Effect of high pressure processing before freezing and frozen storage on functional properties of European hake (*Merluccius merluccius*)

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ABSTRACT

High Pressure Processing (HPP) is a non-thermal technique of growing interest for fish preservation. In this work, the effect on functional properties of European hake (*Merluccius merluccius*) caused by HPP pre-treatment followed by freezing and frozen storage at -10ºC for 5 months were evaluated in an accelerated frozen storage study. Following a central composite design, five pressure levels (150, 169.27, 300, 430.73, or 450 MPa) and frozen storage times (0, 0.32, 2.5, 4.68, or 5 months) were tested. Colour parameters (L*, a* and b*) were measured in raw muscle. Expressible water and mechanical texture parameters were evaluated on both raw and cooked muscles. Results showed that a low-pressure level (150 MPa) allowed an adequate water holding capacity for raw muscle up to 2.5 months of frozen storage time. Values of 150 or 169.27 MPa did not cause significant changes on L* values of raw muscle when compared to non-treated samples. Overall, HPP led to changes on texture parameters of fresh muscle before and after cooking. However, 300 MPa and 5 months of frozen storage gave adhesiveness for raw muscle like that of non-treated fresh muscle. Moreover, 150-300 MPa for 5 months of frozen storage allowed cohesiveness and chewiness values for cooked muscle like those observed on cooked fresh hake showing that the HPP improves the quality of frozen hake.

Keywords: *Merluccius merluccius*, small European hake (eliminar !?), high pressure, freezing, frozen storage, color, funtional properties, quality

1. Introduction

Recently, there is a growing interest for consumption of fresh or minimally processed food. Fish and fish products are highly perishable foods. Their freshness is lost rapidly during post-mortem autolytic degradation produced by microorganims and endogenous enzymes, causing an important loss of quality (Nielsen & Nielsen, 2006). Furthermore, the degradation of proteins creates the favorable conditions for the growth of microorganisms responsible for the formation of compounds which release unpleasant odors (Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & Lamballerie, 2005). Seafood is more susceptible to post-mortem texture deterioration than meat from land animals (Ashie & Simpson, 1996).

Freezing followed by frozen storage is one of the most used methods to preserve the sensory and nutritional properties of fish (Erickson, 1997). During frozen storage, the degree of fish deterioration is influenced by many factors including fish species, storage temperature, time, and endogenous enzymatic activity (Teixeira et al., 2014). In fatty fish species, the presence of highly unsaturated fatty acid and pro-oxidant molecules can lead to important enzymatic and non-enzymatic rancidity (Ramalhosa et al., 2012). Thermal processing can lead to inactivation of microbial growth and lipolytic enzymes. However, it may damage vitamins, flavor compounds, and polyunsaturated fatty acids (Yagiz et al., 2009).

High pressure processing (HPP) is a non-thermal technique of growing interest for the processing and preservation of food. This processing system has advantages over the traditional thermal processing because it retains better the organoleptic and nutritional properties of food while inactivates microbial load leading to shelf-life extension and safety enhancement (Álvarez-Virrueta et al., 2012; Cheftel & Culioli, 1997; Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011; Patterson, Linton, & Doona, 2007; Ríos-Romero et al., 2012; Téllez-Luis, Ramírez, Pérez-Lamela, Vázquez, & Simal-Gándara, 2001). Consumers usually associate the color of seafood with freshness having better flavor and higher quality (Gormley, 1992). HPP has less effect on the fish color than thermal processing (Hayashi, Kawamura, Nakasa, & Okinaka, 1989; Hendrickx, Ludikhuyze, Van Den Broeck, & Weemaes, 1998).

HPP technology allows to inactivate a wide range of food enzymes reducing their impact on food quality and nutritional value. HPP demonstrated its potential application to control enzymes related to fresh seafood texture deterioration (Ashie & Simpson, 1996). Oxidative endogenous enzymes can also be inactivated before further storage and processing of fish products (Murchie et al., 2005). This is particularly important for fatty fish species. Inhibition of endogenous enzymes was observed in Atlantic mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) when HPP pre-treatment followed of freezing and frozen storage was applied (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014a, 2014b). An inhibition of lipid hydrolysis was also observed in these fish species (Torres, Vázquez, Saraiva, Gallardo, & Aubourg, 2013; Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013) and recently also in sardine (*Sardina pilchardus*) (Méndez et al., 2017).

HPP induces modifications on food functional properties. Therefore, it can be used by the industry to create new product textures (Chapleau & De Lamballerie-Anton, 2003). Generally, foods are subjected to pressure levels in the range of 100 to 650 MPa. Variables such as pressure level, pressure holding time, and pressurization rate, show an influence on the functional and sensory properties and microbiological load of fish. Furthermore, the effect of HPP on the fish quality varies with fish species.

HPP treatments also have negative effects on food quality. They affect intermolecular bonds that may induce modifications in water, proteins, polysaccharides, and lipids. The main effect is to produce changes in hydrophobic and electrostatic interactions affecting secondary, tertiary, and quaternary structures in proteins (Chéret et al., 2005).

The pressure levels and holding times needed to reduce the microbial load denature myofibrillar proteins and produce changes in visual appearance, protein functionality, and mechanical properties of fish muscle (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2005). A change in the myofibrillar protein profiles was observed in sea bass fillets. Proteins with molecular weight below 30 kDa increased and those with lower isolectric point values decreased. This could explain the whitish color and the decrease of water holding capacity of fish muscle observed (Teixeira et al., 2014). In chilled coho salmon (*Oncorhynchus kisutch*), an increase of hydrostatic pressure caused a lipid oxidation development augmentation (Aubourg, Tabilo-Munizaga, Reyes, Rodríguez, & Pérez-Won, 2010) and a marked protein damage in the sarcoplasmic fraction (Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2010). Therefore a carefully selection of the conditions used in HHP pre-treatments is necessary to minimize undesirable changes in the fish muscle quality.

