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# Quality changes during the frozen storage of sea bass (*Dicentrarchus labrax*) muscle after pressure shift freezing and pressure assisted thawing

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ABSTRACT

Quality of frozen sea bass muscle stored (1, 3 and 5 months) at two levels of temperature (−15 and −25 °C) after a pressure shift freezing process (200 MPa) — PSF — and/or a pressure assisted thawing process (200 MPa) — PAT — was evaluated in comparison with samples frozen and thawed using conventional methods (air-blast AF and AT, respectively). Frozen storage of high-pressure treated samples did not significantly affect initial quality of frozen muscle. Thus, parameters related to protein denaturation and extractability, water holding capacity and color presented similar values than those obtained for not stored samples. In addition, the improvement of the microstructure achieved by PSF application remains unchanged during frozen storage. On the other hand, conventional treated samples experienced significant changes during frozen storage, such as protein denaturation, and water holding capacity and color modifications. Storage temperatures did not have influence in the quality of PSF and PAT samples, but it showed some effects in AF muscle.

*Industrial relevance:* This work demonstrates the potential application and benefits of high pressure (HP) in the freezing and thawing of fish meat in comparison to conventional methods, due to an improvement on the cellular integrity of the tissue. Although some negative effects are produced during processing with HP, no additional modifications occur during the frozen storage. The studied methodologies seemed to be very suitable for fish freezing and thawing, especially for products which will be frozen stored and/or cooked.



## 1. Introduction

Freezing is a widely used preservation method for fish and other seafood, due to the microbiological growth and some biochemical processes can be minimized. However, deteriorative changes of quality occur during freezing, frozen storage and thawing of fish, such as flavor, odor, texture and color changes (Matsumoto, 1979). One of the main aspects related to the frozen fish deteriorative changes is the myofibrillar protein denaturation, which can lead to textural and functional changes in the frozen fish (Matsumoto, 1979). Ice crystal size is an important factor related to muscle deterioration, because the formation of large ice crystals leads to an extensive mechanical damage; as a consequence, interaction of cellular components — lipids and proteins — with enzymes leads to protein denaturation and lipid degradation (Shenouda, 1980). The size and location of ice crystals depend on the freezing rate and final temperature. Slow freezing induces a low nucleation rate forming only a few nuclei and producing large crystals; while if the freezing rate is high, many nuclei are formed and in consequence the crystals are smaller (Martino, Otero, Sanz & Zaritzky, 1998). During thawing, which generally proceeds more slowly than freezing because of the small temperature difference between the initial freezing point and the room temperature, additional damages to frozen tissue can occur (Shubring, Meyer, Schlüter, Boguslawski & Knorr, 2003; Zhu, Ramaswamy & Simpson, 2004a).

To improve the final quality of frozen foods, process incrementing both the freezing and thawing rates are currently evaluated. In this sense, high-pressure application is a promising alternative. The fact that water remains in a liquid state down to −22 °C at a pressure of 210 MPa (Bridgman, 1912), permits to obtain a very rapid and uniform ice nucleation when a sample is exposed to a high pressure, then cooled to a temperature just above the freezing point at the applied pressure, and finally the pressure is suddenly released. This process, called pressure shift freezing (PSF), leads to an instantaneous and uniform nucleation throughout the sample due to the supercooling resulted, producing small ice crystals (Cheftel & Culioli, 1997; Chevalier, Sequeira-Munoz, Le Bail, Simpson & Ghoul, 2000a; Zhu, Le Bail, Ramaswamy & Chapleau, 2004b). In the case of the thawing process, the application of high pressure allows to enhance the temperature difference between the frozen sample and the surrounding, which produces an increase of the driving force and consequently of the rate of thawing (Zhu et al., 2004a; Cheftel & Culioli, 1997).

High-pressure application during the freezing or thawing of diverse food products has been studied. Effect of pressure shift freezing has been analyzed in pork meat (Martino et al., 1998; Fernandez-Martín, Otero, Solas & Sanz, 2000; Zhu et al., 2004b; Zhu, Le Bail, Chapleau, Ramaswamy & de Lamballerie-Anton, 2004c), bovine muscle (Zhu et

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al., 2004b), and sea products (Chevalier et al., 2000a, Chevalier, Sequeira-Munoz, Le Bail, Simpson & Ghoul, 2000b; Zhu, Le Bail & Ramaswamy, 2003), demonstrating an improvement in the tissue microstructure respect to conventional methods due to the smaller size of the ice crystals. When high pressure has been applied during the thawing process of beef (Makita, 1992; Zhao, Flores & Olson, 1998), pork (Okamoto & Suzuki, 2002) and fish (Chevalier, Le Bail, Chourot & Chantreau, 1999; Murakami, Kimura, Yamagishi, Yamashita, Sugimoto & Satake, 1992; Shubring et al., 2003), the main advantages are a minor thawing time, a reduction of the drip losses and a partial destruction of microbial flora. However, high-pressure application produces deleterious effects, being the more evident the color and texture changes. In particular, fish myofibrillar proteins presented denaturation and aggregation due to high-pressure application (Chevalier et al., 2000b; Ohshima, Ushio & Koizumi, 1993).

In a previous work, authors studied the effect of the high-pressure application during the freezing or/and thawing of sea bass (*Dicentrarchus labrax* L.) (Tironi, de-Lamballerie & Le Bail, 2007). Improvement on the muscle microstructure and, in opposite, a deleterious effect of high pressure on the structure and extractability of proteins as well as on the water holding capacity and color of the muscle, could be evidenced in frozen and rapidly thawed muscle. However, due to several quality modifications occur along the frozen storage, it is important to analyze the fish muscle after this period. At the moment, there are only a few studies presenting results related to the evolution of pressure shift frozen fish muscle during storage (Chevalier et al., 2000a,b). This work is bringing new data in this matter. It is know that glass formation has an important influence within the processing and preservation of foods, due to any type of properties related to molecular mobility, including texture and shelf-life, are affected strongly by the glass transition behavior. Several deterioration processes in foods are significantly reduced due to the compounds involved take many months or even years to diffuse over molecular distances to can react (Slade & Levine, 1991). In a previous work, authors have studied the glass transition of sea bass muscle, obtaining a Tg′ value of around −15 °C (Tironi, de Lamballerie-Anton & Le Bail, 2009). Changes in some sea bass quality parameters during frozen storage at two temperature levels — around (−15 °C) and below (−25 °C) the glass transition temperature — has been evaluated and compared with conventionally frozen and/or thawed samples to have a more general evaluation of the real influence of the high-pressure processes on the final quality of frozen fish.

