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Effects of high pressure on texture and microstructure of sea bass (Dicentrarchus labrax L.) fillets

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Abstract: High-pressure is an innovative non-thermal food preservation technology. We studied the effect of high-pressure treatment up to 500 MPa-5min on physical characteristics of sea bass fillets after 0, 7 and 14 days of refrigerated storage. Color results exhibited an increase of lightness and a slight change of hue, which might be imperceptible in cooked fish. High-pressure treatment induced a decrease of exudation and water-holding capacity. Pressure treatment above 300 MPa provoked fish hardness higher after storage than untreated sample, proving the ability of high-pressure to improve textural quality of chilled stored fish fillet. These assessments were corroborated with microstructure observations. We showed that high-pressure treatment at 500 MPa allowed, after 7 days of storage, a total aerobic count equivalent to that of untreated fresh fish fillet to be obtained. Thus, high-pressure might be considered to be a technology able to improve safety and textural quality of fresh fish fillets.

Keywords: Fish; High-pressure; Texture Profile Analysis; Microstructure; Preservation

1. Introduction

Among the various aspects that contribute to defining the quality of raw fish, freshness is one of the most important. Freshness of fish can be assessed thanks to organoleptic features. Generally, the muscle of fish is known to soften quickly during the *post mortem* storage in refrigerated conditions. One of the main features in appreciating fish is the texture. The appearance and the odour are also very important for consumers.

The muscle of fish has a very particular organization compared to meat, because it is constituted of the alternation of muscular sheets called myotomes separated and maintained by the connective tissue. Moreover, myotomes are composed by a large number of individual muscle fibres in a collagen matrix.

Post mortem softening of fish muscle is caused by the combination of two reactions: biochemical-induced by enzymatic degradation of myofibrils and collagen, and physical due to the separation of myotomes called “gaping”.

After the fish dies, the period of *rigor-mortis* starts. The final quality of fish depends on this phase. Initially, the phenomenon of “gaping” proceeds and is characterised by the separation of the myotomes. This mechanical phenomenon is associated to the contraction of muscle fibres. The skeleton and the connective tissue support this contraction and keep muscle structure. The connective tissue resists until a certain pressure beyond which it weakens itself letting place to “gaping”. “Gaping” is a consequence of the failure of fibres and tissue to connect the muscle blocks along the filet. (Lavety and others 1988; Bremner and Hallet 1985; Bremner 1992; Hallett and Bremner 1988). Then, for the period *post mortem*, many biochemical mechanisms will initiate the degradation of the muscle. These modifications lead to a reduction in hardness (Dunajsky 1979). Protein hydrolysis does not play a significant part. It was observed that the collagen fibres in the pericellular connective tissue were disintegrated. It has been shown in sea bass, that the changes occurring during

57 *post mortem* degradation result in the weakening and disorganisation of the Z line structure,
58 the deterioration of costamers, and a separation between filaments and I bands (Astier and
59 others 1991; Papa and others 1996; Papa and others 1997). Several studies suggest that the
60 gradual disintegration of the extracellular matrix structure in mainly responsible for
61 tenderisation (Ando 1997; Bremner 1992; Montero 1997). The structural links and bonds of
62 proteins are changed during the degradation. These changes are caused by enzymes: in most
63 cases, a result of enzymatic reactions has been determinated, but not activities of the enzymes
64 themselves (Rehbein 1997).

65 When the *post mortem* degenerative process takes place, the degradation of proteins
66 creates ideal conditions for the growth of micro-organisms. In living fish, some micro-
67 organisms remain present on their skin and in the digestive system. After death, they are
68 likely to contaminate the muscle of fish. The compounds formed by different degradations
69 due to the micro-organisms release unpleasant odours. This phenomenon of degradation is
70 dependent on storage temperature. Also, during storage, the color of flesh changes from a
71 bluish translucent color to an opaque and cooked appearance.

72 Among the non-thermal processing techniques, high-pressure processing is of growing
73 interest in food processing. Some products are already available in markets in Japan (fruits
74 juices, jam, rice, seaweed), in the USA (avocado spread and oysters) and in Europe (orange
75 juice in France and ham in Spain). Today, this technology is gaining popularity in the food
76 industry.

77 Generally, products are subjected to high-pressure in the range of 100-1000 MPa. The
78 main advantage of high-pressure is to inactivate pathogenic micro-organisms: so food shelf-
79 life can be prolonged. High-pressure processing for microbial decontamination has been
80 extensively reviewed, but complete microbial inactivation is currently not possible (Knorr
81 1999; Smelt 1998). The advantage of high-pressure treatment over traditional thermal

processing is the result in an almost complete retention of nutritional and organoleptic characteristics. However, chemical bonds of molecules are affected and that may induce modifications of water, proteins, polysaccharides and lipids. The main effect of high-pressure is to provoke changes in hydrophobic and electrostatic interactions with important consequences for the secondary, tertiary and quaternary structures in proteins. In muscle food, high-pressure treatment causes three main kinds of changes in muscle food: enzymatic, protein (mainly on myofibrils) and structural modifications. Pressure treatment of proteins can lead to significant conformational changes which influence functionality (Cheftel and Dumay 1997; Chapleau and de Lamballerie-Anton 2003). The mechanisms of these effects are not yet fully understood.

Recent studies showed that the high-pressure treatment could allow controlling or deactivating some enzymes involved in the degradation of fish muscle to modify the texture and to stabilise the color and the oxidation of the lipids (Angsupanich and Ledward 1998; Ashie and Simpson 1996; Oshima and others 1993).

The objective of this present study was to evaluate and understand the effect of the high-pressure treatment on the different features that characterise the freshness of fish.

