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TESE

Aplicação de Alta Pressão Hidrostática no processamento de vieiras *"Nodipecten nodosus"* (Linnaeus, 1758)

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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APLICAÇÃO DE ALTA PRESSÃO HIDROSTÁTICA NO PROCESSAMENTO DE VIEIRAS *"Nodipecten nodosus"* (LINNAEUS, 1758)

ROSIANE COSTA BONFIM

Sob orientação do Dr.
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Tese submetida como requisito parcial para obtenção do grau de **Doutora em Ciências e Tecnologia de Alimentos,** no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Tecnologia de Alimentos.

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RESUMO GERAL

O processamento de alta pressão hidrostática (APH) é um método não térmico usado para assegurar a segurança microbiológica, mantendo ou incrementando as características sensoriais e nutricionais desejáveis, pois pode aumentar a biodisponibilidade. Na indústria de pescados, a APH é usada para descasque de frutos do mar como ostras e lagostas e extensão da vida de prateleira de peixes, mas t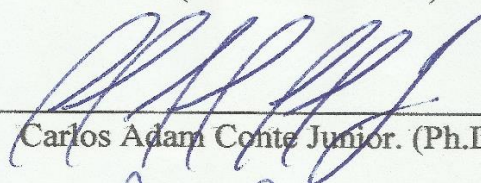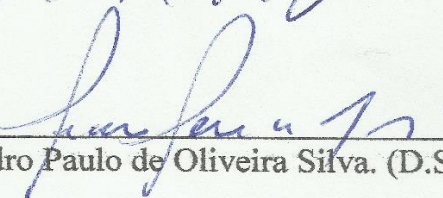em sido pouco investigada para processamento de vieiras. As vieiras são moluscos com alta perecibilidade que são tipicamente vendidas vivas ou congeladas (produto importado). Os objetivos dessa pesquisa foram 1) avaliar o efeito da APH nos atributos de qualidade do músculo vieira; 2) otimizar os principais parâmetros de processamento (nível de pressão e tempo de espera) para reduzir as contagens microbianas, mantendo atributos de qualidade fisico-química, textura e cor produtos e 3) investigar as alterações bioquímicas e microbiológicas e a influência na qualidade promovida pelo processamento por APH no músculo adutor de vieiras estocadas a 4°C por 21 dias. O capítulo I compreende uma revisão bibliográfica focada na aplicação da APH em moluscos bivalves. No capítulo II, foi realizado um planejamento experimental (delineamento Box-Bhenken) de otimização para obtenção da condição mais adequada para redução da carga microbiana e manutenção das características físico-químicas do músculo adutor. Os músculos adutores foram submetidos a pressões variando de 200 a 400 MPa e 0 a 5 min de tempo de processo e foram comparados a um controle. O nível de 200 MPa/5 min foi eficiente no controle da microbiota, no entanto, promoveu modificações físico-químicas no músculo adutor da vieira. APH promoveu um ligeiro aumento na umidade e pH, bem como uma diminuição na capacidade de retenção de água (*water holding capacity*, WHC). A força de cisalhamento relacionada à textura instrumental diminuiu e os parâmetros de cor Brancura (*whiteness*, W) e luminosidade (L *) do músculo aumentaram em nível mais intenso de pressão (400 MPa/5 min). Empregou-se a metodologia de superfície de resposta e a função desejabilidade para realização da otimização simultânea. A desejabilidade apontou as condições de 365 MPa por 2 min como a condição mais adequada para um processamento eficiente. No capítulo III, o tempo de vida de prateleira dos músculos adutores pressurizados a 300 MPa por 2,5 min e 400 MPa/5 min foram avaliados durante 21 dias a 4°C, utilizando-se parâmetros bioquímicos e de qualidade microbiológica, em comparação com controle não tratado por APH. A microbiota das amostras tratadas por pressão não excedeu o limite de 10^6 CFU/g e os parâmetros de qualidade, N-BVT, pH e TBARS apresentaram valores abaixo dos limites estabelecidos pela legislação. Os resultados desse estudo indicam o nível de 300 MPa por 2,5 min como a condição mais adequada para aumentar a vida de prateleira de vieiras refrigeradas.

Palavras-chave: Alta pressão hidrostática; moluscos bivalves; otimização simultânea de processo; vida de prateleira, deterioração bioquímica e microbiológica

ABSTRACT

BONFIM, Rosiane Costa. **Application of high pressure hydrostatic on processing of "Nodipecten nodosus" scallops** 2019. 76f. Thesis (Doctorate in Food Science and Technology) Institute of Technology, Department of Food Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2019.

High Hydrostatic Pressure (HHP) is a non-thermal technology used to increase food safety and shelf life. In the fishery industry, HHP has been used for shelling seafood such as oysters and lobsters and extending shelf life of fishes, but it has been little investigated for processing scallops. Scallops are mollusks with high perishability that are typically sold alive or frozen (imported product). The objectives of this research were 1) to evaluate the effect of HHP on scallop quality attributes; 2) to optimize the main processing parameters (pressure level and holding time) to reduce microbial counts while maintaining attributes related to nutritional quality, texture and color and 3) to investigate biochemical and microbiological changes promoted by HHP and their influence on the quality of scallop adductor muscle stored at 4 ° C for 21 days. In Chapter I a review was carried out focused on the application of HHP to bivalve mollusks. In Chapter II, an experimental optimization plan, Box-Bhenken design, was carried out to obtain the most adequate condition for decreasing microbial load and maintaining physical characteristics of the adductor muscle. The adductor muscles were submitted to pressures ranging from 200 to 400 MPa for 0 to 5 min holding time and were compared to a non-processed control. The level of 200 MPa/5 min was efficient for controlling the microbiota, however, it promoted physicochemical modifications in the adductor muscle of the scallop. HHP promoted a slight increase in moisture and pH as well as a decrease in water retention capacity (WHC). The shear force related to the instrumental texture decreased and the Whiteness (W) and brightness (L*) parameters of the muscle increased at a more severe pressure level (400 MPa/5 min). The response surface methodology and the desirability function were used to perform the simultaneous optimization. Desirability indicated conditions of 365 MPa for 2 min as the most suitable condition for efficient processing. In Chapter III, the shelf life of the pressurized adductor muscles at 300 MPa for 2.5 min and 400 MPa/5 min were evaluated for 21 days at 4 ° C using microbiological and biochemical quality parameters in comparison to the control. The microbiota of samples treated by high pressure did not exceed the limit of 10^6 FCU/g and the quality parameters, N-BVT, pH and TBARS presented values below the established legal limits. The results of this study indicate the level of 300 MPa for 2.5 min as the most adequate conditions to increase the shelf life of refrigerated scallops.

Keywords: High pressure hydrostatic; bivalve mollusks; Simultaneous process optimization; Shelf life; microbial and biochemical spoilage

SUMÁRIO

INTRODUÇÃO GERAL

As vieiras (*Nodipecten nodosus*) são moluscos bivalves, pertencente à família Pectinidae, sendo encontrados naturalmente desde a América Central, Colômbia, Venezuela, Santa Catarina e até o Sul do Rio de Janeiro. São organismos de alto interesse para o desenvolvimento da aquicultura brasileira, apresentando o maior valor agregado dentre os moluscos bivalves com alto potencial para comercialização, devido à boa receptividade e valorização do produto.

A atividade vem se destacando pelo grande potencial para cultivo, devido à alta taxa de crescimento, podendo ser cultivada de forma sustentável através da aquicultura. No entanto, moluscos bivalves, como as vieiras, mexilhões e ostras, são organismos filtradores e bioacumuladores que se alimentam principalmente de microalgas presentes na água do mar, concentrando, em seus tecidos, grande quantidade de substâncias químicas, resíduos orgânicos e inorgânicos, e microrganismos presentes na água. Desta forma, o consumo de moluscos bivalves sem nenhum processamento, ou mesmo tratados com temperaturas brandas, podem trazer grande risco de contaminação ao consumidor. Por outro lado, seu paladar suave pode ser facilmente alterado por tratamentos bruscos de temperatura.

Os mariscos são nutritivos e uma das fontes animais de comida mais rápidas do mundo. Frutos do mar são relatados como ricos em proteínas, ácidos graxos ômega-3, micronutrientes essenciais, consistindo fonte de nutrientes necessários à uma dieta balanceada. Os benefícios para a saúde do consumo de frutos do mar incluem redução do risco de doenças cardíacas e contribuição ao desenvolvimento de neurônios durante a gestação e na infância. Além disso, consistem também de uma fonte de renda importante para os países costeiros em desenvolvimento e desenvolvidos. A contaminação subsequente à captura de frutos do mar pode ocorrer em vários estágios, como processamento, armazenamento e distribuição. Fontes de contaminação incluem água, instalações, equipamentos e manipuladores. As etapas de processamento são particularmente importantes, devido à alta carga microbiana potencial na superfície. O número de microrganismos presentes no produto determina se a contaminação causará deterioração microbiana ou enfermidade. A taxa de deterioração dos frutos do mar é afetada por fatores intrínsecos e extrínsecos, como a composição dos frutos do mar, a atividade de água, o tipo de embalagem e a temperatura de armazenamento.

A qualidade dos frutos do mar depende das causas da deterioração entre outros fatores. A deterioração dos frutos do mar pode ser autolítica, química ou microbiana. Microbiota diversa é observada em frutos do mar. Apenas algumas dessas bactérias conhecidas como organismos específicos de deterioração causam deterioração de frutos do mar. Compreender o mecanismo de deterioração contribui para manter a qualidade e prolongar a vida útil dos frutos do mar. Enquanto as enzimas de frutos do mar nativas são responsáveis pela autólise, atividades microbianas de organismos específicos de deterioração resultam em deterioração dos frutos do mar com mais frequência do que a oxidação química ou autólise. Organismos específicos de deterioração produzem sabores, metabolitos de odor e dominam a microbiota de frutos do mar. Estes organismos de deterioração são principalmente bactérias Gram-negativas. O odor residual produzido inclui sulfeto de hidrogênio, bases voláteis totais e aminas biogênicas devido à atividade de descarboxilação de aminoácidos. Estes compostos, utilizados como marcadores de deterioração, reduzem a aceitabilidade dos consumidores, a palatabilidade dos frutos do mar e o prazo de validade.

O processamento por alta pressão hidrostática (APH) é considerado uma tecnologia não térmica, e possui, entre outras, a capacidade de destruir microrganismos patogênicos, e

apresenta um grande potencial para a indústria de moluscos bivalves. Métodos tradicionais de conservação, como o calor, podem ter efeitos prejudiciais ao gosto, textura e aparência de frutos do mar, causando rejeição dos consumidores. Em contraste aos alimentos processados termicamente, alimentos tratados por alta pressão geralmente mantêm características de sabor, aparência e qualidades nutricionais inalteradas.

A tecnologia de APH tem se mostrado muito adequada ao processamento de pescados, em especial moluscos bivalves e crustáceos. O processamento é comercialmente aplicado na indústria de ostras e crustáceos nos EUA, Canadá, Nova Zelândia, Austrália, Coreia do Sul e Grécia, na abertura de conchas de moluscos e descasque de crustáceos. O processamento sob APH é capaz de promover a separação física da carne de molusco e de crustáceo pela desnaturação proteínas especificas, que ligam a carne à concha e ao exoesqueleto. Assim, após aplicação da APH, o produto pode alcançar 100% de rendimento sem nenhum dano mecânico. No entanto, mudanças na textura, aparência, cor, capacidade de retenção de água (CRA), conteúdo de água durante o armazenamento e no cozimento, são obervadas. Mais recentemente, têm sido estudados os efeitos da APH sobre as enzimas presentes na musculatura do pescado e nos componentes oriundos da degradação do ATP, e interferência dessas ocorrências na vida de preteleira.

Esta tese está dividida em três capítulos, sendo o capítulo I uma revisão bibliográfica sobre a aplicação da tecnologia de alta pressão hidrostática em moluscos bivalves. Este capítulo originou um artigo de revisão intitulado "A review on high hydrostatic pressure for bivalve mollusk processing: relevant aspects concerning safety and quality", aceito e em vias de publicação pela revista Food Science and Technology (Campinas), conforme anexo I. No capítulo II foi realizado um estudo sobre a otimização de parâmetros de processamento de alta pressão para preservar atributos de qualidade de vieiras *N. nodosus*. E, no capítulo III, foi estudado parâmetros bioquímicos e microbiológicos referentes a qualidade de músculos de vieira influenciados pelo processamento por alta pressão armazenados sob refrigeração (4°C) por 21 dias.

OBJETIVOS E OBJETIVOS ESPECÍFICOS

Objetivo Geral

Avaliar a viabilidade técnica da aplicação da tecnologia de alta hidrostática (APH) no beneficiamento de vieiras (*Nodipecten nodosus)* submetidas a armazenamento refrigerado.

Objetivos Específicos

- Produção de material teórico sobre a aplicação da APH especificamente em moluscos bivalves, ressaltando os efeitos da tecnologia sobre as proteínas, microbiologia, aspectos nutricionais e sensoriais;
- Otimização dos parâmetros de processamento, pressão (MPa) e tempo de processo (minuto), e escolha do ponto ótimo em função de atributos de qualidade como: pH, umidade, capacidade de retenção de água, textura, cor e perfil eletroforético de proteínas do músculo adutor;
- Avaliação do efeito da APH sobre aspectos físico-quimicos, bioquímicos e microbiológicos de músculos de vieira estocados por 21 dias sob refrigeração.