## 2. Materials and methods

### 2.1. Materials

Sea bass (*Dicentrarchus labrax* L.) fishes (weight around 800 g, length around 40 cm) were obtained from a fish farm (St-Clément des Baleine, Ile de Ré, France); transported to the laboratory in ice, slaughtered, cleaned, skinned and filleted within 8 h. Each fillet was divided in 3 samples of around 30 g. Samples were placed in polyethylene bag (2 samples/bag), vacuum-packed and stored at 5 °C up to the freezing treatment.

### 2.2. Freezing processes

Two freezing processes were applied: *air-blast freezing* (conventional method) was performed in a pilot freezer (Servathin, Poissy, France) at −30 °C using an air speed 4 m/s, during 2 h (AF samples); *pressure shift freezing* (PSF samples) was carried out in a 3.5 L reactor unit (ACB Pressure Systems, Nantes, France) equipped with temperature and pressure regulator device. The temperature of the transmitting medium (ethanol/water 50/50 %v/v) was maintained at −18 °C. Samples were placed in the vessel and the pressure was increased to 200 MPa at a rate of 3 MPa/s. When the temperature of samples reached −18 °C, pressure was rapidly released (2 s) to initiate the nucleation process. After depressurization, samples were left in the vessel for completion of the freezing under atmospheric conditions.

### 2.3. Frozen storage

Frozen samples were transported in a heat insulating container and stored at two temperature levels: $-15 \pm 2$ °C and $-25 \pm 2$ °C, during 1, 3 or 5 months.

### 2.4. Thawing processes

For each storage time (0, 1, 3 and 5 months), two thawing procedures were performed for each frozen system: *air-blast thawing* AT (conventional method) ($T = 4$ °C, air speed: 4 m/s); and *pressure assisted thawing* PAT applying a pressure of 200 MPa (increasing rate: 3 MPa/s) during 20 min, maintaining the medium transmitting temperature at 20 °C; after that pressure was rapidly released. Thus, for each storage time/temperature condition the following systems were analyzed: AF/AT, AF/PAT, PSF/AT, PSF/PAT. All systems were stored at 4 °C until analysis.

### 2.5. Microstructure analysis

Small pieces (approximately $5 \times 5 \times 10$ mm) were cut transversally to the fibers of frozen muscle inside a chamber at −20 °C. The isothermal freeze substitution technique (Martino & Zaritzky, 1986) was carried out with some modifications in these samples to visualize the size and location of the ice crystals. Pieces (3 pieces/sample, 2 samples/system) were placed in a fixative solution at −20 °C (Carnoy solution: 60% ethanol absolute, 30% chloroform, and 30% glacial acetic acid). In the case of unfrozen samples (F), cuts and fixation were performed in a chamber at 5 °C. After fixation (24–48 h), samples were brought to room temperature, dehydrated with ethanol absolute (2 h), ethanol:toluene (50:50 %v/v) overnight and treated with toluene (4–5 h). Dehydrated samples were consecutively immersed in toluene/paraffin solutions of increasing paraffin concentration at 60 °C (1 h each) and in paraffin pure (2 h). Finally, samples were embedded in paraffin in small molds. Ten μm thick sections (4 sections/piece) were obtained with a microtome (SM-2000-R, Leica Microsystems, Bensheim, Germany) and fixed to glass plates with glycerin albumin in water (1/25 v/v), heating at 57 °C to melting the paraffin. After paraffin removing (toluene, twice, 10 min), solvent elimination (ethanol absolute) and rehydration (ethanol:water and pure water), sections were stained. First, samples were treated for 2 min with Orange G (0.5 g Orange G, 1 ml acetic acid, 99 ml distilled water, filtered at 0.45 μm), which stained muscle proteins orange. After washing with distilled water, a second staining with Aniline blue for 2 min (0.01 g Aniline blue, 1 ml acetic acid, 99 ml distilled water, filtered at 0.45 μm) was performed (for collagen staining) (Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis & Lamballerie, 2006). Stained sections were mounted with Eukitt (Labonord, France). Observations were carried out in a microscope (Leica DML, Germany) equipped with a CCD RGB camera (MACC-C71, Sony, Japan).

### 2.6. Differential scanning calorimetry (DSC)

Muscle samples (810–850 mg wet weight) were analyzed by differential scanning calorimetry (MicroDSC III, Setaram, Caluire, France), using distilled water as reference. Heating between 20 and 100 °C was performed at 1 °C/min and then, a temperature decrease step was applied. Denaturation enthalpies were estimated by measuring the corresponding areas under the DSC transition curve. Specific areas (partial area/total mg of dry sample) were calculated. Duplicate samples were analyzed.

### 2.7. Protein extractability

2.50 g of minced muscle were homogenized with 25.0 ml of 0.03 M Tris–HCl, pH = 7.0 buffer solution in an Ultraturrax for 15 s. After centrifugation at 7000 *g*, 15 min, 4 °C (GR 20.20, Jouan, France) the supernatant was separated. The pellet was treated with 25.0 ml of the same buffer solution, repeating the procedure previously described. The two supernatants obtained were mixed resulting on the *water extractable proteins* (*WEP*). The remaining pellet was homogenized with 25.0 ml of 0.03 M Tris–HCl, 0.6 M KCl, pH = 7 buffer solution. The homogenate was centrifuged (7000 *g*, 15 min, 4 °C), separating the supernatant. This operation was carried out two times and the supernatants mixed. This fraction was called the *salt extractable proteins* (*SEP*). Extraction was performed from three independent samples. Protein concentration in the extracts was determined by the Biuret method (Gornall, Bardawill & David, 1949); and polypeptide composition was analyzed by SDS-PAGE as was previously described (Tironi et al., 2007).

### 2.8. Water holding capacity (WHC)

WHC was determined in raw and cooked (65 °C, 15 min) muscle by centrifugation of 10 g of minced muscle at 10,000 *g* for 15 min at 20 °C (GR 20.20, Jouan, France), inverting then the tubes allowing the liquid drain during 30 min. WHC was expressed as the percentage of retained liquid respect to the initial water content (which was determined by drying at 105 °C, 24 h). Three replicate of samples were analyzed.

### 2.9. Color determinations

Color parameters were determined using a Minolta CM-3500d (Minolta, Carrières-sur-Seine, France), under the CIE Lab system. In this system, L* is related to lightness (0–100 scale, black to white); a* correspond to red color if it is positive and to green color if negative; and b* indicate yellow color if its value is positive and blue color if it is negative. Five measures were carried out on different positions of two samples.

### 2.10. Statistical analysis

DSC, protein extractability, WHC and color data were analyzed by means of the analysis of variance (ANOVA) according to the General Linear Model Procedure. When differences were significant ($p<0.05$), Least Significant Difference (LSD) method was used to evaluate mean values differences, according to the Fisher test. Statistical analysis was performed using a Statgraphics plus version 5.0 software (Statistical Graphics Corp., Princeton NJ, USA).

