were subjected to mechanical deboning and shell removal. Flesh was manually cut into pieces and packaged in polyethylene terephthalate (PET) trays (VR11-6T-T1, Technopress Srl., FC, Italy) containing six mono-portions of 15–20 g approximately, each sealed under vacuum with a high barrier PET film (thickness 40 µm; oxygen transmission rate [OTR] 60 $cm^3/m^2/24$ h/bar; 4°C, 0% RH; Cryovac Sealed Air Corp., NJ, USA).

HPP treatment

The vacuum-packed samples were subjected to HPP treatment performed by HPP Italia s.r.l (Parma, Italy). The samples were placed in a 350-L chamber, filled with water and subjected to 400-, 500- or 600-MPa pressure for 10 min. An untreated sample was used as a control. The samples were coded using initial of the species (M for grey mullet, P for striped prawn and S for rose shrimp) and 0-, 400-, 500- and 600-MPa pressure was applied. For each treatment, 24 packages were prepared.

Storage

After treatment, the samples were stored in the laboratories of the Campus of Food Science of the University of Bologna at 2 ± 1°C. During storage, the samples were subjected to analytical determinations after 0, 1, 6, 9, 14, 21, 28 and 35 days. Storage duration was determined for each sample based on the results of microbiological analysis, considering the end of the shelf life when reaching a microbial load of 6 log cfu/g referred to total mesophilic bacteria.

Three different packages were used for each HPP treatment at each storage period.

Analytical determinations

Microbiological analyses

Microbiological analyses were performed on untreated grey mullet, striped prawn and red shrimp, and the samples were treated with 400-, 500- and 600-MPa pressure. Immediately after HPP treatments for the stated storage period, all the samples were investigated for the presence of *Salmonella* spp. and *Listeria monocytogenes* according to EN ISO 6579-1:2017/A1:2020 and ISO 11290-1:2017.

Microbial groups considered in this research were total mesophilic bacteria (TMB), *lactobacillus* spp., *pseudomonas* spp., sulphite-reducing anaerobic bacteria, total *Coliforms, E. coli*, and coagulase positive *staphylococci*. In all, 10 g of samples were serially diluted using physiological saline solution (0.9% NaCl), and appropriate inoculum was included or spread in different selective culture media, such as plate count agar (PCA; Oxoid-Thermofisher, Milan, Italy) for TMB; De Man, Rogosa and Sharpe agar (MRS; Oxoid-Thermofisher) supplemented with cycloheximide (0.2% p/v) for *lactobacillus* spp.; selective *pseudomonas* agar base (PAB; Oxoid-Thermofisher) for *pseudomonas* spp.; reinforced clostridial agar (RCA; Oxoid-Thermofisher) for sulphite-reducing anaerobic bacteria, violet red bile agar (VRBA; Oxoid-Thermofisher) supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG; Oxoid-Thermofisher) for total coliforms and *E. coli*, respectively; and Baird Parker agar (BP) for coagulase positive *staphylococci*. Plates were incubated for 24/48 h at 30°C for *pseudomonas* spp. (PA) and 37°C for lactobacilli, sulphite-reducing anaerobic bacteria, total coliform, *E. coli* and coagulase positive *staphylococci*. Sulphite-reducing anaerobic bacteria were incubated under anaerobic conditions using a gas generating kit (Oxoid-Thermofisher).

pH values

pH values of the samples homogenised for 60 s with an Ultraturrax (T-25, Ika, Germany) with distilled water (sample–water ratio: 1:2 w/w) were assessed with a pH meter (Crison, Barcellona). The analysis was performed at least in triplicate for each sample.

Colour

Colour parameters lightness (L*), redness (a*) and yellowness (b*) were measured with a spectrophotocolorimeter model ColorFlex™ (Hunterlab, Reston, VA, US). The tristimulus L*, a*, b* measurement mode (International Commission on Illumination [CIE], 1976) was used. The hue angle ($h°$) was calculated as follows:

$$h^{\circ} = \frac{\tan^{-1}\frac{b^*}{a^*}}{2\pi} \times 360$$

For each sample and storage time, an average of at least 15 measurements was calculated.

Texture

The texture was evaluated with a texture analyser model TA.HDi 500 (Stable Micro Systems, Godalming, UK) equipped with a 25-kg load cell. Briefly, 15 g of each sample was inserted in a cylindrical cup (diameter of 3 cm) and a piston was used to compress the sample at 1 mm/s up to 50% of its height. The pressure was held for 60 s. Maximum force was considered as hardness (force, F_1 [Newton, N]) and force at the end of the compression was considered as index of resistance to compression (force F_2 [N]).

The texture was evaluated with at least six replicates for each sample.

Peroxide value (PV)

Lipids were extracted from 25 g of sample with a method described by Bligh and Dyer (1959). The peroxide value was determined by the ferrothiocyanate method (Chapman and Mackay, 1949). Results were expressed as the amount of oxygen (O_2) per kilogram of fat/oil (mEq O_2/kg fat/oil). The analysis was performed with at least triplicates for each sample.

Statistical analysis

The significance of differences was tested by the analysis of variance (ANOVA) using Tukey's honestly significant difference (HSD) test as a *post-hoc* test ($p < 0.05$). Statistical analysis was conducted with Statistica 8.0 for Windows.

Results and Discussion

Microbial inactivation

The effects of HPP treatments (400, 500 and 600 MPa), compared to the untreated controls, on the microbiological quality of packaged grey mullet, striped prawn and rose shrimp are reported in Tables 1, 2 and 3, respectively. In all tested conditions, *Salmonella* spp. and *Listeria monocytogenes* were not detected during the shelf life of the considered products.

In addition, coagulase positive *staphylococci* were not found in untreated and HPP-treated samples of grey mullets and striped prawn. In general, the application of HPP treatments increased the microbiological shelf life of the considered products, and the inactivation effect became more severe with increased pressure. The microbiological threshold to define product shelf life was fixed

Table 1. Evolution of microbial cell loads (log CFU/g) of total mesophilic count (TMC), *lactobacillus* spp., *pseudomonas* spp., total coliforms, sulphite-reducing anaerobic bacteria (AB), and *E. coli* during the refrigerated storage of packaged grey mullet (*Mugil cephalus*) flesh with applied high hydrostatic pressure (HPP) treatments (400, 500 and 600 MPa).

		Mullet	Log CFU/g			
			M-0	M-400	M-500	M-600
Days of storage	0 d	TMC	4.67 ± 0.43	<1**	<1	<1
		Lactobacillus spp.	3.18 ± 0.33	<1	<1	<1
		Sulphite-reducing AB	4.20 ± 0.54	<1	<1	<1
		Pseudomonas spp.	4.45 ± 0.22	<1	<1	<1
		Total coliforms	2.56 ± 0.38	<1	<1	<1
		E. coli	1.12 ± 0.10	<1	<1	<1
	2 d	TMC	5.14 ± 0.44	<1	<1	<1
		Lactobacillus spp.	3.30 ± 0.29	<1	<1	<1
		Sulphite-educing AB	5.15 ± 0.51	<1	<1	<1
		Pseudomonas spp.	5.20 ± 0.45	<1	<1	<1
		Total coliforms	1.80 ± 0.12	<1	<1	<1
		E. coli	1.22 ± 0.10	<1	<1	<1
	6 d	TMC	6.22 ± 0.52	<1	<1	<1
		Lactobacillus spp.	4.00 ± 0.28	<1	<1	<1
		Sulphite-reducing AB	5.54 ± 0.46	<1	<1	<1
		Pseudomonas spp.	5.27 ± 0.35	<1	<1	<1
		Total coliforms	1.85 ± 0.16	<1	<1	<1
		E. coli	1.50 ± 0.20	<1	<1	<1
	12 d	TMC	-*	5.67 ± 0.31	<1	<1
		Lactobacillus spp.	-	<1	<1	<1
		Sulphite-reducing AB	-	<1	<1	<1
		Pseudomonas spp.	-	2.46 ± 0.29	<1	<1
		Total coliforms	-	<1	<1	<1
		E. coli		<1	<1	<1
	19 d	TMC	-	7.52^{a} ± 0.43	4.22^{b} ± 0.41	2.20^{c} ± 0.23
		Lactobacillus spp.	-	1.06 ± 0.32	<1	<1
		Sulphite-reducing AB	-	<1	<1	<1
		Pseudomonas spp.	-	5.11 ± 0.29	<1	<1
		Total coliforms	-	<1	<1	<1
		E. coli		<1	<1	<1
	32 d	TMC	-	-	5.55^{a} ± 0.42	3.10^{b} ± 0.33
		Lactobacillus spp.	-	-	<1	<1
		Sulphite-reducing AB	-	-	<1	<1
		Pseudomonas spp.	-	-	<1	<1
		Total coliforms	-	-	<1	<1
		E. coli		-	<1	<1