CAPÍTULO I

A REVIEW ON HIGH HYDROSTATIC PRESSURE FOR BIVALVE MOLLUSK PROCESSING: RELEVANT ASPECTS CONCERNING SAFETY AND QUALITY

ABSTRACT

Mollusks are considered a nutritious source of food and their consumption has increased worldwide. However, their consumption, mainly of bivalves, has been considered responsible for numerous cases of foodborne diseases. This is related to their food intake, as they are filter-feeders and, consequently, bioaccumulate toxic compounds. High hydrostatic pressure (HHP) is recognized as an efficient technology to control pathogenic and deteriorating microorganisms, with low damage to the sensorial and nutritional properties of foodstuffs. This review addresses the use of HHP on bivalve mollusks, based on recent relevant studies in this field.

Practical application: Information generated from this study provide insights into HHP application and effects on bivalve mollusks, with information on process conditions, its effects on muscle proteins and microorganisms and its impact on extending product shelf-life. These data are extremely important for the development of further industrial applications of this novel, nonthermal, fresh seafood processing technology.

Keywords: Shellfish; Bivalves; Quality; Isostatic

1. INTRODUCTION

Mollusks, particularly bivalves, are often associated with food safety issues, due to recurrent episodes of gastrointestinal infections and food poisoning (MURCHIE et al., 2005). This is due to the physiological characteristics related to their nutrition, as they are filter-feeding animals with the capacity to bioaccumulate toxic chemicals and waterborne pathogens, including human intestinal viruses, certain sewage and wastewater bacteria, and bacteria naturally present in estuarine waters. Furthermore, toxins derived from plankton and dinoflagellates present in marine environments may also bioaccumulate in mollusks, leading to serious neurological consequences for seafood consumers (KINGSLEY et al., 2014).

Most mollusks are consumed whole, including their gastrointestinal tract, either raw or only lightly cooked (LEES, 2000), since more severe heat treatments cause detrimental effects to the taste and appearance of these marine animals, causing consumer rejection (MURCHIE et al., 2005).

The use of high hydrostatic pressure (HHP) on bivalve mollusks is currently under study more and of interest, due to its minimal effects on the sensorial characteristics and nutritional qualities of these organisms. This technology is used for open shucking of oysters and other mollusks, and has proven efficient in reducing microorganism loads, including of certain pathogens, such as *Vibrio parahaemolyticus*.

In contrast to traditional heat treatments, high pressure processing is able to reduce microbial loads without altering product physicochemical properties, since pressure is transmitted uniformly and instantaneously (isostatic process) and temperature variations in the process are low, of about 3ºC per 100MPa (adiabatic), depending on the food composition. These characteristics prevent food deforming or heating and any relevant organoleptic property alterations (RENDUELES et al., 2011).

HHP is able to inactivate microorganisms and enzymes due to protein modifications and/or denaturation, while valuable lower molecular weight components, such as vitamins and volatile compounds, responsible for food nutritional and organoleptic quality, remain unchanged (HEINZ and BUCKOW, 2010). Thus, the process makes it possible to extend the shelf life of food products with minimal effect on their nutritional properties and freshness (TRUONG et al., 2014).

This article presents a review of the HHP process applied to bivalve mollusks, pointing out effects on microbial load, shelf life, physical structure, chemical components and the advantages of this preservation industrial process.

2. LITERATURE REVIEW

2.1 High hydrostatic pressure (HHP)

HHP technology has been widely applied in the production of meat products, dairy products, aquatic products and vegetable and fruit products, as well as various beverage products. The global market for HPP foods reached approximately $9.8 billion in 2015 and is expected to culminate in a market value of $ 54.77 billion in 2025 (apud HUANG et al., 2017).

HHP processing applied to food consists in subjecting the hermetically packaged food to pressures ranging from 100 to 700 MPa, for a certain time, according to the purpose and/or equipment capacity (CHEFTEL, 1995; FARKAS e HOOVER, 2000). Food packed in flexible packages is placed in a compression chamber to undergo the pressurization process. The chamber, which is hermetically sealed, is then filled with the pressure transmitting fluid (usually water), thus expelling all the air inside the chamber. A predefined pressure is then initiated and

maintained for the set time and, at the end of this cycle, the chamber is depressurized (FARKAS end HOOVER, 2000; HOOVER et al., 1989). As the packed food is pressurized inside the pressure chamber, this processing presents little risk of cross-recontamination and even contamination in case of operational failures (PEREIRA and VICENTE, 2010).

HHP is based on the *Pascal* (or isostatic) and *Le Chatelier* principles. The former states that pressure is transmitted uniformly and almost instantaneously throughout the food, regardless of its mass, size or composition (with a certain minimum moisture content required for pressure transmission), while the latter states that any phenomenon (phase transition, molecular conformation change or chemical reaction) accompanied by a reduction in volume is favored by increased pressure (and vice versa) (BARBOSA-CÁNOVAS and RODRÍGUEZ, 2002; CHEFTEL, 1995).

The adiabatic condition of the process causes only a slight temperature variation with increasing pressure, regardless of the size and shape of the food, (the temperature increases approximately 3 °C per 100 MPa, depending on the food constitution), which prevents the food from being effectively deformed or heated (CHAWLA et al., 2011; SMELT, 1998). The pressurizing process is, therefore, independent of the volume and shape of the sample, unlike a thermal process (SOUSA and GONÇALVES, 2013).

However, although the food exhibits reduced compressibility, it shows a certain reduction in volume. According to FARKAS and HOOVER (2000), this reduction may reach up to 15% during the process, but reverts during depressurization, and is due to changes promoted, mainly, in proteins and water molecules (CHEFTEL and CULIOLI, 1997).

2.2   HHP effect on proteins

HHP processing is capable of altering the functional properties of food constituents. Protein conformation effects may lead to disruption, aggregation or gelation, depending on the protein system, applied pressure, temperature and treatment duration (MESSENS et al., 1997).

Under pressure, protein molecules behave according to "*Le Chatelier*" the law, where they suffer volume reduction due to the presence of internal spaces and the better packaging of water molecules (CHEFTEL and CULIOLI, 1997; TRUONG et al., 2014). These changes may promote the reduction of up to 1.0% of the protein volume, through changes in quaternary, tertiary and secondary structures (SILVA et al., 2001).

Ionic bonds and hydrophobic interactions, responsible for maintaining protein tertiary and quaternary structures, are disrupted and more easily broken at pressure levels of around 150 and 200MPa, while the secondary structure requires higher pressures, between 300 and 700MPa (CONSIDINE et al., 2008; HEREMANS and SMELLER, 1998; LULLIEN-PELLERIN e BALNY, 2002; OLIVEIRA et al., 2017). However, the primary structure of a protein, in other words, its native structure, is not influenced by HHP, since covalent bonds display reduced compressibility (CHEFTEL and CULIOLI, 1997).

As a consequence, complex organization structures that contain proteins, such as membranes, are altered due to the breakdown of hydrophobic and electrostatic interactions, as well as the disruption of some hydrogen bonds (CONSIDINE et al., 2008; FARLAS and HOOVER, 2000). Changes in protein structure can be reflect on numerous parameters such as texture, water content and color. Table 1 provides a compilation of the main effects caused by the HHP protein structure modifications in bivalve molluscs.

In bivalve mollusks, the main function of HHP application is shucking. Thus, this process can cause the disruption of non-covalent interactions in tertiary protein structures, leading to denaturation of muscle proteins and connective tissues and, ultimately, causing the release of the adductor muscle (RONG et al., 2018; HSU et al., 2010; CRUZ-ROMERO et al., 2004).

The effect of HHP on shucking has always been dominated by treatment pressure and time (HE et al., 2002). However several shellfish species (including bay scallops, oysters, mussels and clams) display different sensitivities to pressure or time. For example, YI et al. (2013) observed that bay scallops were fully released at 350 MPa/0 min (i.e. immediate decompression), while only 18% were released at 300 MPa/0 min, and that they were more affected by critical pressure thresholds than treatment time.

Texture is a quality parameter affected by HHP, although the measurement of texture parameters in seafood in general is controversial. Few studies concerning bivalve mollusks, in particular, are available, with no consensus. For example, some authors report increased shear strength and hardness of the adductor muscle in these animals and suggest that this may to be due to aggregation and water loss induced by denaturation in the myofibrillar fraction (HSU et al., 2010; YI et al., 2013; CRUZ-ROMERO et al., 2008; LOPEZ-CABALERO et al., 2000). However, others authors report contradictory data, where sample hardness decreased after pressure application (PEREZ-WON et al., 2005).

Mootian et al. (2013) observed an increase in the hardness of pressurized clams at 276MPa and 552MPa and observed that 552MPa/3min disrupted the ultra-structure of the adductor muscle and mantle from continuous tightly packed muscle fibers, to open, broken, or twisted fibers, by Scanning electron images (SEM). However, these data do not agree with the report by Pérez-Won et al. (2005), who reported that the alveolar structure of the scallop adductor muscle was destroyed after exposure to 400 MPa for 10 min, with reduction in the size of interfiber spaces, resulting in a more compact structure. The loss of the honeycomb structure was accompanied by a decrease in shear values, indicating firmness reduction. This data variability is due to the great diversity of species included when using the term shellfish, even between species of the same bivalve mollusk genus, and also due to the variety of methodologies for gauging mollusk texture.

Another very important quality parameter concerning HPP-processed bivalve mollusks is color. According to Cruz-Romero et al. (2004, 2007 and 2008) regarding oysters and Briones-Labarca et al. (2012) for abalones, the L *value increases with increasing pressure, indicating that HHP treatment could lead a to the brighter and less transparent adductor tissue. After high pressure treatment, seafood showed an opaque appearance similar to that obtained by very light cooking (MURCHIE et al., 2005). Muscle paleness after HHP treatment resulted in brightness increases, and it was not only accounted for loss of active pigment, but also for protein coagulation, altering sample surface properties, reflecting reflected light and creating the whitish color (Kruk et al., 2011).

Although some differences are noted between different studies, most have reported decreased a* (loss of red) and increased b* (yellow), which varies according to species and pressurization parameters (Table 1). The parameters that make up color in scallops, for example, can display great variability, as migration of carotenoids from the gonads to the adductor muscle occurs due to not yet fully elucidated genetic mechanisms (LI et al., 2010, DU et al., 2017). According to Rodriguez-Amaya (1993), lipid oxidation is another cause of colour loss in fish products, due to the degradation of highly unsaturated carotenoids such as astaxanthin, one of the major pigments in shellfish and fish products.

Table 1. Main effects caused by protein structure modifications when applying HHP

Reference	Product	Treatment/Conditions	Application /Objective	Main Conclusions
Hsu *et al.*, 2010	Oyster (*Crassostrea gigas*)	150 to 300 MPa for 0, 1 and 2 min + fry cooking at 160ºC for 90 sec	Shucking	Shucking 250 MPa/1min - 92%, 250 MPa/2 min and 300 MPa/0 min - 100% shucking
			Colour	L*↑; a*↓; b*↑; ΔE ↑
			Texture	300MPa/0min increased cutting force
Yi *et al.*, 2013	Scallop (*Aequipecten irradians*)	150 to 400 MPa for 0, 2 and 3 min.	Shucking	200 MPa/3min, 300 MPa/3min, 350 MPa / 2min and 400 MPa 0 min - 100% shucking
			Colour	L*↑; a*↓; b*↑; ΔE ↑
			Texture	Increased hardness by 300MPa / 0min
Rong *et al.*, 2018	Oyster (*C. gigas*)	275, 300, 350 MPa/1min; 100, 200, 250, 275, 300 MPa/3min; 275 and 300MPa/2min.	Shucking	275 MPa for 3 min or 300 MPa for 2 min – 100% shucking
Briones-Labarca *et al.*, 2012	Red abalone (*Haliotis rufescens*)	500 MPa/8 min, 550 MPa/3 min, 550 MPa/5 min, Storage at 4ºC for 60 days	Texture	More compact structure due to protein gelling.
Perez-Won *et al.*, 2005	Scallops (*A. irradians*)	400MPa+ one 10 min pulse; 400MPa+two 5 min pulses; 200MPa+ one 10 min pulse; 200MPa+two 5 min pulses;	Microstructure / Texture	Hardness reduction at 400MPa + one 10 min pulse and 200MPa + one 10 min pulse. Compression of the muscle fibers with rearrangement of the perimysium and reduction of the endomysium.
Lopez-Cabalero *et al.*, 2000	Oysters (*Ostraea edulis*)	400 MPa at 7ºC for 10 min or 400 MPa at 7ºC for 5 min in two consecutive steps.	Texture	400 MPa por 5 and 10 min - Increase in the shear strength in storage
Cruz-Romero *et al.*, 2004	Oyster (*C. gigas*)	100, 300, 500 or 800 MPa for 10 min at 20 ºC	Colour	L*↑; a* ↓- 500 and 800MPa similar to cooked; b* ↑
			Protein profile (SDS-PAGE)	protein denaturation at a pressure level ≥ 300 MPa

Cruz-Romero et al., 2008a	Oyster (*C. gigas*)	100, 300, 500 or 800 MPa for 10 min at 20 °C	Colour	L*↑; a*↓; b*- unaffected
Cruz-Romero et al., 2008b	Oyster (*C. gigas*)	260, 400 or 600 MPa for 5 min at 20 °C+ storage	Texture	Increased cutting force
			Colour	L*↑; a*↓; b*↑ at 260 MPa
Bindu et al., 2015	Mussels (*Perna viridis*)	100, 200, 300 and 400 MPa for 5min at 30 ± 3 ° C	Shucking	300 and 400 MPa –easily detached from the shell
			Texture	increased proportionately with pressure levels
			Colour	L*↑; a*↓; b* ↑
Mootian et al., 2013	Clams (*Mercanaria mercanaria*)	Pressure levels 250 to 552 MPa for hold times ranging between 2 and 6 min	Texture/ Microstructure	Increased hardness in 276Mpa and 552 MPa. 552 MPa for 3 min disrupted the ultra-structure of the adductor muscle and mantle.
			Colour	L*↑ and a* ↓- values stabilized at 276 MPa.
He et al., 2002	Oyster (*C. gigas*)	241, 275 and 310 MPa/ 0min; 241and 275MPa/ 1min; 207, 241 and 275MPa/ 2 min	Shucking	241 MPa for 2 min -88% detachment; 310MPa, 0 min -100% release.