The effect of HPP treatments followed of freezing and frozen storage on the functional and sensory properties of fish species such as Atlantic mackerel (*Scomber scombrus*) (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013) and horse mackerel (*Trachurus trachurus*) (Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vazquez, 2014) were studied. Results of these studied showed a species-dependent effect. This can be due to differences in fat composition.

The European hake (*Merluccius merluccius*) is a lean fish, widely consumed in many countries because of its high nutritional value and recognised health benefits. There is not studies about the effects of HPP pre-treatments of freezing and frozen storage on this fish species. Therefore, the aim of this work was to study the changes on color and functional properties of frozen small European hake (*Merluccius merluccius*) caused by HPP pre-treatments before freezing and frozen storage for up to 5 months. The effect of HPP on functional properties was evaluated in raw and cooked fish.

Quizás haya que reducer algo la mención de nuesrto paper previos, y centrarnos en los más directamente relacionados, y mencionados en Res y Disc.

Y creo que faltaría, en cambio, más énfasis en la problemática propia de merluza. Ahí se puede tomar algo del paper enviado; de la Introducción: el gran problema de la congelación de merluza sería la formación de FA y DMA y su repercussion en la textura. Copia, pega y cambia algo lo que veas de esa Introducción

2. Materials and methods

2.1. Raw fish, processing, storage and sampling

European hake (*Merluccius merluccius*) was obtained at the Vigo harbour (Pontevedra, más conocido es Galicia, no ?, northwest Spain), being caught close to the Galician coast and immediately transported to the “Plataforma Tecnológica Multidisciplinar Alta Pressão” (University of Aveiro, Portugal) in a refrigerated truck for HPP treatment within 6 h after catch. Samples were packed in polyethylene bags and vacuum sealed at 400 mbar. The length and weight of the specimens ranged 27.5-29.5 cm and 180-205 g, respectively.

HPP treatments were performed in a 55-L high pressure unit (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain). The pressure levels studied were 150, 169.27, 300, 430.73 and 450 MPa. In all cases, the holding time of the pressure was 2 min. Water applied as the pressurising medium at 3 MPa s^{-1} yielded 50, 56.42, 100, 143.58 and 150 s as the come-up times for the 150, 169.27, 300, 430.73 and 450 MPa treatments, respectively. While decompression time was less than 3 s. Inlet water was adjusted to holding temperature conditions during HPP treatment at room temperature (20 ºC). After HPP processing, individual small hakes were kept frozen at –20 ºC for 48 h before storage at –10 ºC and sampling after 0, 0.32, 2.5, 4.68 and 5 months of frozen storage. The relatively high temperature (-10ºC), as compared to commercial practices (-18ºC), was chosen as an accelerated storage test to determine in less time the effect of the HPP pre-treatment.

For analysis, fish samples were thawed at 4 ºC for 24 h, eviscerated, bones removed manually and then filleted. Frozen samples with no HPP treatment (frozen controls) were subjected to the same freezing and frozen storage conditions. Fresh fish with no HPP treatment (fresh controls) was also analyzed. The analytical procedures described below were carried out on the muscle, raw or cooked. Cooked fish was prepared in an oven at 200 ºC for 10 min reaching at least 68ºC at the centre point.

2.2. Expressible water content and colour

The expressible water content was determined for raw and cooked samples following the procedure described by Uresti, Lopez-Arias, Ramirez, & Vazquez (2003). Four analyses were realized for each treatment and replicate. Colour was only determined in raw samples following the procedure described by the same authors. A colorimeter ColorStriker meter (Mathai, Hannover, Germany) was used. Values of L*, a*, and b* were calculated based on illuminant C and the 2° standard observer. Nine measures were realized for each treatment and replicate.

2.3. Texture profile analysis (TPA)

Texture profile for each treatment was studied in raw and cooked fish samples. Measures were realized at room temperature. A TA-XTplus texturometer (Stable Micro System, Viena Court, UK) equipped with a 50-mm diameter cylindrical aluminium probe (P/50) was used. Samples were cut into small cubes (2 x 2 x 1.5 cm) which were compressed to 75% of the original height at a 60 mm/min compression speed. Five textural parameters (hardness, adhesiveness, springiness, cohesiveness and chewiness) were determined and seven samples were analysed for each treatment and replicate

(Cortez-Vega, Fonseca, Feisther, Silva, & Prentice, 2013; Palmeira, Mársico, Monteiro, Lemos, & Conte Junior, 2016; Silva, Lourenço, & Pena, 2017).

2.4. Statistical analysis

The experimental design was statistically analyzed using the Design Expert® 7.1.1 software (Stat-Ease, Inc., Minneapolis, MN). The set of experiments followed a central composite design (CCD). A CCD has three groups of design points: (a) two-level factorial or fractional factorial design points; (b) axial points (sometimes called "star" points); (c) center points. This design has 5 levels of the independent variables with desirable statistical properties. The following second order polynomial model was used as a first approach to analyse the experimental data:

$$y^i = b_0^i + b_1^i x_1 + b_2^i x_2 + b_3^i x_1 x_2 + b_4^i x_1^2 + b_5^i x_2^2$$

where x_i (i = 1–2) are the code variables for pressure level and storage time; y^i (i = 1–15) are the dependent variables (raw expressible water, cooked expressible water, L*, a*, b*, raw hardness, adhesiveness, springiness, cohesiveness and chewiness and cooked hardness, adhesiveness, springiness, cohesiveness and chewiness); and, b_0^i, $b_0^i...b_5^i$ are regression coefficients estimated from the experimental data by multiple linear regression. Model terms were selected or rejected based on p-values at 95% confidence level determined by analysis of variance (ANOVA). Partial models of the quadratic model were also obtained and analysed by ANOVA.