*Not analysed because the microbiological threshold fixed at 6 log CFU/g was reached in the previous time of analysis.
**Under detection limit.
In the same row, values with different superscript letters are significantly different ($p < 0.05$).

at the attaining of 6 log CFU/g for total mesophilic count (TMC) even if, according to Regulation 2073/2005, other important microbiological criteria, such as cell load of *Escherichia coli* and positive coagulase *staphylococci*, were considered in the data discussion.

More specifically, the detected microbiological data for grey mullets, untreated and in relation to the pressure applied and storage period are reported in Table 1. As shown, the control sample was spoiled within 6 days of storage at 2°C, reaching a cell load of total mesophilic bacteria of 6.22 log CFU/g with a corresponding level of total coliforms of 1.8 log CFU/g. In contrast, application of 400, 500 and 600 MPa pressure prolonged product shelf life to 12, 19 and 32 days, respectively. The application of HPP treatments ranging between 400 MPa and 600 MPa decreased the cell loads of *E. coli,* compared to the untreated sample, under the detection limit of 1 log CFU/g.

Pseudomonas spp. was strongly affected by the level of applied HPP since cell load of 2.4 CFU/g were detected at 400-MPa pressure after 12 days of treatment to further increase on the 19th day of storage. In all the other treated samples, and for each time of sampling considered, *Pseudomonas* spp. was always under the detection limit (<1 Log CFU/g).

Regarding untreated striped prawn (Table 2), the total mesophilic bacteria reached the threshold level of 6.0 log CFU/g after 6 days of refrigerated storage, while the application of HPP pressure of 500 MPa and 600 MPa determined a significant increase in the product's shelf life to 19 days and 32 days, respectively, and the cell load of *E. coli* was always under the detection limit. However, for the sample treated at 500-MPa pressure, after 19 days, *lactobacilli* and *pseudomonas* spp. were able to recover and reach the respective cell load of 4.49 log CFU/g and 5.72 log CFU/g, different from the samples treated at 600 MPa, where these microbial groups were found to be under the detection limit.

Applying a pressure level of 400 MPa resulted in a shelf life of about 12 days. Similar trends were observed in the shrimp products (Table 3), where the application of a pressure of 600 MPa allowed the shelf-life threshold to reach after 28 days. In contrast, the samples treated with 500-MPa pressure reached the spoilage threshold after 21 days. Interestingly, 400-MPa pressure was not able to inactivate completely the coagulase-positive *staphylococci*, which were able to recover but only after reaching the shelf-life threshold.

The rationale for the use of HPP for fish and fish products is based on its ability to inactivate pathogenic and spoilage microorganisms and microbial enzymes, resulting in an increased shelf life (de Alba *et al.,* 2019) as well as an increased yield of the shucking process of bivalves and crustaceans (Patterson, 2014). In general, according to the literature, HPP between 150 MPa and 450 MPa is commonly applied on fish samples (Perez-Won *et al.,* 2020) because higher pressure, aimed to increase microbial inactivation, is generally associated with significant changes in physicochemical, texture and sensory properties of products, such as increase in discoloration, cooked appearance or lipid oxidation (Truong *et al.,* 2015). However, the data resulting from this research evidenced that HPP treatment at 400 MPa is not able to increase significantly the shelf life of the considered products. Although this level of pressure seemed adequate to inactivate *E. coli* during the shelf life of the considered fish samples, other microbial groups, involved in spoilage and safety issue, such as *pseudomonas* or positive coagulase *staphylococci*, were able to retrieve during storage, highlighting the critical issue of viable but not culturable (VBNC) cells. Although the efficiency of microbial inactivation is influenced by various factors, such as food matrix characteristics and food processing parameters, the physiological diversity within a microbial population also has to be taken into consideration, especially in the validation of the effectiveness of a treatment on a specific food product (Patrignani *et al.,* 2019). Moreover, a further consideration is required in relation to the biogenic amines, potentially present in these types of products. In fact, although histamine was not detected, the application of 400–600-MPa HPP appears to reduce several microbial groups that are able to produce biogenic amines, such as lactic acid bacteria and coliforms, reducing the risk of production of these indicators of food safety and quality indices (Borges et *al*. 2023).

Pseudomonas spp., strict aerobic bacteria, whose growth decreased in vacuum, was able to recover in grey mullet and striped prawn samples treated at 400 MPa during their shelf life. *Pseudomonas* spp., also having a *psychrotrophic* behaviour and able to produce specific H_2S off-flavours, could have a negative impact to produce specific proteases, which could potentially affect the textural properties of food matrix.

pH value

The initial pH values measured in grey mullet, tiger prawn and rose shrimp samples according to HPP treatment and during storage are reported in Table 4. The initial values for the untreated samples were 6.4 ± 0.1, 7.2 ± 0.01, 7.6 ± 0.03 for grey mullet, tiger prawn and rose shrimp, respectively. While pH values for grey mullet were consistent with the literature (Tsogas *et al.,* 2019), for prawn and shrimp, these values were slightly higher, compared to literature (Bindu *et al.,* 2013; Kaur *et al.* 2013).

Table 2. Evolution of microbial cell loads (log CFU/g) of total mesophilic count (TMC), *lactobacillus* spp., *pseudomonas* spp., total coliforms, sulphite-reducing anaerobic bacteria (AB), and *E. coli* during the refrigerated storage of packaged striped prawn with applied high hydrostatic pressure (HPP) treatments (400, 500 and 600 MPa).