* ↑ and ↓: increase or decrease in color index as a function of HP processing parameters.

2.3 HHP effect on microorganisms

High pressures cause morphological, biochemical and genetic changes, especially in membranes, leading to changes in microorganism functioning and reproduction (CHEFTEL and CULIOLI, 1997), including gaseous vacuole compression, cell stretching, cell wall membrane separation, cell wall contraction with pore formation, cytoskeletal modifications and nucleus and intracellular organelles changes (CAMPOS et al., 2003). In addition, HHP increases cell permeability, inhibits energetic reactions and denatures enzymes essential for microorganism growth and reproduction (CALDERÓN-MIRANDA et al., 1998) .

Due to its special characteristics, the cell membrane is the main target of HHP treatment (SMELT, 1998), mainly resulting in permeability and functionality modifications (PAGÁN e MACKEY, 2000). One hypothesis for microbial inactivation by HHP is linked to the decrease of sodium and potassium-dependent ATPase activity, located in the phospholipid layer of the cell membrane and involved in active membrane transport. In this way, ATPase becomes unable to maintain proton transport through the membrane causing internal pH decreases and cell death (CHEFTEL and CULIOLI, 1997).

However, it seems that no single damage to a cellular structure or function is responsible for microorganism inactivation; cell death is due to a multiplicity of accumulated damages in different parts of the cell (HOOVER et al., 1989). Thus, when accumulated damages exceed the ability of a cell to repair itself, cell death occurs (RENDUELES et al., 2011).

Recently Rong et al. (2018) published a study on the use of high throughput sequencing (HTS) to investigate control microbiota and oysters treated with HPP during refrigerated storage. Fresh oysters (hand-shucked) were compared to a 300 MPa treatment for 2 min, due to

its presented shucking efficiency. Shelf life was evaluated and fresh oyster samples became sensorially unacceptably on the eighth day of storage and microbiologically unfit for consumption on the sixth day of storage. Oysters treated with HPP, on the other hand, were valid for 12 days, as HPP promoted a reduction of 1.27 logs cycles (P<0.01). A principal component analysis (PCA) concerning odor analysis by electronic nose demonstrated discrepant positions for fresh, damaged and pressurized samples, confirming the hypothesis that HPP altered the oyster deterioration process during storage, influencing their microbiota. The dominant bacteria present in fresh oysters were *Vibrio*, *Shewanella* and *Pseudoalteromonas*, with *Pseudoalteromonas* and *Shewanella* dominant in spoiled oysters. The HPP treatment altered oyster deterioration microbiota dramatically, with *Psychrobacter* dominant in HPP treated spoiled oysters. Table 2 displays the main research on HHP applications in bivalve mollusks.

Viruses are of great concern in foods, as they are obligatory intracellular parasites and can only replicate inside suitable living host cells. As a result, viruses cannot multiply in the environment or in foods, so traditional factors used to control bacterial levels in food systems (e.g., acidified pH, reduced temperature, or reduced water activity) are ineffective as barriers to viral hazards (JAYKUS, 2000). In the case of viruses, experiments suggest that HHP inactivates viruses through the denaturation of their capsid proteins, which renders them unable to bind to their receptor on the surface of their host cell (KINGSLEY, 2014).

Investigations concerning the potential of HPP in inactivating human norovirus and hepatitis A virus, currently considered the two most significant foodborne virus threats in raw bivalve shellfish, have demonstrated that pressures ≥ 400 MPa will inactivate these viruses in shellfish tissues (KINGSLEY et al., 2002, 2005, 2007, 2009; CALCI et al., 2005; TERIO et al., 2010; LEON et al., 2011; YE et al., 2014).

The efficiency of HHP technology in inactivating microorganisms depends, mainly, on the magnitude of the applied pressure, pressurizing time, process temperature and type of microorganism, as well as cell growth phase, type of food material and the presence of microbial agents, among others (FARKAS and HOOVER, 2000).

HHP processing, alone or alongside other methods, has been investigated as a way to reduce pathogenic microorganism contamination in bivalve mollusks, mainly concerning *Vibrio parahaemolyticus* and *Vibrio vulnificus*. In addition, it is used to reduce spoilage burdens and, thus, extend seafood shelf life (HE et al., 2002; HUGHES et al., 2016; MOOTIAN et al., 2013; PHUVASATE e SU, 2015; YE et al., 2012; Ye et al., 2013). In addition, reports of the potential use of HHP against hepatitis A virus and calicivirus are also found in the literature (CALCI et al., 2005).

Table 2. Compiled on the effect of HHP on the microbiota of bivalve molluscs

Seafood	Microorganisms Group/ Method	Pressure treatments	Main effects	Reference
Oyster (*C. Gigas*)	TVC, APC and H2S-producing bacteria	100, 300, 500 or 800 MPa for 10 min at 20 ℃	Bacterial load was initially reduced at all pressures to levels below the detection limit.	Cruz-Romero *et al.*(2008a)
Oyster (*C. Gigas*)	TVC, APC and H2S-producing bacteria	260, 500 or 800 MPa for 3, 5 or 5 min, respectively, at	TVC, APC and counts of H2S-producing bacteria increased during storage,	Cruz-Romero *et al.*(2008c)

		20 °C and stored at 2ºC	independently of pressure treatment	
Oyster (*C. Gigas*)	Inoculated with titer of the MNV-1 stock ($2x10^{11}$ PFUs/ml). Plaque Assays and RT-PCR	200, 300 or 400 MPa for 5 min.	5-min 400-MPa treatment at 0ºC inactivated MNV-1 within oysters to undetectable levels; HPP might subtly alter the viral capsid proteins but that the RNA remains protected	Li et al. (2009)
Oyster (*C. Gigas*)	Inoculation of 4-5 log CFU/ml of *V. parahaemolyticus*. APC and PPC by the pour-plate method; Total and fecal coliforms*; V. parahaemolyticus* for MNP method and PCR	293 MPa for 90,120,150,180 or 210 seg	293 MPa por 120 seg was capable of 3.52-log reductions of *V. parahaemolyticus*.	Ma & Su (2011)
Oyster (*C. Gigas*)	APC and ANPC	207 to 310 MPa at 0, 1, and 2 min and stored at , 4 ºC and evaluated over 27 d.	Reduction of 2-3 logs with APC and ANPC at reduced level during storage	He *et al*. (2002)
Oyster (commercial)	Numbers of total aerobic bacterial counts (TABC), presumptive *Vibrio spp*. counts (PV), and presumptive *V. vulnificus* counts (PVv); 16S rDNA sequencing.	- HP -treated (250 at 400MPa for 1 at 3 min); - QF (quick frozen) - raw oysters; Stored for 21 days; Three sampling were carried: winter, summer and fall.	Numbers of bacterial flora in HP oysters were reduced in comparison to the controls (raw oysters), however increased in TABC over time (7,14,21 days) at levels higher than raw oysters in two out of the three samplings (fall and winter).	Prapaiwong *et al.* (2009)
Oyster (*C. virgínica*)	Counts of *V. vulnificus*	150 MPa/4 min and 200 MPa/1min at -2, 1, 5, 10, 20, 30, 40 and 45°C.	Conditions for a 5-log reduction of Vibrio vulnificus: ≥250 MPa; ≤ 4 min at -2 or 1°C.	Kural *et al.* (2008)
Oyster (*C. virgínica*)	Inoculation of the Hepatitis A virus (HAV); Plaque	300, 325, 350, 375, and 400 MPa for 1 min at	Reductions of > 1, > 2 and $> 3 \log^{10}$/PFU for 1 min treatments at 350, 375 and 400 MPa at 8, 7	Calci *et al.* (2005)

	Assays and RT-PCR	approximately 9°C	and 10.3 ° C, respectively.	
Oyster (*C. virgínica)*	Inoculation of the Hepatitis A virus (HAV); Plaque Assays.	350, 375 and 400 Mpa for 5 min at 17-22ºC; whole-in-shell oysters and shucked oysters.	2.56 and 2.96 log10 inactivation of HAV, for whole-in-shell oysters and shucked, oysters respectively, after a 400-MPa treatment.	Kingsley *et al*. (2009)
Oyster (*C. virgínica)*	total Vibrionaceae (MPN), *Vibrio parahaemolyticus* (MPN), total coliform, faecal coliform and total aerobic bacteria.	Raw oysters at 600 MPa, in the presence or absence of hot sauce flavouring.	initially reduced aerobic plate counts by 2 $\log^{10}$ when compared to raw untreated oysters and bacterial counts remained low over the 8 days of refrigerated storage	Kingsley *et al*. (2015)
Oyster (*C. virgínica)*	Inoculation of 7-8 log MPN/g of *V. parahaemolyticus* and *V. vulnificus*	225 MPa, 250 Mpa, 275 MPa e 300 MPa for 2 min. Stored at: 21ºC/5h; 35ºC/5h; 4ºC/1day; 4ºC/2 days; 10ºC/1day and -18ºC/ 2 weeks	HHP at 300 MPa/2 min achieved a > 5-log MPN/g reduction of *V. parahaemolyticus,* completely inactivating *V. vulnificus*; HHP at 200 MPa/2 min/-18ºC for 7days- completely inactivating *V. parahaemolyticus*	Ye *et al*. (2013)
Scallop (*Argopecten irradians*)	Total coliforms for MPN and APC methods	150 to 400 MPa for 0, 2 and 3 min.	Level of 200MPa for 3 min produced reductions in the ACP and coliform to undetectable levels.	Yi *et al*. (2013)
Scallop (*A. irradians*)	APC method	400MPa+1 pulse of 10 min; 400MPa+2 pulse of 5 min; 200MPa+1 pulse of 10 min; 200MPa+2 pulse of 5 min;	All UHP treatments reduced the initial load in total plate count of microorganisms to <10 cfu/g.	Pérez-Won *et al*. (2005)
Clams (*M. mercanaria*)	Inoculation of 7 log CFU/g of a cocktail of V. parahaemolyticus	Pressure levels 250 to 552 MPa for hold times ranging between 2 and 6 min.	450 MPa for 4 min and 350 MPa for 6 min reduced the initial concentration of V. parahaemolyticus to a nondetectable level	Mootian *et al*. (2013)

			(<101 CFU/g), achieving >5log reductions.	
Abalone (*Haliotis rufescens*)	APC method	100 or 300 MPa for 5 or 10 min and control	The pressure level of 300MPa per 5 or 1m min extended the shelf life from 14 days to 35 days. However it was not enough to reach the stationary phase.	Hughes *et al*. (2016)

APC = Aerobic plate counts; PPC = Psychrotrophic plate counts; ANPC = Anaerobic plate counts ; CFU = colony-forming units; MPN = most probable number; PFU = plaque-forming units; TVC = Total viable counts; PCR = polymerase chain reaction; RT-PCR = reverse transcription- polymerase chain reaction; TABC = numbers of total aerobic bacterial counts.

2.4   HHP effect on nutritional and sensory aspects

Food conservation by HHP originates from HPP ability to conserve original color, flavor, aroma, quality and nutritional content attributes. Pressurization is able to alter the structure of high molecular weight molecules, such as proteins and carbohydrates, while smaller molecules, such as volatile compounds, pigments, vitamins and other compounds related to sensory, nutritional and health characteristics, are less affected. Thus, this method is able to provide products displaying sensorial characteristics very close to those of the fresh food without the addition of preservatives (additives), while also displaying a favorable effect on texture characteristics and other desirable attributes, such as digestibility (CHAWLA et al., 2011; GINSON et al., 2015).