2. Materials and methods

2.1. Preparation of the fish samples

Fresh sea bass (*Dicentrarchus labrax L.*), 4 years old, average weight 325 g and length 300 mm, were collected from a local aquaculture farm (Les Viviers du Gois, Beauvoir-sur-Mer, France) and brought back alive to the laboratory. The fish were slaughtered by decapitation, skinned and filleted in *pre-rigor* conditions. Only white muscles of the dorsal part of the fillet were collected to carry out experiments, measuring 50 x 150 mm with a thickness of about 13

106 mm. To take in account the fish variation, we used twelve fillets for each level of pressure: 0,
107 100, 200, 300, 400 and 500 MPa.
108
109 *2.2. High-Pressure Processing*
110 High-pressure processing was carried out in a 3.5 L reactor unit (ACB Pressure Systems,
111 Nantes, France) equipped with temperature and pressure regulator device. Prior to pressure
112 processing, previously prepared sea bass fillets were vacuum packed individually in
113 polyethylene bag (La Bovida, France). The level of pressure (100, 200, 300, 400 and 500
114 MPa) was reached at 3 MPa/s, kept constant 5 min, and then released quickly (< 1.5 s).
115 Temperature of transmitting medium in the vessel was settled at 10°C (± 5°C) during pressure
116 treatment. Temperature of the cooling jacket which surrounded the pressure vessel was
117 controlled at 10 °C during pressure treatment. One thermocouple K-type (0.3 mm diameter,
118 Omega, Stamford, CT, USA) positioned close to the sample, allowed the variation of
119 temperature during treatment to be followed. After pressure treatment, samples were stored on
120 ice (maximum 4 h) until analysis.
121
122 *2.3. Storage of fillet*
123 Among 12 control or high-pressure treated fillets, four were analysed the very same day.
124 The eight other were stored at 4°C, four were analysed 7 days later and four 14 days later.
125 Four fillets were used for all tests: color, exudation, WHC, texture and microstructure.
126
127 *2.4. Color measurements*
128 Color measurements were performed on intact fillets after pressure processing and/or
129 storage, using a Minolta CM-3500d (Minolta, Carrières-sur-Seine, France). Measures were
130 achieved in the referential CIE 1976 L*a*b*, L* denotes lightness on a 0 to 100 scale from

131 black to white; a*, corresponds to the indication of red when its value is positive and to green
132 when it is negative; b* corresponds to the indication of yellow when its value is positive and
133 to blue when it is negative. Measurements were repeated ten times on different positions of
134 four fish fillets.
135
136 *2.5. Exudation*
137 Exudation of fillet was determined by weighing samples prior to and after pressure
138 processing. The bags were unwrapped, surface drip was removed using filter paper, and fillets
139 were weighed. Each experimental value represented the mean of four determinations.
140
141 *2.6. Water-Holding Capacity*
142 The Water-Holding Capacity (WHC) was measured individually on coarsely chopped
143 filleted muscle. The samples (10 g) were transferred to centrifugation tubes and centrifuged at
144 10 000 g for 15 min at 20°C (GR 20.22, Jouan, France). The WHC was determined as liquid
145 loss, and expressed by the percentage of weight of liquid that was retained in the fillets. Mean
146 values were calculated from four replicates.
147
148 *2.7. Texture profile analysis*
149 Texture measurements (according to Texture Profile Analysis defined by Bourne 1978)
150 were performed at room temperature with a texture testing machine (Lloyd Instruments
151 LR5K, United Kingdom) equipped with a sensor of 50 N. Twelve cylindrical samples of 20
152 mm in diameter were cut on the 4 dorsal muscles: 3 cylinders of a height of 11 to 14 mm were
153 sampled in the same way of the longitudinal orientation of the muscular fibres. Prior to
154 analysis, samples were allowed to reach room temperature (20°C, 1 h). Each sample was
155 compressed between stainless steel plates (diameter 40 mm) as shown on figure 1. The texture

measurement was composed by two consecutive compressions of 50% parallel to way of muscle fibre orientation, at a constant speed of 1 mm/s, with a delay of 30 s between the two compressions.

From the resulting force-time curve (figure 1), the following parameters were determined: hardness corresponding to the maximum force required to compress the sample; cohesiveness, extent to which the sample could be deformed prior to rupture ($(A_3+A_4)/(A_1+A_2)$, where A_1+A_2 was the total energy required for the first compression and A_3+A_4 was the total energy required for the second compression); springiness ability of sample to recover its original form after the deforming force is removed (L_2/L_1, were L_1 was the lengthening of the first compression and L_2 was the lengthening of the second compression); gumminess was the

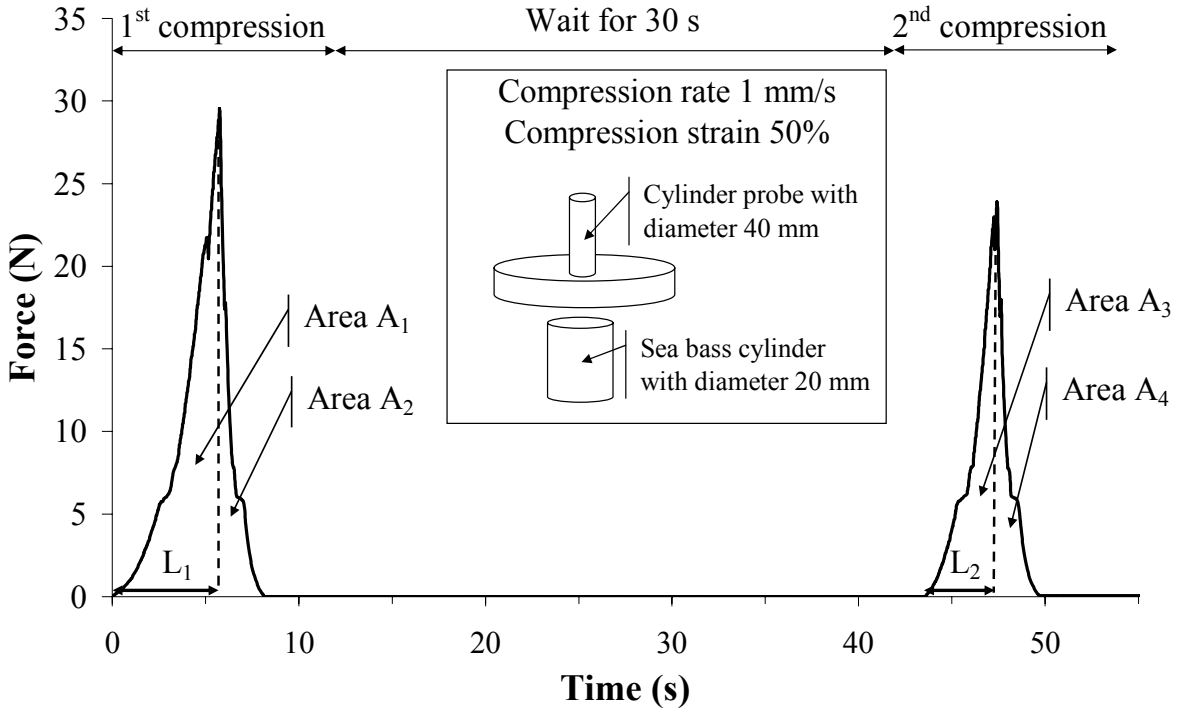


Figure 1: Texture Profile Analysis curve obtained with sea bass fillet

force needed to disintegrate a semisolid sample to a steady state of swallowing (hardness x cohesiveness); resilience was how well a product fights to regain its original position (A_2/A_1, where A_1 was the total energy required for compression of the first compression and A_2 was