3. Results and discussion

3.1. Expressible water

This parameter is related to the fish flesh water holding capacity and affects to the product juiceness perceived by consumers. Therefore, fish processing should have a minimum effect on this parameter to retain an acceptable product sensory quality.

The values of expressible water of raw and cooked samples without HPP (control) for the frozen storage time studied are shown in Table 1. The average values for fresh fish were 33.92 ± 2.24% (w/w) before cooked and 36.66 ± 1.60% (w/w) for cooked samples. The frozen storage of fresh fish led to changes in the expressible water values of both raw and cooked muscle. After frozen storage for 5 months at -10ºC, expressible water increased up to 42.72 % (w/w) before cooking and 40.70 (w/w) after cooking.

The expressible water values of raw muscle subjected at HPP pre-treatments are shown in Table 2. They were higher (34.32-46.88% w/w) than those observed in fresh muscle without HPP pre-treatment. In order to assess the relative influence of pressure level and frozen time on the value of expressible water, a multifactor ANOVA was carried out. A significant model (p <0.0001) was obtained to predict the effect of pressure level and frozen storage time on expressible water. Experiment 1 was excluded because it was detected as an outlier point. ANOVA showed that expressible water was mainly affected by the quadratic pressure level term (F-value=13.52) and the pressure level-frozen storage time interaction term (F=7.79) followed of linear pressure level and linear frozen storage time terms. Quadratic frozen storage exerted less influence. The determination coefficient (r^2) of the model was 0.9289. The prediction of the model obtained for the effect of the pressure level and frozen storage time on expressible water is shown in Figure 1a.

The employment of HPP pre-treatment followed of freezing and frozen storage can lead to a significant increase of expressible water if high pressure levels and frozen storage times are selected. However, fish subjected to a hydrostatic pressure of 150 MPa followed of 2.5 months of frozen storage yielded an expressible water value of 37.86 % (w/w). The use of this pressure and frozen storage time produced a water holding capacity enough for a desirable juiciness.

At 150 MPa, studies on other fish species showed comparable results. Expressible water values lower than 40% (w/w) was obtained for Atlantic mackerel (*Scomber scombrus*) (Aubourg et al., 2013). In sea bass fillets, the water holding capacity of the samples treated at 100 MPa were not different from non-treated samples. With high pressure levels (250 and 400 MPa) and holding times (5-30 min), water holding capacity decreased significantly (Teixeira et al., 2014). An expressible water value of 38.7% was considered optimal for low-salt restructured fish products from Atlantic mackerel (Martelo-Vidal, Mesas, & Vazquez, 2012).

HPP pre-treatments gave expressible water values for cooked muscle between 30.10 and 36.32% (w/w) (Table 2). The effect of pressure level and frozen storage time on expressible water of the cooked fish was evaluated by multifactor ANOVA. However, the model obtained was not significant (F-value = 3.00).

3.2. Flesh colour

The mean colour parameters for fresh muscle were 59.58 for L*, 0.449 for a* and 0.542 for b*. The effect of frozen storage time of raw fish on colour parameters was evaluated. The results obtained are showed in Table 1. The L* values did not significantly change after frozen storage. However, important changes in the values of a* and b* were observed. After fish frozen storage, the values of a* were lower and the values of b* higher than those observed on fresh muscle. For instance, after 5 months of frozen storage, the values of a* and b* were -1.091 and 2.931, respectively.

The values for L*, a* and b* obtained for the HPP pre-treatments and frozen storage times studied are showed in Table 2. The values of L* ranged between 65.44 and 79.06. The effect of HPP pre-treatment and frozen storage time on L* value of fish was evaluated by ANOVA. A significant linear model was obtained (F-value 9.36). The value of r^2 was acceptable (0.7006). The effect of pressure level on L* value of the raw muscle was significant (F-value=18.67). However, the effect of frozen storage time was negligible. The prediction of the model for the effect of the pressure level and frozen storage time on L* value is shown in Figure 1b. The L value significantly increased with pressure level, reaching a value of 79.06 at 450 MPa. Low pressure levels (150 and 169.27 MPa) did not cause differences when compared to non-treated samples.

An increase on the L* value with pressure was also observed on the muscle of Atlantic mackerel (*Scomber scombrus)* (Aubourg et al., 2013), sea bass (*D. labrax*) (Tironi, Lebail, & De Lamballerie, 2007) and horse mackerel (*Trachurus trachurus)* (Torres et al., 2014). This HPP effect could be interesting if whiteness is a desirable parameter by consumers. The effects of HPP pre-treatment and frozen storage time on a* and b* parameters were also evaluated by multifactor ANOVA. Significant models were not obtained.

3.3. Textural profile analysis of raw samples

Table 3 shows the effect of frozen storage time up to 5 months on the texture profile of raw muscle without HPP pre-treatment. Hardness of fresh muscle was 5152 g-force and significantly increased when it was frozen stored. After 4.98 and 5 months of frozen storage, hardness increased to 9314 g-force and 7764 g-force, respectively.

Texture parameters of raw muscle subjected at different HPP pre-treatments are shown in Table 4. Hardness ranged from 7,246 to 14,763 (en general habría que poner la coma a los miles, no ?)g-force. The effect of HPP pre-treatment and frozen storage time on raw fish hardness were evaluated by multifactor ANOVA. A significant model was obtained. Hardness was highly affected by linear (F-value = 93.09) and quadratic pressure level (F-value = 20.35) terms. Terms corresponding to linear and quadratic frozen storage times and pressure level-frozen storage time interaction, exerted a minor effect on the hardness of muscle. Prediction of the model (r^2 = 0.9603) is shown in Figure 2a. High pressure levels caused a strong increase on hardness. The hardness of raw samples (5152 g-force) increased to 14623 g-force at 450 MPa.