		Striped prawn	Log CFU/g			
			P-0	P-400	P-500	P-600
Days of storage	0 d	TMC	4.65^{a} ± 0.44	2.45^{b} ± 0.43	<1	<1
		Lactobacillus spp.	3.95 ± 0.29	<1**	<1	<1
		Sulphite-reducing AB	3.83 ± 0.39	<1	<1	<1
		Pseudomonas spp.	4.63 ± 0.56	<1	<1	<1
		Total coliforms	4.60^{a} ± 0.48	1.30^{b} ± 0.33	<1	<1
		E. coli	2.10 ± 0.33	<1	<1	<1
	2 d	TMC	4.50^{a} ± 0.46	2.96^{b} ± 0.43	<1	<1
		Lactobacillus spp.	3.50 ± 0.36	<1	<1	<1
		Sulphite-reducing AB	3.20 ± 0.51	<1	<1	<1
		Pseudomonas spp.	5.25 ± 0.48	<1	<1	<1
		Total coliforms	4.75 ± 0.49	<1	<1	<1
		E. coli	2.80 ± 0.15	<1	<1	<1
	6 d	TMC	6.30^{a} ± 0.52	2.88^{b} ± 0.55	<1	<1
		Lactobacillus spp.	5.82 ± 0.42	<1	<1	<1
		Sulphite reducing AB	4.42 ± 0.49	<1	<1	<1
		Pseudomonas spp.	5.35 ± 0.35	<1	<1	<1
		Total coliforms	3.75 ± 0.46	<1	<1	<1
		E. coli	2.95 ± 0.15	<1	<1	<1
	12 d	TMC	7.20^{a} ± 0.39	4.61^{b} ± 0.23	2.00^{c} ± 0.15	1.50^{c} ± 0.50
		Lactobacillus spp.	6.80^{a} ± 0.55	5.12^{b} ± 0.34	<1	<1
		Sulphite reducing AB	5.84 ± 0.51	<1	<1	<1
		Pseudomonas spp.	5.30^{a} ± 0.45	3.88^{b} ± 0.39	<1	<1
		Total coliforms	3.72^{a} ± 0.43	1.48^{b} ± 0.46	<1	<1
		E. coli	3.20 ± 0.23	<1	<1	<1
	19 d	TMC	-	8.35^{a} ± 0.48	6.18^{b} ± 0.35	4.80^{c} ± 0.39
		Lactobacillus spp.	-	6.56^{a} ± 0.30	4.59^{b} ± 0.33	<1
		Sulphite-reducing AB	-	<1	<1	<1
		Pseudomonas spp.	-	7.83^{a} ± 0.37	5.72^{b} ± 0.47	<1
		Total coliforms	-	1.95 ± 0.34	<1	<1
		E. coli		<1	<1	<1
	32 d	TMC	-	-	-	6.51 ± 0.39
		Lactobacillus spp.	-	-	-	<1
		Sulphite-reducing AB	-	-	-	<1
		Pseudomonas spp.	-	-	-	<1
		Total coliforms	-	-	-	<1
		E. coli				<1

*Not analysed because the microbiological threshold fixed at 6 log CFU/g was reached in the previous time of analysis.
**Under detection limit.
In the same row, values with different superscript letters are significantly different ($p < 0.05$).

Table 3. Evolution of microbial cell loads (log CFU/g) of total mesophilic count (TMC), *lactobacillus* spp., *pseudomonas* spp., total coliforms, sulphite-reducing anaerobic bacteria (AB), *E. coli* and positive coagulase (PC) *staphilococci* during the refrigerated storage of packaged rose shrimp in relation to the applied high hydrostatic pressure (HPP) treatments (400, 500 and 600 MPa).

		Rose shrimp	Log CFU/g			
			S-0	S-400	S-500	S-600
Days of storage	0 d	TMC	$5.04^{a} \pm 0.47$	$4.46^{a,b} \pm 0.56$	$3.78^{b,c} \pm 0.53$	$3.34^{c} \pm 0.45$
		Lactobacillus spp.	5.34 ± 0.44	<1**	<1	<1
		Sulphite-reducing AB	$4.53^{a} \pm 0.51$	$2.48^{b} \pm 0.35$	$2.38^{b} \pm 0.43$	<1
		Pseudomonas spp.	5.16 ± 0.37	<1	<1	<1
		Total Coliforms	3.53 ± 0.46	<1	<1	<1
		E. coli	2.26 ± 0.45	<1	<1	<1
		PC *staphilococci*	2.20 ± 0.20	<2	<2	<2
	7 d	TMC	$6.48^{a} \pm 0.49$	$5.18^{b} \pm 0.46$	$4.35^{b,c} \pm 0.39$	$3.63^{c} \pm 0.47$
		Lactobacillus spp.	6.05 ± 0.55	<1	<1	<2
		Sulphite-reducing AB	6.08 ± 0.36	<2	<2	<1
		Pseudomonas spp.	5.32 ± 0.42	<1	<1	<1
		Total coliforms	4.70 ± 0.20	<1	<1	<1
		E. coli	3.60 ± 0.15	<1	<1	<1
		PC *staphilococci*	3.40 ± 0.25	<2	<2	<2
	14 d	TMC	-*	$8.15^{a} \pm 0.35$	$4.31^{b} \pm 0.51$	$4.20^{b} \pm 0.56$
		Lactobacillus spp.	-	<2	<1	<1
		Sulphite-reducing AB	-	<2	<2	<1
		Pseudomonas spp.	-	3.56 ± 0.44	<1	<1
		Total coliforms	-	<1	<1	<1
		E. coli		<1	<1	<1
		PC *staphilococci*		2.60 ± 0.41	<2	<2
	21 d	TMC	-	-	5.64 ± 0.42	3.6 ± 0.41
		Lactobacillus spp.	-	-	<1	<1
		Sulphite-reducing AB	-	-	<2	<1
		Pseudomonas spp.	-	-	<1	<1
		Total coliforms	-	-	<1	<1
		E. coli		-	<1	<1
		PC *staphilococci*		-	<2	<2
	28 d	TMC	-	-	$8.43^{a} \pm 0.37$	$5.46^{b} \pm 0.43$
		Lactobacillus spp.	-	-	<1	<1
		Sulphite-reducing AB	-	-	4.32 ± 0.33	<1
		Pseudomonas spp.	-	-	<1	<1
		Total coliforms	-	-	<1	<1
		E. coli		-	<1	<1
		PC *staphilococci*		-	<2	<2
	35 d	TMC	-	-	-	7.49 ± 0.39
		Lactobacillus spp.	-	-	-	<1
		Sulphite-reducing AB	-	-	-	<1
		Pseudomonas spp.	-	-	-	<1
		Total coliforms	-	-	-	<1
		E. coli	-	-	-	<1
		PC *staphilococci*	-	-	-	<2

*Not analysed because the microbiological threshold fixed at 6 log CFU/g was reached in the previous time of analysis
**Under detection limit.
In the same row, values with different superscript letters are significantly different ($p < 0.05$).

Table 4. pH and peroxide values (PV; mEq O_2/kg fat) measured in HPP-treated seafood samples during refrigerated storage.