172 the total energy required for decompression of the first compression); chewiness, the work
173 needed to chew a solid sample to a steady state of swallowing (springiness x gumminess).
174
175 *2.8. Microstructure analysis*
176 For each fillet analysed, two samples of 10x5x5 mm were cut transversally to the muscles
177 fibres from the core of the fillet in the fleshiest part using a blade, then fixed in Carnoy's
178 solutions (60% absolute ethanol, 30% chloroform and 10% glacial acetic acid, v/v) at 4°C.
179 After 24 h, the samples were brought to room temperature and dehydrated with several
180 alcohol solutions, first in absolute ethanol for 2 h and then in 1-butanol for 2 h (repeated three
181 times). Dehydrated samples were then cleared with toluene for 30 min (repeated three times)
182 and embedded in paraffin at 56 – 58°C. Samples were cut with a microtome Leica SM2000
183 (Leica, Germany) in 10 µm thick slices. The sections were stained for 5 min in Orange G (0.5
184 g of Orange G, 1ml acetic acid dissolved in 99 ml distilled water and filtered at 0.45 µm). The
185 sections were washed with distilled water and stained for 5 min in Aniline blue (0.01 g of
186 Aniline blue, 1ml acetic acid dissolved in 99 ml distilled water and filtered at 0.45 µm). The
187 stained samples were washed with distilled water before mounting with Eukitt (Labonord,
188 France). This staining method stained the muscle proteins orange and collagen blue. The
189 samples were examined in a microscope (Leica DML,Germany) fitted with a CCD RGB
190 camera (MACC-C71, Sony, Japan) at 400× magnification.
191
192 *2.9. Microbiological analyses*
193 Aliquots (10 g) of fillets were prepared under sterile conditions and homogenised with a
194 Stomacher for 30 s in 90 ml sterile peptone water (BK 018, Biokar Diagnostics, Beauvais,
195 France). Subsequently, a decimal dilution series of the homogenate was made, from which
196 each 3 replicates were inoculated in depth plate (1 ml) on plate count agar (BK 043, Biokar

Diagnostics, Beauvais, France). The CFU formed after incubation at 30°C for 72 h was counted for dilutions setting 25 to 300 CFU. Results were expressed as the decimal logarithm of CFU per g of fillet sample.

2.10. Statistical analysis

Statistical analysis was performed using a one-way analysis of variance according to the general linear model procedure with least-square means effects to determine significant differences between treatments. Multiple range tests were applied to determine which means were significantly different according to Fisher's Least Significant Differences (LSD). Significant differences were determined with 5% level of significance ($p<0.05$) by Student's test. The vertical bars represent the standard deviation. Statistical analysis was carried out using Statgraphics plus version 5.0 software (Statistical Graphics Corp., Princeton NJ, USA).

3. Results and discussion

3.1. Color

Table 1 shows the evolution of lightness (L*), red-green indice (a*) and yellow-blue indice (b*) according to the pressure and to the time of storage. Before the treatment, the day the fish dies, the muscle has a shiny, smooth, whitish and translucent appearance characterised by a moderate value of L* (34.19±1.51) and weak value of a* (-2.48±0.46) and b*(0.39±0.92). The muscle of sea bass studied is a white muscle whose pigmentation is bound to the presence of heme proteins, carotenoids and melanins. According to our results, the storage of non-pressurised fillets led to an increase of L* value (47.71±0.60) for storage time of 7 days, then L* decreased (42.56±1.90) for 14 days of storage. The indices of red-green and yellow-blue remained almost constant during refrigerated storage. During storage, the appearance of fish fillets became whiter and less grey.

222 **Table 1. L*a*b* color parameters of sea bass fillets treated by high-pressure processing after 0, 7 and 14 days at 4°C.**