## 3. Results and discussion

### 3.1. Microstructural analysis

Isothermal freezing substitution technique permits to evaluate the size and location of the ice crystals in frozen tissues by observing the holes left by them. In a previous work, AF and PSF sea bass was analyzed in comparison with fresh muscle (Tironi et al., 2007), observing very important differences in the ice crystals formation between both freezing techniques. It was possible to conclude that in PSF samples neither shrinkage nor deformation of cells was evident, only some changes in the shape of cells were observed which can be explained by the presence of extracellular ice. Meanwhile, AF muscle presented seriously deformed and shrunken muscle fibers, with large intra and extracellular ice crystals. In the present work, the evolution of

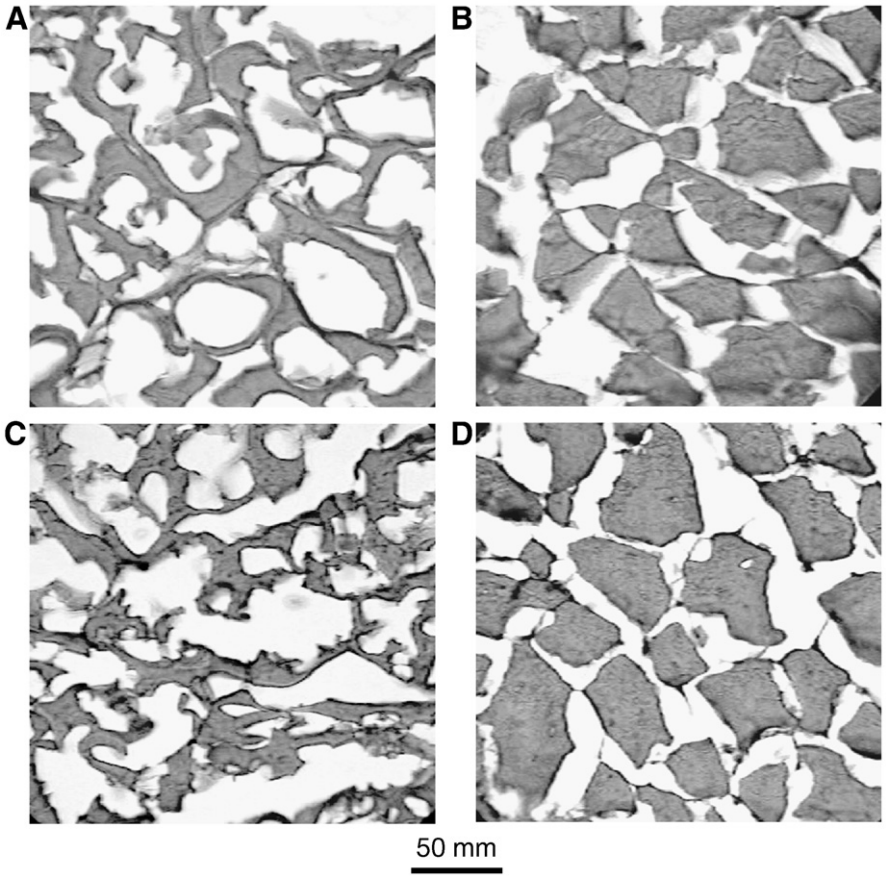


**Fig. 1.** Transversal sections of sea bass muscle: AF after 3 months at −15 °C (A), and 3 months at −25 °C (C); PSF after 3 months at −15 °C (B), and 3 months at −25 °C (D). White = whole left by the ice; gray = fish tissue.

microstructure along the frozen storage is analyzed. Fig. 1A and B shows micrographs corresponding to 3 months at −15 °C (AF and PSF, respectively), while Fig. 1C and D corresponds to 3 months at −25 °C (AF and PSF respectively). It is possible to mention that any of these systems presented evident microstructural changes respect to the corresponding time 0 samples (Tironi et al., 2007). Results were similar for 1 and 5 months of storage at both temperatures. These facts suggested that the ice crystals formed during each freezing process (AF and PSF) did not experience a notorious evolution in size and shape during the frozen storage at any temperature assayed. In function of this, the microstructural improvement obtained by application of a pressure shift freezing process (related to conventional air-blast freezing) would be maintained during the frozen storage at −15 and −25 °C. It is important to notice that temperature fluctuations during frozen storage can induce ice recrystallization, increasing the mean crystal diameter and reducing the number of crystals (Fernández, Otero, Martino, Molina-García, & Sanz, 2008). In this way, the benefits obtained by PSF process could be lost during the frozen storage. However, according to the present microscopic study, this process could not be observed in sea bass muscle. These results are in agreement with those obtained by Chevalier et al. (2000a) in turbot fillets stored at −20 °C during 75 days where, similarly to our work, not increment of the ice crystal size could be evidence neither in AF or PSF samples. These authors attributed their results to the isothermal conditions employed and the "relatively short" storage time; however, storage time was longer in our case. Fernández et al. (2008) observed a similar time evolution in the size of the AF and PSF ice crystals in a liquid food model, however, differences in the shape evolution of crystals have been detected, which can be ascribed to small differences in the initial distribution. This solution model allowed ice crystals to grow in the absence of structural obstacles inherent to the cellular tissue of structured food (Fernández et al., 2008). Thus, recrystallization process could be restrict in muscle food because of the structural elements present in these samples

### 3.2. Differential scanning calorimetry (DSC)

Typical DSC sea bass muscle thermal behavior, as well as the changes in the thermograms due to the diverse freezing/thawing processes applied, were analyzed in a previous work (Tironi et al., 2009). The main facts observed were denaturation of the myosin and the sarcoplasmic proteins when either PSF or PAT were performed, while, AF and AT processes had not influence on the protein structures. However, after frozen storage, some changes could be observed. Fig. 2 shows thermograms corresponding to AF/AT and AF/PAT systems stored at −15 and −25 °C. Conventionally frozen and thawed muscle (Fig. 2A and B) did not show notorious changes in the shape of the peaks, except after 5 months at −25 °C, where the myosin (peak 1) presented one only broad transition and the actin peak did not appear. However, protein denaturation was evident after frozen storage of these systems. Denaturation enthalpy corresponding to peak 1 ($\Delta H_1$) — mainly myosin — decreased as a function of the storage time; diminution was significant ($p<0.05$) after 1 month in the case of storage at −15 °C (Table 1) and after 5 months for the storage at −25 °C (Table 1), with similar values for both temperatures at this time. $\Delta H_2$ — associated with sarcoplasmic proteins — presented a small decrease as a function of the storage time, especially at −15 °C (Table 1), while $\Delta H_3$ (actin) did not show significant changes, except after 5 months at −25 °C where the corresponding peak could not be