	Storage time (days)						
	1	6	9	14	21	28	35
Sample				**pH**			
M-0	6.41 ± 0.13^{a}	6.34 ± 0.03^{b}	6.46 ± 0.05^{a}	6.23 ± 0.03^{b}			
M-400	6.44 ± 0.06^{a}	6.55 ± 0.06^{a}	6.52 ± 0.04^{a}	6.47 ± 0.02^{a}	6.34 ± 0.07^{c}		
M-500	6.44 ± 0.01^{a}	6.50 ± 0.04^{a}	6.45 ± 0.01^{a}	6.46 ± 0.01^{a}	6.51 ± 0.04^{a}	6.31 ± 0.10^{a}	6.51 ± 0.01^{a}
M-600	6.46 ± 0.01^{a}	6.50 ± 0.03^{a}	6.41 ± 0.02^{a}	6.45 ± 0.01^{a}	6.41 ± 0.03^{b}	6.40 ± 0.03^{a}	6.51 ± 0.01^{a}
P-0	7.18 ± 0.01^{b}	7.20± 0.01^{c}	7.54 ± 0.12^{a}	7.44 ± 0.06^{b}			
P-400	7.45 ± 0.01^{a}	7.29 ± 0.03^{b}	7.27 ± 0.01^{b}	7.25 ± 0.01^{c}	7.06 ± 0.01^{c}		
P-500	7.40 ± 0.01^{a}	7.45 ± 0.01^{a}	7.32 ± 0.01^{b}	7.24 ± 0.03^{c}	7.47 ± 0.02^{a}	7.27 ± 0.04^{b}	7.17 ± 0.01^{b}
P-600	7.50 ± 0.02^{a}	7.44 ± 0.08^{a}	7.26 ± 0.02^{b}	7.54 ± 0.02^{a}	7.36 ± 0.03^{b}	7.36 ± 0.01^{a}	7.37 ± 0.01^{a}
S-0	7.59 ± 0.04^{b}	7.68 ± 0.08^{a}		7.73 ± 0.03^{a}			
S-400	7.65 ± 0.05^{a}	7.44 ± 0.03^{b}		7.47 ± 0.03^{b}	7.46 ± 0.01^{a}		
S-500	7.55 ± 0.11^{b}	7.52 ± 0.02^{b}		7.50 ± 0.01^{b}	7.39 ± 0.03^{a}	7.37 ± 0.01^{b}	
S-600	7.74 ± 0.02^{a}	7.61 ± 0.01^{ab}		7.48 ± 0.01^{b}	7.51 ± 0.04^{a}	7.48 ± 0.01^{a}	7.41 ± 0.02
Sample				PV			
M-0	0.88 ± 0.09^{b}	0.55 ± 0.08^{b}	1.40 ± 0.05^{a}	0.33 ± 0.10^{b}			
M-400	1.00 ± 0.06^{b}	0.68 ± 0.10^{b}	0.77 ± 0.06^{b}	1.50 ± 0.11^{a}	1.52 ± 0.07^{a}		
M-500	0.99 ± 0.05^{b}	0.90 ± 0.14^{a}	1.00 ± 0.22^{ab}	1.25 ± 0.11^{a}	1.10 ± 0.06^{a}	1.51 ± 0.14^{a}	0.79 ± 0.04^{b}
M-600	1.28 ± 0.05^{a}	0.92 ± 0.07^{a}	0.92 ± 0.05^{b}	1.14 ± 0.05^{a}	1.57 ± 0.10^{a}	1.06 ± 0.17^{a}	1.75 ± 0.15^{a}
P-0	1.88 ± 0.03^{ab}	1.36 ± 0.15^{b}	1.56 ± 0.16^{b}				
P-400	2.03 ± 0.02^{a}	2.03 ± 0.11^{a}	1.37 ± 0.02^{c}	1.30 ± 0.14^{a}	1.30 ± 0.14^{a}		
P-500	1.54 ± 0.14^{b}	2.5 ± 0.07^{a}	2.68 ± 0.41^{a}	1.57 ± 0.11^{a}	1.92 ± 0.31^{a}	1.89 ± 0.07^{a}	1.78 ± 0.2^{a}
P-600	1.52 ± 0.11^{b}	0.70 ± 0.07^{c}	1.96 ± 0.11^{b}	1.44 ± 0.16^{a}	1.82 ± 0.12^{a}	0.89 ± 0.03^{b}	1.66 ± 0.12^{a}
S-0	0.91 ± 0.14^{a}	0.43 ± 0.02^{b}		0.59 ± 0.01^{b}			
S-400	0.63 ± 0.12^{a}	0.82 ± 0.13^{a}		0.59 ± 0.02^{b}	0.68 ± 0.11^{a}		
S-500	0.66 ± 0.08^{a}	0.86 ± 0.04^{a}		0.82 ± 0.05^{a}	0.72 ± 0.01^{a}	0.62 ± 0.04^{a}	
S-600	0.98 ± 0.10^{a}	0.67 ± 0.03^{ab}		0.85 ± 0.08^{a}	0.57 ± 0.13^{a}	0.49 ± 0.02^{a}	0.65 ± 0.04

Different superscript letters indicate significant differences ($p < 0.05$) between samples of the same specie at the same storage time.

In the present study, despite no difference being observed between grey mullet samples just after treatment, after 6 days, pH was slightly higher in HPP-treated samples, compared to the untreated sample. During storage, a slight decrease was observed for M-0 sample, while for the samples subjected to high pressure, values showed little variability until the end of the storage period.

On the other hand, for tiger prawn, all samples showed higher pH values of about 7.2–7.5 after HPP, compared to the control, without differences in relation to different pressure levels applied. However, while in the case of control sample, an increase in pH was observed during storage, treated samples showed an opposite trend (Figure S1). In sample P-400, pH values decreased by about 0.4 points, while in samples P-500 and P-600, they showed little variability. In rose shrimp, only sample S-600 showed a significant increase in pH just after treatment, compared to the untreated sample. During storage, the control sample showed a slight increase, while for all treated samples, pH values decreased progressively.

Increase in pH after pressurization of fish and seafood tissues was observed in literature (Angsupanich and Ledward, 1998; Bindu *et al.,* 2013; Briones-Labarca *et al.,* 2012; Kaur *et al.,* 2013) and was explained with the induction of protein unfolding by pressure and the following ionisation of denatured proteins.

On the other side, the evolution of pH during storage could be attributed mainly to the activity of spoilage microorganisms that produced both basic (e.g. ammonia, trimethylamine and other biogenic amines) and acidic (e.g. lactic acid in the case of *lactobacillus* spp.) compounds.

Color

Values for luminosity (L^*) and hue angle ($h°$) of the three seafood species are reported in Figure 1. Luminosity of the flesh was significantly increased by about 20 units straight for the three considered species after HPP treatment. This effect was largely observed in many fish species and attributed to protein denaturation. Considering that for each investigated species there was no significant difference in the values of the treated products just after the treatment as a function of the different pressure levels adopted, it was assumed that protein denaturation occurred to a similar extent in all samples for HPP of 400–600 MPa. During storage, all L^* values were very close to the initial values for the control and the pressure-treated samples.

Significant differences were observed for $h°$ values between the control and the pressure-treated samples just after treatment. However, while for grey mullet, $h°$ value increased due to HPP, for shrimp and prawn, the values of this chromatic parameter decreased. Hue angle is calculated using both red and yellow indexes; in all samples a^* was decreased remarkably, while changes in b^* were higher in shrimp and prawn, compared to grey mullet. According to the literature, variation in colour during storage could depend on degradation of myofibrillar proteins and disorganisation of myofibrils caused by enzymatic and non-enzymatic reactions as well as on the possible oxidation of pigments (Yagiz *et al.,* 2007). Hence, change in colour occurring during storage in seafood products subjected to high pressures could differ significantly depending on the species and the adopted treatment conditions. In the present study, the final effect for all samples was general whitening and occurrence of a cooked appearance, which is typical for muscle foods subjected to pressurization (Figure S2).

