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FOOD TECHNOLOGY

Optimization of high pressure processing parameters to enhance the quality attributes of scallops (*Nodipecten nodosus*)

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ABSTRACT: Seafood is one of the most important sources of nutrients. However, they have a short shelf-life and the traditional preservation methods may generate losses in their natural flavour and nutrients. Thisstudy evaluated and optimize the High Pressure Processing (HPP) regarding pressure level (200–400 MPa) and holding time (0–5 min) applied to lion's paw scallop (*Nodipecten nodosus*) to reduce microbial contamination while maintaining desirable characteristics. Response surface methodology with a Box-Behnken design and Desirability function were employed to simultaneously optimize these quality attributes. HPP enhanced microbial quality at 200 MPa/5 min, despite promoting inadequate physical-chemical modifications in the adductor muscle of the scallop. In such processing condition, in spite of a slight increase in muscle humidity which could be of benefit, pH increase was also verified, as well as a decrease in water holding capacity (WHC). At more severe level (400 MPa/5 min), a decrease in the shear force related to instrumental texture and in Whiteness (W) and Luminosity (L*) related to color was observed. Simultaneous optimization provided a value of 365MPa / 2min where physicochemical characteristics would be the more similar to the scallop without facing a preservation process.
Key words: optimization, desirability function, food safety, seafood, physicochemical characteristics.

Otimização de parâmetros de processamento de alta pressão para realçar os atributos de qualidade de vieiras (*Nodipecten nodosus*)

RESUMO: Os frutos do mar são uma das fontes mais importantes de nutrientes. No entanto, possuem vida de prateleira curta e os métodos tradicionais de conservação podem gerar perdas em seu sabor natural e nutrientes. O objetivo deste estudo foi avaliar e otimizar o Processamento por Alta Pressão (APH) em relação ao nível de pressão (200-400 MPa) e tempo de espera (0-5 min) aplicado à vieira pata de leão (*Nodipecten nodosus*) para reduzir a contaminação microbiana, mantendo características desejáveis. Metodologia de superfície de resposta com design Box-Behnken e função de Desejabilidade foram empregadas para otimizar simultaneamente esses atributos de qualidade. A APH melhorou a qualidade microbiana a 200 MPa/5 min, apesar de promover modificações físico-químicas inadequadas no músculo adutor da vieira. Nessa condição de processamento, apesar de um leve aumento da umidade do músculo que poderia ser benéfico, também foi verificado aumento do pH, bem como diminuição da capacidade de retenção de água (CRA). No nível mais severo (400 MPa/5 min), observou-se uma diminuição na força de cisalhamento relacionada à textura instrumental e na Brancura (W) e Luminosidade (L*) relacionada à cor. A otimização simultânea proporcionou um valor de 365MPa/2min em que as características físico-químicas seriam as mais semelhantes às da vieira sem enfrentar um processo de preservação.
Palavras-chave: otimização, função de desejabilidade, segurança de alimentos, frutos do mar, características físico-químicas.

INTRODUCTION

The lion's paw scallop (*Nodipecten nodosus*) is a bivalve mollusk belonging to the family Pectinidae and is a significant fishery resource in Rio de Janeiro, Brazil. The specie is native to the Brazilian coast, occurring in the Atlantic Ocean, from the south of the Yucatan Peninsula in Mexico, along eastern Central America and Caribbean Islands, Colombia, Venezuela coasts, and, discontinuously, along the coast of Brazil. While *N. nodosus* does not naturally form extensive banks on the Brazilian coast, its cultivation has become essential for supplying the consumer market (RUPP & PARSONS, 2016). Scallops are organisms of great interest for the development of global aquaculture, and they are widely renowned for the prized taste and nutritional value of their adductor muscle (YI, 2013). In addition,

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scallops are highly prized for their texture, flavor, and nutritional value, containing bioactive compounds, minerals, vitamins, omega-3 fatty acids, and high-quality proteins. (ARU et al., 2018).

The aquaculture of bivalve shellfish is a global activity contributing significantly to the economic development. These animals play an important role in worldwide mariculture activities with estimates of 17.7 million tonnes produced annually (FAO, 2020). Currently, Santa Catarina is Brazil's largest producer, followed by Rio de Janeiro (DA SILVA et al., 2022); and although, the Brazilian production is currently destined to supply only the domestic market, the potential of national malaco culture is large.

This food is often consumed raw or minimally cooked, which can pose health risks due to scallops' filtration feeding behavior. In addition, shelf life of seafood is reduced (MARTÍNEZ et al., 2017) because of physical-chemical characteristics like near-neutral pH, high water activity, and the presence of active autolytic enzymes, making it susceptible to microbial and oxidative degradation (PRABHAKAR et al., 2020). However, there is limited research on processing scallops for improved safety and durability.

High pressure processing (HPP) is particularly useful for seafood that is commonly consumed raw or minimally cooked in order to protect the health of the consumer (HSU et al., 2010). It is a non-thermal technology that provides the same level of food safety as heat pasteurization, resulting in fresher-tasting minimally processed foods (HSU et al., 2014). This technology reportedly increases shelf life, while minimizing loss of quality. Additionally, it maintains the nutritional value of food; and therefore, does not result in undesirable changes associated with thermal processing (ZHAO et al., 2019; BONFIM et al., 2019).