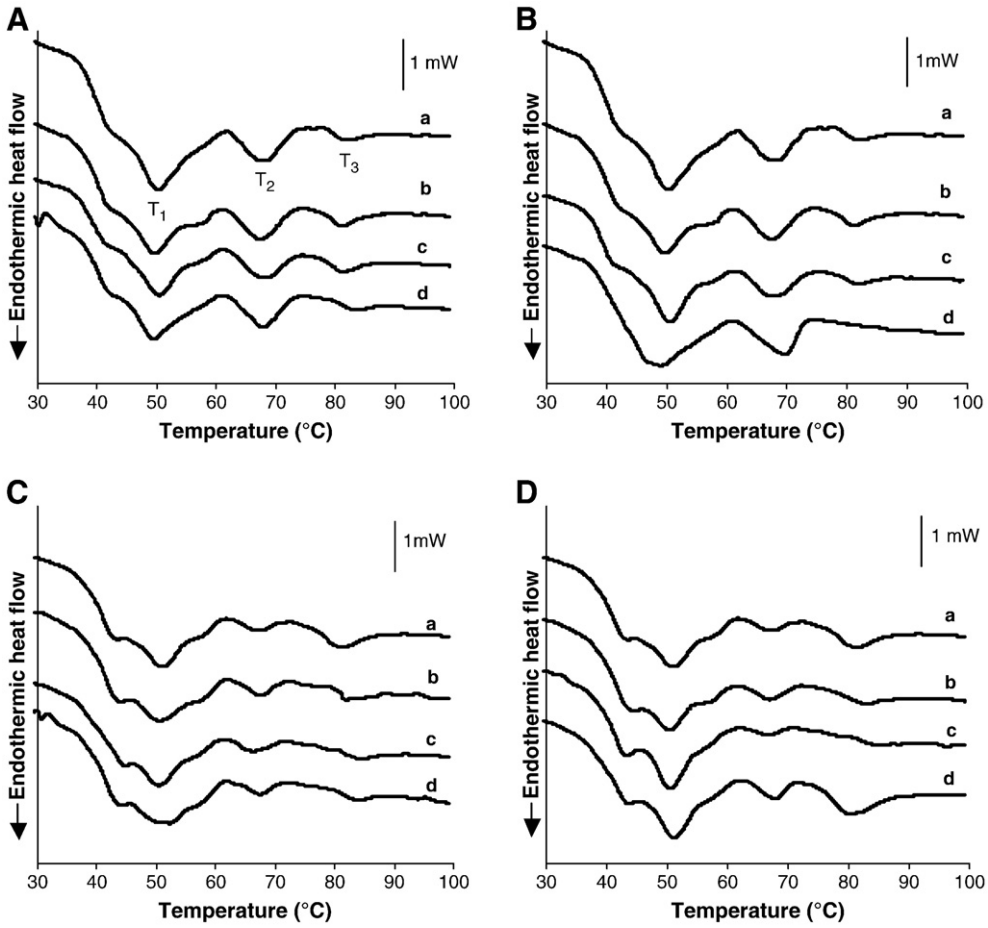


**Fig. 2.** DSC thermograms of air-blast freezing sea bass muscle: A) AF/AT, −15 °C; B) AF/AT, −25 °C; C) AF/PAT, −15 °C; and D) AF/PAT, −25 °C; stored at the corresponding temperature during: (a) 0, (b) 1, (c) 3, and (d) 5 months. Denaturation temperatures corresponding to myosin ($T_1$), sarcoplasmic proteins ($T_2$) and actin ($T_3$) are indicated.

**Table 1**
Denaturation entalphies corresponding to myosin ($\Delta H_1$), sarcoplasmic proteins ($\Delta H_2$), actin ($\Delta H_3$) and total proteins ($\Delta H_t$) of sea bass muscle processing by diverse combinations of freezing and thawing methods and stored at −15 and −25 °C.