An increase in hardness with the pressure level was also observed in other species like Atlantic mackerel (*Scomber scombrus*). The effect of frozen storage time subsequent to HPP treatment was negligible in that case (Aubourg et al., 2013). In cod (*Gadus morhua*), hardness increased with the pressure level while only minor changes were observed during frozen storage (Matser, Stegeman, Kals, & Bartels, 2000). Hardness of horse mackerel (*Trachurus trachurus*) was highly affected by pressure level and frozen storage time (Torres et al., 2014).

The adhesiveness of fresh muscle was -175.7 g·s and decreased with the frozen storage time, reaching a value of -56.9 g·s after 5 months of frozen storage at -10ºC. When HPP pre-treatments were applied, the adhesiveness values ranged from -50.3 to -392.2 g.s. The multifactor ANOVA analysis of the effect of HPP pre-treatment and frozen storage time on adhesiveness of raw muscle resulted in a significant model. Adhesiveness was mainly affected by the linear (F-value = 8.22) and quadratic pressure levels (F-value=6.72) and quadratic frozen storage time (F-value =7.15) terms. A minor influence was observed for the linear frozen storage time and pressure level-frozen storage time interaction terms. The prediction of the model (r^2 = 0.8501) obtained is shown in Figure 2b. The model obtained can be used to select a similar adhesiveness to that of fresh fish.

Comparing with other species, the adhesiveness of horse mackerel (*Trachurus trachurus*) was highly affected by the linear and quadratic pressure level term. However, the effect of frozen storage time on adhesiveness was negligible (Torres et al., 2014). HPP pre-treatments caused a significant negative effect on the adhesiveness of Atlantic mackerel (*Scomber scombrus)* when high pressure levels and long storage times were used (Aubourg et al., 2013).

Springiness of fresh muscle (0.232) increased with the frozen storage time in non-treated samples, reaching a value of 0.377 after 5 months. Springiness values for HPP pre-treated raw muscle ranged from 0.294 to 0.494. Similar ranges were found for HPP pre-treated and frozen Atlantic mackerel (*Scomber scombrus)* (0.189-0.346)

(Aubourg et al., 2013), HPP pre-treated and frozen horse mackerel (*Trachurus trachurus*) (0.224-0.492) (Torres et al., 2014) and restructured fish products (0.20-0.60) from gilthead sea bream (*Sparus aurata*) (Andrés-Bello, García-Segovia, Ramírez, & Martínez-Monzó, 2011).

For springiness, the ANOVA led to an F-value of 6.67 excluding experiment 10 as outlier, which implied that the linear model was significant. The value of r^2 was 0.6558. The evaluation of the F-values showed that springiness was affected by pressure level (F-value = 5.62) and frozen storage time (F-value = 5.07; p-value = 0.0590). Prediction of the model obtained for the effect of the pressure level and frozen storage on springiness is shown in Figure 2c. The two variables affected positively the springiness values. Pressure levels between 150 and 300 MPa gave springiness values in the range 0.301-0.388 like the range observed in frozen muscle controls (0.326-0.377). Contrary, previous studies on horse mackerel (*Trachurus trachurus*) showed that springiness was not affected by the variation of the pressure treatment and frozen storage time (Torres et al., 2014). This fact can show a species-dependent effect.

Cohesiveness of fresh muscle (0.247) increased with the frozen storage time at -10ºC, reaching a value of 0.414 for 5 months. HPP pre-treatment followed of freezing and frozen storage gave cohesiveness values ranging between 0.294 and 0.476. A similar range of cohesiveness was observed for other fish products such as restructured fish products from gilthead sea bream (*Sparus aurata*) where values between 0.30 and 0.40 were obtained (Andrés-Bello et al., 2011).

The prediction linear model was significant (r^2=0.8581). Cohesiveness was affected by both frozen storage time (F-value = 30.01) and pressure level (F-value = 20.49). Prediction of the model is showed in Figure 3a. HPP pre-treatments caused an important increase on cohesiveness when a high pressure and long storage time were selected. At 430.73 MPa and 4.68 months of frozen storage, the cohesiveness value was 0.476. This effect was also observed in another fish species. HPP pre-treatments caused a significant increase on cohesiveness of Atlantic mackerel (*Scomber scombrus*) when high pressure and long storage time were selected (Aubourg et al., 2013).

Chewiness of fresh muscle (320 g) increased to 1213 g for 5 months of storage at -10ºC. HPP pre-treated samples showed a wide range of chewiness values (713-3583 g). The multifactor ANOVA analysis led to a significant linear model (F-value=10.00). Chewiness was more affected by the pressure level (F-value = 17.14) than by the frozen storage time. The value of r^2 for the model was 0.7144. Prediction of the model obtained for the effect of the pressure level and frozen storage on chewiness is shown in Figure 3b. The changes on chewiness with the time of frozen storage were more pronounced when high pressures are selected. For example, at 430.73 MPa, 0.38 and 4.68 months of frozen storage gave chewiness values of 2255 and 3583, respectively.

3.4. Textural profile analysis of cooked samples

Textural parameters for cooked muscle samples without HPP pre-treatment are shown in Table 3. When the samples of fresh controls were cooked, hardness (8060 g-force), springiness (0.42), cohesiveness (0.45), and chewiness (1541) were higher than those of raw samples, mainly chewiness. However, adhesiveness was lower after cooking (-93 g·s). This behaviour was also observed for any time of frozen storage considered.

All textural parameters for cooked muscle were affected by the frozen storage time. However, the effect on springiness and cohesiveness was very small. After freezing and frozen storage, hardness (11661-19601 g-force), springiness (0.42-0.593), cohesiveness (0.45-0.539), and chewiness (3181-5440 g-force) increased and adhesiveness (-14.4-(-32.1) decreased.

The results on the cooked texture profile of HPP are shown in Table 5. Using multifactor ANOVA, no significant models were obtained for hardness, adhesiveness and springiness. The values range for these parameters were: hardness: 8669-18405 g-force, adhesiveness: -29.9-(-259.6), and springiness: 0.336-0.591.