HHP has been applied very efficiently to control pathogens and to reduce deteriorating microbial load in seafood such as oysters, scallops, mussels, abalone, shrimp, octopus, squid, and various species of fish (PÉREZ-WON et al., 2005; MOOTIAN et al., 2013; YI et al., 2013; HSU et al., 2014; TEIXEIRA et al., 2014; BINDU et al., 2015; GINSON et al., 2015; SERMENT-MORENO et al., 2015; HUGHES et al., 2016; TSAI et al., 2022).

In addition to assuring microbiological safety, HHP can promote modifications in protein structures, leading to dissociation, unfolding, denaturation, aggregation, precipitation and gelatinization at different intensities (TRUONG et al., 2016) o alterationing in texture and color of seafood, which can impact consumer acceptance. In the fish industry, HHP gained space by shucking mollusks and crustaceans with reduction labor, muscle trim, and processing time (RONG et al., 2018). Studies with scallops and crayfish showed its efficiency for shucking at specific pressure and time settings (YI et al., 2013; SHAO et al., 2018). Therefore, the present investigated the effect of HPP on scallop muscle quality and optimize processing parameters to reduce microbial counts while maintaining physical-chemical, texture, and color attributes.

MATERIALS AND METHODS

Sample preparation and high-pressure (HP) Treatment

A total of 20 dozens of aquaculture scallops, approximately one year old, were purchased from the "Vieiras da Ilha" marine farm, in Ilha Grande, Rio de Janeiro State, Brazil. The scallops were manually dislodged from their shells, their organs were excised, and they underwent thorough cleaning using a continuous flow of water. Therefore, we only used their adductor muscle for the analyses. The scallops were then vacuum packed using poly nylon bags and held at 4 °C for 12 h prior to high pressure processing.

Pressure treatments were carried out in the high pressure processing lab equipment (Stansted Fluid Power, model S-FL-850-9-W) at Embrapa Food Technology pilot plant, Rio de Janeiro, Brazil. The dimension of the vertical pressure vessel were 4 cm in diameter and 30 cm in length, with a cylindrical holder sample with a useful volume of 250 ml, which contains several holes in its metallic wall throughout the liquid pressurization (70% ethanol) circulated. Samples were subjected to three different pressures of 200, 300 and 400 MPa for 0 and 5 min according to experimental design and compared with untreated sample (control). The term '0 min' refers to preliminary operation in which the pressure in the vessel increased up to the set pressure, followed by immediate decompression.

Experimental design

The experiments were planned and conducted using a 2^2 full factorial with three replicates in the central point, resulting in 7 experiments (BOX & BEHNKEN, 1960). The two factors were the pressure level (P) and the duration of time (t) the pressure was maintained (holding time).

The levels for pressure were 200, 300 and 400 MPa and for holding time were 0 2.5 and 5 min.

Sample analysis

Microbiological analysis

All samples were analyzed for counting of mesophilic and psycrophilic aerobic microorganisms. Twenty five grams of each sample were obtained aseptically and homogenized with two hundred twenty five ml of peptone water (0.1%) added NaCl (1%) in a filter bag using a homogenizer (Nova Ética, São Paulo, Brazil) for 15 s. Further decimal dilutions were made with the same diluent, and duplicates of at least three appropriate dilutions were plated on appropriate media. In order to enumerate the mesophilic and psycrophilic aerobic microorganisms, 0,1 ml of each dilution was pour-plated in Plate Count Agar (Difco, Detroit, MI, USA) with 1% NaCl, as described by SWANSON et al. (2001). After incubation at 25 °C/72 h (for mesophilic counts) and at 7 °C/10 days (for psycrophilic counts), plates with 10–250 colonies were counted, according to CRUZ-ROMERO et al. 2008b. Microbial data were expressed as logarithms of the colony-forming units (log CFU g−1).

Moisture and pH analyses

Moisture content was analyzed according to AOAC (2010). The pH was determined using a digital pH meter (Testo, model 205, Lenzkirch, Germany) equipped with a glass electrode, which was dipped into the adductor muscle. For each of the 7 experiments, three separate samples were collected and analyzed for pH assessment, ensuring accurate results.

Water holding capacity (WHC)

The WHC was evaluated according to the methodology proposed by GÓMEZ-GUILLÉN et al. (2002), for which 2 g of sample was subjected to a centrifugal force (centrifuge Hettich - Zentrifugem, model Routine 38R, Hamburg, Germany) at 4000 x g for 10 minutes at room temperature. Water holding capacity (WHC) was expressed as the percentage of water retained per 100 g of water present in the muscle prior to centrifugation. For each of the experiments, four samples were collected and analyzed for WHC evaluation (quadruplicate).

Color measurement

Color of adductor muscles was analyzed using a colorimeter (CR-400, Konica Minolta Chroma Meter, Osaka, Japan), adjusted to operate with D65 illuminant and observation angle of 10°. The colorimeter was calibrated before each series of measurements using a white ceramic plate (Y = 93.18, x = .3138 and y = .3328). The parameters L^* (lightness, ranges 0–100), a^* (from green ($-a^*$) to red ($+a^*$)), and b^* (from blue ($-b^*$) to yellow ($+b^*$)), were measured using he CIElab color scale (CHEN L. et al., 2022). The measures were automatically obtained after a light shot was discharged perpendicularly to the surface of the muscle. Ten repetitions were done with two readings per muscle. With these parameters, the "hue angle" (H°ab) was calculated, as it was chromaticity, total color difference (ΔE) and whiteness index with the following equations:

Whiteness index= $100- [(100 – L)^2 + a^2 +b^2]^{0,5}$ $\Delta E= [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0,5}$

The smaller the value of ΔE, the closer the samples are in color. Differences in perceivable color can be analytically classified as very distinct (ΔE > 3), distinct (1.5 < ΔE < 3) and small difference (1.5 < ΔE) (ADEKUNLE & OZOEMENA, 2010).