Considering that visual quality is a very important parameter for consumer acceptability, and that these products are intended for raw consumption, the cooked

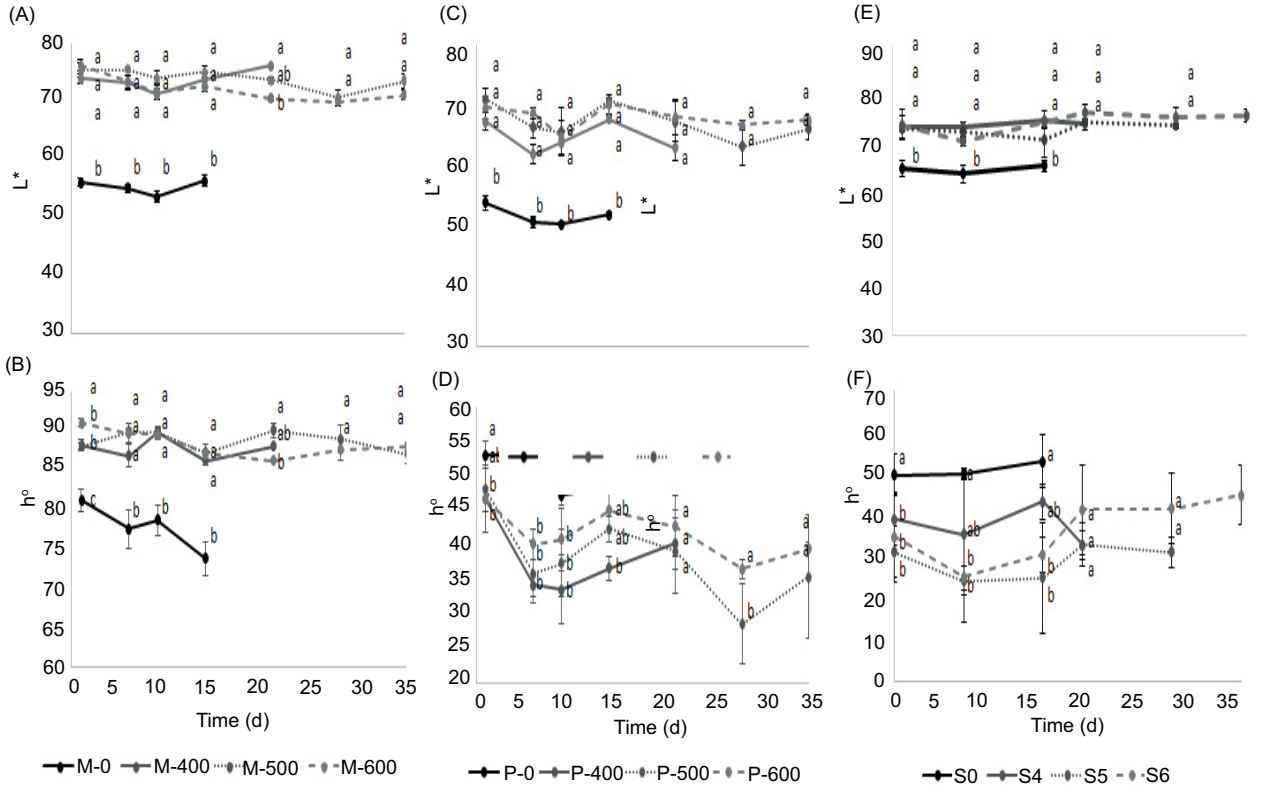

Figure 1. Colour coordinates of luminosity (L^*) and hue angle ($h°$) of (A and B) mullet, (C and D) shrimp and (E and F) rose shrimp. Different letters indicate significant differences between samples ($p < 0.05$).

appearance could represent a problem that could be addressed probably by a marketing and/or informative strategy.

Texture

Texture is a very important parameter for appreciation of seafood products. Even if, in general, the texture profile analysis (TPA) test is carried out on fish fillets, considering the specific characteristics of the investigated product, in the present study compression, followed by the application of steady pressure, was applied. Results of the two parameters analysed, that are, hardness (F1) and index of resistance to compression (F2) (Figure S3), are reported in Figure 2 for all the three considered species. In the present work, the observed effect was different for the three considered species.

For grey mullet (Figures 2A and 2B), a reduction in the initial hardness was observed for higher applied pressures (500 and 600 MPa) just after the treatment. Moreover, the increasing pressure decreased resistance to compression of the tissues in proportion to the applied pressure level (Figure 2B). For both parameters, in the control sample, a decrease was observed during storage, as reported by Chéret *et al.* (2005) for sea bass. The softening of the tissue during refrigerated storage could be attributed to enzymatic activity of proteases that influenced both myofibrillar proteins and connective tissues, bringing about myofibrillar fragility and gaping. A similar behaviour could be observed for sample M-400, but with a more gradual decrease of both values. Indeed, after 9 days, the resistance to compression was significantly higher, compared to the control, possibly because of a partial enzymatic denaturation caused by pressure. However, samples M-500 and M-600 showed quite constant values for both parameters for all storage periods with fewer differences between them.

In striped prawn (Figures 2C and 2D), a slight but significant reduction of hardness was observed for the 500- and 600-MPa treatments, while the resistance parameter was reduced only for 600-MPa pressure. During storage, hardness showed a fluctuating behaviour but tended to increase, compared to the initial value for samples P-0, P-400 and P-500, while it remained practically unchanged for sample P-600. Resistance was shown to increase during storage only in samples P-0 and P-400, although the storage was shorter due to microbiological spoilage.

On the contrary, in rose shrimp (Figures 2E and 2F), hardness was not influenced by any pressure applied, while the resistance to compression was found significantly higher after 600-MPa treatment. During storage, both parameters showed a decreasing trend for all samples. The values were highly variable and very few significant changes were observed.

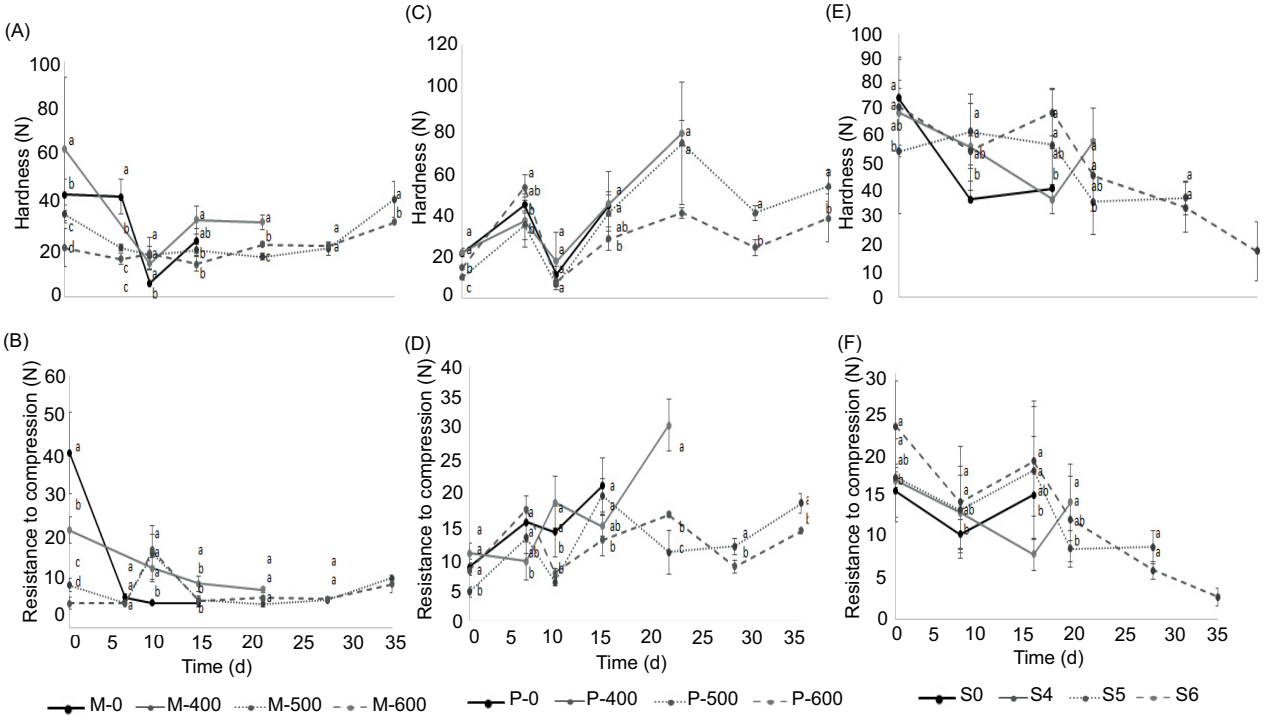

Figure 2. Texture parameters of hardness (N) and resistance to compression (N) of (A and B) mullet, (C and D) shrimp and (E and F) rose shrimp. Different letters indicate significant differences between samples ($p < 0.05$).

