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Effect of high-pressure processing pretreatment on the physical properties and colour assessment of frozen European hake (*Merluccius merluccius*) during long term storage

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ABSTRACT

Fish freshness is lost by autolytic degradation produced by endogenous enzymes. Frozen storage is one of the most used methods to preserve fish properties. However, protein denaturation has shown to be a major problem for frozen European hake (*Merluccius merluccius*), leading to texture losses and off-odour development. The aim of this work was to study the changes produced by high-pressure processing (HPP) before freezing on quality of frozen European hake stored at -21ºC for 12 months. The effect of HPP (150 – 450 MPa) on mechanical properties and expresible water was evaluated in raw and cooked fish samples. The effect on colour (L*, a* and b*) was assessed only in raw fish. Results showed that HPP before freezing is beneficial to maintain expresible water in good levels up to 6 months. The luminosity significantly increased with pressure level. Textural profile of raw samples showed that HPP increased hardness, adhesiveness and springiness of frozen hake. Cooked samples were also affected by HPP, being the best results obtained at 300 MPa for 6 months of frozen storage. Overall, results showed that HPP improves the quality of frozen hake.

Keywords: *Merluccius merluccius*; high pressure processing; frozen storage; physical properties; quality enhancement

1. Introduction

High pressure processing (HPP) is a non-thermal technique of growing interest for the processing and preservation of food. HPP is incresingly used commercially to pasteurize food products such as fruit juices, meat products, and seafood (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014). It eliminates food pathogens at room temperature extending the shelf life through the cold chain (Huang, Wu, Lu, Shyu, & Wang, 2017). The effect of high pressure on microorganisms and proteins/enzymes was observed to be similar to that of high temperature. However, when compared to traditional thermal pasteurization, HPP processing has the advantage that it retains better the organoleptic properties and nutritional value of food (Křížek, Matějková, Vácha, & Dadáková, 2014; Medina-Meza et al., 2014). Moreover, HPP is also an interesting technology for food industry due to its ability to modify functional properties and create new textures (Chapleau & De Lamballerie-Anton, 2003).

HPP is used on several types of seafood though its potential for extending the shelf life of fresh fish has not been fully exploited (Rode & Hovda, 2016). HPP inactivates to different levels a wide variety of enzymes which produce fish spoilage, thus reducing their activity during storage. The decreasing of endogenous enzymes activity was observed in pelagic fish species such as Atlantic mackerel (*S. scombrus*) and horse mackerel (*Trachurus trachurus*) when HPP pre-treatment before freezing and frozen storage was applied (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014b, 2014a). Reduction of lipid deterioration was also observed in these fish species (Torres, Vázquez, Saraiva, Gallardo, & Aubourg, 2013; Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013) and more recently in sardine (*Sardina pilchardus*) (Méndez et al., 2017).

Different pressure levels and holding times can denature myofibrillar proteins and produce changes in protein functionality, visual appearance, and mechanical properties of fish muscle to different extents (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2005). For example, in sea bass (*Dicentrarchus labrax*) fillets treated at 250-400 MPa for 5-30 min, myofibrillar proteins with molecular weights below 30 kDa increased, whereas those with lower isolectric point values decreased. Furthermore, fish muscle become whitish, pH increased, and microbiological load and water holding capacity decreased. Changes in the protein profiles might explain the effect of HPP on fish muscle physical properties (Teixeira et al., 2014). In cold smoked salmon (*Salmo salar*), high pressures (600-900 MPa) and holding times (30 and 60 s) produced a considerable shrinkage of myofibrils resulting in increased space between them (Gudbjornsdóttir, Jonsson, Hafsteinsson, & Heinz, 2010).

Treatment at 300 MPa for 15 min effectively reduced susceptibility to oxidation in Atlantic salmon (*Salmo salar*) and led to total microbial reduction (Yagiz et al., 2009). Moreover, HPP (150 or 300 MPa for 15 min) did not produce changes in terms of total saturated, monoenes, n-3 PUFA and n-6 PUFA polyunsaturated fatty acid profile. The effect of pressure level (135-200 MPa) on sensory and physical properties of chilled coho Salmon (*Oncorhynchus kisutch*) was studied by several researchers (Yagiz et al., 2009). According to odour (rancid, putrid), texture (elasticity, gaping, firmness), and color (L* value) attributtes, 135 MPa showed to be the most effective treatment (Aubourg, Rodríguez, Sierra, Tabilo-Munizaga, & Pérez-Won, 2013). Higher pressure levels reduced the microbial load but caused an increase in the lipid oxidation (Aubourg, Rodríguez, et al., 2013) and marked damage of sarcoplasmic proteins fraction (Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2010).

A compative study of effect of HPP on salmon (*Salmo salar*), cod (*Gadus morhua*), and mackerel (*Scomber scombrus*) on several quality paramenters (microbiological load, pH, lipid oxidation, and acid phosfatase activity), revealed that HPP at 200 or 500 MPa induced different changes on these parameters depending on the type of fish species (Rode & Hovda, 2016). A species-dependent effect was also reported for the effect of HPP followed by freezing and frozen storage on the functional and sensory properties of Atlantic mackerel (*S. scombrus*) and horse mackerel (*T. trachurus*) (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013; Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vázquez, 2014), what can be due to differences in fat composition.

Some studies showed that the application of HPP before refrigerated storage could noticeably reduce the contents of biogenic amines in vacuum-packed pike (*Esox licius*) (Křížek et al., 2014), smoked cod (*Gadus morhua*) (Montiel, De Alba, Bravo, Gaya, & Medina, 2012) and rainbow trout (*Oncorhynchus mykiss*) (Matějková, Křížek, Vácha, & Dadáková, 2013).

European hake (*Merluccius merluccius*) is a gadiform species that has high nutritional value and healthy properties, but frozen storage of this fish is very limited due the protein denaturation that leads to texture losses and off-odour development. Different pathways have been proposed to explain this degradation process such as partial dehydration of proteins during freezing due to the formation of ice crystals, interaction of proteins with formaldehyde and lipids, and modification of the environment in the liquid phase surrounding proteins caused by the concentration of salts (Vázquez, Fidalgo, Saraiva, & Aubourg, 2018). Consequently, it is of interest to explore technological treatments that can overcome such drawbacks in order to increase fish shelf life and, accordingly, its trading value.