Significant models for cohesiveness (F-value=10.75; r^2=0.9307) and chewiness (F-value=7.70; r^2=0.9059) were obtained. Cohesiveness (0.428-0.548) was mainly affected by the pressure level-frozen storage time interaction (F-value=16) and quadratic pressure level terms (F=12.19). The linear and quadratic frozen storage time terms exercised a minor influence and the effect of linear pressure level term was negligible. The prediction of model for the effect of pressure level and frozen storage time on cohesiveness is shown in Figure 4a. The model obtained can be used to select a value of springiness like that of cooked fresh muscle without HPP pre-treatment. Therefore, a pressure of 300 MPa and a time of frozen storage of 5 months gave cohesiveness like that of cooked fresh muscle (not HPP treated).

Chewiness was mainly affected by quadratic pressure level (F=15.76) term followed of linear (F-value=8.01) and quadratic (F-value=7.68) frozen storage time terms. The linear pressure level and pressure level-frozen storage time interaction terms exercised less influence on chewiness. The prediction of model for the effect of pressure level and frozen storage time on chewiness of cooked muscle is shown in Figure 4b. The model obtained can be used to select a value of chewiness like that observed in cooked fresh muscle (non-treated). A pressure of 300 MPa followed by freezing and frozen storage during 5 months yielded a chewiness value like that of cooked fresh muscle without HPP pre-treatment.

4. Conclusions

Frozen storage of European hake (*Merluccius merluccius*) at -10ºC led to important changes on colour parameters of raw muscle and texture profile of both raw and cooked muscles of European hake. However, lightness of raw fish was not affected. The frozen storage for 5 months of European hake caused important changes on the water holding capacity of both raw and cooked muscles. A pretreatment at 300 MPa before frozen storage at -10ºC for 5 months gave cohesiveness like that of cooked fresh muscle showing that the HPP improves the quality of frozen hake.

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Table 1

Effect of frozen storage time at -10ºC on expressible water and colour of European hake (*Merluccius merluccius*) without HPP pre-treatment.

Frozen time (months)	Expressible water % (w/w)	Expressible water % (w/w)	L*	a*	b*
	raw	cooked	raw	raw	raw
0	33.92	36.66	59.58	0.449	0.542
0.32	37.14	33.53	61.73	-0.063	4.567
2.5	35.00	34.26	63.08	-0.876	3.170
4.68	38.63	34.47	60.16	-0.370	3.136
5	42.72	40.70	63.52	-1.091	2.931

Table 2

Effect of high pressure processing (HPP) as a pre-treatment and frozen storage time on expressible water and colour of European hake (*Merluccius merluccius*). Experimental treatment use codes P and F for Pressure (MPa) and frozen storage time (months), respectively.

Treatment	**Expressible water % (w/w)**	**Expressible water % (w/w)**	**L***	**a***	**b***
	raw	**cooked**	**raw**	**raw**	**raw**
1 (P150F2.5)	37.86	32.25	66.15	-1.821	4.106
2 (P169.27F0.32)	34.32	33.87	66.14	-0.659	0.003
3 (P169.27F4.68)	44.46	34.01	65.44	-1.261	2.963
4 (P300F0)	44.14	36.32	72.49	-1.994	0.412
5 (P300F2.5)	43.51	33.31	77.61	-2.258	-0.039
6 (P300F2.5)	41.38	30.84	68.78	-0.676	3.018
7 (P300F2.5)	44.46	30.82	72.29	-1.199	4.051
8 (P300F5)	46.88	34.74	73.21	-1.440	4.540
9 (P430.73F0.32)	37.66	30.10	75.07	-2.833	0.716
10 (P430.73F4.68)	43.20	33.15	73.31	-2.097	2.969
11 (P450F2.5)	40.33	30.24	79.06	-1.971	4.023

En lugar de F, parece más intuitive emplear T, de time)

Table 3

Effect of frozen storage time on the raw and cooked texture profile of European hake (*Merluccius merluccius*) without HPP pre-treatment.

Frozen time (months)	**Hardness (g)**	**Adhesiveness (g·s)**	**Springiness**	**Cohesiveness**	**Chewiness (g)**
	Raw	Raw	Raw	Raw	Raw
0	5152	-175,7	0,232	0,247	320
0.32	6511	-126,1	0,266	0,255	489
2.5	7106	-97,2	0,326	0,343	783
4.68	9314	-97,6	0,358	0,384	1260
5	7764	-56,9	0,377	0,414	1213
	Cooked	Cooked	Cooked	Cooked	Cooked
0	8060	-93,0	0,42	0,45	1541
0.32	19601	-32,1	0,593	0,464	5440
2.5	15875	-25,9	0,524	0,486	4045
4.68	15096	-14,4	0,577	0,537	4678
5	11661	-20,3	0,487	0,539	3181

Corregir puntos y comas en anglosajón

Table 4

Effect on the raw muscle texture of high pressure processing as a pre-treatment for frozen European hake (*Merluccius merluccius*). Experimental treatment use codes P and F for Pressure (MPa) and frozen storage time (months), respectively.

Experiments	**Hardness (g)**	**Adhesiveness (g·s)**	**Springiness**	**Cohesiveness**	**Chewiness (g)**
1 (P150F2.5)	9669	-152,0	0,359	0,294	1005
2 (P169.27F0.32)	7246	-120,1	0,301	0,328	713
3 (P169.27F4.68)	8000	-50,3	0,346	0,358	991
4 (P300F0)	8195	-244,8	0,294	0,301	773
5 (P300F2.5)	10642	-392,2	0,388	0,368	1581
6 (P300F2.5)	9357	-388,7	0,333	0,320	1036
7 (P300F2.5)	9448	-237,7	0,346	0,346	1119
8 (P300F5)	9241	-188,0	0,356	0,406	1333
9 (P430.73F0.32)	13270	-349,7	0,489	0,347	2255
10 (P430.73F4.68)	14763	-129,0	0,494	0,476	3583
11 (P450F2.5)	14623	-287,9	0,401	0,364	2187

Corregir puntos y comas en anglosajón

Table 5

Effect on the cooked muscle texture of high pressure processing as a pre-treatment for frozen European hake (*Merluccius merluccius*). Experimental treatment use codes P and F for Pressure (MPa) and frozen storage time (months), respectively.

