



# Kinetic modelling of texture and colour changes in frozen fish: effect of high pressure processing as a pre-freezing treatment

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## ARTICLE INFO

### Keywords:

European hake  
*Merluccius merluccius*  
 High pressure processing  
 HPP  
 Frozen storage

## ABSTRACT

High pressure processing (HPP) is a promising technology for improving food safety and extending shelf life. This study examined the effects of HPP (150 MPa, 2 min) followed by frozen storage at  $-10$ ,  $-18$ , or  $-30$  °C on the texture and colour of European hake (*Merluccius merluccius*) over 12 months. Texture parameters (toughness and firmness) were assessed in raw and cooked samples, along with instrumental colour ( $L^*$ ,  $a^*$ ,  $b^*$ ). Results showed that storage time generally increased toughness and firmness, though this trend stabilized or reversed after 6 months. Lower storage temperatures, particularly  $-30$  °C, better preserved textural properties. HPP-treated fish showed higher initial toughness and firmness, suggesting enhanced structural integrity and improved texture retention during storage. Colour stability was also greater in HPP-treated samples, with reduced discoloration and better maintenance of lightness ( $L^*$ ) over time. Overall, combining HPP with storage at  $-18$  or  $-30$  °C effectively preserves both the structural and visual quality of frozen hake, offering a promising strategy for shelf-life extension.

## 1. Introduction

The global demand for high-quality frozen fish products has driven the development of preservation techniques that minimize quality deterioration during storage. European hake (*Merluccius merluccius*), a commercially valuable species, is particularly prone to texture softening, water loss, and discoloration due to protein denaturation and enzymatic activity during frozen storage (Pita-Calvo et al., 2018).

In Southern Europe, hake is one of the most consumed white fish species, with Spain accounting for a significant share of European consumption. Ensuring its quality during storage is thus critical for both consumer satisfaction and commercial viability.

Traditional freezing methods, while effective in slowing spoilage, are often insufficient to fully preserve sensory and structural attributes, particularly at moderate sub-zero temperatures (e.g.,  $-10$  °C). From a sustainability perspective, identifying preservation strategies that maintain quality even at moderate sub-zero temperatures could reduce energy consumption during frozen storage, aligning with industry efforts to lower environmental impact (Careche et al., 1999).

High-Pressure Processing (HPP) has emerged as a promising non-thermal preservation technique in the seafood industry. As consumer demand shifts toward clean-label and minimally processed foods, HPP offers an attractive alternative to chemical preservatives and intensive thermal treatments. By subjecting food products to pressures ranging from 100 to 1000 MPa, HPP inactivates spoilage microorganisms and enzymes without the use of high temperatures or chemical preservatives, thereby enhancing both safety and sensory quality. In seafood products, HPP has shown potential to reduce enzymatic degradation, inhibit the formation of off-flavor compounds and lipid oxidation by-products, and stabilize muscle proteins (Carrera et al., 2020; Vázquez et al., 2018).

While some early and foundational studies predominantly originated from European groups, recent advances illustrate that research on HPP of fish and seafood is extensive and globally distributed. For example, Asia-Pacific, particularly China and Japan, has seen rapid adoption and research growth on HPP applications in seafood preservation and quality improvements, driven by regional industry demand and innovation investments (Peng et al., 2023). North America represents

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another major hub, with mature food processing industries and strong regulatory frameworks fostering extensive use of HPP for seafood safety and shelf-life extension (Roobab et al., 2022). Furthermore, Latin American countries such as Chile and Peru have increasingly incorporated HPP technology to add value to native seafood products while expanding export markets.

Lipid degradation is a major concern in frozen fish. HPP has been reported to mitigate lipid oxidation and hydrolysis. HPP reduced the formation of free fatty acids (FFA) in horse mackerel during storage (Torres et al., 2013). It was also found that HPP reduced volatile amines in mackerel muscle, suggesting improved spoilage control (Prego et al., 2021). Similarly, several authors emphasized that HPP treatments, especially in combination with other technologies, can reduce oxidative rancidity and preserve sensory quality (Gómez-Estaca et al., 2014; Pazos et al., 2015).

Protein quality and texture are closely linked in frozen fish. HPP preserved protein structure and sensory traits in rainbow trout fillets (Cioca et al., 2018). The combining brine salting with HPP enhanced texture and chewiness in mackerel (Huang et al., 2022). HPP has also been linked to improved firmness and water retention in Atlantic salmon, cod, trout, and mackerel. These benefits are particularly valuable for delicate species like hake.

Microbial safety is critical in seafood. HPP has been proven to effectively inactivate foodborne pathogens and spoilage organisms, serving as a viable alternative to thermal processing (Erkan, 2014). HPP significantly inhibited microbial growth and histamine production in spotted mackerel, further validating its effectiveness for shelf-life extension (Lin et al., 2021).

Sensory quality, especially texture and colour, is key for consumer satisfaction. It was reported that HPP-treated fish fillets maintained superior sensory characteristics, including texture and flavour. In terms of colour retention, HPP has been found to preserve natural pigments and prevent enzymatic browning, both critical for marketability (Boitsova et al., 2020; Uçak and Gököçlü, 2017).

Several studies have demonstrated HPP's broad applicability. HPP preserved the nutritional value and colour of vacuum-packed fish patties (Zhu et al., 2014). Proteomics was used to analyse the stability of hake proteins under HPP, revealing its capacity to maintain quality (Carrera et al., 2018). Synergistic benefits were also observed when combining HPP with smoking and canning techniques (Erkan et al., 2011; Prego et al., 2021; Prego et al., 2021).

While HPP has been extensively studied in various seafood products, its application in hake remains limited. Given hake's delicate texture and mild flavour, understanding the effects of HPP pre-treatment on its quality attributes during frozen storage is essential. Furthermore, the effectiveness of HPP may vary with storage temperature. Lower temperatures (e.g.,  $-30\text{ }^{\circ}\text{C}$ ) are expected to better preserve structural proteins and inhibit enzymatic activity, but this interaction with HPP has not been systematically studied.

Few studies have evaluated the combination of HPP and different storage conditions for hake. Recent results show that chemical quality indices such as trimethylamine (TMA), dimethylamine (DMA), formaldehyde (FA), and FFAs increase with storage time and temperature, suggesting a need for optimized strategies to control these degradation markers (Carrera et al., 2020).

Modeling the kinetic behaviour of fish muscle under different preservation conditions is challenging due to the complex interactions between protein denaturation, enzymatic activity, and water migration. Developing such models is crucial for reliable shelf-life prediction and quality control.

Kinetic modeling is an established tool in food science to describe how quality attributes such as texture, color, water-holding capacity, and microstructure degrade over storage time and under stress from temperature and freezing. In fish, texture and color are highly dynamic properties influenced by biochemical reactions such as protein denaturation, proteolysis, lipid oxidation, and pigment degradation.