These results are in contrast with increase in hardness measured after applying high pressure, as observed by Bindu *et al.* (2013) in Indian white prawn and by Jantakoson *et al.* (2012) and Kaur *et al.* (2013) in black tiger shrimp. Moreover, the two considered parameters between two crustaceans showed different behaviour during storage. An increasing tendency was observed in prawn, while in shrimp, values decreased progressively for the considered period. Decrease in hardness during storage, attributed to the effect of proteolytic enzymes, was also observed by Kaur *et al.* (2013). However, values of hardness in sample P-600 and resistance in samples P-500 and P-600 were mostly constant. This effect could be explained by a partial inactivation of such enzymes.

In general, an increase in hardness after HPP treatment was observed by many authors on different fish species, such as Rainbow trout and Mahi mahi (Yagiz *et al.,* 2007), cod (Angsupanich and Ledward, 1998), tuna (Zare, 2004). This was explained by the unfolding of actin and sarcoplasmic proteins and the formation of new hydrogen-bonded networks and by an increase in protein–protein interactions and bond formation. On the other hand, Briones-Labarca *et al.* (2012) found no differences in red abalone treated with up to 500-MPa HPP, compared to the control. Chéret *et al.* (2005) observed a softening effect after subjecting sea bass to a pressure of up to 300 MPa, while no change in hardness was observed with 400- and 500-MPa treatment.

Besides modification to myofibrillar proteins, HPP also promoted pH values and modification to hydrogen and hydrophobic bonds that resulted in changes to the structural characteristics of proteins. Moreover, an effect on collagen and the connective tissue of red abalone was observed by Briones-Labarca *et al.* (2012) through scanning electric microscope. This confirmed a significant change in the microstructure of the flesh upon application of high pressure. Hence, the effect of HPP on fish texture was due to modifications to water bonding and holding capacity, activity of enzymes, such as proteases, that could be inhibited or enhanced, and structural modification of myofibrillar and sarcoplasmic protein.

The results obtained in the present study confirmed that the effect on texture strictly depended on the considered species of the raw material and the specific tissue structure. Moreover, the effect observed during storage probably depended on the possible inactivation of proteolytic enzymes, which again probably was matrix-dependent.

The effect of HPP on proteolytic enzymes in different species of fishes was studied by different authors, but results appeared to vary depending on seafood species, pressure level, holding period, and type and structure of the enzyme.

Low level of pressure of about 100 MPa applied at 10°C for 5 min showed to increase enzymatic activity of calpain in sea bass muscles (Chéret *et al.,* 2005), while Teixeira *et al.* (2013) observed that rate of pressurization could also play a role in the activation of the same enzyme in sea bass fillets. In general, increase in pressure level and holding period was shown to inactivate enzymes. However, although seafood muscles were shown to be more sensitive to pressure compared to bovine muscles, the food matrix could strongly affect enzymes (Truong *et al.,* 2015). The application of pressure caused variation in protein structure that could lead to the breakdown of cell membrane and release of proteolytic enzymes in the cytoplasm, favouring their denaturation.

Lipid oxidation

Table 4 shows peroxide values measured in the three considered species subjected to HPP treatment during refrigerated storage. A slight but significant increase in peroxide values was observed after 600-MPa treatment in grey mullet samples. However, while no difference was observed in rose shrimp samples, lower values were measured in prawn samples treated at higher pressures. Moreover, peroxide values remained very low for all the storage periods considered (below 1.75, 2.7 and 0.98 mEq O_2/kg fat for grey mullet, prawn and shrimp, respectively).

In general, an enhancement of lipid oxidation was observed in the literature for many seafood products, particularly when pressures above 300 MPa were applied (Truong *et al.,* 2015). This increase was mainly attributed to the presence of haemoglobin and myoglobin containing iron in their structure that was released because of pressurization, and could promote oxidation. However, Yagiz *et al.* (2009) established that 300-MPa treatment helped to inhibit lipid oxidation of Atlantic salmon, compared to the untreated sample and the sample subjected to 150-MPa pressure during storage. The authors suggested that perturbation to changes to cell membrane structure caused by pressure made phospholipids less susceptible to oxidation even in a fish that was considered fatty. Moreover, presence of astaxanthin (a carotenoid) was believed to have a powerful antioxidant effect.

Studies related to the effect of pressure on the activity of enzymes responsible for lipid oxidation are still scarce. The reduced oxidation level showed by the evolution of its primary index in the present study was due to different reasons. Firstly, the considered seafood species were characterised by a low content of fat (about 2.5, 0.8 and 1.1 g/100 g for grey mullet, prawn and shrimp, respectively) and were not susceptible to lipid oxidation. Secondly, we assumed that further reduction in lipid

oxidation could have been due to modification to cell membranes induced by pressure. Also, a very low presence of oxygen in packages would have surely inhibited oxidative reactions during storage.

Conclusions

The investigation of the effect of HPP treatment on different types of considered seafood products, intended for raw consumption, highlighted a significant increase in microbiological shelf life at the highest applied pressure levels (500 and 600 MPa) for all the considered species.

Even if lower pressure (400 MPa) appeared adequate to inactivate *E. coli*, *pseudomonas*, and/or positive coagulase *staphylococci*, they were recovered during fish product storage, stressing the issue of viable but non-culturable cells (VBNC). On the other side, the application of 600 MPa allowed to extend the microbiological shelf life by up to around 30 days.

In terms of visual quality, the final effect for all samples was general whitening and occurrence of a cooked appearance, typical for muscle foods subjected to pressurization. The texture response was strictly dependent on the considered species of raw material and specific tissue structure. Fat oxidation was minimally affected and remained quite low during storage.

Further studies are in progress in our laboratories to better clarify the physico-chemical and biological causes of detected differences through microstructural assessment and their overall sensorial impact. Moreover, the effect of HPP on the production of biogenic amines is also under evaluation in order to clarify the effectiveness of the proposed technology on the safety aspect.

However, considering the effect on microbiological shelf life, for all the three considered species, attaining a pressure of over 500 MPa appears necessary, particularly because the effect on quality did not show changes at higher pressures. It is important to emphasize that in the optic of the commercialisation of HPP-treated seafood intended for raw consumption, a specific marketing/informative strategy is required, evidencing to the final consumer the important advantages of this non-thermal technology in terms of nutritional and sensorial food properties.

Moreover, in addition to *L. monocytogenes* and *Salmonella* pathogens, the effect of HPP process must be considered against *Vibrio* spp. and *Clostridium botulinum*, especially in vacuum packaging, because these pathogens are responsible for human diseases through the consumption of contaminated fish and fish products. These pathogens have a wide distribution in aquatic environments as well as high mortality rate. In addition, the spore-forming quality of *C. botulinum* fosters its survival in water sediments and fish, certainly influenced by geographical location, feeding habits of the fish species, types of product samples, and the detection method used (Aleksandr *et al.*, 2015). Then again, the data emphasized, following the HPP treatment, a shift in microbial populations responsible for reaching the threshold level. In addition, this aspect requires further investigation to understand product spoilage patterns.

Author Contributions

Conceptualization: S.T; P.R.; Methodology: S.T., F.P., P.R.; Formal analysis: A.C.D.A.S.P., G.B.; Investigation: A.C.D.A.S.P., G.B.; Resources: F.P., P.R.; Data curation: A.C.D.A.S.P., J.G., G.B.; Writing-Original Draft: A.C.D.A.S.P., G.B.; Writing - Review & Editing: S.T., J.G., F.P., P.R.; Supervision: S.T, F.P.; Project Administration: P.R.; Funding Acquisition: F.P., P.R.