| System | Storage time (months) | $\Delta H_1$ (J/g) | | $\Delta H_2$ (J/g) | | $\Delta H_3$ (J/g) | | $\Delta H_T$ (J/g) | |
|---|---|---|---|---|---|---|---|---|---|
| | | −15 °C | −25 °C | −15 °C | −25 °C | −15 °C | −25 °C | −15 °C | −25 °C |
| AF/AT | 0 | 6.16 (0.20)$^{a}$ | 6.16 (0.20)$^{a}$ | 0.99 (0.08)$^{a,b}$ | 0.99 (0.08)$^{a}$ | 0.23 (0.10)$^{b}$ | 0.23 (0.10)$^{b}$ | 7.41 (0.30)$^{a,c}$ | 7.41 (0.30)$^{a,c}$ |
| | 1 | 4.15 (0.13)$^{b}$ | 5.62 (0.23)$^{a,c}$ | 0.94 (0.07)$^{b}$ | 1.08 (0.06)$^{a}$ | 0.15 (0.00)$^{b}$ | 0.42 (0.05)$^{a}$ | 5.10 (0.24)$^{b}$ | 6.80 (0.28)$^{c}$ |
| | 3 | 4.66 (0.61)$^{b,c}$ | 5.45 (0.50)$^{a,c}$ | 0.81 (0.11)$^{c}$ | 0.89 (0.00)$^{b,c}$ | 0.34 (0.05)$^{b,a}$ | 0.30 (0.01)$^{b}$ | 5.57 (0.55)$^{b}$ | 6.42 (0.50)$^{c}$ |
| | 5 | 4.85 (0.09)$^{b,c}$ | 4.75 (0.16)$^{b,c}$ | 0.98 (0.06)$^{b}$ | 1.04 (0.01)$^{a}$ | 0.23 (0.03)$^{b}$ | Nd | 5.90 (0.07)$^{b}$ | 5.78 (0.10)$^{b}$ |
| AF/PAT | 0 | 5.18 (0.19)$^{c}$ | 5.18 (0.19)$^{c}$ | 0.24 (0.01)$^{d,e}$ | 0.24 (0.01)$^{d,e}$ | 0.72 (0.10)$^{c}$ | 0.72 (0.10)$^{c}$ | 6.14 (0.28)$^{c,b}$ | 6.14 (0.28)$^{c,b}$ |
| | 1 | 5.10 (0.15)$^{c}$ | 5.03 (0.35)$^{c}$ | 0.32 (0.07)$^{d}$ | 0.32 (0.05)$^{d}$ | 0.27 (0.12)$^{b}$ | 0.28 (0.09)$^{b}$ | 5.49 (0.05)$^{b}$ | 5.42 (0.28)$^{b}$ |
| | 3 | 4.87 (0.03)$^{c}$ | 5.02 (0.24)$^{c}$ | 0.27 (0.03)$^{d}$ | 0.17 (0.04)$^{d}$ | 0.31 (0.03)$^{b}$ | 0.21 (0.00)$^{b}$ | 5.21 (0.05)$^{b}$ | 5.25 (0.20)$^{b}$ |
| | 5 | 3.99 (0.02)$^{b}$ | 4.71 (0.36)$^{c,b}$ | 0.24 (0.03)$^{d}$ | 0.37 (0.01)$^{d}$ | 0.40 (0.05)$^{a}$ | 1.04 (0.09)$^{e}$ | 4.35 (0.06)$^{d}$ | 5.38 (0.32)$^{b}$ |
| PSF/AT | 0 | 4.77 (0.05)$^{c}$ | 4.77 (0.05)$^{c}$ | 0.12 (0.01)$^{e}$ | 0.12 (0.01)$^{e,f}$ | 0.26 (0.08)$^{b}$ | 0.26 (0.08)$^{b}$ | 5.15 (0.05)$^{b}$ | 5.15 (0.05)$^{b}$ |
| | 1 | 5.34 (0.07)$^{c}$ | 5.17 (0.01)$^{c}$ | 0.30 (0.00)$^{d}$ | 0.14 (0.08)$^{e}$ | 0.61 (0.02)$^{c,d}$ | 0.18 (0.04)$^{b}$ | 5.80 (0.07)$^{b,c}$ | 5.36 (0.10)$^{b,c}$ |
| | 3 | 4.37 (0.11)$^{c,b}$ | 4.33 (0.23)$^{c,b}$ | 0.25 (0.01)$^{d}$ | 0.14 (0.00)$^{e}$ | 0.69 (0.07)$^{c,d}$ | 0.42 (0.00)$^{a}$ | 4.82 (0.14)$^{b,d}$ | 4.59 (0.23)$^{b,d}$ |
| | 5 | 4.06 (0.11)$^{b}$ | 4.75 (0.18)$^{c,b}$ | 0.15 (0.01)$^{e}$ | 0.11 (0.00)$^{e}$ | 0.57 (0.01)$^{a}$ | 0.55 (0.07)$^{a}$ | 4.38 (0.11)$^{d}$ | 5.01 (0.20)$^{b,d}$ |
| PSF/PAT | 0 | 4.63 (0.20)$^{c}$ | 4.63 (0.20)$^{c}$ | 0.18 (0.02)$^{e,d}$ | 0.18 (0.02)$^{e,d}$ | 0.43 (0.06)$^{a}$ | 0.43 (0.06)$^{a}$ | 5.24 (0.25)$^{b}$ | 5.24 (0.25)$^{b}$ |
| | 1 | 4.14 (0.28)$^{c,b}$ | 4.56 (0.05)$^{c,b}$ | 0.16 (0.05)$^{e,d}$ | 0.20 (0.03)$^{e,d}$ | 0.23 (0.09)$^{b}$ | 0.58 (0.06)$^{d}$ | 4.37 (0.21)$^{d}$ | 4.91 (0.01)$^{b,d}$ |
| | 3 | 4.24 (0.28)$^{c,b}$ | 4.03 (0.31)$^{c}$ | 0.18 (0.01)$^{e,d}$ | 0.05 (0.01)$^{f}$ | 0.58 (0.04)$^{d}$ | 0.52 (0.05)$^{d,a}$ | 4.58 (0.26)$^{b,d}$ | 4.22 (0.30)$^{d}$ |
| | 5 | 3.90 (0.48)$^{b,d}$ | 3.51 (0.06)$^{d}$ | 0.12 (0.00)$^{e}$ | 0.12 (0.01)$^{e}$ | 0.54 (0.09)$^{a}$ | 0.47 (0.04)$^{a}$ | 4.17 (0.45)$^{d}$ | 3.80 (0.05)$^{d}$ |

Each value is represented as the mean of two determinations, showing the SD in parentheses. For each parameter, different letters (a, b, etc.) indicate significant differences among systems ($p<0.05$). Nd: not detected.

detected (Table 1). AF/PAT samples (Fig. 2C and D) did not present evident changes in the thermograms respect to the time 0; only after 5 months at −15 °C, myosin peak presented some changes, being the main transition less important (Fig. 2C). In this case, enthalpies did not show changes because of the frozen storage at any temperature (Table 1).

When PSF and AT were applied, no changes were registered neither in the shape of thermograms (Fig. 3A and B) nor in the denaturation enthalpies (Table 1) with not effect of the storage temperature, suggesting not additional structural changes due to the frozen storage. However, in PSF/PAT systems, some changes were evident after storage. At −15 °C, the principal transition (around 50 °C) in the peak 1 became

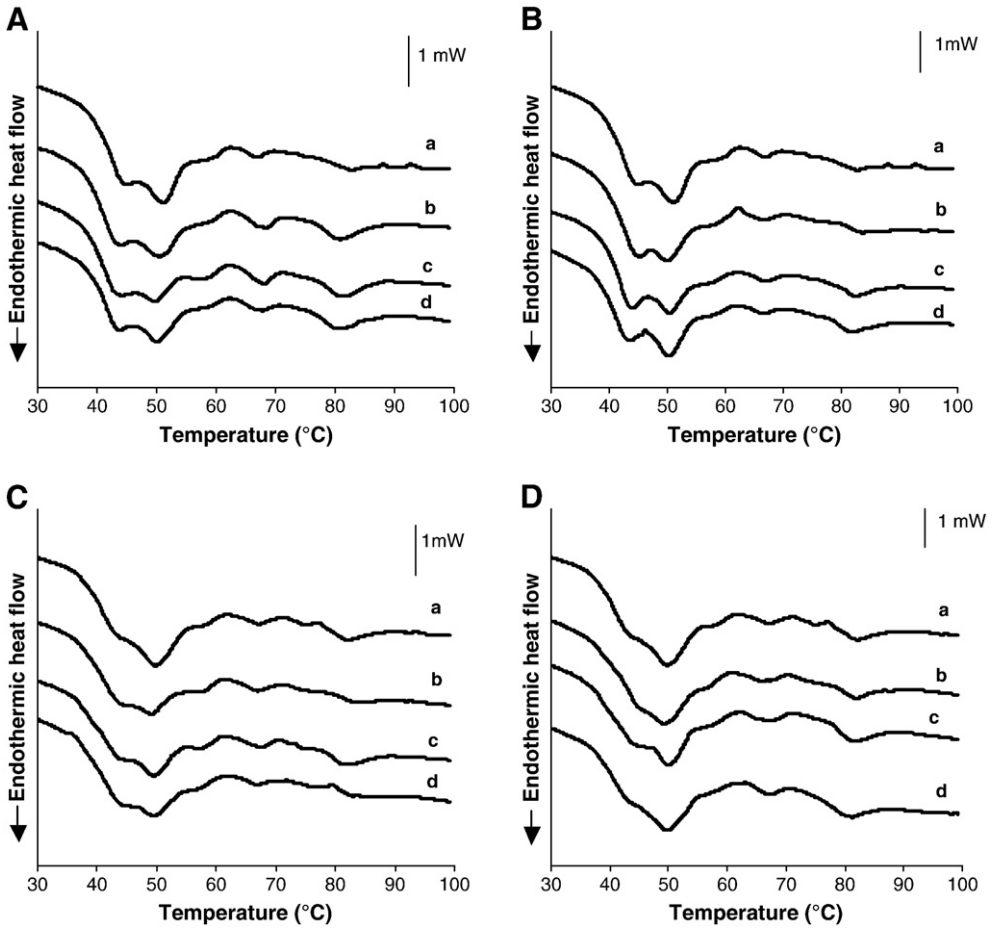


**Fig. 3.** DSC thermograms of pressure shift freezing sea bass muscle: A) PSF/AT, −15 °C; B) PSF/AT, −25 °C; C) PSF/PAT, −15 °C; and D) PSF/PAT, −25 °C; stored at the corresponding temperature during: (a) 0, (b) 1, (c) 3, and (d) 5 months.

less notorious (Fig. 3C); enthalpies remained constant as a function of the storage time, except a small decrease after 5 months at −25 °C (Table 1).