Kinetic models have been applied to predict changes in fish muscle under frozen storage because these processes often follow time- and temperature-dependent reaction pathways. For example, firmness and water-holding capacity in hake have been successfully described using zero-, first-order, and fractional conversion models, with temperature dependence fitting Arrhenius-type equations (Sánchez-Valencia et al., 2014). Another study of tilapia products treated with glazing used general rate law forms, finding that zero-order kinetics best described many quality indices under certain conditions (Wang et al., 2022). Similarly, Weibullian and empirical rate models have been used to capture non-linear degradation behaviors in salmon, gilthead seabream, sea bass and tuna during frozen storage (Aguilera Barraza et al., 2015; Tsironi et al., 2020). These approaches highlight that no single kinetic model universally applies to all quality attributes, and the choice of model should balance goodness-of-fit with mechanistic interpretability. In this context, the present study extends the application of kinetic modeling to explore how HPP as a pre-treatment influences the evolution of texture and color in European hake during frozen storage. Moreover, there are currently no mathematical models to predict the impact of HPP on textural parameters of hake under various storage temperatures, leaving a gap in the predictive understanding of HPP efficacy. Therefore, it is hypothesized that the kinetics of texture and color changes in European hake during frozen storage can be modulated by HPP prior to freezing, through the alteration of the balance between protein degradation and aggregation mechanisms, thereby improving quality retention. This study aims to investigate the effect of a previous 150-MPa HPP pretreatment on the texture and colour of frozen European hake frozen stored at different temperatures ( $-10$  to  $-30\text{ }^{\circ}\text{C}$ ) for 0–12 months.

By addressing a gap in the literature on HPP's application to hake, this research contributes to a better understanding of how non-thermal technologies can enhance product quality and shelf life. The development of predictive models will further aid seafood processors in optimizing storage and processing strategies for hake, ensuring that consumers receive high-quality, safe, and appealing products.

## 2. Materials and methods

### 2.1. Raw fish, processing, storage and sampling

European hake were caught off the Galician Atlantic coast (north-western Spain) and transported on ice to the laboratory. The specimens measured between 28.5 and 31.5 cm in length and weighed between 185 and 215 g. Upon arrival, six individuals were selected, divided into three groups (two fish per group), and analysed independently as fresh fish ( $n = 3$ ).

The remaining fish were packed into flexible polyethylene bags (30 bags total, six fish per bag), vacuum-sealed at 150 mbar using a Vacuum Packaging Machine Culinary (Albipack, Águeda, Portugal), and separated into two groups of 15 bags each. One group was frozen at  $-40\text{ }^{\circ}\text{C}$  for 48 h and then subdivided into three batches of five bags each. These batches were stored at  $-10$ ,  $-18$ , and  $-30\text{ }^{\circ}\text{C}$ , respectively, and served as the control samples for each storage temperature.

The second group underwent high-pressure processing (HPP) at 150 MPa for 2 min using a 55-L high-pressure unit (WAVE 6000/55 HT; NC Hiperbaric, Burgos, Spain). The selected pressure and processing time were based on previous studies reporting that mild pressure levels (100–200 MPa) are sufficient to inactivate proteolytic enzymes while minimizing undesirable protein denaturation and cooked-like appearance in fish muscle (Aubourg et al., 2013; Pita-Calvo et al., 2018; Torres et al., 2014). Water was used as the pressure-transmitting medium at a rate of  $3\text{ MPa s}^{-1}$ , resulting in a come-up time of 54 s and a decompression time of under 3 s. The pressurizing water was kept cold to maintain a treatment temperature of 18.8–21.0  $^{\circ}\text{C}$ , with the fish temperature ranging from 9 to 10  $^{\circ}\text{C}$ . Following HPP, these samples were also frozen at  $-40\text{ }^{\circ}\text{C}$  for 48 h and then divided into three batches (five

bags each), stored at  $-10$ ,  $-18$ , and  $-30$  °C, respectively.

For both control and HPP-treated groups, samples were taken at month 0 (after the initial 48 h freezing at  $-40$  °C), and after 3, 6, 9, and 12 months of frozen storage. Sampling at  $-10$  °C was discontinued after 9 months due to significant product deterioration. At each sampling point, three replicates ( $n = 3$ ) per condition were independently analysed. Each analysis was conducted on extracts from the white muscle, pooled from two fish per sample. All solvents and reagents used were of analytical grade (Merck, Darmstadt, Germany). Fig. 1 summarises the workflow of the processing.

## 2.2. Mechanical and colour properties

European hake samples were thawed for 12 h at  $5$  °C and equilibrated at room temperature for 30 min in plastic bags to avoid dehydration before mechanical properties were measured. Cooked fish samples were obtained heating at oven at  $200$  °C for 15 min. Mechanical properties were determined using a TA-XTplus (Stable Micro System, Viena Court, UK). Warner–Bratzler analysis was performed using an aluminium Warner-Bratzler probe. Samples were compressed to 80 % of their initial height using a compression speed of 0.5 mm/s. Firmness and toughness (work of shear) were recorded for each treatment and six samples were analysed from each treatment. Colour was determined in raw samples by the procedure described previously (Uresti et al., 2003). A colorimeter ColorStriker (Mathai, Hannover, Germany) was used.  $L^*$ ,  $a^*$ , and  $b^*$  values were determined.

## 2.3. Statistical analysis

Non-linear regression analyses of texture data were performed with a commercial optimization routine dealing with the Newton's method (Solver, Microsoft Excel, 2000; Microsoft Corporation, Redmond, WA) by minimizing the sum of the squares of deviations between experimental and calculated data, as reported previously (Rodríguez-Chong et al., 2004). Data obtained from colour parameters were subjected to the analysis of variance (ANOVA) method to explore differences resulting from the effect of the previous HPP, temperature and storage time. The colour parameters were statistically analysed by the Design Expert® 7.1.1 software (Stat-Ease, Inc., MN, USA).

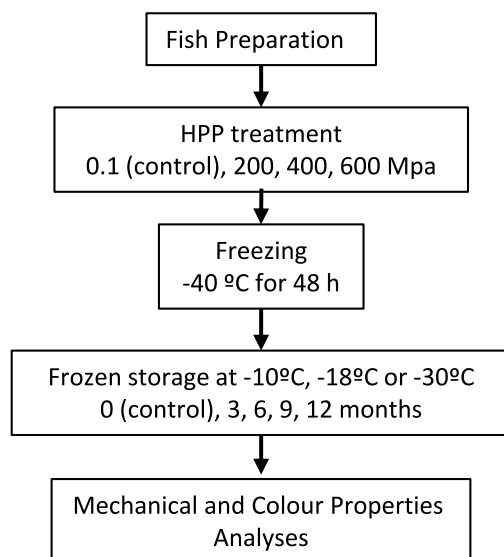
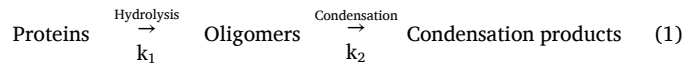


Fig. 1. Flow diagram of the experimental workflow for European hake (*Merluccius merluccius*).