Texture analysis

The shear force required to cut the sample was evaluated according to the methodology described by (BELTRÁN-LUGO et al., 2006). For the texture measurement, a Stable Micron System texturometer TA-XT2, coupled to the Warner Bratzler (WB) device was used, operating at a speed of 20cm/min at a distance of 40mm. Shear force measurements were carried out perpendicularly to the muscle fibers as this has been shown to result in higher repeatability and reduced variability (TAYLOR et al., 2002). The recorded peak force was expressed in Newton (N). Ten muscles were analyzed per treatment.

Gel electrophoresis

Protein extraction

For the extraction of myofibrillar proteins 5g of the processed muscle and the control was used. Subsequently, the sample was homogenized in blender with 30mL of extractive solution (Phosphate Buffer K2HPO4 / KH2PO4 20mM + KCl 0.45M pH7.5). After blender homogenization, the material was filtered (Whatman No. 5) and the permeate transferred into Falcon tubes, which were kept under refrigeration for 1 hour and centrifuged at 6000 RPM for 15 minutes at 4 ° C. A 200 µL aliquot of the extract was collected along with 10 µL of sample buffer, for further application in electrophoresis gel.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The electrophoresis of proteins in a polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS/PAGE) was performed according to the method proposed by (LAEMMLI, 1970; ALAK et

al., 2021), using the BIORAD PROTEAN II xi Cell vertical electrophoresis system.

Acrylamide at the concentration of 12% on the running gel and 4% on the application gel was used. The electrophoretic run was performed over a period of seven hours and at voltage of 100V. The proteins in the gels were stained with 10% (v/v) acetic acid, 40% (v/v) methyl alcohol and 1% (v/v) Coomassie Brilliant Blue R250 overnight. The gel was decolorized in a solution containing 10% (v/v) acetic acid and 40% (v/v) methyl alcohol, the solution being renewed every 30 minutes until a clear development was obtained. The molecular mass of the protein fractions was calculated by constructing the standard curves with molecular weights of the markers against the respective distances traveled in the gel.

The molecular mass markers were those of the BIO-RAD LABORATORIES brand (Richmond, USA), with high molecular weight: myosin (201,653 kDa), ovalbumin (47,873 KDa), β-galactosidase (114,505 KDa), BSA-serum albumin bovine (72,516 KDa) and low molecular weight: phosphorylase B (102,567 kDa), ovalbumin (47,873 kDa), carbonic anhydrase (34,143 kDa), soybean trypsin inhibitor (26,890 kDa) and lysozyme (17,074 kDa).

Simultaneous optimization

The simultaneous optimization was performed using the desirability function proposed by DERRINGER & SUICH (1980). The Derringer´s desirability function allows to find experimental conditions (factor levels) to simultaneously achieve the optimum value for all evaluated variables considering the set priorities, and it has been widely used in distinct areas of food processing (GRANATO et al., 2010; DUONG & BALABAN, 2014; DENOYA et al., 2016; KAUSHIK et al., 2016; FERNANDEZ et al., 2018).

The desirability function was set to maximize the responses moisture and the WHC and to achieve a target pH of 6.3. The maximization of moisture and the WHC is desirable because they are directly related with the muscle juiciness and the pH was target at 6.3 to approximate with the control sample.

Statistical analysis

Response surface methodology (RSM) was used to estimate the effects of pressure and holding time on attributes (responces) of the scallop adductor muscle.

For each response variable (Y), the linear, quadratic, and simple interaction effects of the factors were tested (Equation 1). Each response variable (Y) was evaluated by ANOVA for linear regression using the F test at a significant level of 5%, additionally the adjustment of the models were tested using R^2 (coefficient of determination) and lack of fit test. The dependent variables were moisture, water holding capacity (WHC), and pH.

$$Y_i = \beta_o + \beta_{11}X_1 + \beta_{12}X_1^2 + \beta_{21}X_2 + \beta_{22}X_2^2 + \beta_{31}X_1.X_2 \quad (1)$$

Where X1 and X2 are the independent variables for pressure and holding pressure time; Y_i (i= 1 - 3) are the dependent variables (moisture, WHC and the pH); and β_i are regression coefficients. Model terms were selected at P-values < 0.05 by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Tables 1 and 2 show the experimental mean values of the evaluated quality and color attributes of muscle scallops, respectively. Among all the evaluated responses, some were not affected by treatment (chromatic parameters a^* and b^*), others were affected but did not fit the quadratic model (microbiologic, L^*, W, ΔE and textural parameters) while others were affected by treatment and well fitted the model (moisture, pH and WHC). The regression coefficients of the fitted models for each response variable are described in table 3. Coefficients of determination (R^2) and lack of fit for each equation are also presented. R^2 in all cases was higher than 0.8, indicating that the equations obtained for each response variable explained the variation adequately.

Microbial quality

The mesophilic and psycrophilic counts after the treatment of scallop adductor muscle treated wuth HHP are shown in table 1. Microbial counts of adductor muscles exhibited a low microbiological load as indicated by mesophiles (3.7 log10 cfu / g) and psycrophiles (2.9 log10 CFU/ g) in control samples.