223

		Pressure processing during 5 min					
Color parameter	**Storage time (day)**	**Control**	**100 MPa**	**200 MPa**	**300 MPa**	**400 MPa**	**500 MPa**
L*	0	34.19±1.51 $^{a\alpha}$	37.45±1.52 $^{b\alpha}$	46.42±3.32 $^{c\alpha}$	61.54±1.61 $^{d\alpha}$	66.39±1.20 $^{e\alpha}$	71.30±1.49 $^{f\alpha}$
	7	47.71±0.60 $^{a\beta}$	48.94±1.70 $^{a\beta}$	54.32±1.94 $^{b\beta}$	63.51±1.26 $^{c\beta}$	72.04±1.50 $^{d\beta}$	70.24±1.59 $^{e\alpha}$
	14	42.56±1.90 $^{a\gamma}$	45.12±1.31 $^{b\gamma}$	51.63±3.35 $^{c\gamma}$	60.83±1.65 $^{d\alpha}$	65.55±1.95 $^{e\alpha}$	70.25±2.10 $^{f\alpha}$
a*	0	-2.48±0.46 $^{a\alpha}$	-3.75±0.38 $^{b\alpha}$	-5.23±0.60 $^{c\alpha}$	-5.88±0.57 $^{d\alpha}$	-5.41±0.36 $^{c\alpha}$	-4.79±0.38 $^{e\alpha}$
	7	-2.23±0.21 $^{a\alpha}$	-2.89±0.15 $^{b\beta}$	-4.05±0.28 $^{c\beta}$	-5.04±0.40 $^{d\beta}$	-4.85±0.40 $^{d\beta}$	-4.17±0.41 $^{c\beta}$
	14	-3.33±0.28 $^{a\beta}$	-3.48±0.21 $^{a\gamma}$	-4.56±0.21 $^{b\gamma}$	-5.76±0.43 $^{c\alpha}$	-5.07±0.31 $^{d\beta}$	-4.41±0.44 $^{b\beta}$
b*	0	0.39±0.92 $^{a\alpha}$	-0.67±1.03 $^{a\alpha}$	-2.25±1.00 $^{b\alpha}$	0.16±1.64 $^{a\alpha}$	2.58±1.51 $^{c\alpha}$	4.74±1.66 $^{d\alpha}$
	7	-0.93±0.57 $^{a\beta}$	-1.67±0.52 $^{ab\beta}$	-2.65±1.01 $^{bc\alpha}$	-2.92±1.25 $^{c\beta}$	0.58±1.48 $^{d\beta}$	2.99±1.37 $^{e\beta}$
	14	0.78±1.14 $^{ab\alpha}$	-0.43±0.79 $^{ac\alpha}$	-0.71±1.06 $^{c\beta}$	-1.33±1.37 $^{c\gamma}$	1.51±2.01 $^{b\alpha\beta}$	4.75±1.81 $^{d\alpha}$
h	0	171.45±21.50 $^{a\alpha}$	190.65±15.85 $^{bc\alpha}$	203.19±10.65 $^{c\alpha}$	177.98±15.53 $^{ab\alpha}$	155.62±12.88 $^{d\alpha}$	136.60±9.30 $^{e\alpha}$
	7	201.66±12.96 $^{a\beta}$	209.63±8.18 $^{a\beta}$	212.47±11.83 $^{a\alpha}$	209.71±10.85 $^{a\beta}$	175.85±18.32 $^{b\beta}$	145.93±12.82 $^{c\alpha}$
	14	168.25±16.14 $^{a\alpha}$	187.08±12.51 $^{b\alpha}$	188.63±12.85 $^{b\beta}$	192.07±12.35 $^{b\alpha}$	164.96±18.61 $^{a\alpha\beta}$	134.58±12.30 $^{c\beta}$
C*	0	2.68±0.47 $^{a\alpha}$	3.94±0.21 $^{b\alpha}$	5.78±0.51 $^{c\alpha}$	6.08±0.57 $^{c\alpha}$	6.13±0.72 $^{c\alpha}$	6.82±1.25 $^{d\alpha\beta}$
	7	2.48±0.13 $^{a\alpha}$	3.37±0.23 $^{b\beta}$	4.93±0.36 $^{c\beta}$	5.90±0.76 $^{d\alpha}$	5.08±0.37 $^{c\beta}$	5.24±0.84 $^{c\beta}$
	14	3.55±0.47 $^{a\beta}$	3.58±2.11 $^{a\gamma}$	4.72±0.22 $^{b\beta}$	6.03±0.61 $^{cd\alpha}$	5.58±0.75 $^{c\alpha\beta}$	6.61±0.84 $^{d\alpha}$

All values were means ± standard deviation of ten values
Same letters (a, b, c, d) in the same row indicate no significant differences between means ($p \leq 0.05$)
Same symbol (α, β, γ) in the same column indicate no significant differences between means ($p \leq 0.05$)

Whatever pressure level, application of pressure on fillet induces an increase of L* value. From 300 MPa, L* is almost two times higher than the untreated sample: the fish looks like it is cooked. For the untreated sample and 100 MPa and 200 MPa samples, L* value changes in a significant way with the time of storage: it is higher on day 7 and decreases between days 7 and 14, but final day 14 value is higher than for the untreated sample. However, from 300 MPa, pressure-induced modifications of L* value change very slightly during storage. Thus lightness of sea bass fillet raises under high-pressure treatment and from 300 MPa, the increase is particularly important but does not change with time storage.

Table 1 shows a* changes with high-pressure level, from -2.48 for untreated sample to -4.79 for 500 MPa, and b* changes in diverse ways around its initial value 0.39. Changes of hue are better described considering λ value in CIE Yxy system. We noticed in table 1 that main wavelength increases in a significant way to greenish and almost yellowish at 400 MPa: hue changes from bluish to greenish and almost yellowish at 500 MPa. Of course fish appearances is neither blue nor green but these color terms indicate the trend of white translucent color. Changes of hue characteristics are not modified during storage. These results are in accordance with Ashie and Simpson (1996), who put in evidence an increase of L* from 0 to 300 MPa in bluefish, and with Oshima and others 1992).

The fish fillet color is linked with heme based pigment, physical structure of muscle and amount of unbound water which influences light scattering. As sea bass is a white fish, we can suppose that changes of pigments under high-pressure treatment are of minor importance, so we can attribute these changes to modifications of protein matrix. In the same way, evolution of color during storage can be associated with enzymatic and non enzymatic reactions resulting in degradation of myofibrillar proteins and disorganisation of myofibrils (Haard, 1992; Jiang, 2000).

3. 2. Exudation during storage

Figure 2 shows the evolution of fish fillet natural exudation with pressure treatment for the 3 storage times. Whatever pressure level, high-pressure treatment does not induce any variation of exudation first day. However considering results of exudation after storage, we can consider two groups. For samples treated at 100 MPa and 200 MPa exudation increases two times the seventh day and four times the fourteenth day. For samples treated at 300 MPa, 400 MPa and 500 MPa, exudation changes very few during storage. Then from 300 MPa yield is improved the seventh and the fourteenth day storage. The increase of exudation with storage time for control, 100 MPa and 200 MPa can be related with enzymatic and microbiological degradation of muscle which modifies muscle structure and leads to an important water release. High-pressure treatment above 200 MPa induces an irreversible denaturation of proteins than can reach a gelation. This phenomenon allows better natural water retention. High-pressure treatment above 200 MPa also induces modifications of proteases activity then causing indirectly an increase of water retention.