## 3. Results and discussion

This study presents a kinetic modeling approach to quantify the impact of storage time and temperature on the texture parameters of hake fillets, comparing samples with and without HPP pretreatment. Such models are commonly used to study the hydrolysis of carbohydrate polymers (Aguilar et al., 2002; Bustos et al., 2003; Gámez et al., 2004; Rodríguez-Chong et al., 2004). It was hypothesized that both frozen storage and HPP can influence enzymatic activity in fish muscle, leading to protein degradation and the occurrence of the following reactions:



Both reactions are assumed to follow first-order kinetics. Here,  $k_1$  is the rate constant for the formation of hydrolysis products (monomers or oligomers), while  $k_2$  is the rate constant for the formation of condensation products (structural reorganization), which represent new covalent bonds within proteins. Both constants have units of reciprocal time. Hydrolysis products may also include amino acids or amines.

Assuming these reactions affect the texture of the fish samples, a relationship can be derived by solving the system of differential equations for isothermal conditions. The resulting model is:

$$T = M \frac{k_1}{k_1 - k_2} (e^{-k_1 t} - e^{-k_2 t}) + P \cdot e^{-k_2 t} \quad (2)$$

Where  $T$  is the texture parameter (e.g. firmness or toughness),  $M$  and  $P$  are theoretical minimum and maximum values for the texture parameter, and  $t$  is storage time.

$M$  and  $P$  should not be interpreted as literal physical limits of firmness or toughness, but rather as asymptotic bounds within the mathematical framework of the two-step kinetic model. Their role is to ensure convergence of the differential equations to an upper and lower bound during curve fitting. This approach is consistent with kinetic modeling in other food systems, where boundary parameters serve as empirical scaling constants rather than direct physical quantities (Oliveira et al., 2017; Qiu et al., 2013).

While the model originates from studies on carbohydrate hydrolysis, its application in protein-rich systems such as fish muscle can be rationalized based on the dual nature of textural changes under processing and storage. In fish, texture evolves through two concurrent and partially opposing phenomena: (i) structural breakdown of proteins and enzymatic proteolysis, which reduce firmness, and (ii) protein aggregation, cross-linking, and water redistribution, which can transiently increase toughness or firmness. These processes are analogous to the hydrolysis ( $k_1$ ) and condensation ( $k_2$ ) terms in the two-step kinetic scheme, where  $k_1$  reflects degradative changes (loss of myofibrillar integrity, protease activity, denaturation) and  $k_2$  captures re-aggregation or gelation phenomena that counteract softening.

Several studies confirm that HPP directly modifies protein structure in fish muscle by causing partial unfolding, denaturation, and aggregation, all of which are closely linked to texture evolution during frozen storage. HPP has been shown to alter myofibrillar proteins and proteolytic enzyme activity in ways that modulate both softening and hardening phenomena (Chéret et al., 2006). Similarly, high-pressure treatments in silver carp were reported to follow first-order inactivation kinetics of myofibril-bound proteinases, directly linking pressure effects to the kinetic control of texture deterioration (Qiu et al., 2013). In European hake, HPP before freezing has been shown to increase hardness and springiness during long-term frozen storage, indicating the relevance of aggregation and cross-linking processes that can be captured with a condensation-like term in the model (Pita-Calvo et al., 2018). More broadly, reviews on HPP effects on fish muscle note that between 200 and 300 MPa, partial protein denaturation, proteolysis, water redistribution, and gelation are the dominant mechanisms leading to either texture loss or hardening (Oliveira et al., 2017).

Therefore, although the model was originally applied to carbohydrate hydrolysis, its mathematical form provides a useful framework for describing the competing degradative and aggregative mechanisms that govern fish muscle texture under HPP and frozen storage. In our study,  $k_1$  represents the net rate of proteolysis/denaturation-induced softening, while  $k_2$  reflects cross-linking and aggregation processes that increase resistance to deformation. This abstraction allows us to capture the biphasic behavior of firmness and toughness in a mechanistically interpretable and statistically robust way.

In this work, Eq. (2) was applied to model texture parameters at different storage temperatures. This model reflects a mechanistic understanding of how processing and storage influence protein structure and texture. Application of a kinetic model to fish texture that incorporates both degradation (hydrolysis) and structural reorganization (condensation) is not common in the literature. Most models focus only on degradation or empirical texture changes.

### 3.1. Changes in raw texture parameters of frozen hake

The model was applied to both control frozen samples and frozen samples pretreated with HPP. The estimated kinetic parameters for raw firmness are presented in Table 1, and Fig. 2 compares the experimental data with the model predictions. Table shows F-test Prob. values as complementary probabilities to p-values.

Analysis of the fitted rate constants ( $k_1$  and  $k_2$ ) suggests that the rate of condensation reactions is equal to or exceeds that of hydrolysis reactions. The coefficient of determination ( $R^2$ ) indicated strong agreement between experimental and predicted values across all regressions.

The model predicts that raw firmness (N/mm) increased to a peak value of 2.27 N/mm during frozen storage at  $-10\text{ }^\circ\text{C}$  for the first 3 months. After this period, hydrolysis reactions became predominant, leading to a decline in firmness to 1.44 N/mm. Nevertheless, these values remained higher than those observed at  $-18\text{ }^\circ\text{C}$  and  $-30\text{ }^\circ\text{C}$ , where firmness ranged from 0.41 to 0.71 N/mm.

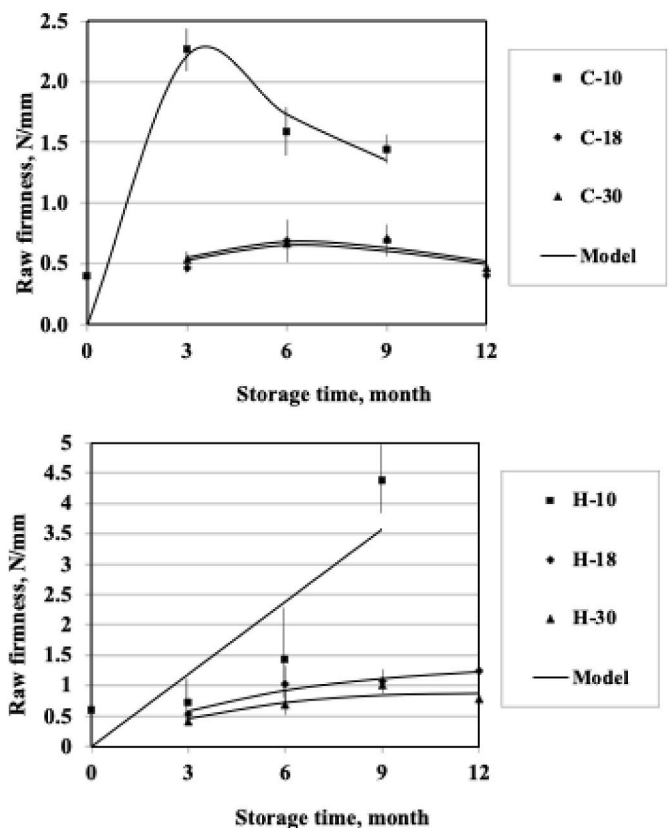
Pre-treatment with HPP enhanced raw firmness during frozen storage. The effect was particularly pronounced at  $-10\text{ }^\circ\text{C}$ , reaching a maximum of 4.37 N/mm after 9 months. In contrast, frozen storage at lower temperatures ( $-18\text{ }^\circ\text{C}$  and  $-30\text{ }^\circ\text{C}$ ) helped to limit the firmness increase, maintaining values below 1.12 N/mm even after 12 months. Overall, the results clearly show that storage at lower temperatures, especially at  $-30\text{ }^\circ\text{C}$ , leads to reduced raw firmness, indicating slower structural changes in the muscle tissue.