The processing at 200MPa for 0 min promoted a small reduction in counts of mesophilic and psycrophilic bacteria in relation to the control sample. The 200MPa/5min treatment was sufficient to reduce the count to an unmeasurable level, primarily due to the longer holding time. Other treatments with HHP reduced the growth of mesophilic and psycrophilic microorganisms (Table 1) to a non-determinable level. This reduction in bacterial load may be attributed to the breakdown of plasma membrane, protein denaturation, and changes in the cell wall permeability of the bacteria, among other effects (TRUONG et al., 2016; CHEN et al., 2021).

Table 1 - Experimental values for HHP (High Hydrostatic Pressure)-treated scallop muscle adductor quality attributes.

	Moisture (%)	pH	WHC (%)	Force (N)	Mesophilic	Psycrophilic
Control	78.14 ± 0.74a	6.28 ± 0.04ab	94.52 ± 1.21abc	7.55 ± 1.76c	3.7 ± 0.0	2.9 ± 8.5
P200T0	79.32 ± 0.50ab	6.20 ± 0.01a	95.34 ± 0.71bc	6.11 ± 0.76abc	3.6 ± 0.7	2.5 ± 9.9
P200T5	80.02 ± 0.24bc	6.44 ± 0.01c	90.03 ± 2.90a	6.57 ± 0.97abc	ND	ND
P300T2,5-1	80.46 ± 0.04bc	6.49 ± 0,00c	95.69 ± 0.15c	6.19 ± 0.88abc	ND	ND
P300T2,5-2	80.69 ± 0.20c	6.50 ± 0.01c	95.49 ± 0.47bc	6.95 ± 0.62abc	ND	ND
P300T2,5-3	80.62 ± 0.15bc	6.51 ± 0.01c	94.88 ± 2.90bc	5.99 ± 0.50ab	ND	ND
P400T0	80.52 ± 0.04bc	6.69 ± 0.01d	95.70 ± 1.19c	5.66 ± 0.73a	ND	ND
P400T5	80.64 ± 0.11bc	6.32 ± 0.03b	91.00 ± 1.06ab	7.24 ± 1.58bc	ND	ND

Results are expressed as means plus/minus standard deviation.
a,b,c,d Means with different superscripts indicate significant differences ($P < 0.05$) amongst treatments.
Control: untreated muscle; P200T0; P200T5; 3 Central points (P300T2,5/1,2,3); P400T0; P400T5.
Mesophilic and psycrohilic population: log CFU g-1.
ND: not detected.

The data presented here are consistent with the literature. YI et al. (2013) in a scallop shucking study (*Argopecten irradians*) detected reduction of the mesophilic microbiota at unassessable level with application of 200MPa for 3 min. In line with this, LIU et al. (2022) verified a reduction in the mesophyll count in oysters treated at 200, 400 and 600MPa for 3 minutes. A decrease in total microbiota after HP treatment of seafood in the range of 200–600 MPa has previously been reported (CRUZ-ROMERO et al., 2008a; GINSON et al., 2015; REYES et al., 2015; CHEN et al., 2021; TSAI et al., 2022).

Effect of HP-treatment on color of scallop muscle

Color is one of the main attributes of foods that influence acceptability by consumers and purchasing decision (PATHARE et al., 2013; SUEMITSU & CRISTIANINI, 2019). The color of seafood muscle is related not only with carotenoids and heme pigments, namely myoglobin and hemoglobin (KAUR et al., 2016), but also with the muscle physical structure and the amount of unbound water that influences light scattering (CHÉRET et al., 2005).

Significant changes were observed in the surface color of scallop adductor muscle following HPP (Table 2). The scallop samples lost their transparency with increased pressure intensity and holding time as clearly indicated by increased L^* values. Higher pressure levels resulted in brighter and less transparent adductor tissue, consistent with findings in other fishes and seafood including scallops, red abalone, shrimp and oysters (CRUZ-ROMERO et al., 2004; BRIONES-LABARCA et al., 2012; YI et al., 2013; CHEN et al., 2022; LIU et

Table 2 - Experimental values for color parameters of scallop adductor muscle treated with HHP (High Hydrostatic Pressure).

	L^*	a^*	b^*	W	ΔE
Control	56.88 ± 2.87a	-0.51 ± 0.29a	5.78 ± 2.92a	56.42 ± 3.06c	-
P200T0	56.83 ± 1.94a	-0.46 ± 0.24a	4.97 ± 2.64a	56.47 ± 1.85c	3.47 ± 2.18a
P200T5	62.00 ± 2.55b	-0.41 ± 0.46a	6.33 ± 3.07a	61.34 ± 2.15b	7.16 ± 2.92ab
P300T2,5-1	63.91 ± 3.48b	-0.71 ± 0.50a	6.06 ± 2.64a	63.29 ± 3.08b	6.56 ± 4.14ab
P300T2,5-2	63.91 ± 3.48b	-0.82 ± 0.33a	5.33 ± 1.84a	62.72 ± 2.95b	7.21 ± 4.55ab
P300T2,5-3	64.54 ± 3.41bc	-0.73 ± 0.30a	5.65 ± 2.67a	63.6 ± 3.55b	8.81 ± 4.60bc
P400T0	61.76 ± 3.15b	-0.74 ± 0.54a	6.26 ± 2.35a	61.17 ± 2.93b	5.67 ± 3.64ab
P400T5	67,99 ± 3,55c	-0.74 ± 0.54a	5.10 ± 3.42a	67.39 ± 3.18a	11.84 ± 4.39c

Results are expressed as means plus/minus standard deviation a,b,c,d Means with different superscripts indicate significant differences ($P < 0.05$) amongst treatments.
Control: untreated muscle; P200T0; P200T5; 3 Central points (P300T2,5/1,2,3); P400T0; P400T5.