Using a relatively high temperature of frozen storage (-10ºC), a previous accelerated frozen storage study (5 months) was carried out by our group to assess the effect of HPP pre-treatment before freezing and frozen storage on mechanical and physical properties of frozen small European hake (*Merluccius merluccius*) (Pita-Calvo, Guerra-Rodríguez, Saraiva, Aubourg, & Vázquez, 2017), allowing to gain insights about the effect of HPP in a shorter experimental time period. The results obtained were very promising, but extrapolation to a more usual frozen storage temperature (-21ºC) used commercially is not straightforward and it is necessary to study the effect of HPP at this temperature. Therefore, the aim of this work was to study the changes produced by HPP pre-treatments on quality of frozen European hake stored at -21ºC for 12 month, studying mechanical properties and expresible water in raw and cooked fish and colour in raw fish.

2. Materials and methods

2.1. Raw fish, processing, storage, and sampling

European hake (*M. merluccius*) was purchased at the Vigo harbour (Galicia, northwest Spain). Fish was transported to "Plataforma Tecnológica Multidisciplinar Alta Pressão" (University of Aveiro, Portugal) in a refrigerated truck. Samples packed in polyethylene bags were vacuum sealed at 400 mbar. The length of the specimens varied between 27.5 and 29.5 cm and its weight between 180 and 205 g.

Fish processing was carried out in a HPP equipment (55-L-WAVE 6000/55HT; Hyperbaric, Spain). The pressure levels tested were 150, 169.27, 300, 430.73, and 450 MPa. These pressure levels were reached at 50, 56.42, 100, 143.58, and 150 s, respectively, using a rate of 3 MPa s^{-1}. The holding time of the pressure was 2 min and the decompression time less than 3 s. Processing was carried out at room temperature using pressurizing water at 20 ºC. After HPP treatment, hake specimens were frozen at –21ºC for 48 h and frozen storage at this temperature as described earlier (Aubourg, Torres, et al., 2013). Samples without HPP processing (control samples) were also tested; sampling was carried out for 12 months of frozen storage following the experimental design showed below. Cooked fish samples were obtained heating at oven at 200 ºC for 15 min.

2.2. Expressible water and colour parameters

The expressible water (E_w) values were determined in raw and cooked samples following the procedure described in the literature (María J. Martelo-Vidal, Guerra-Rodríguez, Pita-Calvo, & Vázquez, 2016). Four analyses were carried out for each treatment from several samples.

Colour was determined in the raw fish flesh as described previously (Rocio M. Uresti, López-Arias, Ramírez, & Vázquez, 2003). Values of L*, a*, and b* were determined based on illuminate C and the 2° standard observer in reflection mode using a colorimeter ColorStriker (Mathai, Hannover, Germany). Nine measures were obtained for each treatment from several samples.

2.3. Texture profile analysis (TPA)

Texture profile for each treatment was analyzed in raw and cooked fish samples. A texturometer (TA-XTplus, Stable Micro System, UK) equipped with an aluminium cylindrical probe (P/50-50 mm diameter) was used. Samples were cut into small pieces (2 x 2 x 1.5 cm) being compressed to 75% of their original height using a compression speed of 60 mm/min. For each treatment, five textural parameters (hardness, adhesiveness, springiness, cohesiveness, and chewiness) were evaluated. Ten analyses using several samples were carried out for each treatment (Cortez-Vega, Fonseca, Feisther, Silva, & Prenticea, 2013; Palmeira, Mársico, Monteiro, Lemos, & Conte Junior, 2016; Silva, Lourenço, & Pena, 2017).

2.4. Statistical analysis

The experimental design was statistically analyzed by the Design Expert® 7.1.1 software (Stat-Ease, Inc., MN, USA). The set of experiments followed a central composite design (CCD) which handles three groups of design points: (a) two-level factorial design points; (b) axial or "star" points, and (c) center points. This design uses five levels of the independent variables with desirable statistical properties. In this case, in order to study the range of pressures from 150 to 450 MPa, the design gave the following levels: 150, 169.27, 300, 430.73, and 450 MPa. The range of frozen time was from 0 to 12 months. The design gave the following levels: 0, 0.778, 6, 11.322, and 12 months (0, 23, 180, 337 and 360 days). The models used and the statistical approach are described earlier (Méndez et al., 2017). Cook´s distance was applied the detect outliers (Cook, 1977).

3. Results and discussion

3.1. Expressible water

Expressible water (*Ew*) is an inverse measure of the water holding capacity of fish flesh that has an important influence on the consumer's judgment about fish quality. Consequently, any processing method applied to fish must avoid changes or affect minimally the sensory quality of the product. The values of E_w were measured in raw and cooked samples. Average values for control samples (no HPP treatment) are shown in Table 1. Controls frozen at time 0 gave average values of 33.92 ± 2.24% (w/w) before cooking and 36.66 ± 1.60% (w/w) for cooked samples. The frozen storage of raw fish for 12 months caused an important decrease in the water holding capacity as the value of E_w obtained increased up to 39.36 ± 3.08% w/w. On the contrary, after cooking, controls showed a slight lower *Ew* (33.15% w/w). In the previous study with hake at -10ºC, after 5 months of frozen storage the E_w values were 42.72% (w/w) before cooking and 40.70% (w/w) after cooking (Pita-Calvo et al., 2017). The change in the frozen temperature from -10ºC to -21ºC caused a beneficial decrease in *Ew* and consequently an increase in water holding capacity as expected.

The *Ew* values of raw fish samples subjected to HPP pre-treatments and frozen are shown in Table 2. These values are usually higher (35.18-48.13% w/w) than those observed in controls. The relative influence of pressure level and frozen time on the value of expressible water was assessed by a multifactor ANOVA. A significant model (p <0.0045) was obtained to predict the effect of pressure level and frozen time on *Ew*. Treatment 3 was excluded because it was detected as an outlier. Statistical results by ANOVA showed that expressible water was mainly affected by the pressure-frozen storage time interaction (F-value = 7.16) followed by the frozen time term (F-value = 6.85). The quadratic terms of pressure level and frozen storage had also an important effect. The value of r^2 was 0.8918, and the adequate precision obtained was 9.18 indicating an adequate signal. Equation 1 predicts the response of *Ew* for raw samples (*Ew*_raw):

$$Ew_{raw}(\%) = 20.14 + 0.11\ Pressure(MPa) + 0.89\ Frozen\ time(month) - 0.004\ Pressure \cdot Frozen\ time - 0.001\ Pressure^2 + 0.08\ Frozen\ time^2 \qquad \text{(Eq. 1)}$$

The prediction of the model obtained for the effect of the pressure level and frozen storage time on *Ew* of raw fish is shown in Figure 1a. The employment of HPP pre-treatment followed by freezing and frozen storage leads to a significant initial increase of expressible water at high pressure levels, but this value was reduced with time. Contrarily, the use of low pressure lead to a continuous increase of *Ew* with the frozen storage time. Therefore, the use of high pressures (450 MPa) is recommended to maintain *Ew* at lower levels at long frozen times (6 months).