The estimated kinetic parameters for raw toughness are presented in Table 2, and Fig. 3 compares the experimental data with the model predictions. The determination coefficient ( $R^2$ ) demonstrated a high

**Table 1**

Kinetic and statistical parameters of raw firmness in frozen hake stored at different temperatures and previous high-pressure processing treatment on frozen hake.

	Stored $-10\text{ }^\circ\text{C}$	Stored $-18\text{ }^\circ\text{C}$	Stored $-30\text{ }^\circ\text{C}$
<b>Raw Firmness (Control)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0825	0.1613	0.1606
$k_2$ ( $\text{min}^{-1}$ )	3.8212	0.1613	0.1606
M0	128.59	1.785	1.865
P0	0.00	0.000	0.000
$r^2$	0.9754	0.9062	0.8227
Adjusted $r^2$	0.9631	0.8593	0.7341
F-test Prob.	0.7416	0.2583	0.1694
<b>Raw Firmness (HPP)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0000	0.000	0.0851
$k_2$ ( $\text{min}^{-1}$ )	0.0000	0.183	0.0831
M0	14370	682.2	2.325
P0	0	0.0	0.000
$r^2$	0.7743	0.9467	0.8011
Adjusted $r^2$	0.6615	0.9200	0.7017
F-test Prob.	0.8199	0.8923	0.6351



**Fig. 2.** Predicted and observed raw firmness values of frozen European hake (*Merluccius merluccius*) muscle for control (C) and high-pressure processing (H) pretreatment, stored at  $-10$ ,  $-18$ , and  $-30\text{ }^\circ\text{C}$  for 12 months.

**Table 2**

Kinetic and statistical parameters of raw toughness in frozen hake stored at different temperatures (control) and previous high-pressure processing treatment on frozen hake.

	Stored $-10\text{ }^\circ\text{C}$	Stored $-18\text{ }^\circ\text{C}$	Stored $-30\text{ }^\circ\text{C}$
<b>Raw toughness (Control)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0776	0.1232	0.0589
$k_2$ ( $\text{min}^{-1}$ )	0.1895	0.0000	0.0589
M0	49.97	9.67	23.81
P0	7.00	0.00	0.00
$r^2$	1.0000	0.9208	0.9599
Adjusted $r^2$	1.0000	0.8812	0.9399
F-test Prob.	0.9994	0.9262	0.8680
<b>Raw toughness (HPP)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0791	0.0884	0.073
$k_2$ ( $\text{min}^{-1}$ )	0.0790	0.0883	0.073
M0	29.22	26.58	24.78
P0	3.50	0.00	0.00
$r^2$	0.9093	0.9066	0.8014
Adjusted $r^2$	0.8640	0.8599	0.7021
F-test Prob.	0.9392	0.6408	0.5028

level of consistency between the observed experimental results and the values predicted by the model in all cases. Furthermore, the F-test probability values supported the reliability and statistical robustness of the model in accurately representing the experimental trends, excepting for  $-18\text{ }^\circ\text{C}$  and  $-30\text{ }^\circ\text{C}$  in HPP treated samples where the values obtained were too low, indicating a few data points to can predict reliable.

The model predicts that raw toughness (N/mm·s) increased to a peak value of 12.88 N/mm·s during storage at  $-10\text{ }^\circ\text{C}$  for 6 months. After this period, hydrolysis reactions became predominant, leading to a gradual decline in toughness to 12.21 N/mm·s. Nevertheless, these values

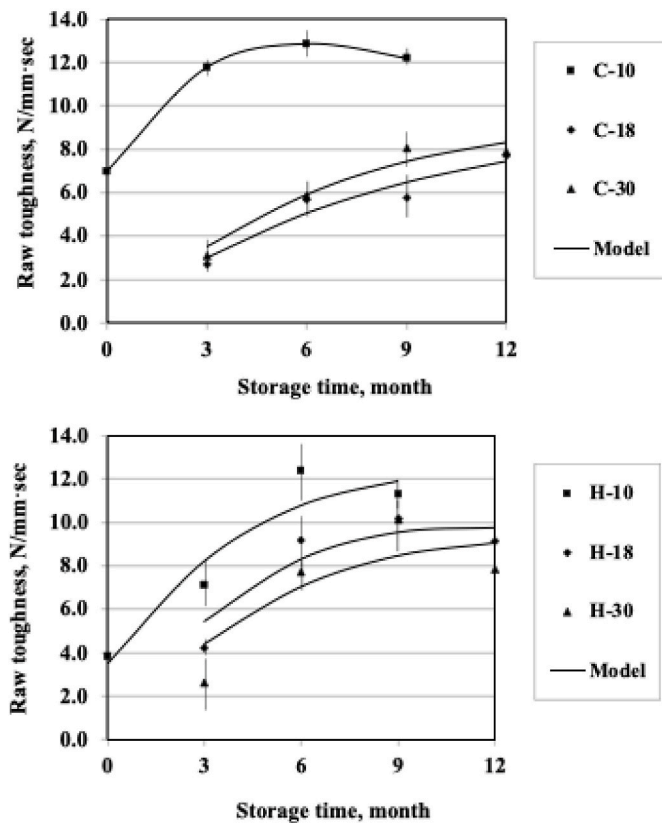


Fig. 3. Predicted and observed raw toughness values of frozen European hake (*Merluccius merluccius*) muscle for control (C) and high-pressure processing (H) pretreatment, stored at  $-10$ ,  $-18$ , and  $-30$  °C for 12 months.

remained higher than those observed at  $-18$  °C and  $-30$  °C, where raw toughness increased continuously up to 7.92 N/mm·s after 12 months of storage. Similar values were observed at  $-18$  °C and  $-30$  °C throughout the storage period, indicating that ultra-low frozen storage at  $-30$  °C offers no significant advantage over  $-18$  °C for preserving this texture parameter.

Pre-treatment with HPP enhanced raw toughness during frozen storage. The differences between storage temperatures were modest: at 9 months, the raw toughness values were 11.27 N/mm·s ( $-10$  °C), 10.15 N/mm·s ( $-18$  °C), and 10.13 N/mm·s ( $-30$  °C). After this point, the effect of hydrolysis reactions increased, resulting in a progressive decrease in toughness across all conditions.