Table 3 - Regression coefficients, R2 values and fit test results for scallop adductor muscle response variables undergoing HPP (High Hydrostatic Pressure).

Regression coefficient	Moisture (%)	WHC (%)	pH
P (linear)	0.4562*	0.3296	0.0938*
t (linear)	0.2062	-2.5032*	-0.0338*
P2 (quadratic)	0.2322*	1.1697*	0.0435*
t2 (quadratic)	Ne**	Ne**	Ne**
P*t	80.2786*	93.7965*	6.4403*
R2	0.9239	0.9874	0.9085
Lack of fit	0.1301	0.5435	0.0082

*Significant at 0.05 level.
Ne**: no effect.
Reduced equations for process parameters:
Moisture (%): $Y_1 = 80{,}2786 + 0.4562X_1 + 0{,}2322X^2_1 + 0.2062$.
pH: $Y_2 = 6.4230 + 0.0938X_1 + 0.0435X^2_1 - 0.0338X_2 - 0.1512X_1X_2$.
WHC: $Y_2 = 93.7966 - 0.3296X_1 + 1.1670X^2_1 - 2.5033X_2$.
WHC: Water holding capacity.

al., 2022). Similarly, for a given pressure level, the L* value increased with increasing processing time, according to SEQUEIRA-MUNOZ et al. (2006) on the application of HHP to carp. The parameters redness (a* value) and yellow/blue color indicator (b* value) did not present a significant difference ($P \leq 0.05$) in relation to the control (Table 2).

Authors assume that changes in the L* value of HHP treated samples are due to modifications in the protein matrix, such as denaturation and coagulation of myofibrillar and sarcoplasmic proteins (CRUZ-ROMERO et al., 2004; CRUZ-ROMERO et al., 2007; CRUZ-ROMERO et al., 2008c; TEIXEIRA et al., 2014; HUGHES et al., 2015). Protein coagulation changes sample surface properties and increases light reflection, which results in white color (KRUK et al., 2011). Lipid oxidation is another possible reason suggested for color changes in fish products, due to degradation of highly unsaturated carotenoids such as astaxanthin CRUZ-ROMERO et al., 2008c; KAUR et al., 2016).

Whiteness values (W) in samples treated with 200 MPa per 0 min of holding time were not significantly different from the control sample. The treatments 200Mpa/5min, 300Mpa/ 2.5min and 400Mpa / 0min showed a significant increase in the degree of whiteness in relation to the control, but not among them. However, the samples lost their translucency and became whiter with a higher pressure level (400 Mpa) and a retention time of 5 min, revealing a cooking appearance. MURCHIE et al. (2005) described that seafood after high pressure processing may present opacity appearance similar to that obtained by very light cooking.

In a study with chicken meat, KRUK et al. (2011) observed that HHP resulted in increased muscle brightness due to the loss of active pigment together with coagulation of proteins that altered the surface properties, thus increasing light reflection and whitening color. These effects can contribute to consumer's acceptance of raw eating seafood, but can be negative for frozen HHP-shucked adductor muscles of products purchased for subsequent cooking (YI et al., 2013).

It Is possible that HPP also increases the oxidizing potential of the medium, and consequently myoglobin oxidation occurs, in addition to other oxidative processes such as lipid and protein oxidation, which also affects the color (OLIVEIRA et al., 2017).

The ΔE values presented significant differences ($P \leq 0.05$) between HP treated and untreated scallop samples (Table 2), which can be considered very distinct ($\Delta E > 3$) based on a classification scale suggested for total color difference (ADEKUNLE & OZOEMENA, 2010) (see instrumental color methodology), even when using low intensity processes (lowest pressure / time levels).

Effect of HP-treatment on cutting strength of scallop muscle

Cutting resistance strength of the scallop adductor muscle is shown in table 1. A significant reduction in the shear force was observed in all

treatments except for the 400MPa for 5min, which presented a lower value with non-significant difference from the control ($P > 0.05$). Similar results were found by (PÉREZ-WON et al., 2005), in which hardness of muscle decreased after a single 10 min. pulse regardless the pressure level (200 MPa or 400 MPa). Nevertheless, scallops treated with step pulses did not change compared to unpressurised samples. ZHANG et al. (2015) also observed lower hardness in pressurized squid at 200, 400 and 600 MPa per 1 cycle of 20 min or two of 10 min.

However, many authors reported increased shear strength or hardness after pressurizing seafood. CRUZ-ROMERO et al. (2008a) observed that HPP treatment at 260 MPa, 400MPa or 600 MPa for 5 min at 20 °C increased cutting strength of oysters; HSU et al. (2010) reported the increase of shear strength in oysters at 300MPa for 0 min; YI et al. (2013) reported that HP treatment at 350 MPa for 0 min increased hardening in scallops.