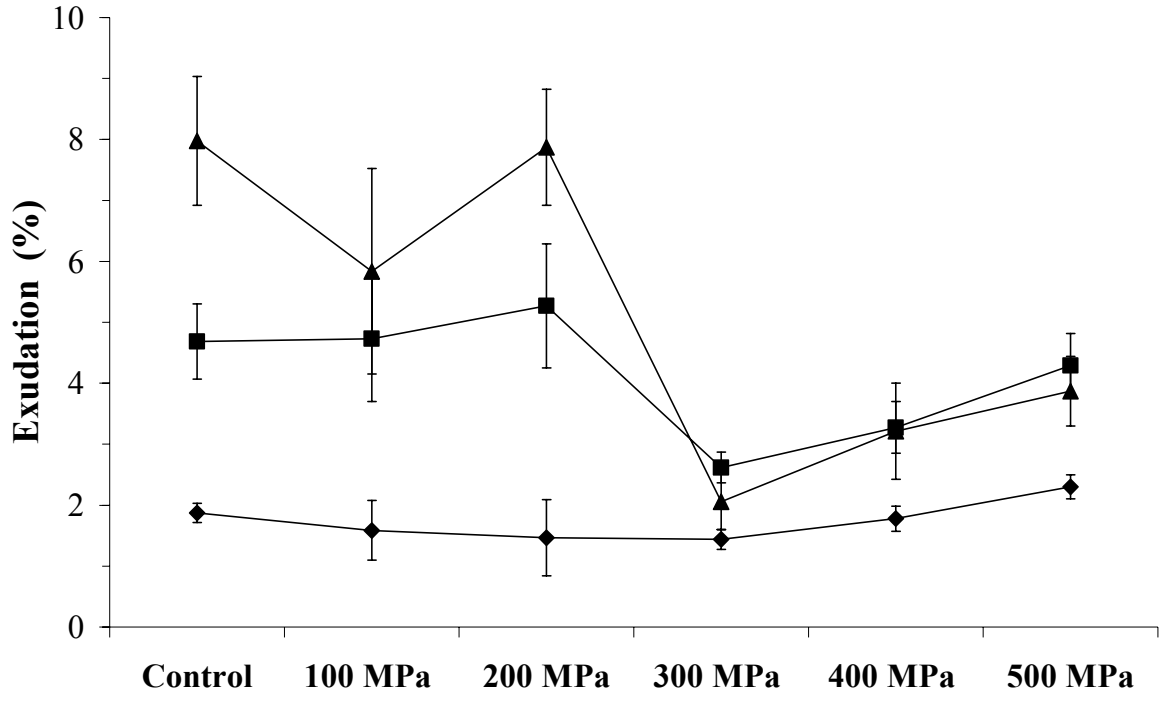


Fig. 2. Evolution of sea bass fillet exudation treated by high-pressure (5 min) after storage at 4°C during 0 (◆), 7 (■) and 14 (▲) days.

3.3. Water-Holding Capacity (WHC)

The evolution of WHC with pressure applied is presented figure 3. WHC evaluation requires a grinding and a centrifugation producing a forced water release. For untreated sample, storage induced a significant increase of WHC. As we previously underscored the increase of natural exudation with time of storage, it might appear an opposite result. In fact, WHC evaluate the ability of proteins to bind water after grinding. This particular property increases with storage. Figure 4 also shows that WHC decreases when pressure level increases, and that time of storage has no more a significant effect when sample is pressurized. Bremmer (2002) put in evidence this decrease of WHC in sole and Pollack, so our results are accordance because sea bass can also be considered as a non fat fish. Grinding before WHC evaluation destroys previously cited protein gel due to high-pressure treatment, and denatured proteins are no more able to bind intrinsic water.

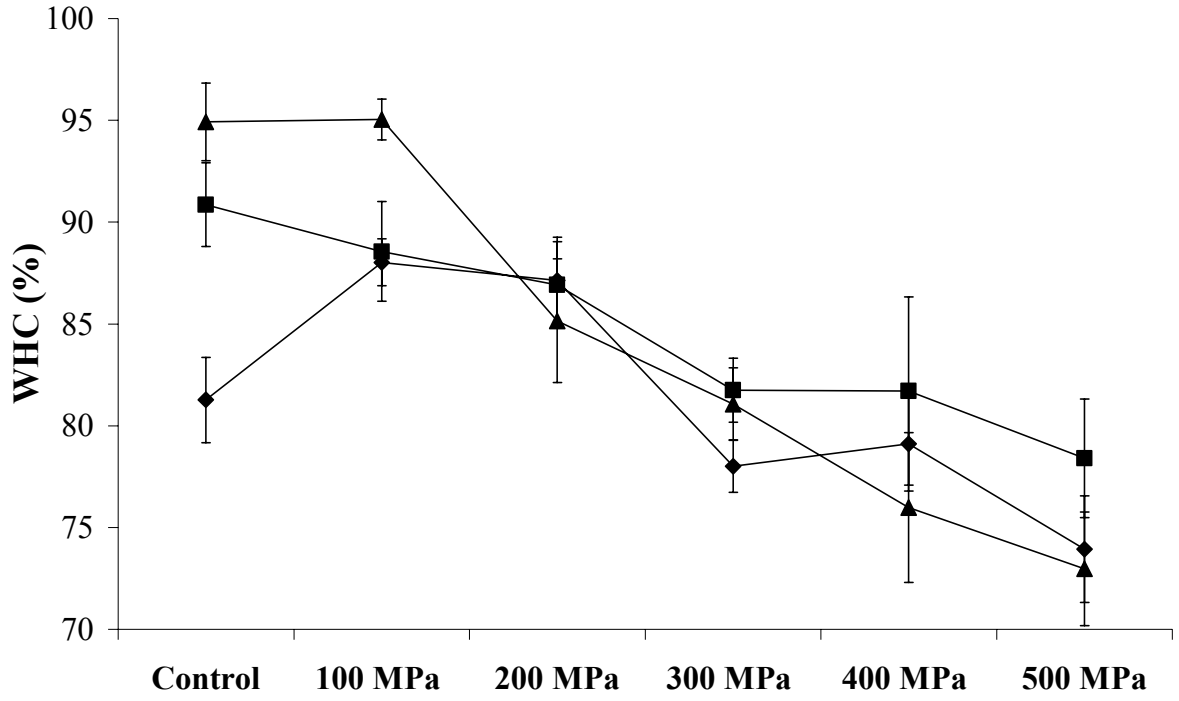


Fig. 3. Effect of storage at 4°C during 0 (◆), 7 (■) and 14 (▲) days, on water-holding capacity of sea bass fillets treated by high-pressure (5 min).

Denaturation of myofibrillar proteins, which are mainly involved in WHC, increases with increasing pressure. Thus, it is normal to observe a decrease of WHC with increasing pressure.

3.4. Modification of texture induced by high-pressure and ageing

Texture Profile Analysis (TPA) was carried out to determinate the effect of pressure treatment and storage for 0, 7 and 14 days on the texture of fish muscle. The results of textural measurements are summarized in table 2: hardness, cohesiveness, springiness, gumminess, resilience and chewiness.