Some authors have reported that HHP may increase the total amount of carotenoids available in vegetable matrices (PATRAS et al., 2009; PATRAS et al.; PLAZA, et al., 2006; SÁNCHEZ-MORENO et al., 2005), and theorized that pressure modifies the permeability of the cell membrane and denatures carotenoid-bound proteins, at the same time making proteins more available (BARBA et al., 2015). However, no studies along these lines are available for mollusks and fishes.

Kingsley et al. (2015) evaluated consumer acceptance of HHP (whole shell) treated oysters at 300, 400 and 500 MPa at 22ºC and 400, 500 and 600 MPa at 6ºC. All HHP-treated samples received the highest scores applying a hedonic scale for attributes such as appearance and texture in relation to control samples, indicating the possibility of the use of this technology for oyster processing.

However, marine foods are characterized by high levels of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are highly susceptible to oxidation and oxidative lipid degradation during storage, directly affecting the quality of these products, impacting taste, color, texture and nutritional value. Lipid systems are the biological components most sensitive to pressure, mainly due to the predominance of hydrophobic bonds, which are very susceptible to the process (MEDINA-MEZA et al., 2014).

A pressure level of 300 MPa leads to a small effect on lipid oxidation, which increases linearly at higher pressures. In the case of shellfish and marine animals in general, the presence of PUFAs promotes radical production, leading to accelerated oxidation in subsequent storage periods. However, HHP can be combined with other methods that perform post-pressurizing antioxidant functions (MEDINA-MEZA et al., 2014).

2.5 Advantages, challenges and perspectives

HHP technology displays many advantages over conventional methods, incuding uniform pressure distribution throughout the food with minimal increases in temperature, possibility of safely extending product shelf life with only minimal nutritional and sensorial losses, and only requiring a small amount of energy for the compression of a solid or a liquid, compared to heating the product at 100°C. In addition, the technology is applicable to packaged foods, thus preventing unnecessary and obsolete recontamination or the need for aseptic packaging processes (PEREIRA and VICENTE, 2010).

HHP consumes relatively low energy and requires low amounts of potable water, thus reducing its carbon footprint and decreasing effluent production, since the pressure transmission liquid (usually water) can be recycled. Consequently HHP can be considered an environmentally sustainable process (TRUONG et al., 2014).

Bermúdez-Aguirre and Barbosa-Cánovas (2011) pointed out that the number of HHP devices worldwide has developed at an annual exponential rate in several countries over the last 20 years, and that, currently, the use of HHP by food industries and the sale of pressurized products is a reality, ranging from fruits and vegetables to seafood and eggs, with wide consumer acceptance. In addition, Huang and colleagues (Huang et al., 2017) recently published a report on HHP growth and relevance in the food sector, and highlighted that this technology is the most commonly applied non-thermal processing technique in the world.

The Food and Drug Administration (Adal) and US Department of Agriculture (USDA) have approved the technology as a food preservation method, and the US National Advisory Committee on Microbiological Criteria for Foods regards HHP as a Non-thermal pasteurization process that can replace conventional pasteurization (Wang et al., 2013).

However, the method still presents obstacles to large-scale applications in the food industry, mainly in relation to the high initial capital to be invested. Besides the cost of the equipment, the use of HHP for shellfish and marine animals can be hampered due to seasonality. Although fish and shellfish can be consumed throughout the year, there may be peaks in production and consumption during certain periods. Thus, in order to meet product demand during harvesting periods, the company may be required to install more than one HHP unit, besides utilizing alternative products considering seasonality.

Although HHP is able to preserve the nutritional and sensory characteristics of foods, the full effects of treatment require individual study, due to the complexity of each food composition and the possibilities for changes and intrinsic reactions that may occur during pressurization. Therefore, several studies have been carried out to investigate microorganism and enzyme inactivation kinetics, biopolymer structures (proteins, polysaccharides), as well as the effect on specific constituents of food products (juices, dairy products, meats, fish, fruits and vegetables). This aspect is related to the fact that HHP is associated with and a promising tool not only for food preservation, but also because of its potential to promote positive effects on technological properties and to preserve the functional and nutritional characteristics of the food constituents. However, these effects, properties and characteristics should be studied both in the integrated system and concerning individual components.

3. CONCLUSIONS

The international fishing industry currently applies HHP in commercial oyster processing. However, further studies are required concerning its effects on biochemical and microflora characteristics in order to overcome the health risks associated with bivalve mollusk consumption. HHP displays many advantages, as it is a non-thermal technology compared to conventional treatments in relation to the preservation of food nutritional composition and sensory quality. Moreover, it is a clean technology, since it presents a significantly lower carbon footprint than thermal methods.

The limiting factor to its implementation remains the high capital cost of the technology. However considering its benefits, the resulting potential value aggregation and prospects of technological development, it is expected that implementation costs will become more accessible, expanding HHP use for the processing of bivalve mollusks and other shellfish in general.

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CAPÍTULO II

OPTIMIZATION OF HIGH PRESSURE PROCESSING PARAMETERS TO PRESERVE QUALITY ATTRIBUTES OF SCALLOPS (*NODIPECTEN NODOSUS*)

ABSTRACT

Seafood is one of the most important sources of nutrients and other health related compounds, has high biological value protein, it is a source of vitamins (E, D, A) and good fats as the omega-3 family and it is also rich in minerals such as calcium, phosphate , zinc and iron. However, they have a short shelf-life and the traditional methods applied to preserve them generate losses in their natural flavor and nutrients. The aim of this study was to optimize the pressure level (200–400 MPa) and holding time (0–5 min) of High Pressure Processing (HPP), in order to achieve reducing the level of microbial contamination while maintaining its natural attributes. Response surface methodology with a Box- Behnken design and Desirability function were employed to simultaneously optimize these quality attributes. Results showed that HPP enhances microbial quality at 200MPa/5min. However, it promoted physico-chemical modifications in the adductor muscle of the scallop. HHP promoted a slight increase in humidity and pH, as well as a decrease in water holding capacity (WHC). The shear force related to instrumental texture decreased and color paramenters Whiteness (W) and luminosity (L*) of the muscle increased at more severe level (400MPa/5min). Simultaneous optimization provided a value of 365MPa / 2min where physicochemical characteristics would be more desirable.

Keywords: Optimization of multiple answers; Desirability function; High hydrostatic pressure; Bivalve mollusk

1. INTRODUCTION

The lion's paw scallop (*Nodipecten nodosus*) is a bivalve mollusk that belongs to the family Pectinidae and is one of the most important fishery resources of Rio de Janeiro, Brazil. The species 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 and, discontinuously, along the coast of Brazil, until the state of Santa Catarina. However, on the Brazilian coast *N. nodosus* does not form extensive natural banks and its cultivation has become important to supply the consumer market of this type of food (MINCHIN, 2006; RUPP and PARSONS, 2006) . The potential for cultivation of this native species is very high, due to its rapid growth, good receptivity in the consumer market and relative ease of production (MANZONI, 1994).

The culture and production of bivalve shellfish in sea farms, mainly for commercial purposes, is a global concern that contributes significantly to the economic development of many countries. These animals play an important role in worldwide mariculture activities with estimates of 17.1 million tonnes produced annually (FDA, 2018).The State of Rio de Janeiro occupies the first national place as producer (ABELIN *et al.*, 2016) and although currently the Brazilian production is to supply only the domestic market, large is the potential of national malacoculture.

However, this food is culturally consumed raw or minimally cooked. Considering to be a filtering animal, where it feeds through the filtration of particles dispersed in the surrounding body of water, it becomes a health risk the consumption of these organisms. In addition, the shelf life of seafood is greatly reduced (MARTÍNEZ *et al.*, 2017) due to physico-chemical characteristics such as pH near neutral, high water activity, nigh content not only of unsaturated fatty acids but also of free amino acids, and presence of active autolytic enzymes, making it prone to microbial and oxidative degradation (LOUGOVOIS and KYRANA, 2005). However, little research has been reported on processing of scallops, in order to give it greater safety and durability.

High pressure processing (HPP) treatment 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 consists of a nonthermal technology that can ensure the same level of food safety as heat pasteurization and produces 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 any undesirable changes associated with thermal processing (BERMÚDEZ-AGUIRRE e BARBOSA-CÁNOVAS, 2011).

HHP technology 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 fish (PÉREZ-WON *et al.*, 2005; YAGIZ *et al.*, 2007; CRUZ-ROMERO *et al.*, 2008; CRUZ-ROMERO *et al.*, 2008; ERKAN *et al.*, 2010; MOOTIAN *et al.*, 2013; YI *et al.*, 2013; HSU *et al.*, 2014; BINDU *et al.*, 2015; GINSON *et al.*, 2015; SERMENT-MORENO *et al.*, 2015; HUGHES *et al.*, 2016).

In addition to promoting microbiological safety, HHP is capable of promoting modifications in the quaternary, tertiary and secondary structures of proteins, leading to dissociation, unfolding, denaturation, aggregation, precipitation and gelatinization at different intensities (MESSENS *et al.*, 1997; LULLIEN-PELLERIN and BALNY, 2002) and consequently causing changes in the texture and color of seafood, which may interfere with the acceptance by the final consumer.

In the fish industry, HHP gained space with the shucking of molluscs and crustaceans with advantages like the reduction labor, muscle trim, and processing time (HE *et al.*, 2002; HSU *et al.*, 2010). In a study with scallops, Yi *et al.* (2013) showed the efficiency of the technology in separating the adductor muscle from the shell with up to 100% utilization with 200MPa / 3min or 300MPa / 0min (YI *et al.*, 2013). And recently, a study showed the efficiency of HHP in the shucking of red swamp crayfish, using 200MPa for 5 minutes (SHAO *et al.*, 2018).

Therefore, the present study aims to investigate the effect of HPP on quality attributes of scallop muscle and to optimize the main processing parameters (pressure level and holding time) in order to achieve reduce microbial counts while maintaining nutritional quality attributes, texture and color of the product.

2. MATERIAL AND METHODS

2.1 Sample preparation and high-pressure (HP) treatment

A total of 20 dozen aquaculture scallops, approximately one year old, were purchased from the "Vieiras da Ilha" marine farm, in Ilha Grande, Rio de Janeiro State. The scallops were cut by hand, organs removed and cleaned with running water. The scallops were then vacuum packed using polynylon bags and held at 4°C for 12 h prior to high pressure processing.

Pressure treatments were carried out in the high pressure processing machine (Stansted Fluid Power, model S-FL-850-9-W) in the Embrapa Food Technology pilot plant. The dimension of the pressure vessel with approximately 4 cm in diameter and 30 cm in length, having a total volume of 250 ml and useful volume of 345 ml with several holes in the wall through which 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 planning and compared with untreated samples (control).

2.2 Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the main effects and interactions of HPP parameters on quality attributes of the scallop adductor muscle. A total of 7 experiments were conducted according to a Box- Behnken design with two factors: pressure level (P) and holding time at working pressure (t).

In this research, pressure was studied at three levels (200, 300 and 400 MPa) and holding time at two levels (0 and 5 min). The term '0 min' refers to treatments where the samples were brought to pressure followed by immediate decompression. Furthermore, the central point of the design was triplicated in order to validate the model by means of an estimate of experimental variance. Table 1 shows the coded and actual values of the factors of the experimental design and their levels.

For each response variable the linear, quadratic, and simple interaction effects of the factors were compared with each other. Each response variable (Y) was analyzed as a function of the two independent factors (P, t) and the significance of the equation coefficients for each response variable was obtained by multiple regression analysis using the F test with a $p < 0.05$:

The experiment was designed following the procedures of (BOX and BEHNKEN, 1960) (Table 1). Total of 2 independent variables, namely pressure (MPa) and time (min) were considered for a 3 - level (+ 1, 0, − 1) design. The effects of these independent variables on moisture, water holding capacity (WHC) and the pH were investigate desing response surface

methodology (RSM). A total 7 experiments (including 3 central points) were studied. the following second-order polynomial equation was used to fit the experimental data.

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

where X_i (i= 1 - 2) are the code variables for pressure level and holding pressure time; Y_i (i= 1 - 3) are the dependent variables (moisture, WHC and the pH); and, β_i are regression coefficients estimated from the experimental data. Model terms were selected at p-values< 0.05 by analysis of variance (ANOVA).

Table 1. Coded and actual values of independent variables in the Box-Benhken design

| Test | Pressure | | Holding time | |
	Coded values (x1)	Actual values (MPa)	Coded values (y1)	Actual values (min)
1	-1	200	-1	0
2	-1	200	1	5
3	1	400	-1	0
4	1	400	1	5
5	0	300	0	2.5
6	0	300	0	2.5
7	0	300	0	2.5

2.3 Sample analysis
2.3.1 Microbiological analysis

All samples were analyzed for numbers 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 Ágar (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. Microbial data were transformed into logarithms of the number of colony-forming units (log CFU g−1).

2.3.2 Moisture and pH analyses

Percent moisture were determined using AOAC methods (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2010).The pH was determined using a digital pH meter (Testo, model 205, Lenzkirch, Germany) equipped with a glass electrode (calibrated at pH four and seven), which was dipped into the adductor muscle. The analyzes were run in triplicate.