LOPEZ-CABALLERO et al. (2000) suggested that the increase shear strength in oyster tissue after HPP might be due to denaturation-induced aggregation and water loss. HPP affects proteins by disrupting non-covalent interactions (electrostatic and hydrophobic) leading to the formation of new bonds within and between protein molecules (MESSENS et al., 1997; MARTÍNEZ et al., 2017).

HHP does not break covalent bonds, but it can modify weak energy bonds like hydrogen and hydrophobic bons, impacting protein structures (DING et al., 2022). However, the effects of HPP lack consistent data, potentially due to variations in parameters, fish species, and methodology used (BELTRÁN-LUGO et al., 2006; OLIVEIRA et al., 2017). Texture is influenced by several factors such as chemical composition (DUNAJSKI, 1980) and structure (TAYLOR et al., 2002).

The results obtained for instrumental texture are in agreement with the results commented next on SDS-PAGE analysis in which protein denaturation induced by HPP was observed (Figure 1).

Effect of HP-treatment on scallops muscle Adductor proteins

The electrophoretic profile of the myofibrillar proteins of the adductor muscle of scallops obtained in SDS-PAGE is shown in figure1. Bands with the respective molecular weights (MW) were observed: 202.02, 157.42, 112.88, 87.96, 46.71 and 43.41 kDa. The effect of pressure was quite visible on actin (43.41 kDa). In the control sample this band is strongly colored and, in the lines corresponding to the treatments P200T0 and P200T5, a band of PM 46.71 kDa was observed. This band appears more flushed in the P200T0 treatment and decreases the intensity, almost disappearing in the treatment with same level of pressure with the time of 5 minutes (P200T5). It suggested an actin unfolding effect at 200 MPa/0min (P200T0) and the beginning of degradation when the same level of pressure remained for 5 min. (P200T5). In the treatments with pressure levels of 300MPa and 400MPa it is also observed that the actin band became clearer. The MW 202.02 band, corresponding to the myosin heavy chain (MHC), appeared strongly stained in all treatments, suggesting that there was no denaturation due to pressure at any level studied.

The effects of HPP on proteins are related to the rupture of non-covalent interactions (electrostatic and hydrophobic) within protein molecules, and to the subsequent restoration of intra and intermolecular bonds within or between protein molecules (MESSENS et al., 1997; MARTÍNEZ et al., 2017), as previously discussed. According to CHEFTEL (1992), disulfide bonds are formed during pressurization, due to the proximity of sulfhydryl groups.

Also, other authors have reported that several factors including treatment time, pressure level and temperature (MESSENS et al., 1997; TEIXEIRA et al., 2014; LUO et al., 2021) influence in the degree of denaturation.

Moisture, pH and WHC of HP-treatment scallop muscle

Table 1 shows the mean values and standard deviations of the moisture content, WHC and pH for untreated and HHP-treated scallop adductor muscle samples. A slight but significant ($P \leq 0.05$) increase in moisture content was observed in samples treated by HHP in relation to the control. This result was in accordance with the previous literatures reported by BELTRÁN-LUGO et al. (2006), CRUZ-ROMERO et al. (2004), BRIONES-LABARCA et al. (2012), YI et al. (2013) and CHEN et al. (2022) who observed that HHP led to an increase of the moisture of treated seafood at pressure levels higher than 200 MPa.

The effects of the pressure level on the adductor muscle moisture content were not strictly linear since the equation contains both a significant ($P \leq 0.05$) positive linear coefficient and also a significant ($P \leq 0.05$) positive quadratic coefficient. As in all cases when the quadratic term is significant, there is a critical value (in this case only for pressure) that must be considered. The surface plot of moisture corresponding to pressure and holding time (Figure 2a) provides evidence

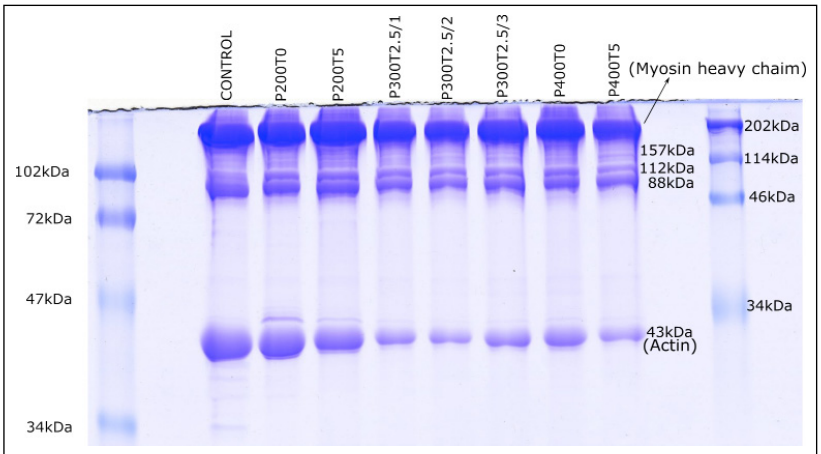

Figure 1 - SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) of scallop adductor muscle proteins. Molecular weight patterns are shown on the first and last lanes.

that the increase in pressure was responsible for the increase of moisture in the muscle.