For untreated sample, all six texture criteria decrease in a significant way between the first and the seventh day of storage, showing that muscle becomes softer. During storage at 4°C, enzymatic degradation induces many physical mechanisms, among them the gaping, which contribute to muscle tenderization (Dunajsky, 1979). Proteases implied are mainly calpains, cathepsins and collagenases. Calpains and cathepsins act on the myofibrillar proteins and promote myofibril fragility, and collagenases act on the connective tissue and contribute to gaping (Montero, 1997).

303 Table 2. **Texture Profile Analysis (TPA) parameters of sea bass fillets treated by high-pressure processing after 0, 7 and 14 days at 4 °C.**

304

			Pressure processing during 5 min				
TPA parameter	**Storage time (day)**	**Control**	**100 MPa**	**200 MPa**	**300 MPa**	**400 MPa**	**500 MPa**
Hardness	0	29.82±2.13 $^{a\alpha}$	24.17±3.99 $^{bc\alpha}$	22.78±2.86 $^{b\alpha}$	26.04±3.89 $^{c\alpha}$	31.24±2.24 $^{a\alpha}$	32.70±4.70 $^{a\alpha\beta}$
	7	25.53±4.32 $^{a\beta}$	27.50±3.49 $^{a\alpha}$	20.44±2.72 $^{b\alpha}$	26.39±2.22 $^{a\alpha}$	38.04±2.76 $^{c\beta}$	33.14±4.00 $^{d\beta}$
	14	25.25±3.50 $^{a\beta}$	24.55±4.49 $^{a\alpha}$	23.26±3.85 $^{ab\alpha}$	21.09±1.51 $^{b\beta}$	35.01±2.94 $^{c\gamma}$	28.93±3.38 $^{c\alpha}$
Cohesiveness	0	0.52±0.04 $^{a\alpha}$	0.53±0.06 $^{a\alpha}$	0.55±0.03 $^{ab\alpha}$	0.56±0.03 $^{ab\alpha}$	0.55±0.08 $^{ab\alpha}$	0.58±0.04 $^{b\alpha}$
	7	0.25±0.04 $^{a\beta}$	0.42±0.05 $^{bcd\beta}$	0.41±0.04 $^{bc\beta}$	0.40±0.05 $^{b\beta}$	0.45±0.07 $^{cd\beta}$	0.46±0.05 $^{d\beta}$
	14	0.30±0.04 $^{a\gamma}$	0.36±0.04 $^{b\gamma}$	0.40±0.04 $^{bc\beta}$	0.43±0.06 $^{c\beta}$	0.50±0.06 $^{d\alpha\beta}$	0.56±0.03 $^{e\alpha}$
Springiness	0	0.69±0.04 $^{a\alpha}$	0.63±0.05 $^{bc\alpha}$	0.61±0.04 $^{b\alpha}$	0.65±0.04 $^{cd\alpha}$	0.69±0.04 $^{ad\alpha}$	0.74±0.06 $^{e\alpha}$
	7	0.51±0.03 $^{a\beta}$	0.55±0.06 $^{ab\beta}$	0.59±0.05 $^{bc\alpha}$	0.54±0.05 $^{ab\beta}$	0.57±0.06 $^{b\beta}$	0.62±0.03 $^{c\beta}$
	14	0.47±0.04 $^{a\gamma}$	0.51±0.05 $^{b\beta}$	0.57±0.05 $^{cd\alpha}$	0.54±0.05 $^{bc\beta}$	0.60±0.03 $^{d\beta}$	0.66±0.04 $^{e\beta}$
Gumminess	0	15.32±1.22 $^{ab\alpha}$	12.67±1.99 $^{cd\alpha}$	12.60±1.52 $^{c\alpha}$	14.49±1.92 $^{ad\alpha}$	17.19±3.05 $^{be\alpha}$	18.97±2.33 $^{e\alpha}$
	7	6.35±1.47 $^{a\beta}$	11.39±1.23 $^{b\alpha}$	8.40±1.25 $^{c\beta}$	10.46±1.34 $^{b\beta}$	17.07±2.31 $^{d\alpha}$	15.21±2.35 $^{e\beta}$
	14	7.51±1.53 $^{a\beta}$	8.91±2.10 $^{ab\beta}$	9.36±2.00 $^{b\beta}$	9.13±1.42 $^{ab\beta}$	17.44±2.06 $^{c\alpha}$	16.24±1.16 $^{c\beta}$
Resilience	0	0.34±0.04 $^{ab\alpha}$	0.35±0.04 $^{b\alpha}$	0.32±0.04 $^{abc\alpha}$	0.31±0.02 $^{bc\alpha}$	0.30±0.06 $^{c\alpha}$	0.32±0.03 $^{abc\alpha}$
	7	0.13±0.02 $^{a\beta}$	0.23±0.04 $^{b\beta}$	0.18±0.02 $^{c\beta}$	0.18±0.03 $^{c\beta}$	0.22±0.04 $^{bd\beta}$	0.20±0.03 $^{cd\beta}$
	14	0.14±0.02 $^{a\beta}$	0.19±0.03 $^{b\gamma}$	0.17±0.03 $^{b\beta}$	0.18±0.03 $^{b\beta}$	0.23±0.03 $^{c\beta}$	0.25±0.02 $^{c\gamma}$
Chewiness	0	10.62±1.12 $^{ab\alpha}$	8.04±1.58 $^{cd\alpha}$	7.68±1.15 $^{c\alpha}$	9.45±1.31 $^{bd\alpha}$	11.92±2.46 $^{b\alpha}$	14.11±2.12 $^{e\alpha}$
	7	3.27±0.92 $^{a\beta}$	6.24±1.06 $^{b\beta}$	4.97±1.00 $^{b\beta}$	5.70±0.91 $^{b\beta}$	9.84±1.93 $^{c\beta}$	9.50±1.53 $^{c\beta}$
	14	3.49±0.69 $^{a\beta}$	4.57±1.24 $^{b\gamma}$	5.23±0.94 $^{b\beta}$	4.92±1.02 $^{b\beta}$	10.52±1.50 $^{c\alpha\beta}$	10.63±0.31 $^{c\beta}$

For each criterion, all values were means ± standard deviation of ten values
Same letter (a, b, c, d, e) in the same row indicate no significant differences between means ($p \leq 0.05$)
Same greek letter (α, β, γ) in the same column indicate no significant differences between means ($p \leq 0.05$)