2.3.3 Water holding capacity (WHC)

The WHC was evaluated in triplicate using the technique proposed by GÓMEZ-GUILLÉN *et al.* (2002), for which they used 2 g of sample. It was subjected to a centrifugal force (centrifuge Hettich - Zentrifugem, model Routine 38R, Hamburg, Germany) of 4000 x g for 10 minutes, 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. The analyzes were run in quadruplicate.

2.3.4 Color measurement

Color of adductor muscles was estimated by tristimulus colorimetry (FRANCIS and CLYDESDALE, 1975), 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. 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 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}

ΔE= [(ΔL)^2 + (Δa)^2 + (Δb)^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 and OZOEMENA, 2010).

2.3.5 Texture analysis

The shear force, the 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 perpendicular 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.

2.3.6 Gel electrophoresis

- *Protein extraction*

For the extraction of myofibrillar proteins 5g of the previously processed muscle was used. Subsequently, the sample was homogenized in blender with 30mL of extractive solution (Phosphate Buffer K_2HPO_4 / KH_2PO_4 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 after that time were 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*) *analysis*

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), 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 under a voltage of 100V. The proteins of 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 decolorised 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).

2.4 Simultaneous optimization

The simultaneous optimization was obtained by the desirability function proposed by DERRINGER and SUICH (1980). The Derringer desirability function allows the analyst to find experimental conditions (factor levels) to simultaneously achieve the optimum value for all evaluated variables, including the researcher's priorities during the optimization procedure and has been widely used in distinct areas of food processing (GRANATO *et al.*, 2010; DUONG e BALABAN, 2014; DENOYA *et al.*, 2016; KAUSHIK *et al.*, 2016; FERNANDEZ *et al.*, 2018).

The individual convenience functions for each response variable were manipulated to achieve optimal values (GRANATO *et al.*, 2010). In this study, moisture and WHC demanded maximization because it is directly linked to muscle juiciness and the pH value closer to the value of the control sample (pH 6.3).

3. RESULTS AND DISCUSSION

Table 2 shows experimental mean values of quality attributes evaluated of muscle scallops. Among all the evaluated responses, some were not affected by treatment (chromatic parameters a* and b*), others were affected but do not fit the quadratic model (microbiologic, L*, W, ΔE and textural parameters) while others were affected by treatment and fit 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.

Table 2. Experimental values for HHP-treated muscle adductor quality attributes.

Treatments	Moisture (%)	pH	WHC (%)	Force (N)	L*	a*	b*	W	ΔE
Control	78.14 ± 0.74ª	6.28 ± 0.04ab	94.52 ± 1.21abc	7.55 ± 1.76c	56.88 ± 2.87a	-0.51 ± 0.29a	5.78 ± 2.92ª	56.42 ± 3.06c	-
P200T0	79.32 ± 0.50ab	6.20 ± 0.01a	95.34 ± 0.71bc	6.11 ± 0.76abc	56.83 ± 1.94a	-0.46 ± 0.24a	4.97 ± 2.64ª	56.47 ± 1.85c	3.47 ± 2.18a
P200T5	80.02 ± 0.24bc	6.44 ± 0.01c	90.03 ± 2.90a	6.57 ± 0.97abc	62.00 ± 2.55b	-0.41 ± 0.46a	6.33 ± 3.07ª	61.34 ± 2.15b	7.16 ± 2.92ab
P300T2,5-1	80.46 ± 0.04bc	6.49 ± 0,00c	95.69 ± 0.15c	6.19 ± 0.88abc	63.91 ± 3.48b	-0.71 ± 0.50a	6.06 ± 2.64ª	63.29 ± 3.08b	6.56 ± 4.14ab
P300T2,5-2	80.69 ± 0.20c	6.50 ± 0.01c	95.49 ± 0.47bc	6.95 ± 0.62abc	63.14 ± 2.99b	-0.82 ± 0.33a	5.33 ± 1.84ª	62.72 ± 2.95b	7.21 ± 4.55ab
P300T2,5-3	80.62 ± 0.15bc	6.51 ± 0.01c	94.88 ± 2.90bc	5.99 ± 0.50ab	64.54 ± 3.41bc	-0.73 ± 0.30a	5.65 ± 2.67ª	63.6 ± 3.55b	8.81 ± 4.60bc
P400T0	80.52 ± 0.04bc	6.69 ± 0.01d	95.70 ± 1.19c	5.66 ± 0.73a	61.76 ± 3.15b	-0.74 ± 0.54a	6.26 ± 2.35ª	61.17 ± 2.93b	5.67 ± 3.64ab
P400T5	80.64 ± 0.11bc	6.32 ± 0.03b	91.00 ± 1.06ab	7.24 ± 1.58bc	67,99 ± 3,55c	-0.74 ± 0.54a	5.10 ± 3.42ª	67.39 ± 3.18a	11.84 ± 4.39c

Médias com letras maiúsculas iguais na mesma coluna não diferem significativamente entre si (p <0,05) pelo Teste de Tukey

Médias com letras minúsculas iguais na mesma linha não diferem significativamente entre si (p <0,05) pelo Teste de Tukey

Table 3. Regression coefficients, R^2 values and fit test results for adductor muscle response variables undergoing HPP.

Regression coefficient	Moisture	WHC	pH
P (linear)	**0.4562***	0.3296	**0.0938***
t (linear)	0.2062	**-2.5032***	**-0.0338***
P^2 (quadratic)	**0.2322***	**1.1697***	**0.0435***
t^2 (quadratic)	Ne**	Ne**	Ne**
P*t	**80.2786***	**93.7965***	**6.4403***
R^2	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

3.1.1 Microbial quality

The mesophilic and psycrophilic counts were detected after the adductor muscle HHP shown in Table 4. 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 level of 200MPa per 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. However, it should be noted that this behavior was due to the holding time applied to the sample. Other treatments with HHP reduced the growth of mesophilic and psycrophilic microorganisms (table 4) to a non-determinable level. Lessening of bacterial load after HP treatment could be due to the breakdown of plasma membrane, denaturation of proteins and alteration in the permeability of the cell wall of the bacteria (CHEFTEL and CULIOLI, 1997; RENDUELES *et al.*, 2011).

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 an unassessable level with application of 200MPa for 3 min. A decrease in total microbiota after HP treatment of seafood in the range of 200–600 MPa has previously been reported (LOPEZ-CABALLERO *et al.*, 2000; HE *et al.*, 2002; LINTON *et al.*, 2003; CRUZ-ROMERO *et al.*, 2008; BRIONES *et al.*, 2010; GINSON *et al.*, 2015).

Table 4. Count of mesophilic and psycrophilic microorganisms in adductor muscle of scallop processed by high hydrostatic pressure.

Treatments	Mesophilic (log CFU g^{-1})	Psycrophilic (log CFU g^{-1})
Control	3.7 ± 0.0	2.9 ± 8.5
200/0	3.6 ± 0.7	2.5 ± 9.9
200/5	ND	ND
300/2,5-1	ND	ND
300/2,5-2	ND	ND
300/2,5-3	ND	ND
400/0	ND	ND
400/5	ND	ND

log CFU g^{-1}, colony forming units
ND, no detected (detection limit < 1 log10 cfu/g).
Each value is the mean ± standard deviation (n = 2).

3.1.2 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 and CRISTIANINI, 2019). The color of seafood muscle is related not only with carotenoids and heme pigments, namely myoglobin and hemoglobin (HUI *et al.*, 2006), 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. As the pressure level increased, the samples obtained a higher L* value, indicating that the HHP-treatment could cause the brighter and less transparent adductor tissue, which was in accordance with data obtained from scallops, oysters and red abalone (YI et al., 2013, CRUZ-ROMERO et al., 2004 and 2007 and BRIONES-LABARCA et al., 2012, respectively). Similarly, for a given pressure level, the L* value increased with increasing processing time, according to SEQUEIRA-MUNHOZ et al., 2006, with application of HHP in carp. The parameters of redness (a* value) and yellow/blue color indicator (b* values) did not present a significant difference (p ≤0.05) in relation to the control (table 2).

Authors assume that changes in the L* value of HHP treated samples are due to changes in the protein matrix, such as denaturation and coagulation of myofibrillar and sarcoplasmic proteins (ANGSUPANICH and LEDWARD, 1998; CHEVALIER et al., 2001; CHÉRET et al. 2005; CRUZ- ROMERO et al., 2004; 2007; 2008; ERKAN et al. 2010; YAGIZ et al. 2009 HUGHES et al., 2015). The protein coagulation changes sample surface properties and increases light reflection and 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 (RODRIGUEZ-AMAYA, 1993; CRUZ-ROMERO et al 2008).

Whiteness values (W) in samples treated with 200MPa per 0 min retention time were not significantly different from the control samples. 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 baked

appearance. MURCHIE *et al.* (2005) in his review describes that seafood after high pressure processing may present opacity appearance similar to that obtained by very light cooking.

A study with chicken meat (KRUK, 2011) it was observed that the pallor of the muscle treated by HHP resulted in increased brightness and was not only responsible for the loss of active pigment, but also for the coagulation of proteins that altered the surface properties of the sample and then reflected the light and created the whitening color. In terms of consumer's acceptances, for raw eating seafood, it may be important for sale. However, it may not be disconcerting for frozen HHP-shucked adductor muscles which are purchased for subsequent cooking at home (YI *et al.*, 2013).

The ΔE values, an indicator of total color difference, showed that there were significant (P≤0.05) differences in color between HP treated and untreated samples (table 2). Using the classification scale for total color difference (ADEKUNTE et al. 2010) it can be concluded that big differences in color were obtained for HP-treated scallop at, even at the lowest pressure / time levels applied (ΔE>3).

Moreover, it is possible that HPP increases the oxidizing potential of the medium, and consequently myoglobin oxidation occurs, as well as other oxidative processes such as lipid and protein oxidation, affecting color (OLIVEIRA *et al.*, 2017).

3.1.3 Effect of HP-treatment on cutting strength of scallop muscle

Cutting strength of the adductor muscle the scallops are shown in Table 2. 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 PERES-WON et al (2005), hardness of muscles decreased with one pulse for 10 min regardless the pressure level (200MPa or 400MPa). However, 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 report increased shear strength or hardness after pressurizing of seafood. CRUZ-ROMERO et al. (2008) observed that HPP treatment at 260 MPa, 400MPa or 600 MPa 5 min at 20°C increased cutting strength of oysters; Hsu et al (2009) agreed to increase 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- CABALERO et al (2000) suggested that the increase of shear strength of oyster tissue might be due to aggregation and water loss induced by denaturation in the myofibrillar fraction. 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 reformation of intra and intermolecular bonds within or between protein molecules (GALAZKA et al., 1996; MESSENS et al., 1997; MARTINEZ et al., 2017). And, although covalent bonds are not broken by HHP application, weak energy bonds like hydrogen and hydrophobic bonds can be irreversibly modified, thus leading to important consequences for the secondary, tertiary and quaternary structures in proteins (ASHIE, 1996; HURTADO et al., 2001; ORTEAS et al, 2010).

However, there is no consistent data to support a clear effect of HPP. It is possible that the effects are dependent on process parameters, fish species, and methodology used (OLIVEIRA, 2017; BELTRAN-LUGO, 2006). Texture is influenced by several factors such as chemical composition (DUNAJSKI, 1979) and structure (TAYLOR et al., 2002).

These results are in agreement with the results commented next on SDS-PAGE analysis in which protein denaturation induced by HPP was observed (Fig. 1).

3.1.4 Effect of HP-treatment on scallops muscle adductor proteins

The polyacrylamide gel electrophoresis (SDS-PAGE) is an analytical method in which proteins are separated according to their size. The electrophoretic profile of the myofibrillar proteins of the adductor muscle of scallops obtained in SDS-PAGE in reducing medium is shown in figure1. Bands with the respective molecular weights (MW) were observed: 202.02, 157.42, 112.88, 87.96, 46.71 and 43.41kDa. The effect of pressure was quite visible on actin (43.41kDa). In the control sample this banding is strongly colored and in the lines corresponding to the treatments P200T0 and P200T5 a band of PM 46.71kDa 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).Suggesting an actin unfolding effect at 200MPa/0min (P200T0) and the beginning of degradation when the same level of pressure remained for 5minutes (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), appears 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 reformation of intra and intermolecular bonds within or between protein molecules (GALAZKA et al., 1996; 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 sulphydryl groups.