One of the expected effects of HPP is the increase in protein hydration (OLIVEIRA et al., 2017). Thus, it is possible that the slight increase in moisture content is due to changes in the structure of the protein molecules (CRUZ-ROMERO et al., 2004; OLIVEIRA et al., 2017). Water holding capacity (WHC) has special importance in seafood products whether by affecting product yield or its direct relation with functional and sensory attributes of the final product and, consequently, consumer perception (LUO et al., 2021). The lowest WHC values, compared to the control sample, were for samples treated at 200 MPa/5 min and 400 MPa/5 min, by showing the influence of pressurizing time in this parameter. For WHC the effects of the pressure level and the holding time were not strictly linear since the equation contains both negative lineal coefficients (first order term) significant ($P \leq 0.05$) for holding time and positive quadratic coefficient significant ($P \leq 0.05$) for pressure (Table 3). Thus, as can be observed in Figure 2b, WHC values decreased with increasing holding time.

A possible explanation for the increase in WHC after pressurizing would be that HPP promotes cross-link interactions through hydrogen bonds and hydrophobic interactions which could retain water molecules (ZHANG et al., 2015). MARTÍNEZ et al. (2017) reported a similar behavior when analyzing pressurized crabs; the WHC values were higher than the control at 100 MPa and 300 MPa for 5 min, but at the pressure level of 600 MPa/5 min, the sample showed lower WHC. And they suggested that at this pressure, the electrostatic interactions, which stabilize the quaternary and tertiary structure of

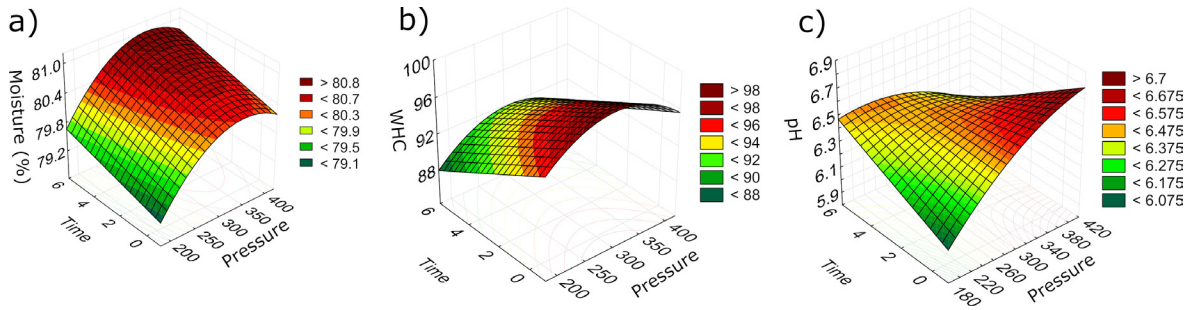

Figure 2 - Effect of Pressure level (P: 200–400 MPa) and holding time (t: 0–5 min) on moisture (a), WHC (Water Holding Capacity) (b) and pH (c) of scallop adductor muscle.

proteins and activate the reactions of sulfhydryl-disulfide bond exchange, can be disrupted. And these structural changes have resulted in the dissociation of proteins. Different fish species may also respond differently to HPP, with some showing increased WHC and others showing a reduction (CHRISTENSEN et al., 2017; OLIVEIRA et al., 2017). The influence of HPP on WHC appears to be influenced by the specific processing conditions and protein characteristics (OLIVEIRA et al., 2017; BONFIM et al., 2019).

The pH of the adductor muscle of "in natura" in the present study was on average 6.28. This data is in agreement with PACHECO-AGUILAR et al. (2008) evaluated in the same species, who found the value of pH 6.3. Higher values were found by BELTRÁN-LUGO et al. (2006) for the same species, ranging from 6.59 - 6.80 due to seasonality.

HP-treated scallop adductor muscle showed significantly ($P \leq 0.05$) increased pH relative to untreated scallop (Table 1), consistent with previous reports for oysters (CRUZ-ROMERO et al., 2004; BINDU et al., 2013; TEIXEIRA et al., 2014; CHEN et al., 2021). The pH was affected by both pressure level and waiting time. The effect of pressure was not strictly linear, since the equation contains both a significant positive linear and quadratic coefficient ($P \leq 0.05$). The effect of holding time in this case was strictly linear, with a significant negative regression coefficient ($P \leq 0.05$).

The muscular pH of consuming animals is due to metabolic routes that occurs in the post-mortem period. Like this, post-mortem glycolysis of fish muscle results in the accumulation of octopine, lactate and H+, which in turn lowers muscle pH (HILTZ & DYER, 1971) and with this reduces the net surface charge on muscle proteins, and causes their partial denaturation (GRAM & HUSS, 1996). The decrease in pH can lead to some WHC loss (GRAM & HUSS, 1996). Additionally, pH strongly influences the microbiology of fish muscle, specially pH sensitive spoilage bacteria (GRAM & HUSS, 1996).

Thus, authors suggested that the variation in pH can be attributed to conformational changes in muscle proteins associated with their denaturation, due to more or less exposure of acidic and basic amino acids groups (TEIXEIRA et al., 2014; CHEN et al., 2021).

Optimization of HPP conditions

Although, the scallop adductor muscle is considered a delicacy, an ingredient that is widely used in high gastronomy preparations and has an appreciable taste and texture, scallops are bivalve mollusks, organisms that have the alimentary habit of filtering suspended particles in the water column where they are cultivated, and this may represent health risks for consumers. Moreover, they are commonly consumed raw or partially cooked. Thus, the HHP applied to this type of food can favor the microbiological quality, reducing spoilage and even pathogenic microorganisms depending on the level of pressure and holding time employed.