305 Application of high-pressure processing induces modifications of TPA criteria, as
306 summarized in table 2. For samples at day 0, hardness decreases significantly from 100 MPa
307 to 300 MPa and remains constant after 400 MPa and 500 MPa treatment; cohesiveness,
308 springiness and resilience are almost constant, and gumminess and chewiness decreases from
309 100 to 300 MPa and increase after 400 MPa and 500 MPa treatment. Globally, TPA criteria
310 are diversely affected by high-pressure, and effect of high-pressure changes markedly around
311 300 MPa, in accordance with exudation and WHC results.
312 At day 0 decrease of hardness for samples treated at 100, 200 or 300 MPa, is in accordance
313 with results of Ashie and Simpson (1996) on bluefish, and differ from results of Anguspanish
314 and Ledward (1998) who observed an increase of cod fillet hardness for 400 MPa -20 min. In
315 regard to evolution of texture during storage, hardness does not change for 100 and 200 MPa
316 treated samples, it decreases the fourteenth day for 300 MPa, and it changes slightly for 400
317 and 500 MPa. Finally, we can assess than pressure treatment above 300 MPa – 5 min is
318 necessary to obtain after 7 or 14 days a hardness equivalent to the untreated sample at day 0,
319 and that samples treated at 100 or 200 MPa do not evolve during storage.
320 *Post mortem* changes of fish texture are mainly caused by modifications of myofibrillar
321 proteins, due both to proteases action and to variation of physical and chemical conditions.
322 High-pressure treatment induces a reversible pH fall (Hayert and others 1999) and a
323 modification of hydrogen and hydrophobic bonds which brings about changes in protein
324 structure. Effect of high-pressure on fish texture ensues from these modifications of water
325 bond, protease activity, aggregation or gelation of myosin and sarcoplasmic proteins
326 (Anguspanish and Ledward 1998; Heremans 1982).
327
328 *3.3. Effect of pressurisation on the microstructure*

329 The histological images of muscular fibres section are shown on the figure 4. Control
330 fibres at day 0 appear as shrunken cells, with a great extracellular space: this space is not the
331 result of gaping since preparation of sample is realized quickly after slaughtering. This
332 phenomenon is ascribed to the fixing and dehydration methods which could weaken links
333 between fibres and myocommata. Storage of fish fillets induces numerous cracks inside the
334 fibres, and a deterioration of pericellular connective tissue is visible after 14 days of storage.
335 This evolution of myofibrils structure is the result of natural degradation from proteases and
336 microorganisms. This observation is linked with evaluated hardness decrease during storage,
337 due to the action of proteases on collagen and myofibrillar proteins.
338 The fibres appearance is not changed when samples has been pressurized at 100 MPa.
339 From 200 MPa, the more the pressure treatment is high, the more the fibres look tightened
340 and round. The extracellular space decreases when pressure increases, in relation to the
341 compaction of muscle and the possible protein gel network formation. Moreover, the sample
342 preparation has less effect on extracellular spaces when the proteins began to form a gel.
343 Cheftel and Culioli (1997) reported that pressure above 200 MPa often causes protein
344 gelation, whenever pressure and protein concentration are high enough. These observations
345 corroborate the increase of hardness observed at 400 and 500 MPa: denser structure muscle
346 presents higher hardness values. Globally, the structure is not modified in comparison with
347 works of Gudmundsson and Hafsteinsson quoted by Bremner, 2002. From 200 MPa, we do
348 not observe any crack apparition during storage, and muscle remains more compact that for
349 control sample. Connective tissue presents an irregular distribution. Indeed the probable
350 proteases inactivation and the gelation of actomyosin above 200 MPa lead to a good
351 myofibrils structure preservation.