Also, other authors have reported that several factors including treatment time, pressure level and temperature (LULLIEN-PELLERIN and BALNY, 2002; MESSENS et al., 1997) influence in the degree of denaturation.
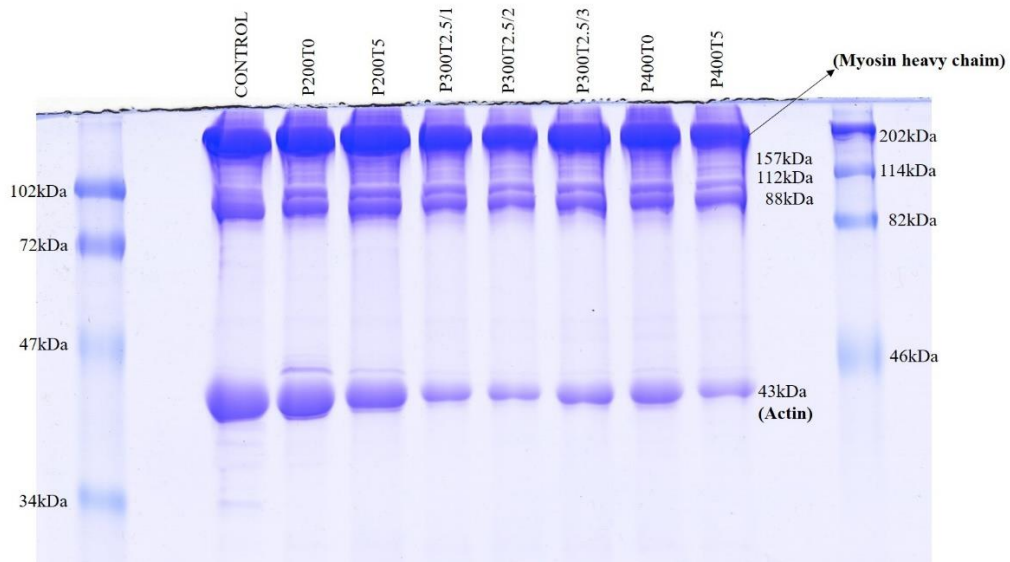
Figure 1. SDS-PAGE of scallop adductor muscle proteins. Molecular weight patterns are shown on the first and last lanes.

3.1.5 Moisture, pH and WHC of HP-treatment scallop muscle

Table 2 shows the mean values and standard deviations of the moisture content, WHC and pH for both 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) and YI et al. (2013), 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 (Fig. 2a) provides evidence 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). However, considering that the process does not enable contact of the samples with water the increase in water content of the samples is not expected. There is no evidence that HPP can increase the water mass of the sample, and the increase of the analytical result of moisture can be related to a greater efficiency of the analytical method in extracting the water. 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 (GEHRING *et al.*, 2011).

The values of the pressurized samples were significantly ($p \leq 0.05$) higher than the control. The lowest WHC values, compared to the control sample, were for samples treated at 200MPa/5min and 400MPa/5min. by showing that the influencing pressurizing time in this parameter. At 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 Fig. 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 (URESTI et al., 2004). MARTINEZ et al. (2017) found a similar behavior when analyzing pressurized crabs; the WHC values were higher than the control at 100MPa and 300MPa for 5 min, but at the pressure level of 600MPa/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 proteins and activate the reactions of sulphydryl-disulfide bond exchange, can be disrupted. And these structural changes have resulted in the dissociation of proteins.

CHRISTENSEN et al. (2017), when comparing three species of pressurized fish (mackerel, salmon and cod), concluded that, in general, HHP promoted changes in proteins to the point of influencing WHC. However, the same behavior was not observed in all species. The mackerel at 200MPa/2 min presented reduction of WHC and at 500MPa/2 min there was an increase. In cod, no significant difference was observed and in salmon there was a significant reduction of WHC values with pressurization. Like this, in the face of the antagonistic effects

of increased hydration and reduction of water holding capacity, it is possible that both are dependent on the intensity of HPP (OLIVEIRA *et al.*, 2017).

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 (2009) evaluated in the same species, who found the value of pH 6.3. Higher values were found by BELTRAN-LUGO (2006) for the same species, ranging from 6.59 - 6.80 due to seasonality.

HP-treated scallop adductor muscle showed significantly (P≤0.05) increased pH relative to untreated scallop (table 2), consistent with previous reports for oysters (LOPEZ-CABALLERO *et al.*, 2000; HE *et al.*, 2002; CRUZ-ROMERO *et al.*, 2004; BINDU *et al.*, 2013; TEIXEIRA *et al.*, 2014). 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 t 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 (ERICKSON, 2002; HILTZ and DYER, 1971), and with this reduces the net surface charge on muscle proteins, and causes their partial denaturation (HUSS, 1995). The decrease in pH can lead to some WHC loss (HUSS, 1995). Additionally, pH strongly influences the microbiology of fish muscle, specially pH sensitive spoilage bacteria (GRAM and HUSS, 1996).

Thus, authors have 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 (RAMIREZ-SUAREZ et al., 2006; TEIXEIRA et al., 2014).
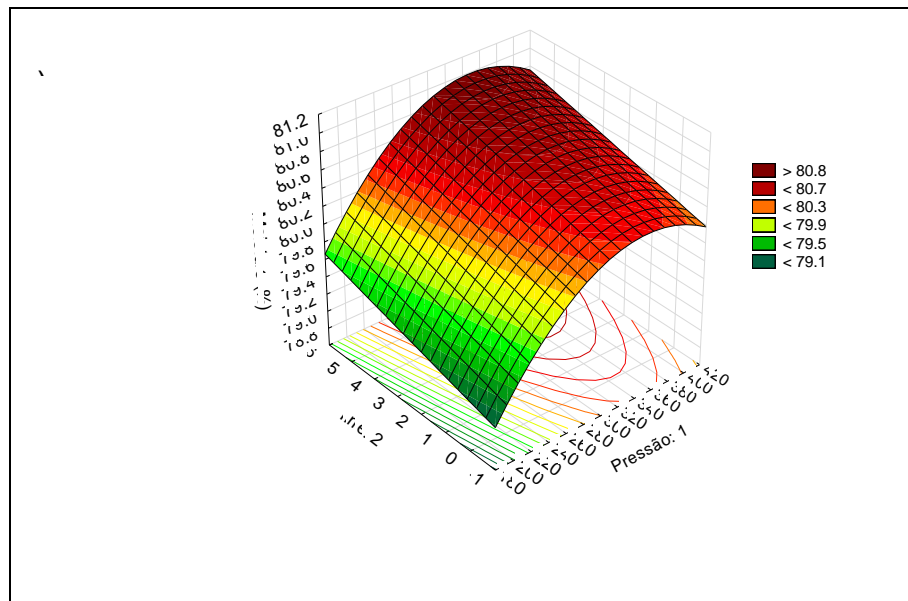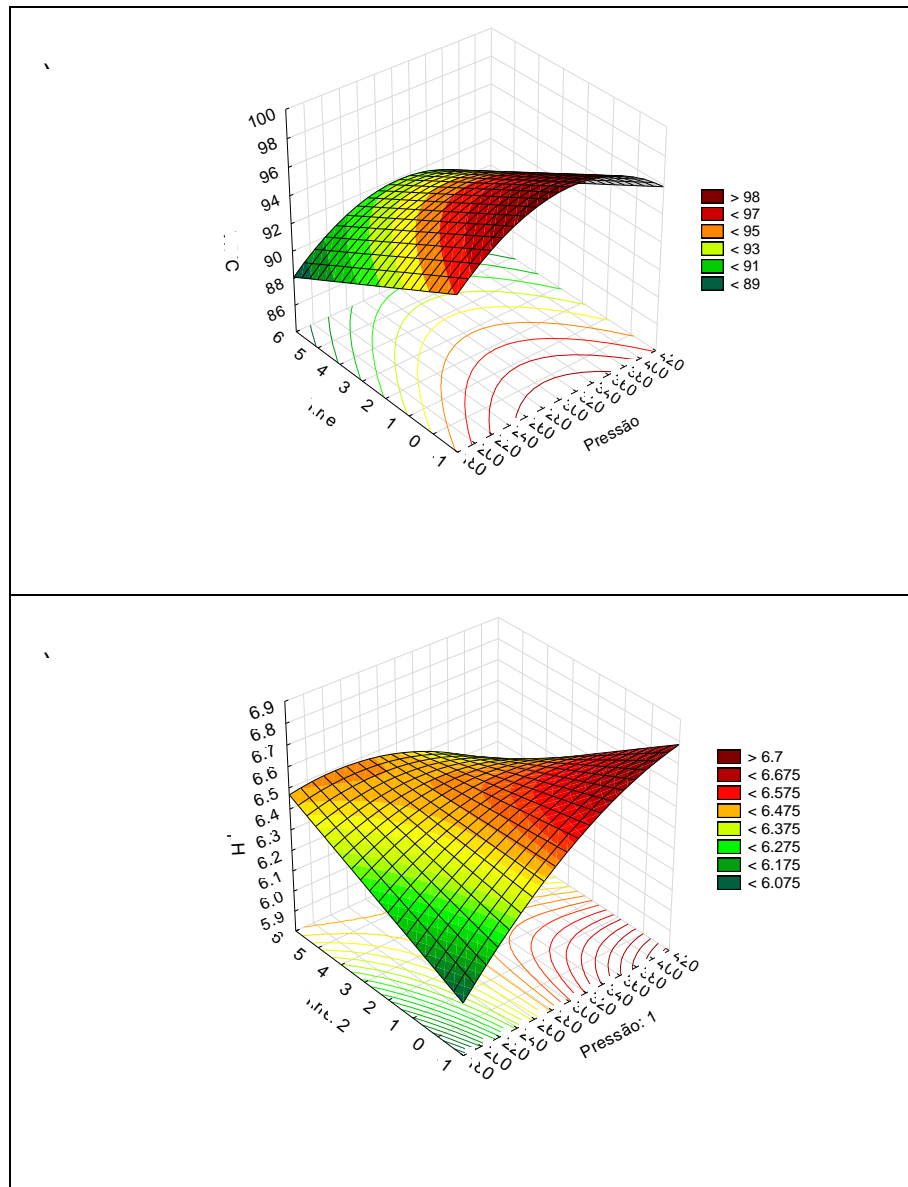
Figure 2. Effect of Pressure level (P: 200–400 MPa) and holding time (t: 0–5 min) on moisture (a), WHC (b) and pH (c) of scallop adductor muscle

3.2 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 molluscs, organisms that have the alimentary habit of filtering suspended particles in the water column where they are cultivated, and this may represent a public health problem. Moreover they are commonly consumed raw or partially cooked. Thus, the HHP applied to this type of food can favor the microbiological quality, reducing microorganisms deteriorating and even pathogenic 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 so as 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 nior 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. Fig. 3 also shows each individual convenience function and global wish function profiles.

The criteria selected for optimization of process parameters were: moisture content and WHC maximization; and pH near 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 365MPa 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 360MPa for 2 min exceeds the pressure level and waiting time required to bring the mesophilic and piscicrotrophic microbial counts to an undetectable level under the conditions of that study. Thus, we can suggest that the HHP 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, because the pH directly influences the development of the microbiota.

Profiles for Predicted Values and Desirability
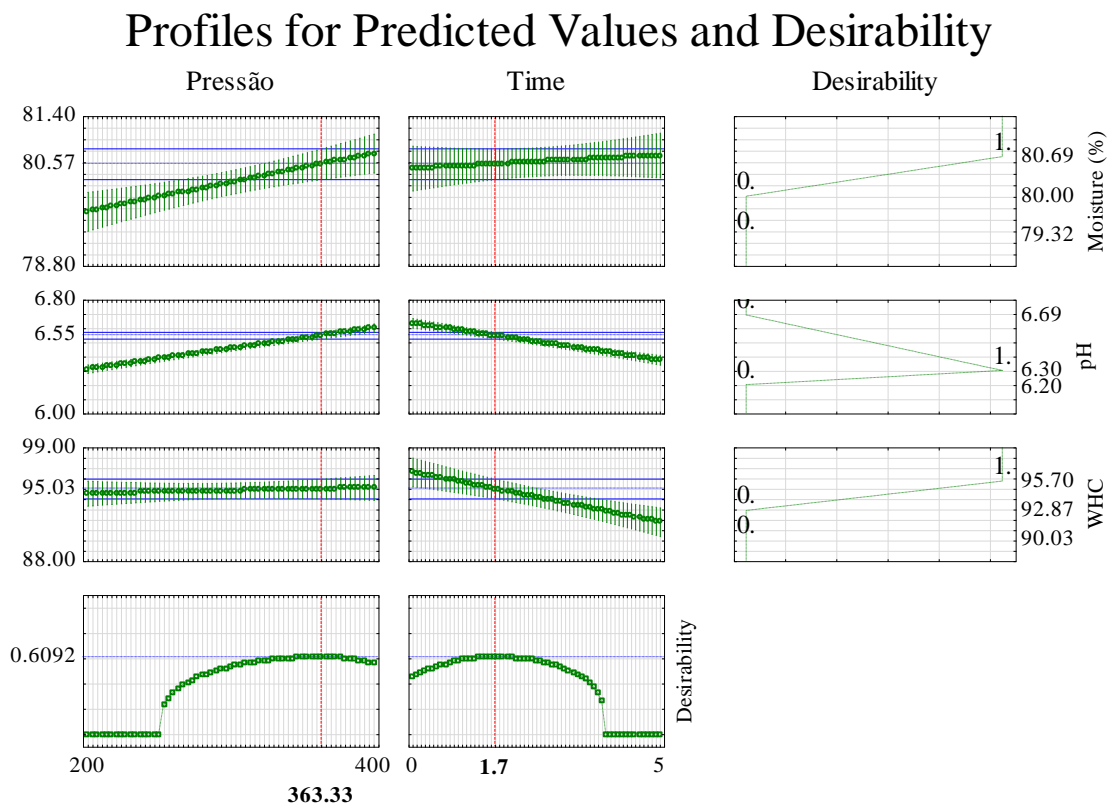
Figure 3. Simultaneous optimization of process conditions for HPP of adductor muscle scallop.