References

Aleksandr N., Margarita T., Inga E., Olga V., Vadims B. and Aivars B. 2015. Major food borne pathogens in fish and fish product: a review. Ann Microbiol. 66(1): 1–15. https://doi.org/10.1007/s13213-015-1102-5

Angsupanich K. and Ledward D.A. 1998. High pressure treatment effects on cod (Gadus Morhua) muscle. Food Chem. 63(1): 39–50. https://doi.org/10.1016/S0308-8146(97)00234-3

Bindu J., Ginson J., Kamalakanth C.K., Asha K.K and Srinivasa Gopal T.K. 2013. Physico-chemical changes in high pressure treated indian white prawn (Fenneropenaeus indicus) during chill storage. Innov Food Sci Emerg Technol. 17: 37–42. https://doi.org/10.1016/j.ifset.2012.10.003

Bligh E.G. and Dyer W.J. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 37(8): 911–17. https://doi.org/10.1139/o59-099

Borges A.F., Cózar A., Patarata L., Gama L.T., Alfaia C.M., Fernandes M.J., et al. 2020s. Effect of high hydrostatic pressure challenge on biogenic amines, microbiota, and sensory profile in traditional poultry- and pork-based semidried fermented sausage. J Food Sci. 85(4): 1256–1264. https://doi.org/10.1111/1750-3841.15101

Briones-Labarca V., Perez-Won M., Zamarca M., Aguilera-Radic J.M. and Tabilo-Munizaga G. 2012. Effects of high hydrostatic pressure on microstructure, texture, colour and biochemical changes of red abalone (Haliotis rufecens) during cold storage time. Innov Food Sci Emerg Technol. 13: 42–50. https://doi.org/10.1016/j.ifset.2011.09.002

Brutti A., Rovere P., Cavallero S., D'Amelio S., Danesi P. and Arcangeli G. 2010. Inactivation of anisakis simplex larvae in raw fish using high hydrostatic pressure treatments. Food Cont. 21(3): 331–333. https://doi.org/10.1016/j.foodcont.2009.05.013

Campus M. 2010. High pressure processing of meat, meat products and seafood. Food Eng Rev. 2(4): 256–273. https://doi.org/10.1007/s12393-010-9028-y

Chapman R.A. and Mackay K. 1949. The estimation of peroxides in fats and oils by the ferric thiocyanate method. J Am Oil Chem Soc. 26(7): 360–363. https://doi.org/10.1007/BF02651444

Chéret R., Chapleau N., Delbarre-Ladrat C., Verrez-Bagnis V. and Lamballerie M.D. 2005. Effects of high pressure on texture and microstructure of sea bass (Dicentrarchus Labrax L.) fillets. J Food Sci. 70(8): e477–e483. https://doi.org/10.1111/j.1365-2621.2005.tb11518.x

de Alba M., Pérez-Andrés J.M., Harrison S.M, Brunton N.P., Burgess C.M. and Tiwari B.K. 2019. High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets. Innov Food Sci Emerg Technol. 55: 80–87. https://doi.org/10.1016/j.ifset.2019.05.010

Jantakoson T., Kijroongrojana K. and Benjakul S. 2012. Effect of high pressure and heat treatments on black tiger shrimp (Penaeus Monodon Fabricius) muscle protein. Int Aquatic Res. 4(1): 1–12. https://doi.org/10.1186/2008-6970-4-19

Kaur B.P., Kaushik N., Rao P.S. and Chauhan O.P. 2013. Effect of high-pressure processing on physical, biochemical, and microbiological characteristics of black tiger shrimp (Penaeus Monodon): high-pressure processing of shrimp. Food Bioprocess Technol. 6(6): 1390–1400. https://doi.org/10.1007/s11947-012-0870-1.Matser

Patrignani F., Mannozzi C., Tappi S., Tylewicz U., Pasini F., Castellone V., et al. 2019. (Ultra) high pressure homogenization potential on the shelf life and functionality of kiwifruit juice. Front Microbiol. 10(Feb). https://doi.org/10.3389/fmicb.2019.00246

Patterson M.F. 2014. Food technologies: high pressure processing. In: Encyclopedia of Food Safety, Vol. 3. Academic Press, Cambridge, MA, pp. 196–201.

Perez-Won M., Lemus-Mondaca R., Herrera-Lavados C., Reyes J.E., Roco T., Palma-Acevedo A. and Aubourg S.P. 2020. Combined treatments of high hydrostatic pressure and co2 in coho salmon (Oncorhynchus Kisutch): effects on enzyme inactivation, physicochemical properties, and microbial shelf life. Foods. 9(3): 273. https://doi.org/10.3390/foods9030273

Teixeira B.A., Fidalgo L., Mendes R., Costa G., Cordeiro C., Saraiva J.A., et al. 2013. Changes of enzymes activity and protein profiles caused by high-pressure processing in sea bass (Dicentrarchus labrax) fillets. J Agric Food Chem. 61(11): 2851–2860. https://doi.org/10.1021/jf3049643

Truong B.Q., Buckow R., Stathopoulos C.E. and Nguyen M.H. 2015. Advances in high-pressure processing of fish muscles. Food Eng Rev. 7(2): 109–129. https://doi.org/10.1007/s12393-014-9084-9

Tsogas K., Vatavali A., Dimitriou A., Badeka E., Kontakos S. and Kontominas M.G. 2019. Combined effect of light salting and vacuum packaging on the microbiological, chemical, and sensory attributes of mullet fillets (Mugil cephalus) during refrigerated and frozen/refrigerated storage. J Food Proc Preserv. 43(8): e14009. https://doi.org/10.1111/jfpp.14009

Yagiz Y., Kristinsson H.G., Balaban M.O. and Marshall M.R. 2007. Effect of high pressure treatment on the quality of rainbow trout (Oncorhynchus mykiss) and mahi mahi (Coryphaena hippurus). J Food Sci. 72(9): 509–515. https://doi.org/10.1111/j.1750-3841.2007.00560.x

Yagiz Y., Kristinsson H.G., Balaban M.O., Welt B.A., Ralat M. and Marshall M.R. 2009. Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon. Food Chem. 116(4): 828–835. https://doi.org/10.1016/j.foodchem.2009.03.029

Zare Z. 2004. High Pressure Processing of Fresh Tuna Fish and Its Effects on Shelf Life. High Pressure Processing of Fish. McGill University, Quebec, Canada. https://api.semanticscholar.org/CorpusID:99272819