In the previous study at -10ºC, a similar pattern for the prediction model was obtained. Fish exposed to a pressure of 150 MPa and after 2.5 months of accelerated frozen storage yielded an expressible water value of 37.86% (w/w). Studies carried out on other fish species showed comparable results. For instance, *Ew* values lower than 40% (w/w) were obtained for Atlantic mackerel (*Scomber scombrus*) when a pressure of 150 MPa was used (Aubourg, Torres, et al., 2013). In sea bass fillets (*Dicentrarchus labrax*), water holding capacity of the samples treated at 100 MPa were similar compared to non-treated samples. With higher pressure levels (250 or 400 MPa) and holding times (5-30 min), water holding capacity/*Ew* decreased/increased significantly (Teixeira et al., 2014). Other researchers found a decrease of water holding capacity when the pressure level increases (100-500 MPa) in sea bass fillets (*Dicentrarchus labrax*) (Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagins, & De Lamballerie, 2005).

Comparing with no-HPP products, an expressible water value of 38.7% was considered optimal for low-salt restructured fish products from Atlantic mackerel (Martelo-Vidal, Mesas, & Vázquez, 2012). The expressible water values of processed hake after cooking are shown in Table 2. Controls values of no-HPP treated cooked fishes were included in the 28.96 - 36.66% range, while HPP cooked samples yielded similar *Ew* values (24.90-36.32%).

The effect of pressure level and frozen storage time on *Ew* of cooked fish was evaluated by multifactor ANOVA. A significant model ($p < 0.0085$) was obtained to predict the effect of pressure level and frozen time on Ew. Treatments 3 and 10 were excluded as outlier points. *Ew* of cooked fish was mainly affected by the quadratic terms of frozen storage time (F-value = 20.82) and pressure level (F-value = 8.24), followed by the pressure-frozen storage time interaction (F-value = 4.95) and the pressure level linear term (F-value = 2.31). The value of r^2 was 0.9093, and the adequate precision obtained was 5.46 indicating an adequate signal. Equation 2 predicts the response of *Ew* for cooked samples (Ew_cooked):

$$Ew_{cooked}(\%) = 49.111 - 0.111\,Pressure(MPa) - 0.745\,Frozen\ time(month) - 0.006\,Pressure \cdot Frozen\ time + 0.0002\,Pressure^2 + 0.225\,Frozen\ time^2 \qquad \text{(Eq. 2)}$$

The prediction of the model obtained for the effect of the pressure level and frozen storage time on *Ew* of raw fish is shown in Figure 1b.

3.2. Fish colour

The average colour values for control samples at time 0 were 59.58 for L*, 0.449 for a*, and 0.542 for b* (Table 1). The L* values increased slightly for 12 months up to 63.15. The b* values also increased with frozen storage time (2.35-5.10), showing a higher yellowness of samples.

The values of L* for samples subjected to HPP pre-treatments and along frozen storage for L* ranged between 61.25 and 78.73 (Table 2). These values were higher than that found in contro fish frozen at time 0, showing that the HPP increased the luminosity. The effect of HPP pre-treatment and frozen storage time on L* value of raw fish was studied using ANOVA. A significant quadratic model was obtained (F-value = 92.72), being the L* value of the raw muscle mainly affected by the term of pressure level (F-value = 237.4), followed by the quadratic term of pressure (F-value = 47.03). The effect of pressure was positive whereas that of quadratic pressure was negative. However, the effect of the linear and quadratic terms of frozen storage time was less important and that of the pressure-frozen time interaction was found negligible. The value of r^2 was 0.9983, and the adequate precision obtained was 24.30 indicating an adequate signal. Equation 3 predicts the response on L* for raw samples (L):

$$L^* = 38.62 + 0.177\ Pressure(MPa) + 0.548\ Frozen\ time(months) - 0.0001\ Pressure \cdot Frozen\ time - 0.0002\ Pressure^2 + 0.051\ Frozen\ time^2$$

(Eq. 3)

The prediction of the model for the effect of the pressure level and frozen storage time on L* value is shown in Figure 2a. The L* value significantly increased with pressure level, reaching a value of 78.14 at 450 MPa. Low pressure levels (150 and 169.27 MPa) resulted in L* values (61.25-64.03) only slightly higher than that of control fish at time 0 (59.58). A significant increase of the L* value with pressure was also observed when hake was HPP treated and frozen stored at -10º C for 5 months (Pita-Calvo et al., 2017). An L* value of 79.06 was obtained at 450 MPa and frozen at -10ºC, in accordance with the result at -21ºC.

An increase on the value of L* with pressure was also observed on the muscle of Atlantic mackerel (*Scomber scombrus*) (Aubourg, Torres, et al., 2013), sea bass (*D. labrax*) (Chéret et al., 2005; Tironi, LeBail, & De Lamballerie, 2007), horse mackerel

(*Trachurus trachurus*) (Torres et al., 2014), and Atlantic salmon (*Salmo salar*) (Yagiz et al., 2009). In cold-smoked cod, L* values increased significantly with HPP processing when compared with untreated samples (Montiel et al., 2012). This HPP effect could be interesting if whiteness is a desirable parameter by consumers.

In the control samples (no-HPP), the values of a* parameter were around 0, in the range -0.71 – 0.47. In HPP pre-treated samples, the a* values ranged between -1.33 and -3.34. The effect of HPP pre-treatment and frozen storage time on a* was also evaluated by multifactor ANOVA. However, the model was not significant ($p < 0.095$, $r^2 = 0.44$).

A wide variability was observed for b* values (-0.56 – 4.93) in HPP pre-treated samples and a significant quadratic model was obtained ($p < 0.013$). The prediction of the model for the effect of the pressure level and frozen storage time on b* is shown in Figure 2b. The linear (F-value = 1.11) and quadratic (F-value = 34.4) terms of frozen storage showed the greatest influence on b* value followed by the linear (F-value = 6.24) and quadratic term for pressure level (F-value = 5.01). The term for pressure-frozen storage interaction was negligible.

The value of r^2 was 0.905, and the adequate precision obtained was 7.26 indicating an adequate signal. Equation 4 predicts the response on b* for raw samples:

$$b^* = -5.15 + 0.034\ Pressure(MPa) + 0.950\ Frozen\ time(months) + 0.0004\ Pressure \cdot Frozen\ time - 5.259\ Pressure^2 - 0.086\ Frozen\ time^2 \quad \text{(Eq. 4)}$$

3.3. Textural profile analysis of raw samples

Table 3 shows the effect of frozen storage time for 12 months on the texture profile of raw muscle without HPP pre-treatment. Hardness of fresh muscle was 5152 g and increased when it was frozen stored (6173-9073 g).