4. CONCLUSION

HPP considerably changed the quality of scallop adductor muscle, although varying with pressure 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 of fillets, 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, where in less severe pressure levels promoted the increase of the value of WHC and in 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, with heats of ΔE above 3.0.

Cutting strength showed lower values in all treatments, demonstrating that the applied treatments promoted the softening of the scrum 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 a new 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 high quality and maximum reduction of spoilage causing factors.

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

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CAPÍTULO III

**EFEITO DO PROCESSAMENTO POR APH EM MÚSCULO DE VIEIRAS
"NODIPECTEN NODOSUS" (LINNAEUS, 1758) ESTOCADO SOB REFRIGERAÇÃO**

RESUMO

Este estudo objetivou avaliar o efeito do tratamento com alta pressão hidrostática (APH) nas características e possível extensão da vida de prateleira de vieiras durante o armazenamento refrigerado a 4°C por 21 dias. Músculos adutores foram processados a 300MPa por 2,5 min e 400MPa por 5 min e comparados com amostras controle durante o armazenamento. Os tratamentos foram analisados para microrganismos mesófilos, pscicrotróficos e bactérias produtoras de H_2S, nitrogênio de base volátil total (N-BVT), substâncias reativas ao ácido tiobarbitúrico (TBARS), pH, degradação do ATP e valor K. As contagens microbianas das amostras pressurizadas não excederam 10^6 UFC/g, os níveis de N-BVT permaneceram abaixo de 30 mg/100 g e o pH, embora tenha aumentado, não excedeu 6,85 nas amostras processadas a 300MPa. A APH nos níveis estudados acelerou a oxidação lipídica, expressada no aumento de TBARS, porém não excedeu o limite de 2mg/kg. Estes resultados indicam que o processamento a 300 MPa por 2,5 min pode aumentar de modo relevante o tempo de armazenamento refrigerado do músculo adutor de vieiras.

Palavras-chave: Alta Pressão Hidrostática; Vida de Prateleira; Degradação de Nucleotídeos

ABSTRACT

This study was carried out to evaluate the effect of treatment with high hydrostatic pressure (HPA) on shelf life and shelf life extension during refrigerated storage at 4 ° C for 21 days. Adductor muscles were processed at 300MPa for 2.5 min and 400MPa for 5min and compared to control samples stored at 4 ° C for 21 days. The treatments were analyzed for mesophilic, pscycrotrophic and H2S producing bacteria, total volatile nitrogen (N-BVT), thiobarbituric acid reactive substances (TBARS), pH, degradation of ATP and K value. Microbial counts of pressurized samples did not exceed 10^6 CFU/g, N-BVT levels remained below 30 mg/100 g and pH although increased did not exceed 6.85 for 21 days at 300 MPa. APH at the levels studied accelerated lipid oxidation, expressed in the increase of TBARS, but did not exceed the limit of 2mg/kg of sample. These results indicate that processing at 300 MPa for 2.5 min can significantly increase the chilled adductor muscle storage time of scallops.

Keywords: High hydrostatic pressure; Shelf Life; Nucleotide Degradation

1 INTRODUÇÃO

A vieira *N. nodosus* é considerada uma iguaria e um componente importante das refeições gourmet. Assim como os frutos do mar em geral, as vieiras são alimentos ricos em ácidos graxos da família ômega-3, possuem proteínas de alto valor biológico, minerais e vitaminas essenciais e fazem parte de uma dieta saudável. Características adicionais do músculo, como textura macia, excelente sabor e crescente aceitação nos mercados nacional e internacional, tem alavancado seu valor como produto gourmet (PONCE-DÍAZ *et al.*, 2011). Estas vantagens fazem da vieira uma excelente candidata para a aquicultura. No entanto, apesar dos benefícios nutricionais e de saúde, os mariscos são altamente perecíveis devido a características físico-químicas como pH quase neutro, alta atividade de água, ser rica não apenas em ácidos graxos insaturados, mas também em aminoácidos livres, e exibir enzimas autolíticas ativas, tornando-a propensa à degradação microbiana e oxidativa (ARU *et al.*, 2018).

Como nos peixes, após a morte, as vieiras passam pelo seguintes estágios: *rigor mortis*, dissolução do *rigor mortis*, autólise e deterioração bacteriana. O processo autolítico ocorre como resultado de alterações enzimáticas endógenas no músculo, enquanto a deterioração é um produto do crescimento bacteriano (OCAÑO-HIGUERA *et al.*, 2006; PACHECO-AGUILAR *et al.*, 2008). Assim, a degradação do trifosfato de adenosina (ATP) é uma importante alteração química em frutos do mar e comumente utilizada como indicador de frescor (HOWGATE, 2006; PACHECO-AGUILAR *et al.*, 2008). O valor K, um indicador de frescor, é definido como a razão entre a soma de hipoxantina ribosídeo ou inosina (HxR) e hipoxantina (Hx) sobre o somatório de adenosina trifosfato (ATP), adenosina difosfato (ADP), adenosina monofosfato (AMP), inosina 5'-monofosfato (IMP), hipoxantina ribosídeo ou inosina (HxR) e hipoxantina (Hx), conforme identificação adiante. O valor K provou ser um indicador químico importante para a frescura dos pescados em geral e de vieira em particular, de acordo com estudos de frescura de vieiras Catarina (OCAÑO-HIGUERA *et al.*, 2006), Pata de leão (PACHECO-AGUILAR *et al.*, 2008) e dourada (HUIDOBRO *et al.*, 2001), durante o armazenamento refrigerado.

De modo geral, a degradação do ATP segue a seguinte sequência: ATP → ADP → AMP → IMP → HxR → Hx (HONG *et al.*, 2017). No entanto, existem controvérsias a respeito da rota bioquímica de degradação do ATP em invertebrados, onde é sugerida a seguinte rota: ATP → ADP → AMP → Adenosina → HxR → Hx (HATAE *et al.*, 1995; MASSA *et al.*, 2003; QIU *et al.*, 2016). Desta forma, a degradação dos nucleotídeos em pescado pode seguir duas vias, uma que envolve a formação de inosina monofosfato e outra que considera uma sequência de desfosforilação até adenosina. No entanto, em algumas espécies podem ocorrer as duas vias e, em outras, pode haver a prevalência da formação de adenosina, ocorrendo acúmulo de AMP e ausência ou baixa concentração de IMP (CONTRERAS-GUZMÁN, 1994). Portanto, permanece inconclusivo se o valor K pode ser usado como indicador de frescor em invertebrados.

A conversão de ATP a IMP ocorre rapidamente pela ação de enzimas endógenas presentes no músculo. No entanto a degradação do IMP a HxR e Hx é mais lenta e ocorre por ação de enzimas autolíticas e microbianas, sendo a taxa de formação por bactérias geralmente superior a autolítica. Desta forma, o IMP é acumulado na etapa inicial de degradação, sendo o principal responsável pela definição do aroma e sabor (umami) do pescado fresco. À medida que a qualidade do pescado decresce verifica-se aumento dos níveis de HxR, que é mais ou menos insípida, e de Hx, que tem efeito direto sobre o sabor amargo do pescado em deterioração (MASSA *et al.*, 2002; HONG *et al.*, 2017; LI *et al.*, 2017; LI *et al.*, 2019).

A microbiota presente em moluscos é dependente da espécie, seus hábitos alimentares, meio ambiente e do modo de captura (GRAM e HUSS, 1996). Bactérias de deterioração, em

pescado, são comumente Gram negativas e produzem odores e sabores característicos, como resultado de suas atividades metabólicas. Condições de temperatura de armazenamento, manuseio e embalagem afetam o crescimento microbiano e, portanto, o prazo de validade dos frutos do mar (ODEYEMI *et al.*, 2018). Os grupos dominantes de bactérias em moluscos armazenados sob refrigeração são Pseudomonas spp., *Shewanella putrefaciens* e *Moraxella/Acinetobacter* e, em menor extensão, *Aeromonas* e *Psychobacter* (ASHIE *et al.*, 1996; CRUZ-ROMERO *et al.*, 2008). Portanto, a qualidade das vieiras durante o acondicionamento e estocagem está associada a mudanças bioquímicas e sensoriais que são afetadas principalmente pela temperatura de armazenamento (KAWASHIMA e YAMANAKA, 1992) e pelo desenvolvimento de microbiota que pode levar à deterioração (OCAÑO-HIGUERA *ET AL.*, 2006).

O processamento por APH tem sido aplicado em moluscos e crustáceos com a finalidade de "desconche" (HE *et al.*, 2002; HSU *et al.*, 2010; YI *et al.*, 2013; RONG *et al.*, 2018; SHAO *et al.*, 2018) e muitos estudos têm sido realizado sobre a eficiência da tecnologia em diminuir a carga microbiana (PÉREZ-WON *et al.*, 2005; CRUZ-ROMERO *et al.*, 2008) e a capacidade de estender a vida de prateleira de moluscos (CRUZ-ROMERO *et al.*, 2004; BÜYÜKCAN *et al.*, 2009; BRIONES *et al.*, 2010; HUGHES *et al.*, 2016). Porém, estudos sobre a influência da APH na degradação do ATP e o impacto na qualidade de vieiras são escassos. Huijuan *et al.* (2018), em estudo sobre aplicação da APH em carne suína, verificaram que a tecnologia poderia influenciar os mecanismos bioquímicos de acumulação da inosina 5'-monofosfato (IMP), e que pressão de 300MPa poderia promover a formação de IMP, devido a maximização da atividade da adenosina monofosfato desaminase, uma enzima que catalisa o monofosfato de adenosina (AMP) para o IMP.

O presente estudo teve como objetivo investigar as alterações bioquímicas e microbiológicas e a influência na qualidade promovida pelo processamento por APH no músculo adutor de vieiras estocadas à 4°C por 21 dias.

2. MATERIAL E MÉTODOS

2.1 Preparação das amostras e processamento por alta pressão hidrostática

Um total de 60 dúzias de vieiras, com aproximadamente um ano de idade, foram adquiridas da fazenda marinha "Vieiras da Ilha", na Ilha Grande, estado do Rio de Janeiro. As vieiras foram desconchadas à mão, o músculo adutor removido e limpo com água corrente. As vieiras foram então embaladas a vácuo, utilizando sacos de polynylon, e mantidas a 4 °C imediatamente antes do processamento a alta pressão.

Os tratamentos sob pressão foram realizados em máquina de processamento de alta pressão (Stansted Fluid Power, modelo S-FL-850-9-W) situado na planta piloto da Embrapa Agroindústria de Alimentos (Guaratiba, RJ – Brasil). A dimensão do vaso de pressão com aproximadamente 4 cm de diâmetro e 30 cm de comprimento, com um volume total de 377 ml e o cilindro de amostras com volume útil de 250 ml com vários orifícios na parede através dos quais circula o líquido (etanol a 70%) sobre pressão. As amostras foram submetidas a duas diferentes condições (300 MPa/2,5 min e 400 MPa/5 min) e posteriormente comparadas com amostras não tratadas (controle), durante armazenamento a 4 ° C por 21 dias. Todas as análises foram realizadas nos dias 0, 3, 6, 10, 13, 17 e 21, exceto análises de nucleotídeos, somente realizadas até o dia 17 de estocagem.

2.2   Análises microbiológicas

A avaliação microbiológica das amostras foi realizada através da contagem total de microrganismos mesófilos, pscicrotróficos e bactérias produtoras de sulfeto de hidrogênio (H_2S). Em cada dia de amostragem, 25g de amostra de cada tratamento foi adicionada de 225 ml de água peptonada 0,1% (Difco, Detroit, MI, USA) adicionada de 1% de NaCl (Sigma Aldrich, Alemanha) e homogeneizada em homogeneizador (Nova Ética, Brasil) por 15 segundos. Diluições decimais apropriadas à contaminação foram realizadas com o mesmo diluente e plaqueadas em seus respectivos meios de cultura. Para enumerar os microrganismos aeróbios mesófilos e psicrotróficos 0,1 ml de cada diluição foi espalhada em Agar Padrão de Contagem em placas (PCA) adicionada de 1% de NaCl, como descrito por SWANSON et al. (2001) . Após incubação a 25°C por 72 h (para contagens de mesófilos) e a 7°C por 10 dias (para contagem de psicrotróficos), foram contadas placas de 10 a 250 colônias.

Para a contagem de bactérias H_2S, 1 ml da diluição apropriada foi inoculada em aproximadamente 10 ml de Agar de Ferro Lyngby (peptona bacteriológica 20 g (Sigma-Aldrich, Alemanha), "Lab lemco" em pó 3g (Oxoid, Alemanha), extrato de levedura em pó 3 g (Himedia , India), citrato férrico 0,3 g (Sigma-Aldrich, Alemanha), tiossulfato de sódio 0,3 (Sigma-Aldrich, Alemanha), L-cisteína 0,6 g (Sigma-Aldrich, Alemanha), NaCl 5 g (Sigma-Aldrich, Alemanha), e ágar bacteriológico 14 g (Merck, Alemanha), e após mistura e solidificação, cada placa foi coberta com uma camada do mesmo meio. Após incubação a 20°C por 4 dias conforme CRUZ-ROMERO *et al.* (2008). Placas com 10-250 colônias características (colônias negras devido à precipitação de FeS) foram contadas. Os dados microbianos foram transformados em logaritmos do número de unidades formadoras de colônias (log CFU g^{-1}). O limite de detecção foi de 10 UFC g^{-1} (1,0 log CFU g^{-1}).