Supplementary

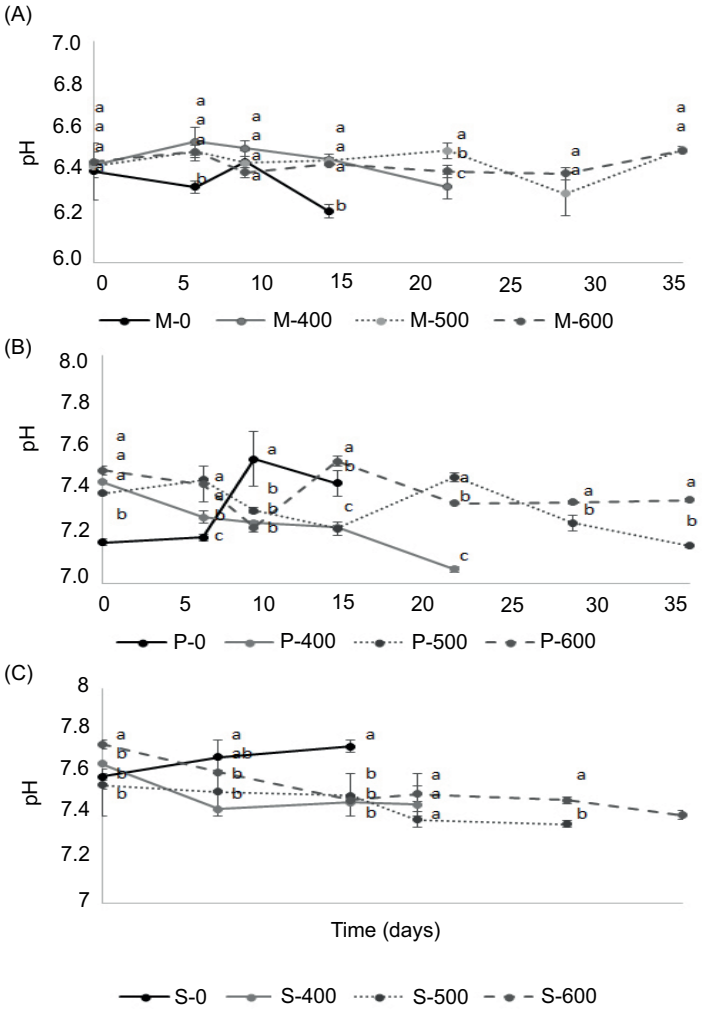

Figure S1. pH measured in HPP-treated seafood samples during refrigerated storage.

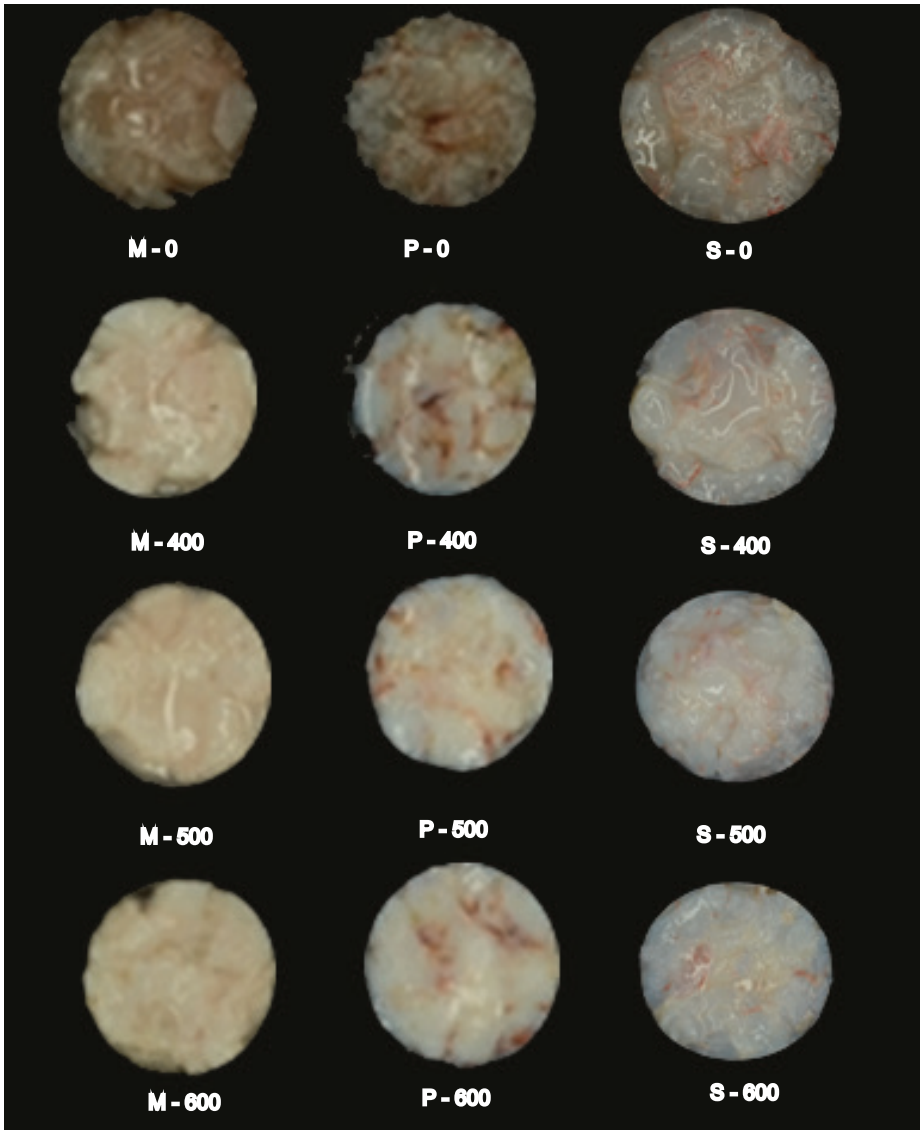

Figure S2. Digital images of grey mullet (M), striped prawn (P) and rose shrimp (S) samples treated at 400-, 500- and 600-MPa pressure, compared to the untreated samples.

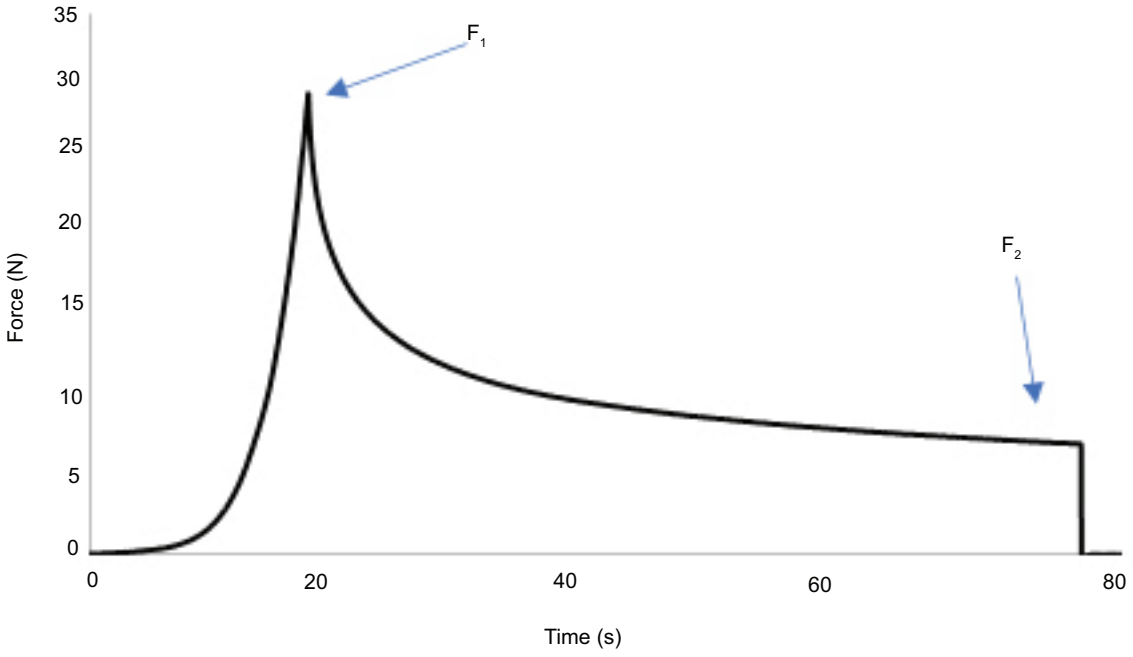

Figure S3. Results of the analysed hardness (F1) and index of resistance to compression (F2).