Overall, the results demonstrate that  $-18$  °C is sufficient to maintain acceptable raw toughness in HPP-treated fish samples during up to 12 months of frozen storage, and that lowering the temperature further to  $-30$  °C does not provide additional benefits in this regard. While our study emphasizes the benefits of HPP as a pre-freezing treatment, HPP can induce partial protein denaturation, which in some cases reduces water-holding capacity and increases drip loss during storage. Additionally, higher pressures or longer holding times may impart a cooked-like appearance and affect sensory properties negatively (Oliveira et al., 2017). These limitations are highly dependent on the species, pressure–time combinations, and storage conditions.

### 3.2. Changes in cooked texture parameters of frozen hake

The same model was applied to cooked samples. The estimated kinetic parameters for cooked firmness are presented in Table 3, and Fig. 4 compares the experimental data with the model predictions. Analysis of the fitted rate constants ( $k_1$  and  $k_2$ ) indicated that the rate of condensation reactions exceeded that of hydrolysis for both frozen hake and

Table 3

Kinetic and statistical parameters of cooked firmness in frozen hake stored at different temperatures (control) and previous high-pressure processing treatment on frozen hake.

	Stored $-10$ °C	Stored $-18$ °C	Stored $-30$ °C
<b>Cooked firmness (Control)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0237	0.0160	0.1247
$k_2$ ( $\text{min}^{-1}$ )	2.1494	0.4336	0.1320
M0	128.6	23.5	1.75
P0	1.0	0	0.09
$r^2$	0.1091	0.7069	0.4065
Adjusted $r^2$	-0.3400	0.5612	0.1124
F-test Prob.	0.5846	0.2843	0.6375
<b>Cooked firmness (HPP)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0000	0.000	0.1408
$k_2$ ( $\text{min}^{-1}$ )	0.0117	0.000	0.0663
M0	7938.9	609.7	1.19
P0	1.4	1.4	1.43
$r^2$	0.9012	0.3880	0.0787
Adjusted $r^2$	0.8518	0.0820	-0.3820
F-test Prob.	0.9338	0.136	0.1429

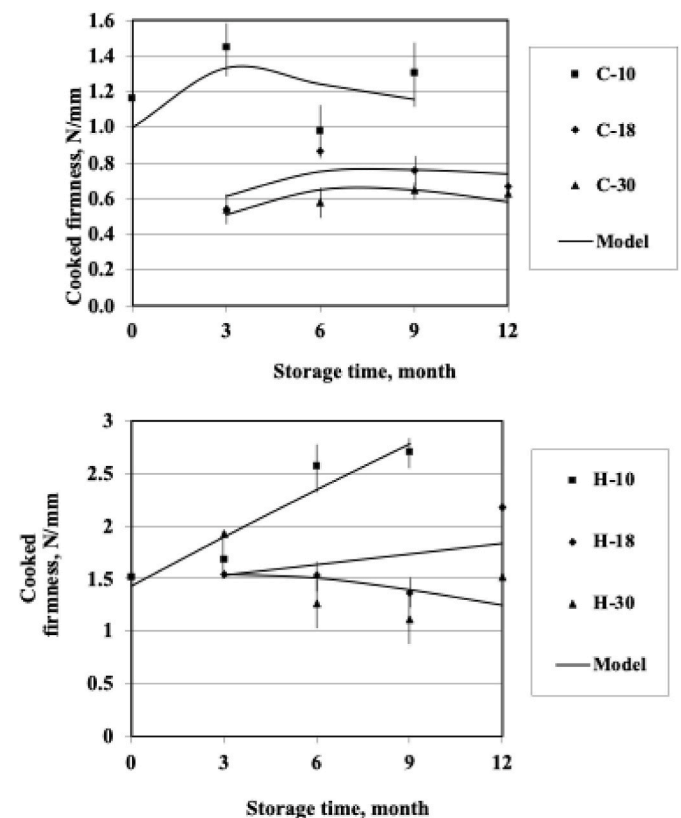


Fig. 4. Predicted and observed cooked firmness values of frozen European hake (*Merluccius merluccius*) muscle with control (C) and high-pressure processing (H) pretreatment, stored at  $-10$ ,  $-18$ , and  $-30$  °C for 12 months.

HPP-treated frozen hake, except for the models corresponding to HPP-treated samples stored at  $-18$  °C and  $-30$  °C. The coefficient of determination ( $R^2$ ) showed strong agreement between experimental and predicted values across all regressions, except for these two HPP-treated conditions. Similarly, the F-test probability values confirmed the statistical significance and reliability of the model in describing the experimental data, again apart from the HPP-treated frozen hake stored at  $-18$  °C and  $-30$  °C, where model performance was notably lower.

The model predicts that cooked firmness increased to a peak value of 1.45 N/mm in hake stored at  $-10$  °C during the first 3 months. After this period, hydrolysis reactions became dominant, resulting in a decline in

firmness to 1.31 N/mm by month 12. Nevertheless, these values remained higher than those observed at  $-18\text{ }^{\circ}\text{C}$  and  $-30\text{ }^{\circ}\text{C}$ , where firmness ranged between 0.54 and 0.67 N/mm throughout the storage period.

Pre-treatment with high-pressure processing (HPP) significantly enhanced the cooked firmness of frozen hake, particularly at  $-10\text{ }^{\circ}\text{C}$ , where it reached a maximum of 2.57 N/mm after 9 months of storage.

The estimated kinetic parameters for cooked toughness are presented in Table 4, and Fig. 5 compares the experimental data with the model predictions.

Similarly, the model predicts that cooked toughness (N/mm-s) increased to a peak value of 12.68 N/mm-s during frozen storage at  $-10\text{ }^{\circ}\text{C}$  after 3 months. Beyond this point, hydrolysis reactions became predominant, leading to a marked reduction in toughness to 8.76 N/mm-s after 12 months. Despite this decrease, toughness values at  $-10\text{ }^{\circ}\text{C}$  remained consistently higher than those at  $-18\text{ }^{\circ}\text{C}$  and  $-30\text{ }^{\circ}\text{C}$ , where toughness values remained relatively stable around 6 N/mm-s throughout the storage period. This suggests that ultra-low frozen storage at  $-30\text{ }^{\circ}\text{C}$  offers no additional advantage over  $-18\text{ }^{\circ}\text{C}$  for preserving this texture parameter.

HPP pre-treatment also improved cooked toughness during frozen storage. The enhancement was most pronounced at  $-10\text{ }^{\circ}\text{C}$ , where toughness increased steadily over time. In contrast, at  $-18\text{ }^{\circ}\text{C}$  and  $-30\text{ }^{\circ}\text{C}$ , the increases were more modest and stabilized earlier. Overall, the results indicate that  $-18\text{ }^{\circ}\text{C}$  is sufficient to maintain acceptable levels of cooked toughness in HPP-treated hake during 12 months of frozen storage, and that lowering the temperature to  $-30\text{ }^{\circ}\text{C}$  does not yield further improvements.