Experiments	**Hardness (g)**	**Adhesiveness (g·s)**	**Springiness**	**Cohesiveness**	**Chewiness (g)**
1 (P150F2.5)	17904	-29,9	0,54	0,504	4995
2 (P169.27F0.32)	15435	-51,8	0,54	0,496	4227
3 (P169.27F4.68)	12182	-65,7	0,517	0,548	3555
4 (P300F0)	12805	-259,6	0,428	0,428	2393
5 (P300F2.5)	12890	-183,0	0,468	0,471	2851
6 (P300F2.5)	15649	-156,6	0,467	0,465	3435
7 (P300F2.5)	14672	-120,7	0,517	0,468	3622
8 (P300F5)	8669	-237,3	0,336	0,45	1497
9 (P430.73F0.32)	18405	-132,2	0,591	0,436	4959
10 (P430.73F4.68)	13950	-197,8	0,546	0,537	4295
11 (P450F2.5)	15149	-215,8	0,539	0,483	4122

Corregir puntos y comas en anglosajón

FIGURE LEGENDS

Fig 1. Model prediction for the effect of pressure level (MPa) and frozen storage time (months) on a) expressible water of raw muscle and b) lightness parameter (L) of European hake (*Merluccius merluccius*).

Fig. 2. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on a) hardness, b) adhesiveness, c) springiness of raw muscle of European hake (*Merluccius merluccius*).

Fig. 3. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on a) cohesiveness and b) chewiness of raw muscle of European hake (*Merluccius merluccius*).

Fig. 4. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on a) cohesiveness and b) chewiness of cooked muscle of European hake (*Merluccius merluccius*)

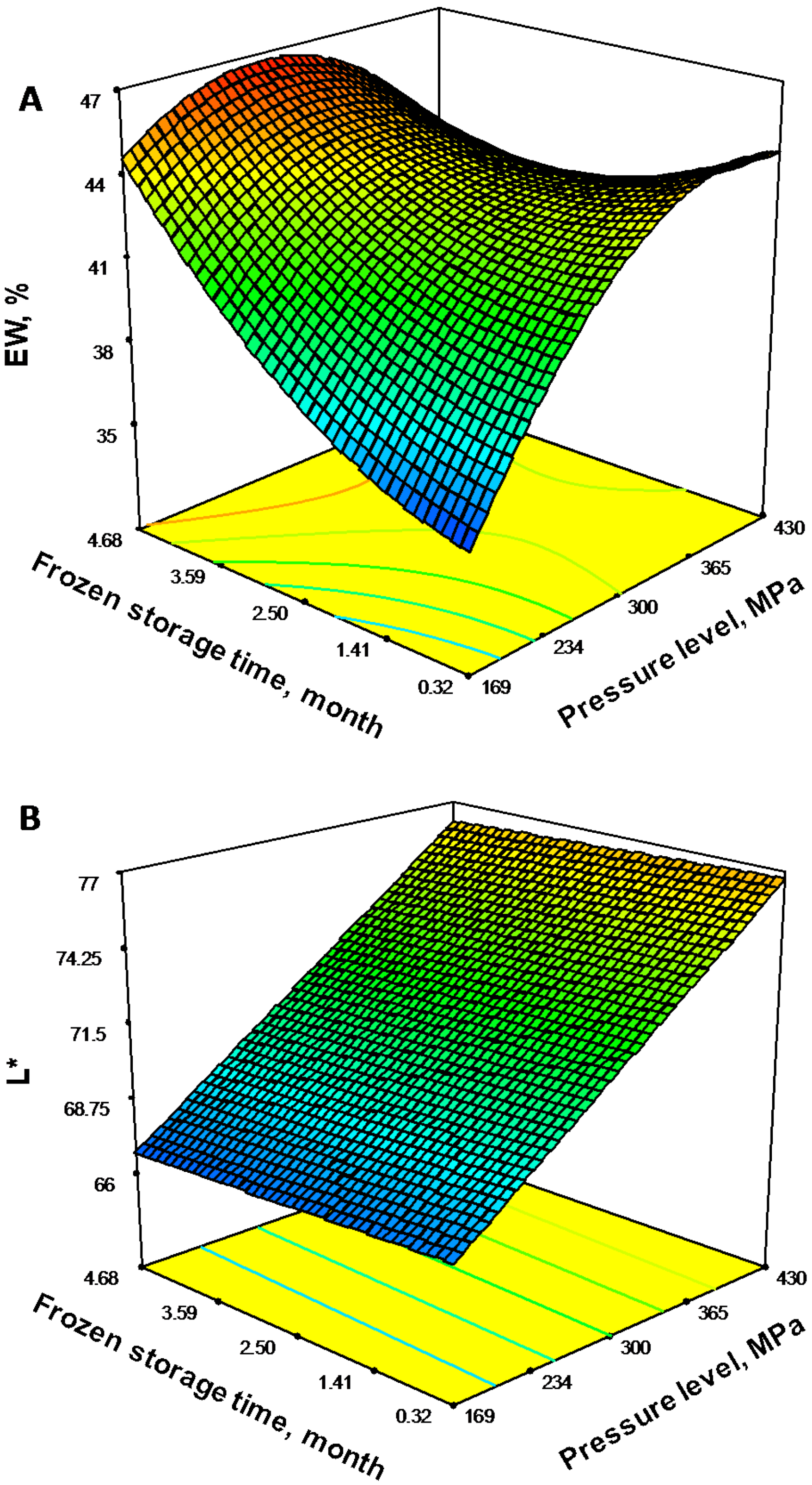
A
EW, %
47
44
41
38
35
4.68
3.59
2.50
1.41
0.32
Frozen storage time, month
169
234
300
365
430
Pressure level, MPa
B
L*
77
74.25
71.5
68.75
66
4.68
3.59
2.50
1.41
0.32
Frozen storage time, month
169
234
300
365
430
Pressure level, MPa