Texture parameters of raw muscle subjected to HPP pre-treatments are shown in Table 4. Hardness ranged from 6557 to 13483 g. The effect of HPP pre-treatment and frozen storage time on raw fish hardness was evaluated by multifactor ANOVA. The Box-Cox plot for power transforms suggested that a power transformation with lambda = -2.59 is needed. It was applied, and a significant lineal model was obtained ($p < 0.004$) with an r^2 value of 0.75. Hardness was mainly affected by pressure level (F-value=12.23). Prediction of the model is shown in Figure 3a. Equation 5 predicts the response on hardness for raw samples:

$$Hardness\_raw^{-2.59}\ (g) = -1.398^{-13} - 2.562^{-13}\ Pressure(MPa) + 1.913\ ^{-12} Frozen\ time(months) \qquad \text{(Eq. 5)}$$

High pressure levels caused a strong increase on hardness and the frozen storage time decreased it. At 450 MPa, the experimental value of hardness was 13044 g. In the previous study at accelerated frozen storage at -10ºC, a high-pressure level also caused an important increase of hardness (14623 g). An increase in hardness with the pressure level was also observed in other fish species like Atlantic mackerel (*Scomber scombrus*). The effect of frozen storage time subsequent to HPP treatment was negligible in that case (Aubourg, Torres, et al., 2013), while hardness of horse mackerel (*Trachurus trachurus*) was highly affected by pressure level and frozen storage time (Torres et al., 2014).

The adhesiveness of control muscle (no-HPP treatment) was -175.7 g·s. It decreased with the time of frozen storage until reaching a value of -116.1 g·s for 11.23 months and then increased to -182.7 g·s after 12 months of storage. When HPP pre-treatments were applied, the adhesiveness values ranged from -93.0 to -552.0 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 linear model. The value of r^2 was 0.778, and the adequate precision obtained was 9.88 indicating an adequate signal. Equation 6 predicts the response on adhesiveness for raw samples:

$$\text{Adhesiveness}_{\text{raw}}(\text{g}\cdot\text{s}) = 19.43 - 1.102\ Pressure(MPa) + 8.67\ Frozen\ time(months)$$

(Eq. 6)

The prediction of the model for the effect of the pressure level and frozen storage time on adhesiveness value is shown in Figure 3b. Adhesiveness was mainly affected by the pressure term (F-value = 25.5) and only minor changes were observed with frozen storage (F-value = 2.53). At 450 MPa, an adhesiveness value of -378.7 g·s was found. It can be observed that low pressures (150-169.27 MPa) are necessary to obtain an adhesiveness similar to that of frozen hake at time 0. Comparing with the previous study at -10 ºC as the frozen accelerated storage temperature, adhesiveness was also significantly affected by pressure level and frozen storage (Pita-Calvo et al., 2017).

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

Springiness of raw control samples (0.232) increased to 0.417 for 0.768 months of frozen storage and then decreased for higher storage times (0.272-0.333). An increase of springiness was also found when fish samples were processed by HPP and frozen storage. Values ranged 0.294-0.413. A similar range of springiness values (0.294-0.494) was also obtained for HPP and frozen storage at -10 ºC (Pita-Calvo et al., 2017). Similar ranges were found for other species like Atlantic mackerel (*Scomber scombrus)* (0.189-0.346) or horse mackerel (*Trachurus trachurus*) (0.224-0.492) (Aubourg, Torres, et al., 2013; Torres et al., 2014) subjected to HPP processing and frozen storage.

The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage time on springiness of raw muscle resulted in a significant linear model (F-value of 12.9). Treatment 4 was excluded because it was detected as an outlier. The value of r^2 was 0.787, and the adequate precision obtained was 10.10 indicating an adequate signal. Equation 7 predicts the response on springiness for raw samples:

$$\text{Springiness}_{\text{raw}} = 0.202 + 0.001\ Pressure(MPa) + 0.004\ Frozen\ time(months)$$

(Eq. 7)

The prediction of the model for the effect of the pressure level and frozen storage time on springiness is shown in Figure 3c. Springiness was mainly affected by pressure level (F-value = 24.6). A lineal model for this parameter was also obtained for HPP and frozen storage at -10 ºC (Pita-Calvo et al., 2017). However, springiness was statistically affected by both pressure and frozen storage. Studies on horse mackerel (*Trachurus trachurus*) led to a no significant model showing that springiness was not affected by the pressure level and frozen storage time (Torres et al., 2014), suggesting a species-dependent effect.

Cohesiveness of control muscle (0.247) increased slightly during frozen storage at -21 ºC (0.257 - 0.311). HPP samples showed cohesiveness values ranging between 0.301 and 0.406. A similar range of cohesiveness was observed for restructured fish products from gilthead sea bream (*Sparus aurata*) where values between 0.30 and 0.40 were obtained (Andrés-Bello, García-Segovia, Ramírez, & Martínez-Monzó, 2011). The multifactor ANOVA was applied in order to study the effect of pressure and frozen storage on cohesiveness, but the model was not significant.

Chewiness of control muscle (320 g) increased considerably when it was frozen stored at -21 ºC for 0.768 months (1034 g) and then decreased when longer storage times were used (570 - 817g). HPP pre-treated samples showed a wide range of chewiness values (711-2254 g), always higher than those of control samples did. The multifactor ANOVA analysis led to a significant linear model (F-value = 5.6), being chewiness affected mainly by pressure level, but the value of r^2 was too low (0.58). For the previous study of accelerated storage at -10 ºC, chewiness (713 - 3583 g) was also mainly affected by pressure level (Pita-Calvo et al., 2017). In Atlantic mackerel (*Scomber scombrus*), HPP pre-treatment led to a significant increase on chewiness when high levels of pressure and long pressure holding times were selected (Aubourg, Torres, et al., 2013).

3.4. Textural profile analysis of cooked samples

Textural parameters for cooked muscle samples without HPP pre-treatment are shown in Table 3. When control fish was cooked the following textural values were obtained: hardness, 8060 g; adhesiveness, -93.0 g·s; springiness, 0.420; cohesiveness, 0.450; and chewiness, 1541 g. All textural parameters for the control cooked muscles were affected by the frozen storage. The effect was higher for adhesiveness (-87.6 - (-197.0) g·s), hardness (10637 - 16737), and chewiness (2759 - 5271), especially for the latter. The effect on springiness (0.449 - 0.572) and cohesiveness (0.485 - 0.534) was minor.