Results show that after storage, the very evident differences registered between systems because of the high-pressure processing (time 0) diminished due to a deteriorative evolution of the conventional treated muscle, while the final characteristics of the AF/PAT, PSF/AT, PSF/PAT systems remained more stable. It is important to notice that the results also suggest that muscle after a double HP treatment (PSF/PAT) would present less storage stability (at −25 °C) of the myosin respect to the PSF/AT system.

### 3.3. Protein extractability

Water and salt extractable proteins evolution during the frozen storage was evaluated. Decrease of protein concentration in both extracts had been noted on PSF and PAT systems at time 0 (Tironi et al., 2007). Fig. 4 shows the evolution of WEP — sarcoplasmic proteins — as a function of the storage time at −15 and −25 °C. Results show that there was no significant modifications of this parameter during the 5 months of storage ($p>0.05$), as well as no effect of the storage temperature, for any system (conventionally and high-pressure treated). When SDS-PAGE profiles of WEP fractions were compared with those corresponding to not stored samples (Tironi et al., 2007), no modifications because of the frozen storage were evidenced (data not shown).

Different results were obtained in the salt extractable fractions containing myofibrillar proteins. In the case of AF/AT muscles, SEP decreased significantly ($p<0.05$) after 1 and 3 months of storage at both temperatures, increasing up to the starting values after 5 months (Fig. 5A). These facts suggest an aggregation process of the myofibrillar proteins during the first months of frozen storage followed by some protein degradation process. The corresponding electrophoretic profiles of AF/AT soluble fractions did not show differences respect to time 0 (data not shown). Due to similar protein mass were loaded in each lane, this fact suggests that the aggregation process would affect all the myofibrillar proteins, remained in the extractable fraction a similar protein composition but in a minor concentration. AF/PAT system did not show significant differences ($p<0.05$) in the SEP due to the time or temperature of storage respect to time 0 (Fig. 5A). PSF systems presented a small decrease ($p<0.05$) in the SEP fraction concentration as a function of the storage time at both temperatures (Fig. 5B). Comparable results have been obtained on PSF (140 MPa) turbot fillets stored at −20 °C (Chevalier et al., 2000b). Electrophoretic patterns of SEP fractions at time 0 presented notorious differences

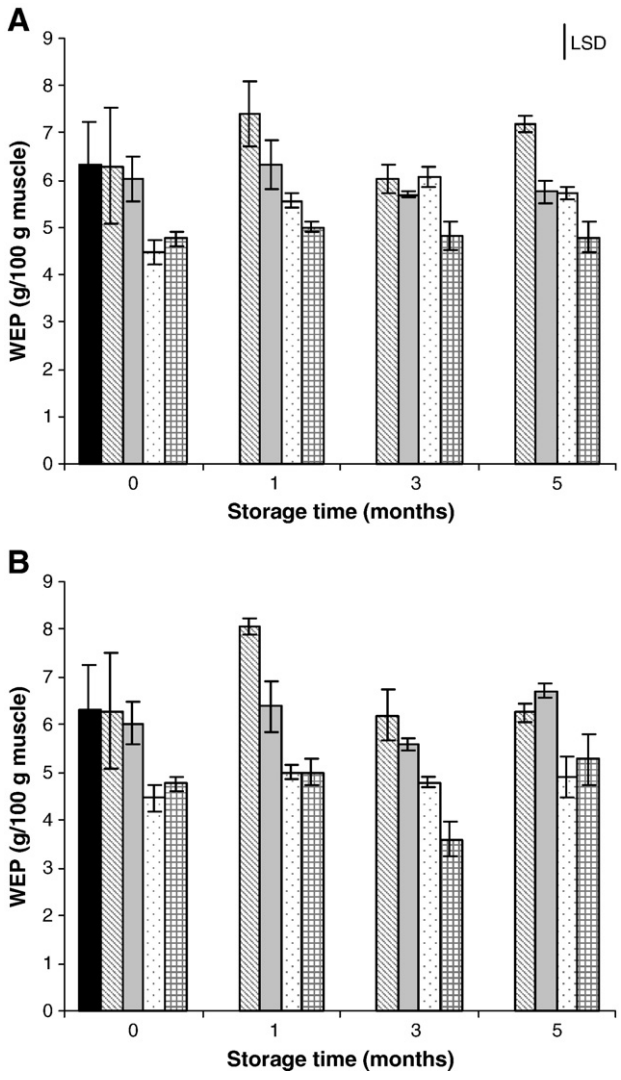


**Fig. 4.** Sea bass water extractable proteins WEP as a function of the frozen storage time: A) −15 °C; and B) −25 °C. ■ F; ▨ AF/AT; ▩ AF/PAT; ⊡ PSF/AT; and ⊟ PSF/PAT.