However, it is known that HHP can influence the protein structure and consequently promote changes in muscle pH, texture and WHC values. Therefore, when proposing HHP processing, the effects on these parameters should be evaluated and adjusted to obtain the maximum favorable characteristics within a limit that provides a food with improved technological characteristics and also safe consumption.

Response variables with at least a statistically significant coefficient in the effects considered in the regression models (moisture, pH and WHC) were selected for simultaneous optimization of the process condition. As previously detailed, HPP affected each response differently. Therefore, this tool is fundamental to reach a compromise solution that allows to obtain good results for all variables under study. Figure 3 shows the profiles predicted at the different levels analyzed for each independent variable (pressure level and holding time), keeping constant the level of the other independent variable at the estimated optimal value. Figure 3 also shows each individual convenience function and global desirability function profiles.

The criteria selected for optimization of process parameters were: moisture content and WHC maximization; and pH close to the control ($P = 6.3$). Based on the above criteria, the predicted ideal process condition leading to the maximum value of the overall convenience function for the process under study was a combination of a pressure level of 363.33 MPa and a holding time of 1.7 min (which would correspond to practical operational values at 365 MPa and 2 min).

The desirability provided the most appropriate level of pressure and holding time for processing the scallop adductor muscle while maintaining the ideal physicochemical characteristics proposed here. This value of 360 MPa for 2 min exceeds the pressure level and waiting time required to bring the mesophilic and psychotropic microbial counts to an undetectable level under the conditions of that study. Thus, we can suggest that the HHP

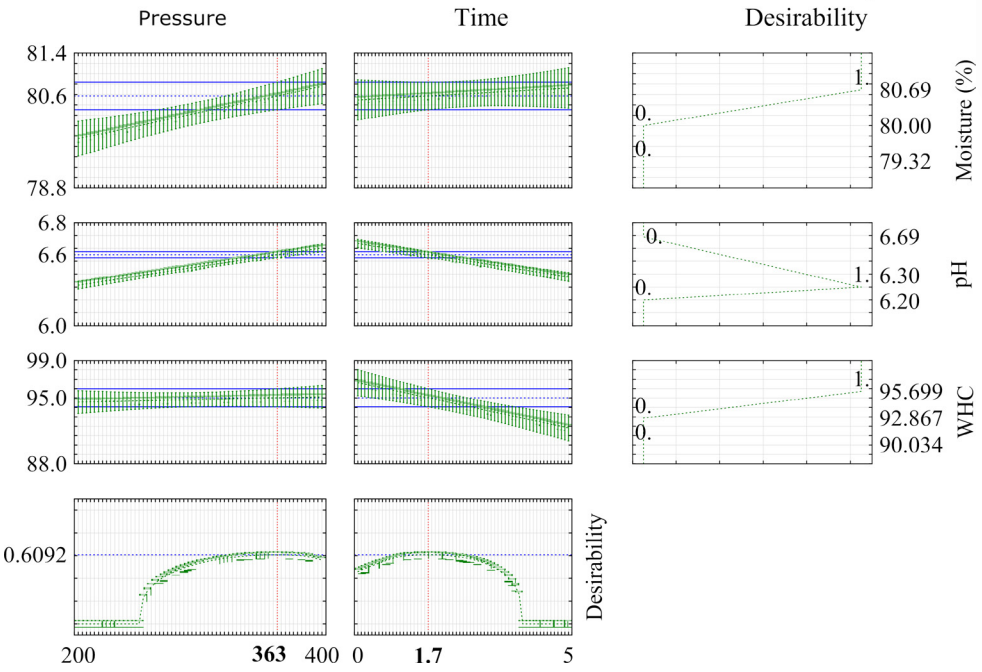

Figure 3 - Simultaneous optimization of process conditions for HPP of adductor muscle scallop.

applied to *N. nodosus* scallops, under the conditions studied, could significantly reduce the microbial load and maintain the desirable characteristics of succulence, and also maintain the pH equivalent to the control sample, since pH directly influences the development of the microbiota.

CONCLUSION

High Pressure Processing (HPP) considerably changed the quality of scallop adductor muscle, which varied according to the processing variables (pressure level and pressure holding time). In general, the treatments showed significant increases in moisture content and pH values. The increase in pressure level and holding time enabled improvements in microbiological quality, being the highest reduction in bacterial counts observed in the treatment at 200 MPa during 0min. The WHC presented antagonistic effects between the pressure levels and holding time, in which the less severe pressure level of 200 MPa promoted the greater increase of the value of WHC and the greater holding time of 5 min promoted a decrease of the parameter. The color of muscle were negatively affected in treatments at 200 and 400 MPa during 5 min, considering the standard characteristics of control sample, resulting in ΔE value above 3.0.

Cutting strength showed lower values in all treatments compared to the control, demonstrating that the applied treatments promoted the softening of the adductor muscle. Changes in the protein profiles (SDS–PAGE analyses) might explain the effect of HPP on adductor muscle physical properties. Depending on the principal goal for the application of HPP (e.g. microbiological safety or improved texture), different HPP conditions should be chosen. The optimization analysis suggests that HHP applied at 365 MPa and 2 min would lead to a product with proper characteristics and maximum reduction of spoilage microorganisms.

Future studies are needed to investigate the effect of HPP treatments in the quality attributes of scallop muscle during storage, mainly by including sensory evaluation and acceptance, which will enable to assess the potential of HPP to extend their shelf-life and potential use by the seafood industry.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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