The effect of HPP processing and frozen storage time on texture profile of cooked hake is shown in Table 5. Using multifactor ANOVA, no significant models were obtained for hardness, springiness, cohesiveness, and chewiness. The ranges of values for these parameters were: hardness, 8904 - 17435 g; springiness, 0.360 - 0.604; cohesiveness, 0.428 - 0.551; and chewiness, 1590 - 5143 g.

The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage time on adhesiveness of cooked muscle resulted in a significant quadratic model (F-value = 11.5). Treatment 3 was excluded because it was detected as an outlier. The value of r^2 was 0.935, and the adequate precision obtained was 9.59 indicating an adequate signal. Equation 8 predicts the response on adhesiveness for cooked samples:

$$\text{Adhesiveness}_{\text{cooked}} = 84.48 - 1.454\, Pressure(MPa) + 19.164\, Frozen\ time(months) + 0.103 Pressure \cdot Frozen\ time + 0.001\, Pressure^2 - 4.859 Frozen\ time^2$$

(Eq. 7)

The prediction of the model for the effect of the pressure level and frozen storage time on adhesiveness of cooked hake is shown in Figure 4. Adhesiveness was mainly affected by the quadratic (F-value = 43.4) and linear (F-value = 22.7) terms of frozen time.

4. Conclusions

The models obtained can be used to select the pressure level and time of frozen storage which produce a desired texture. Results showed that HPP pretreatment is recommended to maintain *Ew* in good levels for frozen times 6 months. The luminosity (L*) value increased significantly with pressure level. Textural profile of raw samples showed that HPP increased hardness, adhesiveness and springiness of frozen hake. Cooked samples were also affected by HPP, showing the best results at 300 MPa for 6 months of frozen storage.

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Table 1 Effect of frozen storage time at -21ºC on expressible water and colour of European hake (*Merluccius merluccius*) without HPP pre-treatment (controls).

Frozen time (months)	Expressible water % (w/w)		L*	a*	b*
	Raw	Cooked	Raw	Raw	Raw
0	33.92±2.24	36.66±1.60	59.58±4.48	0.45±1.67	0.54±1.49
0.768	31.41±2.04	31.66±2.24	63.20±3.41	-0.71±0.21	4.36±2.02
6	35.08±2.22	28.96±1.87	62.39±2.07	0.47±1.02	5.10±1.95
11.232	34.40±2.12	32.34±1.92	58.55±3.15	-0.72±1.42	2.35±1.05
12	39.36±2.41	33.15±2.41	63.15±3.66	0.47±1.17	3.66±1.32

Table 2 Effect of high pressure processing and frozen storage time at -21ºC on expressible water and colour of European hake (*Merluccius merluccius*).

Treatment	Expressible water, % (w/w)		L*	a*	b*
	Raw	Cooked	Raw	Raw	Raw
1 (P150t6)	39.17±1.66	35.18±1.82	61.25±2.37	-1.51±0.55	2.31±0.89
2 (P169.27t0.768)	35.18±0.92	35.20±1.58	64.03±2.82	-1.68±0.95	-0.36±1.23
3 (P169.27t11.232)	37.66±1.20	31.81±2.54	62.39±3.25	-2.64±0.75	-0.55±0.75
4 (P300t0)	44.14±1.56	36.32±2.09	72.49±2.35	-1.99±0.84	0.41±0.62
5 (P300t6)	40.84±1.71	24.90±2.75	75.76±1.75	-2.38±0.73	3.40±1.42
6 (P300t6)	44.92±1.70	27.80±0.76	74.77±2.30	-1.33±1.20	4.93±1.39
7 (P300t6)	41.84±3.28	29.58±3.04	74.97±2.33	-2.22±0.45	2.93±1.22
8 (P300t12)	48.13±3.54	34.78±2.04	72.22±1.10	-1.68±0.45	1.37±0.74
9 (P430.73t0.768)	44.20±1.03	33.94±2.10	78.73±3.63	-2.39±0.64	0.81±1.76
10 (P430.73t11.232)	43.27±2.68	30.39±2.49	76.67±1.92	-3.34±0.30	1.93±0.95
11 (P450t6)	41.34±1.33	29.90±0.93	78.14±1.493	-2.88±0.53	3.32±1.11

Table 3 Effect of frozen storage time on the raw and cooked texture profile of European hake (*Merluccius merluccius*) without HPP pre-treatment (controls).

Frozen time (months)	Hardness (g) Raw	Adhesiveness (g·s) Raw	Springiness Raw	Cohesiveness Raw	Chewiness (g) Raw
0	5152±1254	-175.7±16	0.232±0.097	0.247±0.026	320±21
0.768	9073±1850	-170.67±42	0.417±0.085	0.257±0.031	1034±54
6	7455±1204	-138.3±65	0.272±0.024	0.288±0.017	594±34
11.232	7450±1409	-116.1±40	0.333±0.041	0.311±0.041	817±84
12	6173±1294	-182.7±74	0.311±0.021	0.292±0.062	570±65
Frozen time (months)	Hardness (g) Cooked	Adhesiveness (g·s) Cooked	Springiness Cooked	Cohesiveness Cooked	Chewiness (g) Cooked
0	8060±1750	-93.0±12	0.420±0.032	0.450±0.021	1541±65
0.768	16737±1265	-197.0±32	0.572±0.042	0.534±0.035	5271±210

6	13720±1750	-104.7±40	0.557±0.024	0.485±0.041	3811±145
11.232	10637±1040	-104.7±65	0.449±0.062	0.501±0.068	2759±135
12	13800±1354	-87.6±25	0.570±0.085	0.533±0.084	4206±122

Table 4 Effect of high pressure processing on the raw muscle texture for frozen European hake (*Merluccius merluccius*).

Treatment	Hardness (g)	Adhesiveness (g·s)	Springiness	Cohesiveness	Chewiness (g)
1 (P150t6)	6557±860	-97.8±20	0.304±0.050	0.352±0.032	711±194
2 (P169.27t0.768)	8044±1347	-149.0±54	0.295±0.081	0.329±0.046	802±201
3 (P169.27t11.232)	7095±1049	-93.0±35	0.310±0.101	0.357±0.084	788±084
4 (P300t0)	8195±995	-244.8±17	0.294±0.070	0.301±0.074	773±140
5 (P300t6)	7532±1013	-142.5±45	0.337±0.112	0.365±0.064	930±164
6 (P300t6)	7361±859	-356.8±64	0.321±0.154	0.347±0.095	845±141
7 (P300t6)	8256±987	-282.6±76	0.364±0.044	0.345±0.012	1083±145
8 (P300t12)	8507±1256	-229.7±80	0.399±0.147	0.374±0.065	1328±184
9 (P430.73t0.768	13483±1371	-552.0±94	0.413±0.126	0.373±0.040	2137±214

10 (P430.73t11.232)	8585±972	-324.0±65	0.325±0.104	0.361±0.038	1045±159
11 (P450t6)	13044±1201	-378.7±40	0.409±0.143	0.406±0.073	2254±207

Table 5 Effect of high pressure processing on the cooked muscle texture for frozen European hake (*Merluccius merluccius*).