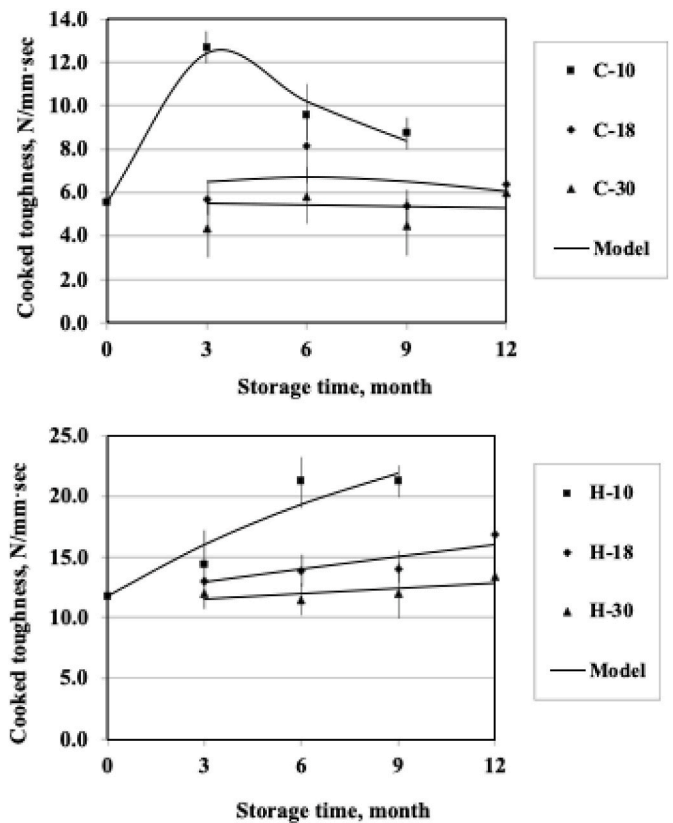
### 3.3. Changes in colour parameters during frozen storage

The results for the colour parameters  $L^*$ ,  $a^*$ , and  $b^*$  are presented in Table 5 it can be observed that there are minimal differences in the colour parameters. At  $-30\text{ }^{\circ}\text{C}$ , colour is better preserved in both HPP-treated and control fish. Similar to the approach used for texture parameters, a kinetic model was initially tested to describe the effect of storage time on colour changes. However, the models were not statistically significant. The likely reason for this difference lies in the fundamentally distinct mechanisms governing texture versus color changes in fish muscle. Texture evolution can often be represented by sequential degradative and aggregative reactions (proteolysis, denaturation, aggregation, cross-linking), which fit well into a two-step kinetic framework (Chéret et al., 2006; Qiu et al., 2013). In contrast, color changes in fish during frozen storage are multifactorial, often driven by pigment oxidation (e.g., myoglobin or hemoproteins), enzymatic

**Table 4**

Kinetic and statistical parameters of cooked toughness in frozen hake stored at different temperatures (control) and previous high-pressure processing treatment on frozen hake.

	Stored $-10\text{ }^{\circ}\text{C}$	Stored $-18\text{ }^{\circ}\text{C}$	Stored $-30\text{ }^{\circ}\text{C}$
<b>Cooked toughness (Control)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0656	0.0640	0.0025
$k_2$ ( $\text{min}^{-1}$ )	4.9906	0.1100	0.0083
M0	1135.7	16.7	7.08
P0	5.6	5.6	5.60
$r^2$	0.9772	0.0700	0.2975
Adjusted $r^2$	0.9658	-0.3950	-0.0540
F-test Prob.	0.9824	0.0191	0.0060
<b>Cooked toughness (HPP)</b>			
$k_1$ ( $\text{min}^{-1}$ )	0.0594	0.0144	0.0058
$k_2$ ( $\text{min}^{-1}$ )	0.0124	0.0000	0.0000
M0	29.2	26.6	27.4
P0	11.8	11.8	11.0
$r^2$	0.9055	0.8075	0.5331
Adjusted $r^2$	0.8583	0.7113	0.2997
F-test Prob.	0.8772	0.7088	0.6183



**Fig. 5.** Predicted and observed cooked toughness values of frozen European hake (*Merluccius merluccius*) muscle for control (C) and high-pressure processing (H) pretreatment, stored at  $-10$ ,  $-18$ , and  $-30\text{ }^{\circ}\text{C}$  for 12 months.

browning, and ice crystal-induced microstructural changes that alter light scattering (Oliveira et al., 2017). These processes are not necessarily sequential or unidirectional, but rather overlapping and influenced by factors such as oxygen availability, lipid oxidation, and pigment denaturation.

Therefore, the failure of the mechanistic kinetic model for color does not invalidate its use for texture, but instead highlights that color evolution follows more complex, non-sequential pathways. In such cases, empirical models such as polynomials provide a better fit to experimental data, even if they lack a mechanistic basis. Consequently, empirical models based on polynomial equations were applied instead, incorporating two numerical factors (storage time and storage temperature) and one categorical factor (HPP pre-treatment vs. no pre-treatment).

For luminosity ( $L^*$ ), the best-fitting model was linear. ANOVA results confirmed that the model was statistically significant, with a p-value (Prob > F) of 0.0417. Among the tested factors, HPP pre-treatment had the most significant effect on  $L^*$  values ( $p = 0.0161$ ), followed by storage time, which had a weaker influence ( $p = 0.1049$ ).

For samples without HPP pre-treatment, the model was:

$$L^* = 51.76 + 0.1623 \times \text{Storage Time} + 0.0063 \times \text{Temperature} \quad (3)$$

For HPP-pretreated samples, the model was:

$$L^* = 53.81 + 0.1623 \times \text{Storage Time} + 0.0063 \times \text{Temperature} \quad (4)$$

As shown in Fig. 6, the model predictions demonstrate an increase in  $L^*$  values with both HPP pre-treatment and longer storage time. This trend indicates that HPP may enhance the lightness of the samples during frozen storage.

The  $a^*$  parameter was fitted using a two-factor interaction (2FI) model. ANOVA results showed that the most influential factor was the

**Table 5**

Colour parameters (L, a, b\*) of hake as affected by storage temperature, time, and HPP pre-treatment (150 MPa, 2 min). Values are expressed as mean  $\pm$  standard deviation.