Figure 1

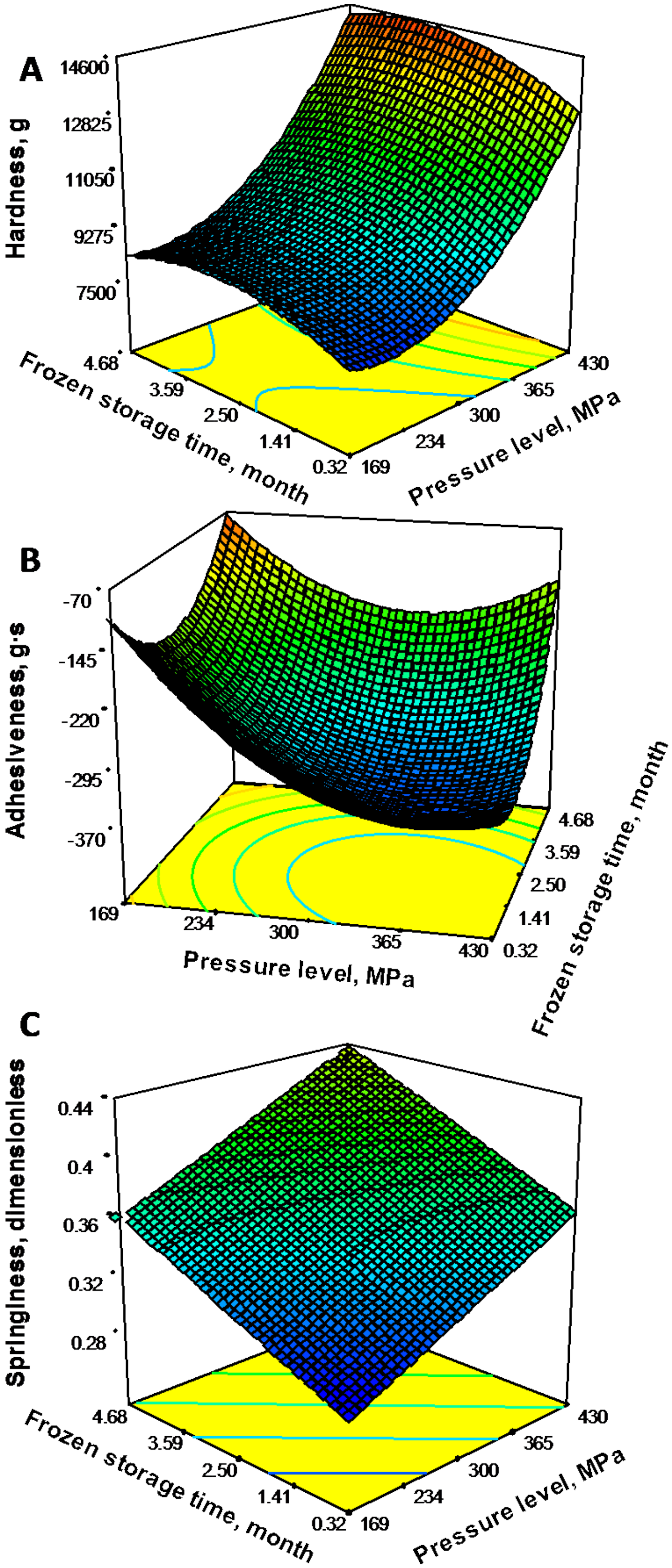
A
Hardness, g
14600
12825
11050
9275
7500
Frozen storage time, month
4.68
3.59
2.50
1.41
0.32
169
234
300
365
430
Pressure level, MPa
B
Adhesiveness, g·s
-70
-145
-220
-295
-370
169
234
300
365
430
Pressure level, MPa
0.32
1.41
2.50
3.59
4.68
Frozen storage time, month
C
Springiness, dimensionless
0.44
0.4
0.36
0.32
0.28
Frozen storage time, month
4.68
3.59
2.50
1.41
0.32
169
234
300
365
430
Pressure level, MPa