Treatment	Hardness (g)	Adhesiveness (g·s)	Springiness	Cohesiveness	Chewiness (g)
1 (P150t6)	15340±2546	-81.8±32	0.511±0.105	0.551±0.034	4293±340
2 (P169.27t0.768)	12962±1875	-81.8±49	0.448±0.120	0.536±0.078	3152±210
3 (P169.27t11.232)	9492±3150	-39.0±12	0.421±0.124	0.493±0.095	2081±198
4 (P300t0)	12805±1441	-259.6±96	0.428±0.098	0.428±0.042	2393±198
5 (P300t6)	8904±1098	-101.1±18	0.360±0.103	0.463±0.035	1590±94
6 (P300t6)	12955±1292	-130.3±23	0.433±0.110	0.437±0.038	2599±145
7 (P300t6)	11544±1340	-59.3±15	0.467±0.165	0.453±0.042	2608±132
8 (P300t12)	13188±1642	-329.9±61	0.437±0.149	0.548±0.054	3496±200
9 (P430.73t0.768)	15493±1874	-224.1±21	0.579±0.184	0.469±0.057	4256±310

10 (P430.73t11.232)	11809±1645	-184.1±13	0.481±0.163	0.468±0.065	2866±175
11 (P450t6)	17435±1209	-97.0±8	0.604±0.179	0.488±0.041	5143±245

Figure legends

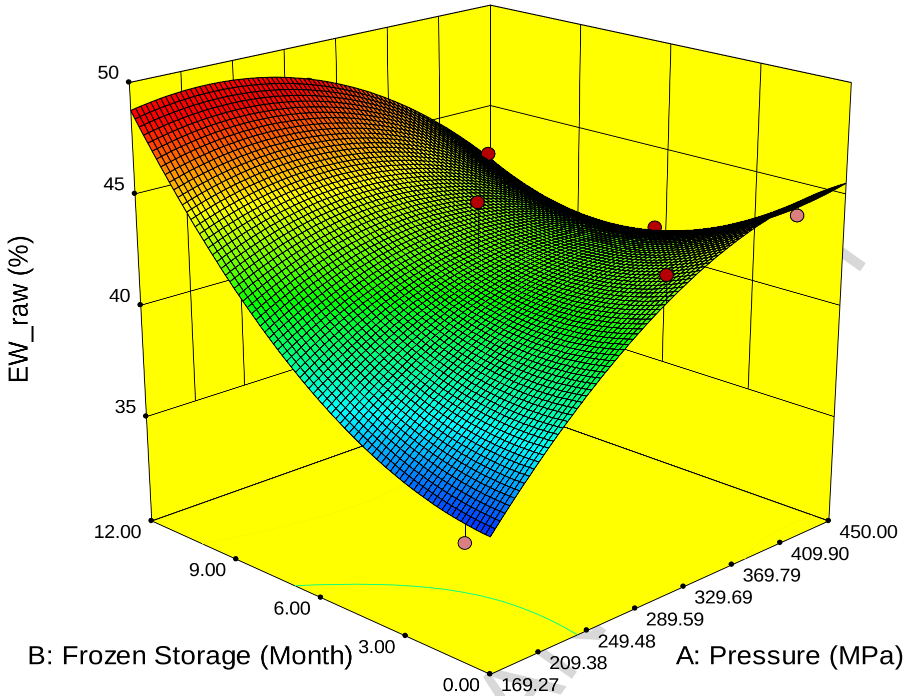
Figure 1. Model prediction for the effect of pressure level (MPa) and frozen storage time (months) on expressible water (*Ew*) of A) raw muscle and B) cooked muscle of European hake (*Merluccius merluccius*).

Figure 2. Model prediction for the effect of pressure level (MPa) and frozen storage time (months) on colour of European hake (*Merluccius merluccius*): A) lightness (L*) and B) b* parameters.

Figure 3. 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*).

Figure 4. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on adhesiveness of cooked muscle of European hake (*Merluccius merluccius*).

A)


50
45
40
35
EW_raw (%)
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

B)