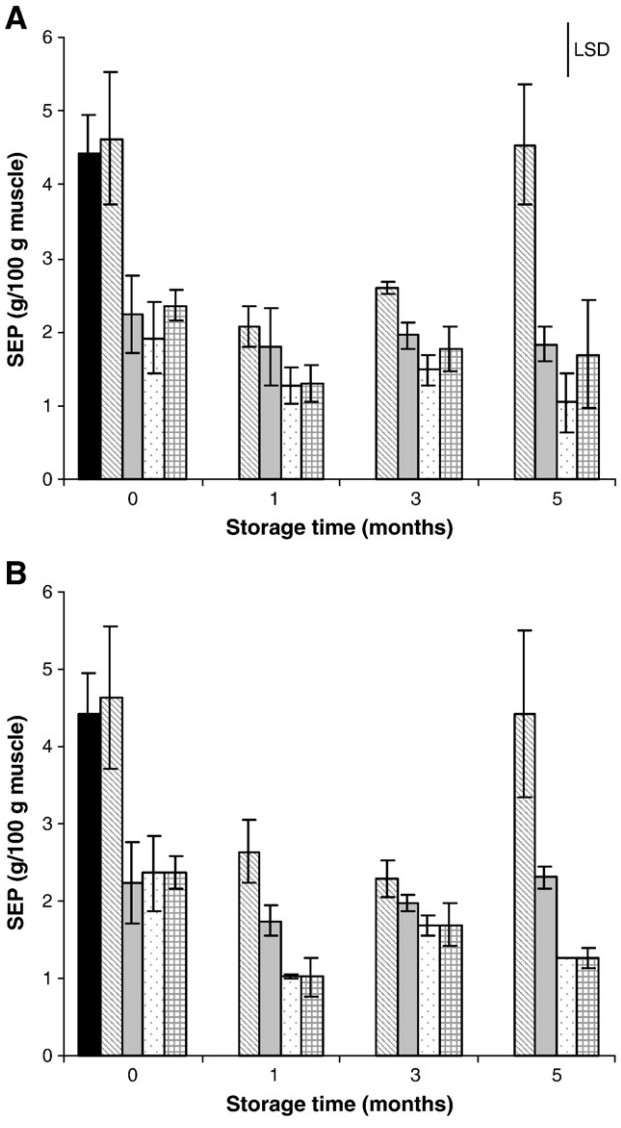


**Fig. 5.** Sea bass water extractable proteins SEP as a function of the frozen storage time: A) −15 °C; and B) −25 °C. ■ F; ▨ AF/AT; ▩ AF/PAT; ⊡ PSF/AT; and ⊟ PSF/PAT.

respect to the conventionally treated muscle, decreasing the intensity of the myosin heavy chain and other myofibrillar proteins (Tironi et al., 2007). Frozen storage did not produce additional modifications on the SDS-PAGE profile of the SEP fraction of high-pressure treated muscle (AF/PAT, PSF/AT and PSF/PAT) (data not shown).

### 3.4. Water holding capacity (WHC)

WHC of raw sea bass muscle conventionally treated (AF/AT) did not present significant changes during the frozen storage at −15 °C (Fig. 6A), showing a decrease ($p<0.05$) after 5 months at −25 °C (Fig. 6B). In the case of the AF/PAT system, this parameter did not change significantly ($p>0.05$) respect to the time 0 for any storage time at both temperatures. For pressure shift frozen systems (PSF/AT and PSF/PAT) a decrease on the WHC after 1 month of storage was registered, without significant differences due to the storage temperature (Fig. 6A and B).

After cooking, WHC behavior as a function of the storage time was different. No significant changes at both temperatures were evident (Fig. 7A and B), presenting all systems comparable values at all storage time evaluated. Thus, this fact confirms that the effect of the application of high pressure on the WHC can be minimized after a cooking process; and in addition, this behavior remains unchanged after 5 months of raw muscle frozen storage.

### 3.5. Color

Sea bass muscle color changes due to PSF and PAT have been previously informed (Tironi et al., 2007). In the present work, additional changes because of the frozen storage were evaluated. L* presented a significant increase ($p<0.05$) after 1 month of storage at both temperatures for AF/AT systems, without important changes in the other samples (Fig. 8A and B). a* showed not significant changes as a function of the time and temperature of frozen storage, for all systems except an increase for PSF/PAT at both temperatures (Fig. 8C and D), suggesting a more red color of the muscle probably related to some fish specimens differences. In the case of parameter b*, it increased after 1 (at −25 °C) and 3 (at −15 and −25 °C) months of storage for the AF/AT system (Fig. 8E and F). Increase of b* — indicating a more yellow color of the muscle — has been largely registered during the frozen storage of fish muscle, due to the appearance of yellow pigments derived of the lipid oxidation and other browning processes (Haard, 1992). High-pressure frozen systems presented a significant increase of this value — with respect to the corresponding samples at time 0 — after 5 months of storage at both temperatures.

## 4. Conclusions

In summary, results obtained in the present work are promissory for the application of freezing and thawing processes using high

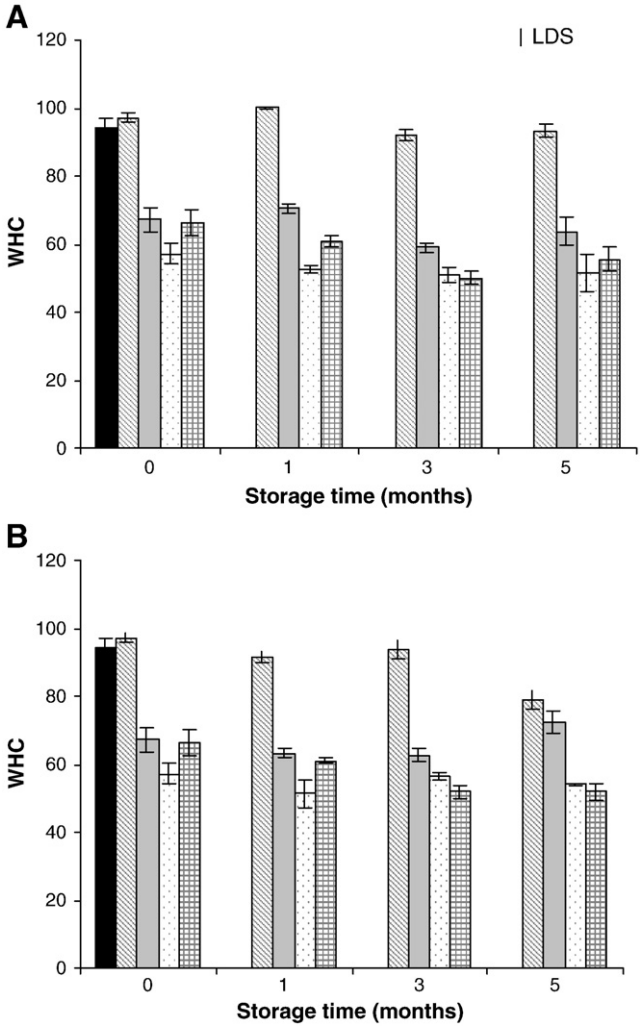


**Fig. 6.** WHC of raw sea bass muscle as a function of the frozen storage time: A) −15 °C; and B) −25 °C. ■ F; ▨ AF/AT; ▩ AF/PAT; ⊡ PSF/AT; and ⊟ PSF/PAT.