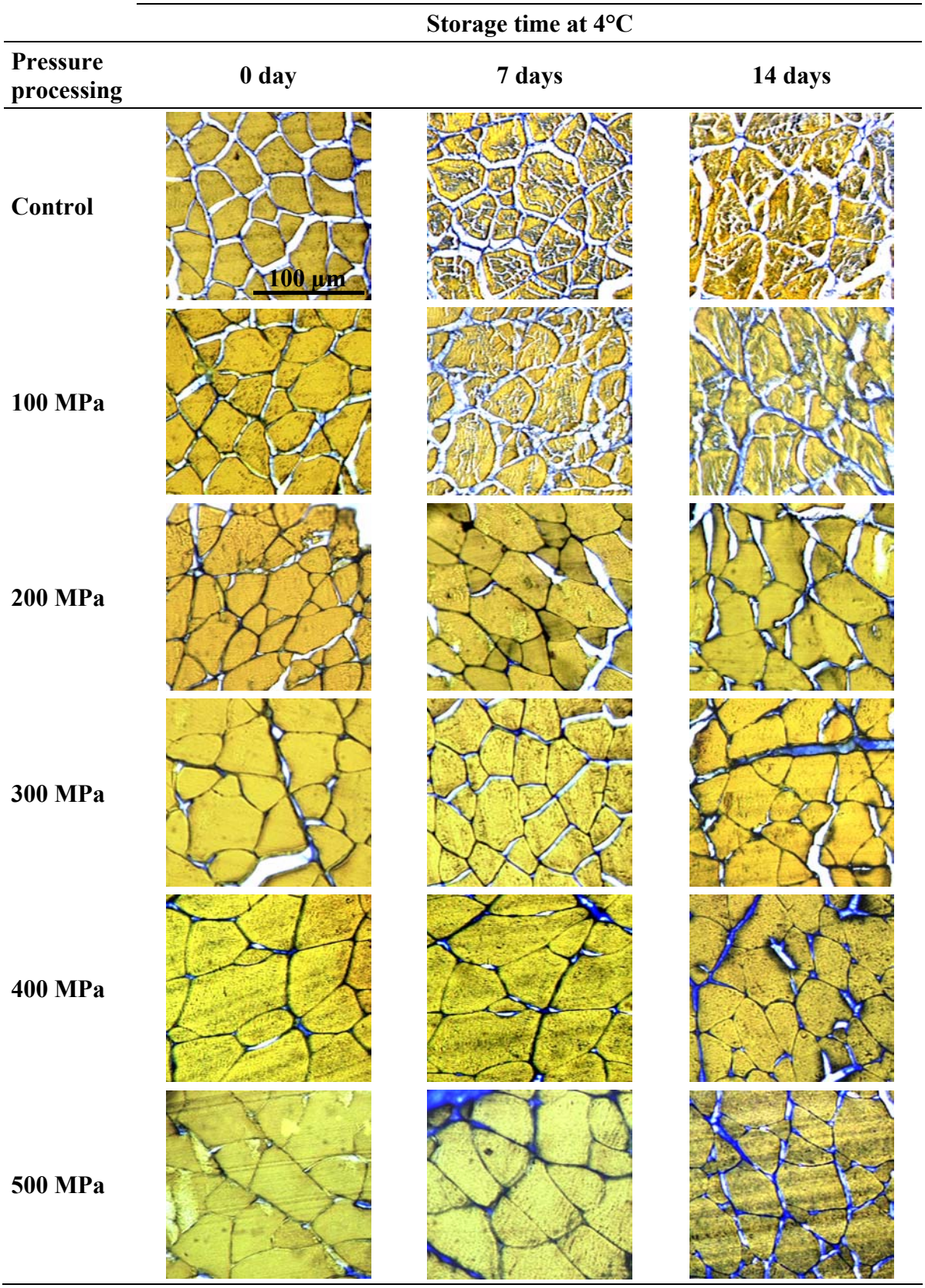
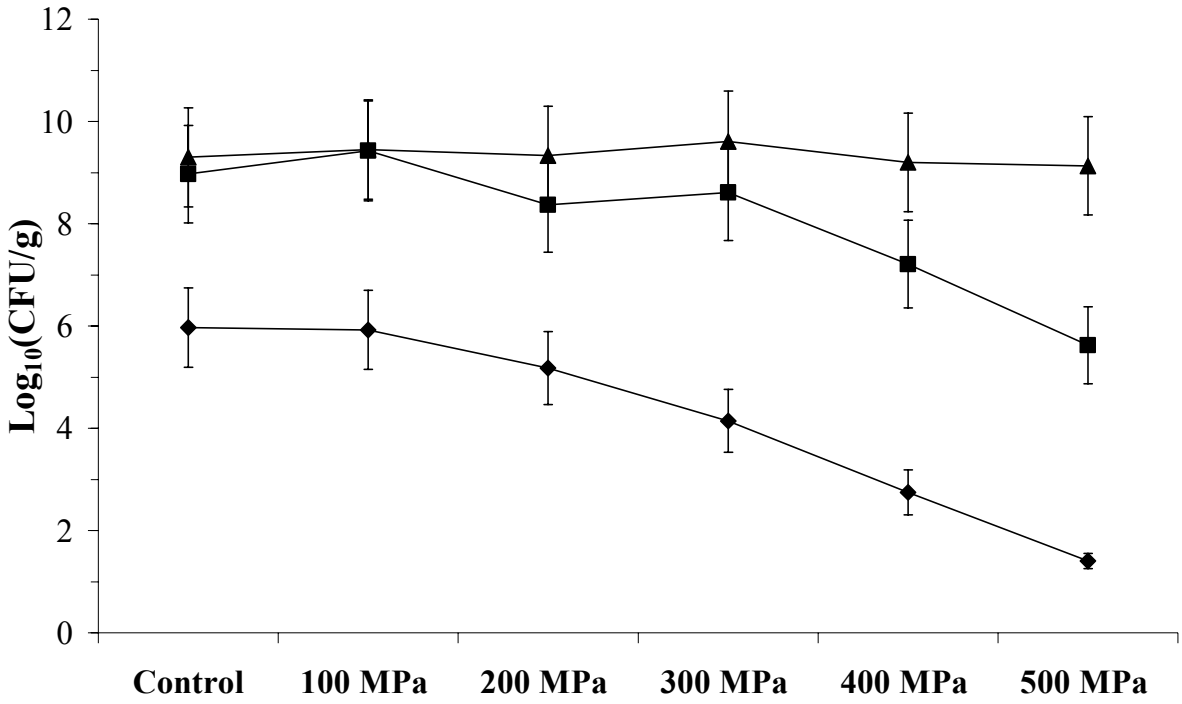


Fig. 4. Transverse sections of sea bass muscle treated by high-pressure processing and stored during 0, 7 and 14 days at 4°C. Muscle protein stains orange and collagen blue.

3.4. Impact of pressure processing on microbiological quality of fish muscle

The microbial flora is present on the skin and in the digestive system of the living fish. These micro-organisms contaminate the muscle after the fish dies. Thus in spite of hygiene precautions, total aerobic count of untreated sample reached 10^6 CFU/g at day 0, and around 10^9 CFU/g after 7 and 14 days of storage at 4°C. These results are in accordance with data from Food and Agriculture Organisation of the United Nations (Huss, 1995) reporting 10^8 to 10^9 CFU/g of fish flesh.

Figure 5 shows a significant decrease of total aerobic count when pressure increases from 200 MPa, reaching only 30 CFU/g for 500 MPa treatment. These results confirm the effect of high-pressure on the reduction of microbial growth (Cheftel, 1995), proving that high-pressure treatment is a powerful tool to improve microbiological quality of fresh food. When sample has been treated at 500 MPa, its total aerobic count after 7 days of storage is the same that the one of control sample at day 0: the shelf life has been extended for one week. However, whatever pressure treatment, at day 14, the total aerobic count reaches 10^9 CFU/g, which is probably the maximal contamination. Then, we underscored that high-pressure treatment above 300 MPa improved microbiological quality of fish fillet for at least seven days.



377
378

379

380 **Fig. 5. Total aerobic count variation during storage at 4°C during 0 (◆), 7 (■) and 14**
381 **(▲) days, of sea bass fillets treated by high-pressure processing**

382

383 **4. Conclusion**

384 Our results showed that the quality of the sea bass fillet can be improved by the high-pressure
385 treatment and that we had a remarkable structural preservation. From a microbiological point
386 of view, high-pressure increases the shelf-life. Moreover above 200 MPa high-pressure
387 decreases natural exudation, allowing an increase of the yield of net weight after storage.
388 Above 300 MPa high-pressure treatment allows also to keep hardness of fillet in a good range
389 for consumer who rejects soft fish flesh. Nevertheless color of fish changes with high-
390 pressure treatment, but this difference probably vanishes after cooking: as fish is mainly eaten
391 after cooking, this drawback could be minor in comparison with the enhancement of

392 microbiological and textural quality. Further studies on proteases and their behaviour under
393 pressure are necessary to understand the ageing of fish fillets after high-pressure treatment.
394
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399

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