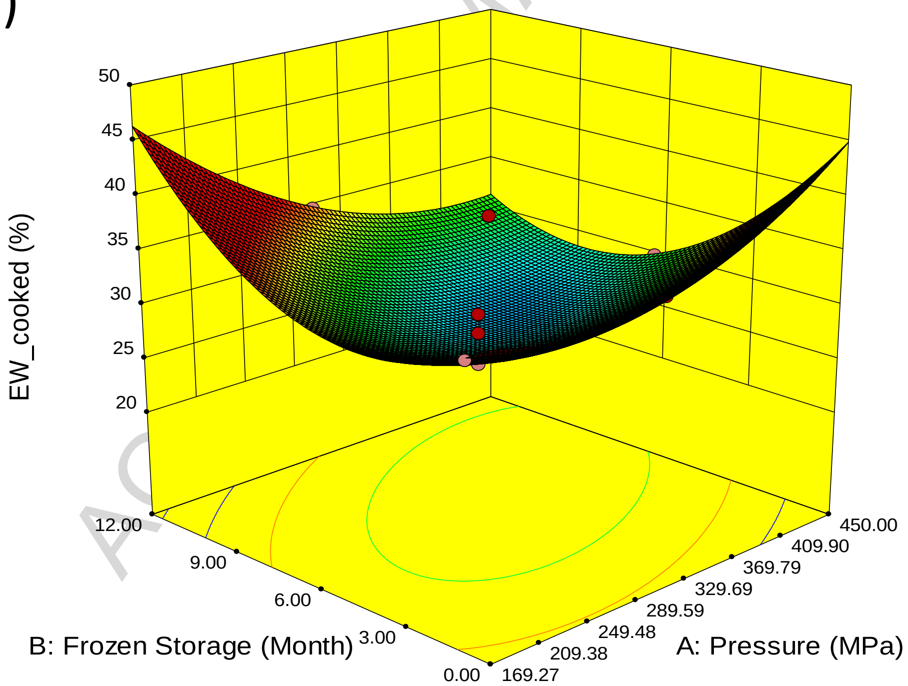
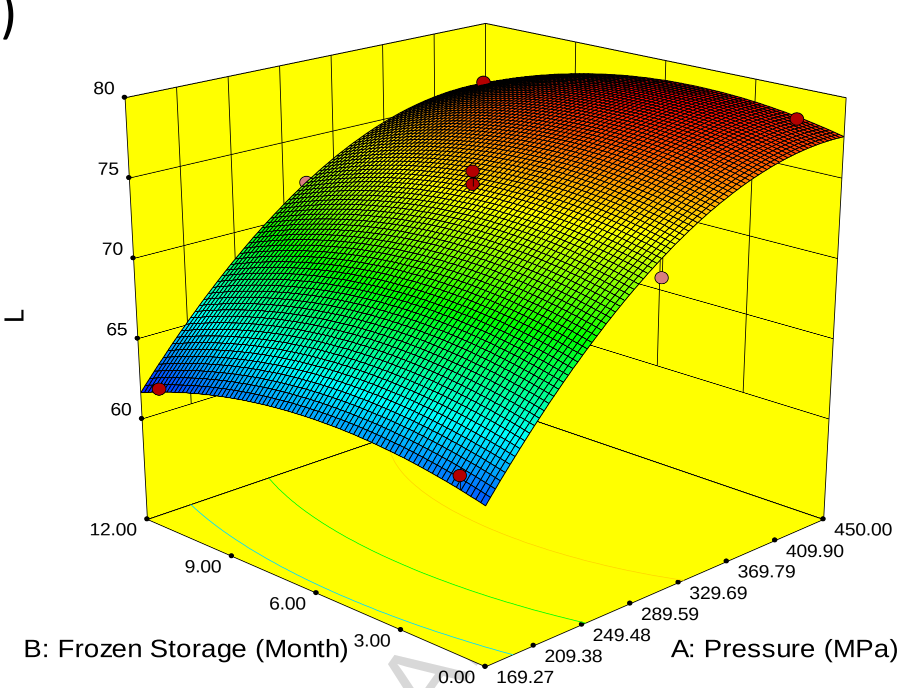

50
45
40
35
30
25
20
EW_cooked (%)
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

Figure 1

A)


80
75
70
65
60
L
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

B)