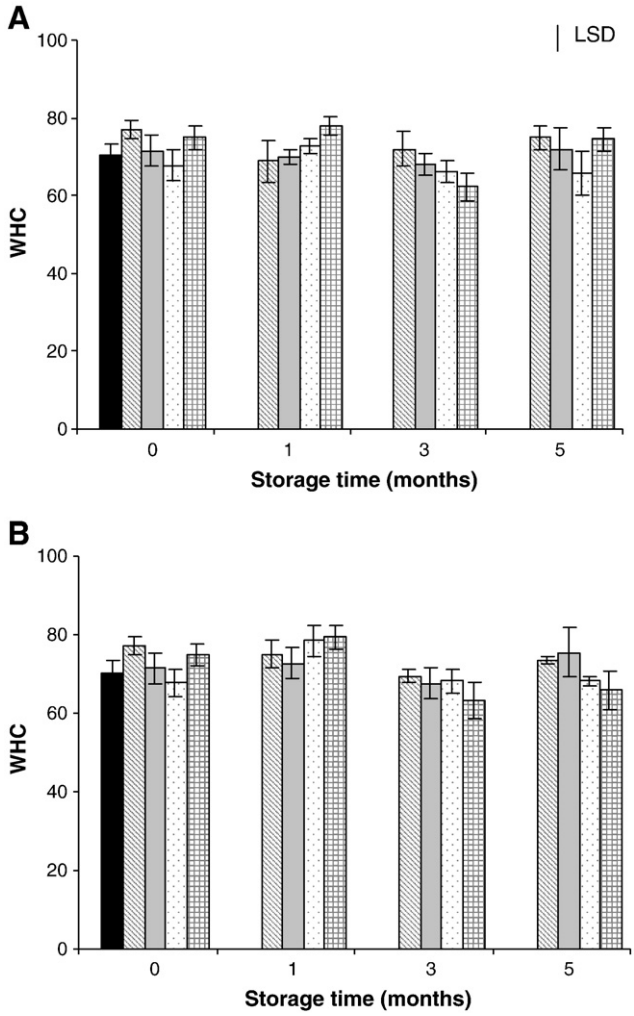


**Fig. 7.** WHC of cooked sea bass muscle as a function of the frozen storage time: A) −15 °C; and B) −25 °C. ■ F; ▨ AF/AT; ▩ AF/PAT; ⊡ PSF/AT; and ⊟ PSF/PAT.

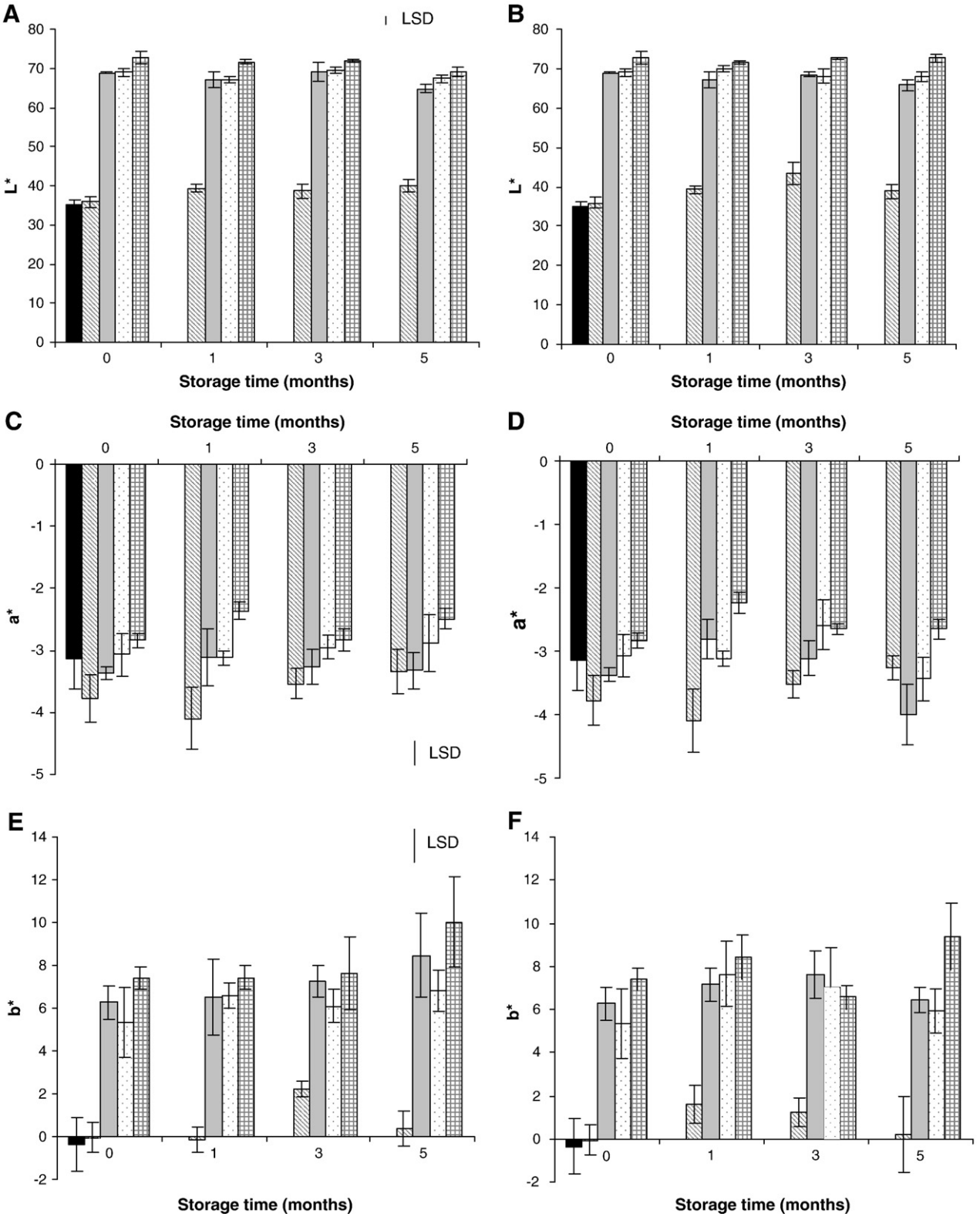


**Fig. 8.** Color parameters as a function of the storage time of sea bass muscle: A) and B) L*, −15 and −25 °C, respectively; C) and D) a*, −15 and −25 °C, respectively; and E) and F) b*, −15 and −25 °C, respectively. ■ F; ▨ AF/AT; ▩ AF/PAT; ⬚ PSF/AT; ⬚ PSF/PAT.

pressure. Although negative effects exist — especially on protein structures and muscle color — other beneficial ones can be obtained using these technologies. Improvement on the cellular integrity due to smaller ice crystal achieved by pressure shift freezing was maintained during the overall frozen storage time. Although an important protein denaturation is produced by the high-pressure treatment, after frozen storage differences between conventional and high-pressure treated samples decreased due to a denaturation and aggregation process in conventionally frozen muscle. It is important to mention that, after cooking, important functional properties of the muscle such as the water holding capacity did not present differences between the different technology combinations. In conclusion, high-pressure

treatments are an interesting alternative to the fish freezing and thawing. Their successful application will depend on the posterior processing and requirements for the fish muscle.

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