Figure 2

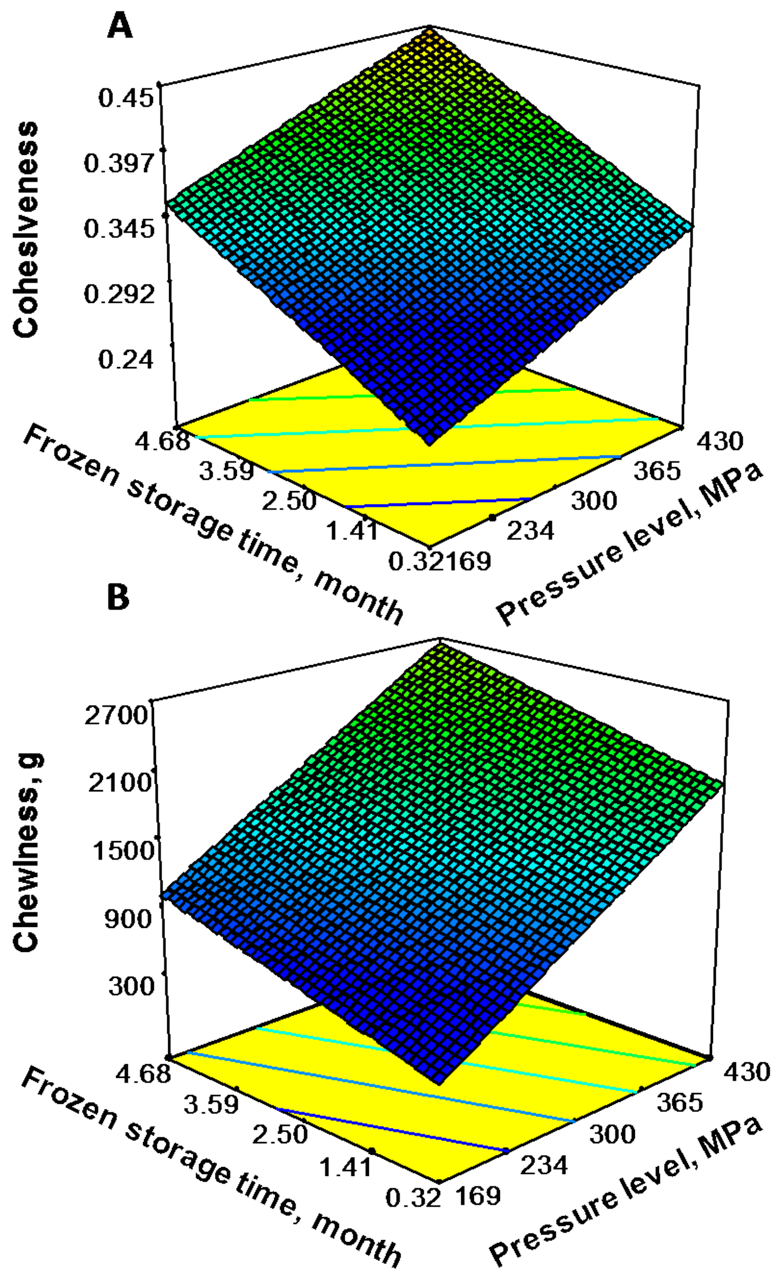
A
Cohesiveness
0.45
0.397
0.345
0.292
0.24
Frozen storage time, month
4.68
3.59
2.50
1.41
0.32
Pressure level, MPa
169
234
300
365
430
B
Chewiness, g
2700
2100
1500
900
300
Frozen storage time, month
4.68
3.59
2.50
1.41
0.32
Pressure level, MPa
169
234
300
365
430

Figure 3

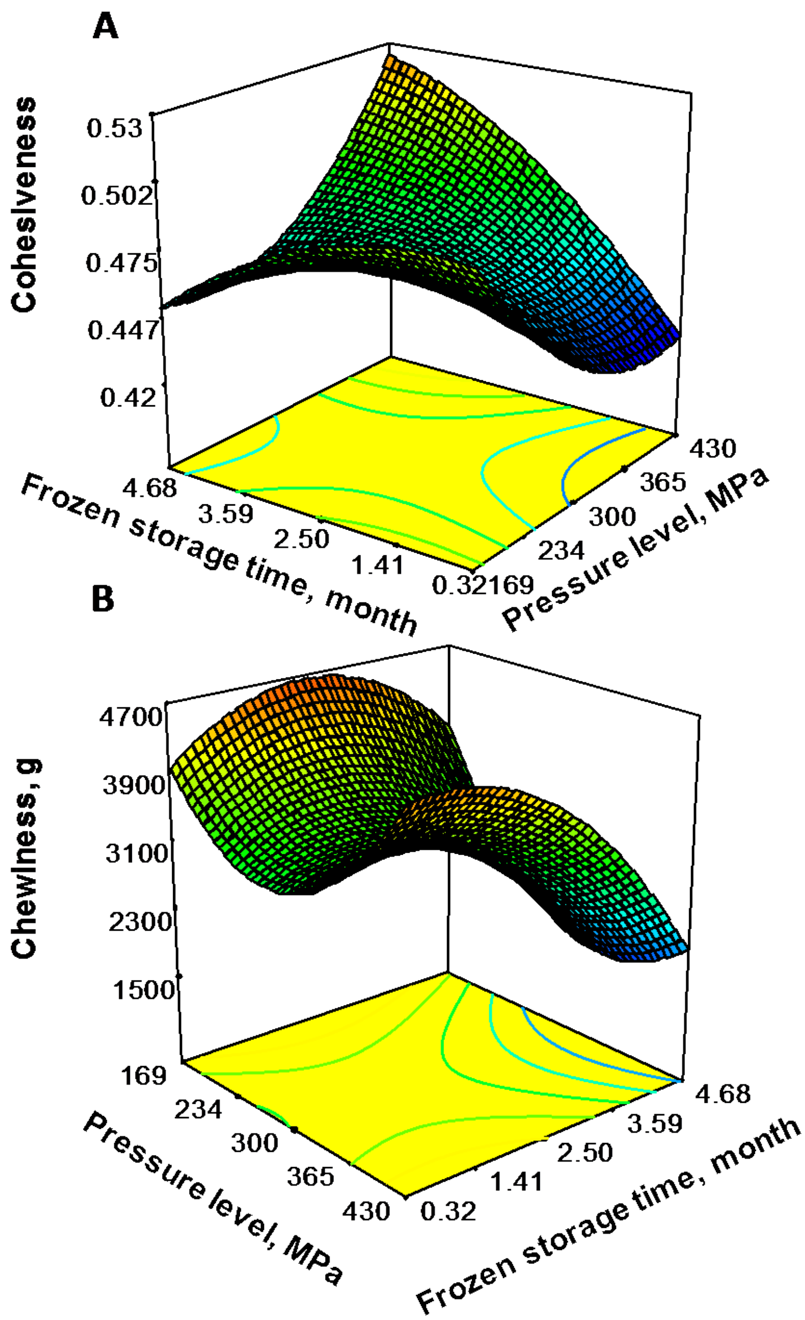

Figure 4

Creo que se pone months, en lugar de month. Eliminar “dimensionless” en Fig 2