Storage Time (month)	Storage Temperature (°C)	HPP pretreatment	L	a	b
0	-30	HPP	51.04 $\pm$ 2.06	-2.58 $\pm$ 0.75	-0.63 $\pm$ 1.68
0	-18	HPP	51.04 $\pm$ 2.06	-2.58 $\pm$ 0.75	0.63 $\pm$ 1.68
0	-10	HPP	51.04 $\pm$ 2.06	-2.58 $\pm$ 0.75	0.63 $\pm$ 1.68
3	-10	HPP	57.00 $\pm$ 2.65	-1.96 $\pm$ 0.67	2.03 $\pm$ 1.38
6	-10	HPP	53.38 $\pm$ 2.55	0.14 $\pm$ 1.81	4.62 $\pm$ 1.88
9	-10	HPP	56.49 $\pm$ 3.33	-1.79 $\pm$ 1.42	3.78 $\pm$ 3.11
3	-18	HPP	55.80 $\pm$ 5.26	-1.78 $\pm$ 0.98	3.34 $\pm$ 1.88
6	-18	HPP	57.08 $\pm$ 3.25	-1.36 $\pm$ 0.68	2.74 $\pm$ 1.99
9	-18	HPP	52.78 $\pm$ 2.64	-0.36 $\pm$ 1.70	8.46 $\pm$ 3.58
12	-18	HPP	56.58 $\pm$ 1.48	-0.64 $\pm$ 1.57	5.45 $\pm$ 2.36
3	-30	HPP	57.14 $\pm$ 2.45	-1.67 $\pm$ 0.84	3.11 $\pm$ 1.69
6	-30	HPP	56.67 $\pm$ 2.37	-1.65 $\pm$ 1.19	2.67 $\pm$ 1.17
9	-30	HPP	53.30 $\pm$ 1.79	-1.48 $\pm$ 0.52	5.02 $\pm$ 1.67
12	-30	HPP	54.84 $\pm$ 2.64	-1.83 $\pm$ 0.51	3.40 $\pm$ 1.78
0	-30	No-HPP	51.75 $\pm$ 2.36	1.19 $\pm$ 0.28	2.26 $\pm$ 1.27
0	-18	No-HPP	51.75 $\pm$ 2.36	1.19 $\pm$ 0.28	2.26 $\pm$ 1.27
0	-10	No-HPP	51.75 $\pm$ 2.36	1.19 $\pm$ 0.28	2.26 $\pm$ 1.27
3	-10	No-HPP	50.36 $\pm$ 1.52	-1.15 $\pm$ 1.22	3.32 $\pm$ 2.29
6	-10	No-HPP	54.96 $\pm$ 1.93	-0.29 $\pm$ 1.11	3.29 $\pm$ 1.50
9	-10	No-HPP	53.00 $\pm$ 1.37	-0.87 $\pm$ 0.35	5.07 $\pm$ 2.00
3	-18	No-HPP	53.16 $\pm$ 1.55	-0.65 $\pm$ 1.40	5.02 $\pm$ 1.94
6	-18	No-HPP	57.76 $\pm$ 3.25	-1.05 $\pm$ 0.74	3.30 $\pm$ 1.05
12	-18	No-HPP	51.85 $\pm$ 2.57	-1.24 $\pm$ 0.39	4.31 $\pm$ 0.67
3	-30	No-HPP	52.90 $\pm$ 2.62	-1.72 $\pm$ 0.45	4.73 $\pm$ 0.90
6	-30	No-HPP	60.91 $\pm$ 3.60	-0.46 $\pm$ 0.58	3.01 $\pm$ 0.54
12	-30	No-HPP	52.71 $\pm$ 2.12	-0.57 $\pm$ 0.93	4.44 $\pm$ 1.82

interaction between storage time and HPP pre-treatment ( $p = 0.0008$ ), followed by the main effect of HPP pre-treatment ( $p = 0.00389$ ).

For samples without HPP pre-treatment, the resulting model was:

$$a = 0.4093 - 0.0911 \times \text{Storage Time} - 0.0030 \times \text{Temperature} - 0.0056 \times \text{Storage Time} \times \text{Temperature}^{\dagger} \quad (5)$$

For samples with HPP pre-treatment, the model was:

$$a = -2.3562 + 0.2434 \times \text{Storage Time} - 0.0030 \times \text{Temperature} + 0.0055 \times \text{Storage Time} \times \text{Temperature}^{\dagger} \quad (6)$$

Fig. 6 illustrates the predicted trends for a\*. The slightly negative values suggest a pale, greyish appearance with a slight greenish hue, which may be linked to oxidation of muscle pigments, such as myoglobin, or the formation of metmyoglobin, commonly associated with colour deterioration in stored or processed fish.

For yellowness (b\*), the best-fitting model was linear. ANOVA results confirmed the statistical significance of the model, with a p-value (Prob > F) of 0.0004. Among the variables tested, storage time had the most significant effect on b\* values ( $p = 0.00001$ ), followed by HPP pre-treatment, which showed a weaker but still significant influence ( $p = 0.0419$ ).

The resulting model for samples without HPP pre-treatment was:

$$b^* = 2.3185 + 0.2858 \times \text{Storage Time} + 0.0072 \times \text{Temperature} \quad (7)$$

For HPP-pretreated samples, the model was:

$$b^* = 1.2642 + 0.2858 \times \text{Storage Time} + 0.0072 \times \text{Temperature} \quad (8)$$

Fig. 6 shows the predicted trends for b\*. An increase in b\* values over storage time was observed, indicating a shift toward yellow tones, which may be associated with oxidation processes or protein/lipid degradation. HPP pre-treatment moderated this increase, suggesting a protective effect against discoloration during frozen storage.

### 3.4. Comparison with chemical changes

It was demonstrated that HPP pretreatment at 150 MPa effectively suppressed the accumulation of degradative nitrogenous and lipid-

derived compounds (dimethylamine, formaldehyde, trimethylamine, and free fatty acids) during frozen storage at both  $-18^{\circ}\text{C}$  and  $-10^{\circ}\text{C}$  (Carrera et al., 2020). They also reported a modest increase in peroxide values across all temperatures but observed a clear inhibition of fluorescent tertiary-oxidation products immediately after freezing and, notably, at month 9 in samples held at  $-10^{\circ}\text{C}$ .

In our study, a kinetic texture-decay model was applied to the HPP-pretreated and control samples over 12 months of frozen storage (up to month 9 at  $-10^{\circ}\text{C}$ ). We found that HPP pretreatment significantly slowed the softening rate (i.e., higher toughness and firmness values) and colour deterioration compared with controls, particularly at  $-10^{\circ}\text{C}$ . These findings are consistent with the inhibition of advanced oxidation markers reported by Carrera et al. (2020), suggesting that HPP helps stabilize the muscle matrix and lipid-protein interfaces under mild frozen conditions, thereby delaying both chemical and physical degradation.

These observations align closely with other results where the chemical quality of hake muscle subjected to HPP pretreatment (150–450 MPa) followed by frozen storage at  $-10^{\circ}\text{C}$  for 5 months was evaluated (Vázquez et al., 2018). That work demonstrated a significant reduction in the formation of dimethylamine (DMA), formaldehyde (FA), free fatty acids (FFAs), and trimethylamine (TMA), especially at higher pressures. Although peroxide and TBARS levels were not significantly altered by HPP, their study confirmed the capacity of HPP to mitigate degradative pathways. Interestingly, our texture and colour data extend these chemical insights by demonstrating that HPP-induced protection at the molecular level is translated into improved macroscopic properties, such as firmness retention and reduced discoloration during extended frozen storage.

Furthermore, while Vázquez et al. (2018) reported minimal impact of HPP on primary oxidation (peroxides), now it was observed that HPP-treated samples stored at  $-18^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  maintained structural integrity and colour stability, even when early peroxide formation occurred. Structural integrity was defined as the preservation of myofibrillar proteins, connective tissue, and the overall organization of the muscle fiber network.