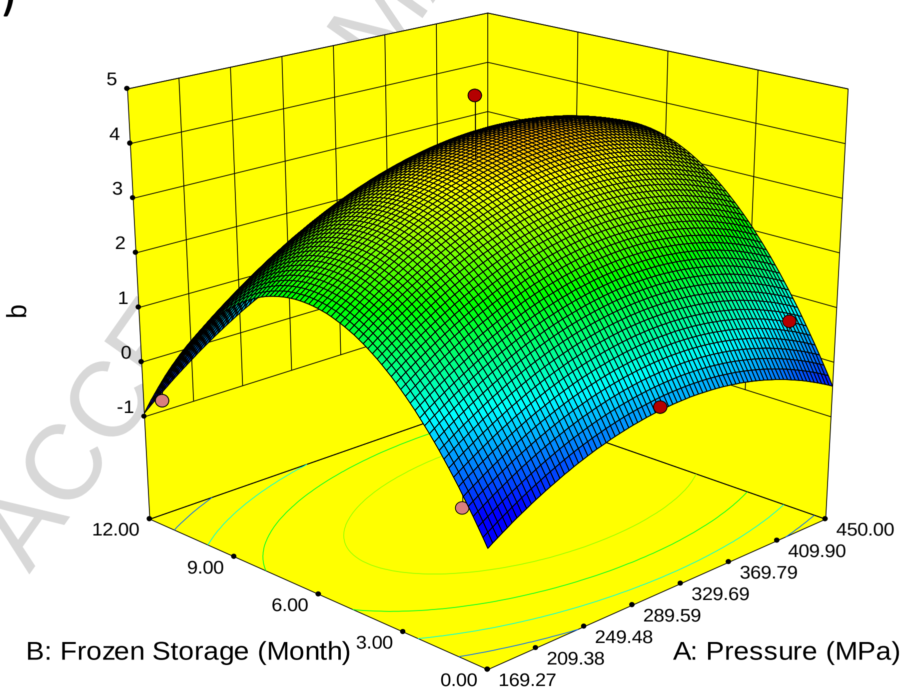

5
4
3
2
1
0
-1
b
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

Figure 2

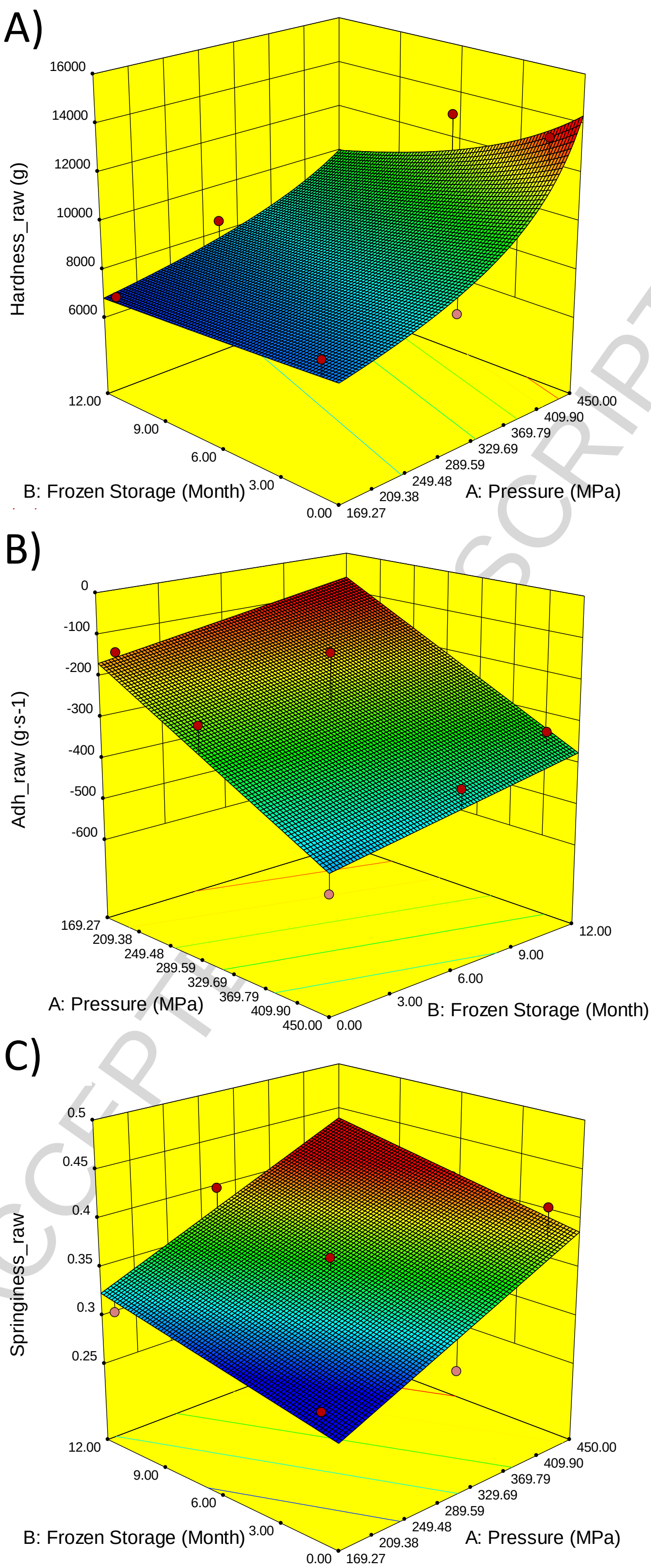
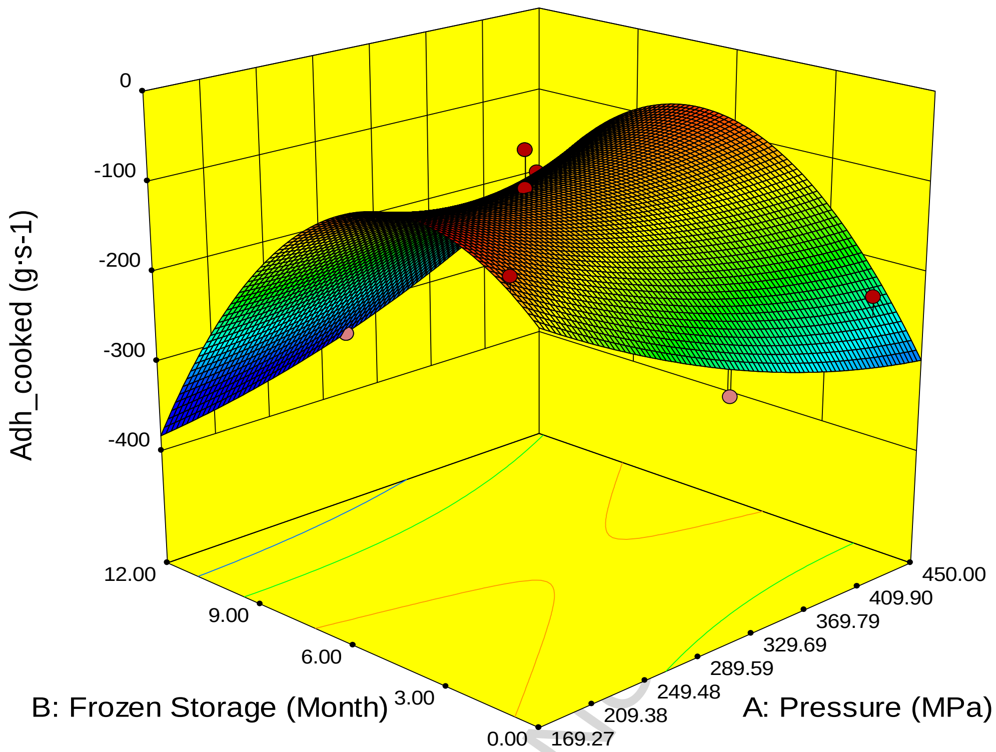
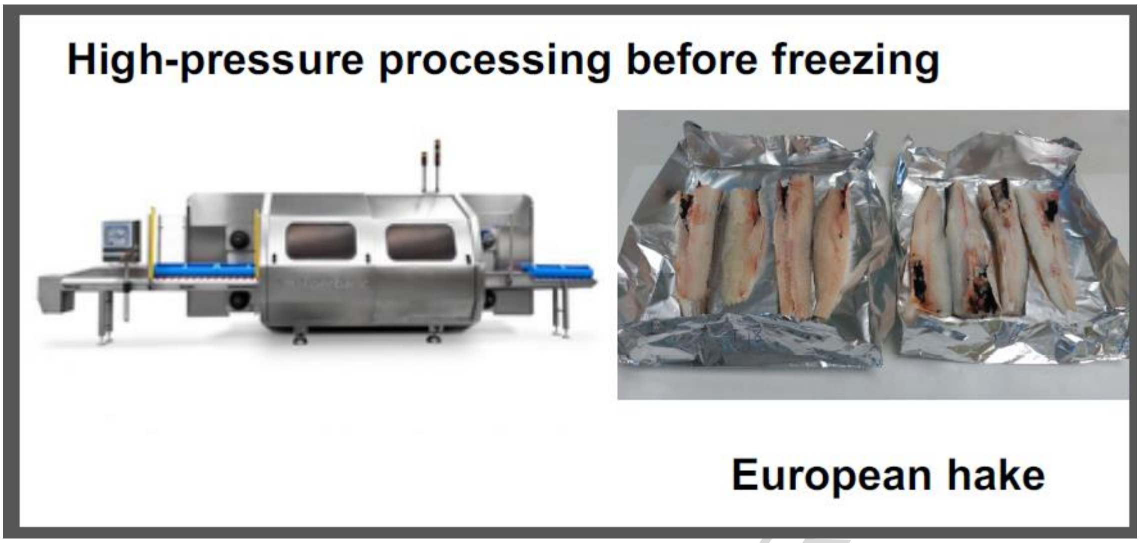

A)
Hardness_raw (g)
16000
14000
12000
10000
8000
6000
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)
B)
Adh_raw (g·s-1)
0
-100
-200
-300
-400
-500
-600
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)
0.00
3.00
6.00
9.00
12.00
B: Frozen Storage (Month)
C)
Springiness_raw
0.5
0.45
0.4
0.35
0.3
0.25
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

Figure 3.



0
-100
-200
-300
-400
Adh_cooked (g·s-1)
12.00
9.00
6.00
3.00
0.00
B: Frozen Storage (Month)
169.27
209.38
249.48
289.59
329.69
369.79
409.90
450.00
A: Pressure (MPa)

Figure 4



Graphical abstract

Highlights

- High pressure processing before frozen storage of hake was assessed.
- Effect of pressure levels on physical properties and colour was evaluated.
- 300 MPa for 6 months at -21ºC storage resulted in good texture and colour
- High pressure processing improves the quality of frozen hake