This suggests that HPP combined with deep frozen storage can decouple early oxidative markers from textural degradation, possibly

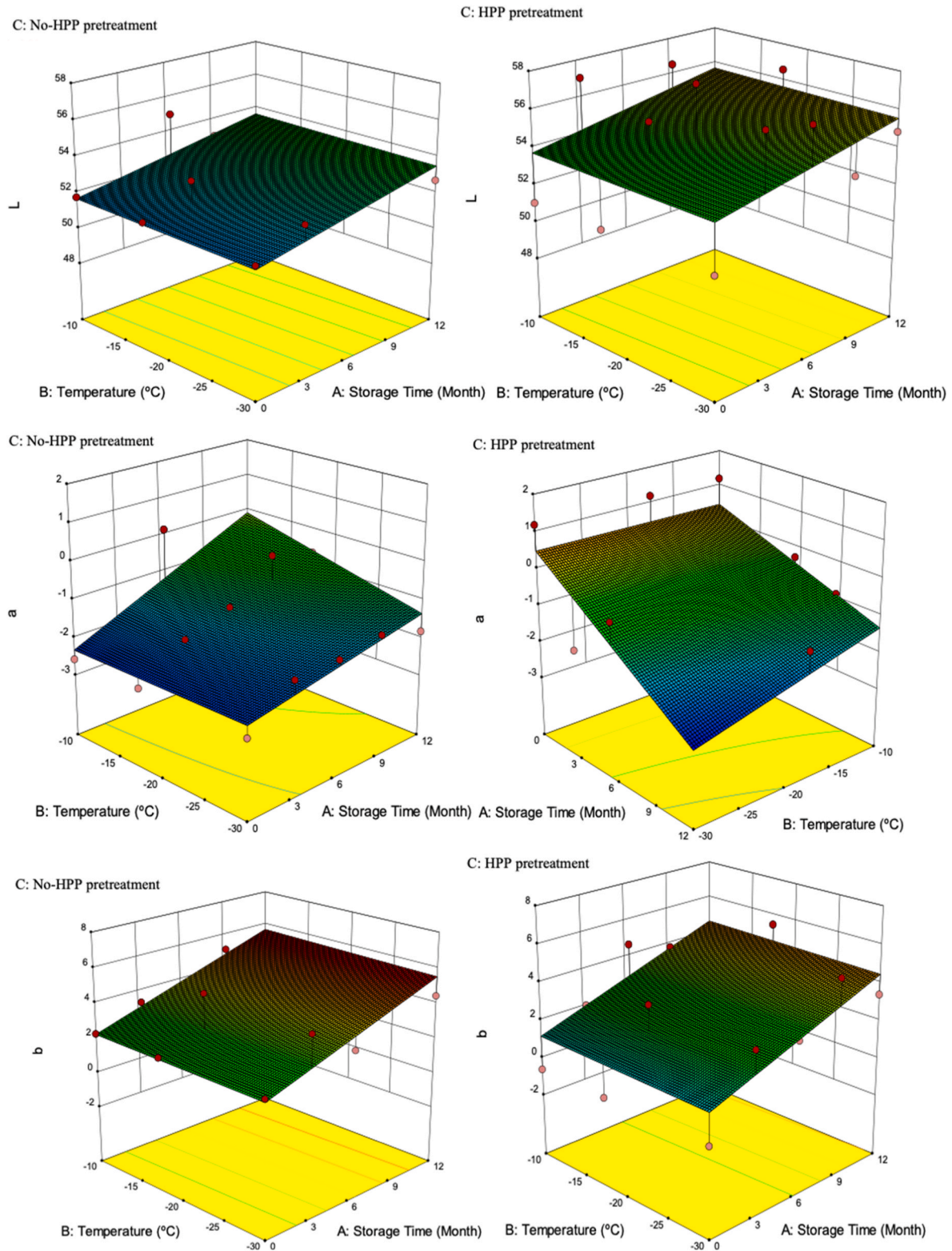


Fig. 6. Predicted effects of storage temperature, duration, and control (No-HPP) and HPP pretreatment on colour parameters of raw European hake (*Merluccius merluccius*) muscle.

due to the limited propagation of lipid oxidation in stabilized tissue matrices.

Taken together, these findings support that HPP pretreatment not only modulates key chemical degradation pathways, including lipid and nitrogenous compound formation, but also yields tangible benefits in texture and colour preservation. The consistent pattern of protection

observed at frozen storage at  $-10\text{ }^{\circ}\text{C}$  across studies highlights the critical interaction between pressure-induced structural changes and the temperature-sensitive kinetics of spoilage during long-term frozen storage.

Future studies should investigate the morphological changes in muscle fibers and connective tissue induced by HPP and frozen storage.

Coupling kinetic modeling with microstructural analyses would enhance the interpretation by linking macroscopic textural properties to microscopic alterations in myofibrils and connective tissue. In addition, factors such as temperature fluctuations, freeze–thaw cycles, and variability during long-term storage play critical roles in fish quality deterioration and should also be examined.

#### 4. Conclusions

This study demonstrates that storage time, temperature, and HPP pretreatment significantly influence the texture and colour stability of frozen European hake. In general, raw and cooked texture parameters (specifically toughness and firmness) increased with storage time, although this trend became less consistent beyond 6 months. Lower storage temperatures, particularly  $-30\text{ }^{\circ}\text{C}$ , were associated with reduced values for these texture parameters, indicating a protective effect against textural hardening during long-term frozen storage.

HPP pretreatment at 150 MPa for 2 min led to consistently higher values of toughness and firmness in both raw and cooked samples, suggesting a structural reinforcement effect induced by pressure. This impact was especially pronounced at  $-10\text{ }^{\circ}\text{C}$ , where both texture and colour changes were better preserved compared to untreated controls, highlighting the synergistic influence of frozen storage and HPP in maintaining product quality.

The combined analysis of storage conditions and HPP treatment revealed complex interactions influencing textural behaviour. While pressure treatment enhanced initial textural strength, the degree of preservation during storage was modulated by temperature and time. These findings underscore the importance of optimizing frozen storage protocols in conjunction with HPP to ensure maximum quality retention in frozen fish products.

In terms of colour stability, HPP-treated fish showed greater lightness retention and smaller shifts in  $a^*$  and  $b^*$  values throughout storage, indicating a protective effect against discoloration. This suggests that HPP not only preserves textural integrity but also contributes to improved visual quality during frozen storage.

Overall, HPP at 150 MPa for 2 min combined with storage at  $-18\text{ }^{\circ}\text{C}$  or  $-30\text{ }^{\circ}\text{C}$  emerges as a promising strategy for extending the shelf-life of frozen hake by preserving both structural and visual attributes.

#### CRedit authorship contribution statement

**Esther Guerra-Rodríguez:** Writing – original draft, Investigation. **Patricia Cazón:** Writing – review & editing, Formal analysis. **Santiago P. Aubourg:** Writing – review & editing, Supervision, Conceptualization. **Manuel Vázquez:** Writing – review & editing, Project administration, Conceptualization.

#### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Chatgpt to correct spelling and grammatical errors. After using this tool/service, they reviewed and edited the content as needed and assume full responsibility for the content of the publication.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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