2.3   Catabolismo de nucleotídeos e Valor K

A extração dos nucleotídeos foi realizada conforme metodologia descrita por VAL *et al.* (1994). Uma alíquota de 50 mg da amostra foi coletada e homogeneizada com 1mL de ácido perclórico ($HClO_4$) a 8% em ultrassom por 10 minutos. Posteriormente, adicionou-se 200µL de hidróxido de potássio (KOH) 6M e homogeneizou-se em vórtex durante 20 segundos. Posteriormente, centrifugou-se a 6300,0g por 3 minutos e o sobrenadante foi filtrado em filtro millipore 0,45µm (Merck, Alemanha) em vial.

Compostos relacionados ao ATP (ATP, ADP, AMP, IMP, HxR e Hx) foram analisados por cromatografia líquida de alta eficiência (HPLC) segundo PACHECO *et al*., (2014). A separação dos nucleotídeos foi realizada em coluna Thermo C18 (100 x 4,6mm; 2,4µm) com eluição gradiente de acetonitrila e tampão fosfato (0,04M KH2PO4 e 0,06M K2HPO4, pH 7,0), filtrado previamente em filtro aquoso de 0,45 µm antes do uso. O gradiente programado segundo STOCCHI *et al.* (1985) com modificações conforme condições descritas na tabela 1. A detecção foi em detector de arranjo de fotodiodo 2996 (Waters®) em 254nm. O fluxo da fase móvel foi 1,3 mL/min e o volume de injeção da amostra foi de 5µL. Os padrões avaliados foram adenosina trifosfato, adenosina difosfato, adenosina monofosfato, inosina monofosfato, inosina e hipoxantina (Sigma Aldrich, Japão). Foram misturados formando um mix de padrões (20,83 a 166,6 µg/ml) para quantificar os nucleotídeos nas amostras. Os resultados foram expressos em µmol/g de amostra.

Tabela 1. Condições técnicas empregadas para a análise por HPLC da degradação de nucleotídeos

Tempo (min)	Fluxo (ml min^{-1})	Solvente A (%)	Solvente B (%)	Solvente C (%)
0,00	1,10	100,0	0,00	0,00
2,55	1,10	100,0	0,00	0,00
4,00	1,10	95,0	5,00	0,00
5,50	1,10	87,5	12,5	0,00
5,60	1,10	0,00	12,5	87,5
6,00	1,10	0,00	60,0	40,0
9,00	1,10	0,00	60,0	40,0
9,10	1,10	0,00	0,00	100,0
9,25	1,10	0,00	0,00	100,0
9,50	1,10	100,0	0,00	0,00
15,00	1,10	100,0	0,00	0,00

Fase A: Tampão fosfato (0,04M KH2PO4 e 0,06M K2HPO4); Fase B: Acetonitrila; Fase C: Água

O valor K foi calculado de acordo com SAITO *et al* (1959) pela seguinte equação:

$$\text{Valor K\%} = [(HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx)] \times 100$$

Onde:
- K < 20%: pescado muito fresco, adequado para ser consumido cru.
- 20 < K < 40%: pescado considerado fresco para ser consumido após o cozimento.
- K > 40%: pescado inadequado para consumo.

2.4 Medição de pH

O pH foi determinado utilizando um medidor de pH digital (modelo 205, TESTO, Brasil) equipado com um eletrodo de vidro (calibrado em pH quatro e sete), que foi inserido diretamente no músculo adutor.

2.5 Oxidação lipídica – Substância reativas ao ácido tiobarbitúrico (TBARS)

A oxidação lipídica foi determinada através de substâncias reativas ao ácido tiobarbitúrico (TBARS), seguindo o método de YIN *et al.* (1993). Os valores de absorvância foram medidos a 532 nm, utilizando um espectrofotómetro UV-1800 (Shimadzu, Kyoto, Japão). Os resultados foram expressos em mg de malonaldeído (MDA) / kg de músculo adutor usando uma curva padrão ($R2 = 0,997$) feita com sete concentrações diferentes de MDA variando de 1 a 500 µmol.

2.6 Nitrogênio das Bases Voláteis Totais (N-BVT)

O TVB-N foi determinado pelo método de microdifusão de Conway, conforme protocolo estabelecido pela Association of Official Analytical Chemists (AOAC, 1920). Os resultados foram expressos em mg TVB-N/100 g.

2.7   Análise estatística

As análises foram realizadas com o software estatístico XLSTAT versão 2016.02.28451 (ADDINSOFT, 2016). Estatística descritiva (média, desvio padrão e coeficiente de variação), one-way ANOVA, comparação múltipla com o teste de Tukey. O nível de significância foi estabelecido em 5%.

3.   RESULTADOS E DISCUSSÃO

3.1   Análises Microbiológicas

O limite superior microbiano para frutos do mar é geralmente considerado como sendo 10^6 unidades formadoras de colônia (UFC)/g, com rejeição sensorial ocorrendo a 10^8 UFC / g, ou antes dependendo do produto alimentar, devido às alterações organolépticas causadas pelo organismo específico de deterioração (GRAM e HUSS, 1996).

A contagem microbiana inicial de mesófilos e piscicrotróficos do músculo adutor de viera (não pressurizado) foi de $1,9x10^3$ e $<1,0x10^1$ UFC g $^{-1}$ respectivamente, aumentando significativo (P<0,05) no decorrer do período de estocagem refrigerada, conforme podemos observar na tabela 1. As amostras não pressurizadas alcançaram o limite superior de contagem entre os dias 6 e 10 dias de refrigeração. Embora, sensorialmente o odor desagradável (por exemplo, cheiro de peixe velho contaminado com amônia, o cheiro semelhante a iodo de algas em decomposição) somente tenha sido percebido no dia 13 de estocagem (10^7). A contagem de bactérias produtoras de H2S no dia 0 de estocagem foi não detectável, e somente apresentou contagem (colônias negras) no sexto dia de estocagem ($2,5x10^4$ UFC g $^{-1}$).

A microflora presente em peixes e moluscos depende da espécie, hábitos alimentares, meio ambiente e o modo de captura (LLANOS *et al.*, 2002). No entanto, as condições durante o armazenamento determinam quais bactérias são responsáveis pela deterioração (GRAM e HUSS, 1996; LINTON *et al.*, 2003). Assim, a baixa contagem inicial de bactérias mesófilas no presente estudo é decorrente da alta qualidade da água de cultivo. As vieiras foram cultivadas em fazenda marinha localizada em uma área preservada ecologicamente em Ilha Grande no município de Angra dos Reis, Rio de Janeiro.

Os níveis de pressão estudados, 300MPa/2,5min e 400MPa/5min, reduziram significativamente (p<0,05) a contagem de mesófilos a nível não detectável no tempo 0 (tabela 2). O tratamento a 300MPa/2,5min ocasionou uma pequena redução na contagem de mesófilos no terceiro dia de estocagem e manteve a contagem estável (10^3) até o 17° dia de estocagem, enquanto o nível 400MPa reduziu a contagem a <1 log10 cfu/g, indicando um comprometimento do crescimento e uma redução da fase exponencial devido à maior pressão de processamento. YI *et al.* (2013) verificou uma redução significativa na contagem inicial de mesófilos (2,98 log10 cfu/g) quando aplicou o nível de pressão de 300MPa/0min (1,69 log10 cfu/g) ao estudar a eficiência da APH no "desconchamento" de vieiras *Argopecten irradians* e em 200MPa/3min essa contagem foi reduzida a não detectado (<1 log10 cfu/g).

Bactérias produtoras de sulfeto de hidrogênio (H2S) somente foram detectadas no sexto dia de estocagem nas amostras controle (tabela 2). A fase de retardamento inicial das bactérias produtoras de H2S pode ser resultado da inibição por *Pseudomonas spp*. Pois *Pseudomonas spp* e *Shewanella putrefaciens* são microrganismos fortemente competitivos entre si e devido a capacidade da primeira produzir sideróforos (compostos orgânicos que atuam na captação do ferro) e esta interação pode ser o principal fator que regula o desenvolvimento da microbiota de deterioração em produtos do mar (GRAM e MELCHIORSEN, 1996; BRIONES *et al.*, 2010).

A resistência dos microrganismos é altamente variável, dependendo principalmente do tipo de organismo e da matriz alimentar, e bem como magnitude da pressão aplicada, tempo de pressurização. Sugere-se que a inativação de microrganismos por APH seja devido ao o efeito causado direto na morfologia, reações bioquímicas, mecanismo genético membranas e paredes celulares (HOOVER, 1989; FARKAS e HOOVER, 2000; RENDUELEs *et al.*, 2011).

Tabela 2. Alterações nos valores de mesófilos, piscicrotroficos e produtoras de H_2S nos músculos adutores pressurizados e controle antes no armazenamento refrigerado a 4°C.

| Contagem (log10 cfu/g)	Tratamentos	Tempo de estocagem (dias)						
		0	3	6	10	13	17	21
Mesófilo	Controle	$3,28 \pm (0.03)^{Ab}$	$3,14 \pm (0.12)^{Acd}$	$4,09 \pm (0.10)^{Abc}$	$7,38 \pm (0.00)^{Aab}$	$7,04 \pm (0.00)^{Aab}$	$7,91 \pm (0.50)^{Aa}$	$8,39 \pm (0.26)^{Aa}$
	300MPa	$0,00 \pm (0.00)^{Bd}$	$3,22 \pm (0.04)^{Bcd}$	$3,04 \pm (0.00)^{Bbc}$	$3,81 \pm (0.07)^{Bab}$	$3,04 \pm (0.00)^{Bab}$	$3,45 \pm (0.03)^{Ba}$	$4,85 \pm (0.55)^{Ba}$
	400MPa	$0,00 \pm (0.00)^{Cd}$	$0,00 \pm (0.00)^{Ccd}$	$0,50 \pm (0.58)^{Cbc}$	$0,00 \pm (0.00)^{Cab}$	$1,00 \pm (0.00)^{Cab}$	$0,25 \pm (0.50)^{Ca}$	$0,50 \pm (0.58)^{Ca}$
Pscicrotrófico	Controle	$0,50 \pm (0.58)^{Ac}$	$0,50 \pm (0.58)^{Ac}$	$4,44 \pm (0.17)^{Abc}$	$7,40 \pm (0.00)^{Aa}$	$5,20 \pm (0.16)^{Aab}$	$6,40 \pm (0.00)^{Aa}$	$8,73 \pm (0.10)^{Aa}$
	300MPa	$0,00 \pm (0.00)^{Bc}$	$0,00 \pm (0.00)^{Bc}$	$0,00 \pm (0.00)^{Bbc}$	$3,75 \pm (0.04)^{Ba}$	$3,06 \pm (0.04)^{Bab}$	$3,47 \pm (0.01)^{Ba}$	$4,38 \pm (0.00)^{Ba}$
	400MPa	$0,00 \pm (0.00)^{Cc}$	$0,00 \pm (0.00)^{Cc}$	$0,00 \pm (0.00)^{Cbc}$	$0,00 \pm (0.00)^{Ca}$	$0,50 \pm (0.58)^{Cab}$	$0,00 \pm (0.00)^{Ca}$	$0,25 \pm (0.50)^{Ca}$
Produtoras de H2S	Controle	$0,00 \pm (0.00)^{Ab}$	$0,00 \pm (0.00)^{Ab}$	$4,39 \pm (0.01)^{Aa}$	$0,00 \pm (0.00)^{Ab}$	$0,00 \pm (0.00)^{Ab}$	$0,00 \pm (0.00)^{Ab}$	$0,00 \pm (0.00)^{Ab}$
	300MPa	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Ba}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$
	400MPa	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Ba}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$	$0,00 \pm (0.00)^{Bb}$

Médias com letras maiúsculas iguais na mesma coluna não diferem significativamente entre si (p <0,05) pelo Teste de Tukey
Médias com letras minúsculas iguais na mesma linha não diferem significativamente entre si (p <0,05) pelo Teste de Tukey
Tratamentos: Controle (sem pressão); 300Mpa por 2,5 minutos e 400Mpa por 5minutos

3.2   Catabolismo de nucleotídeos e Valor K

Uma das mais importantes alterações bioquímicas post-mortem no músculo do organismo marinho é a degradação de ATP, que juntamente com seus produtos de degradação tem sido amplamente estudada e aplicada para monitorar o frescor e vida útil do músculo do pescado (VAZQUEZ-ORTIZ *et al.*, 1997). A Figura 1 mostra os cromatogramas da mistura de padrões de ATP, ADP, AMP, IMP, Inosina e Hipoxantina (A) e do extrato da amostra controle no primeiro dia de estocagem (B)
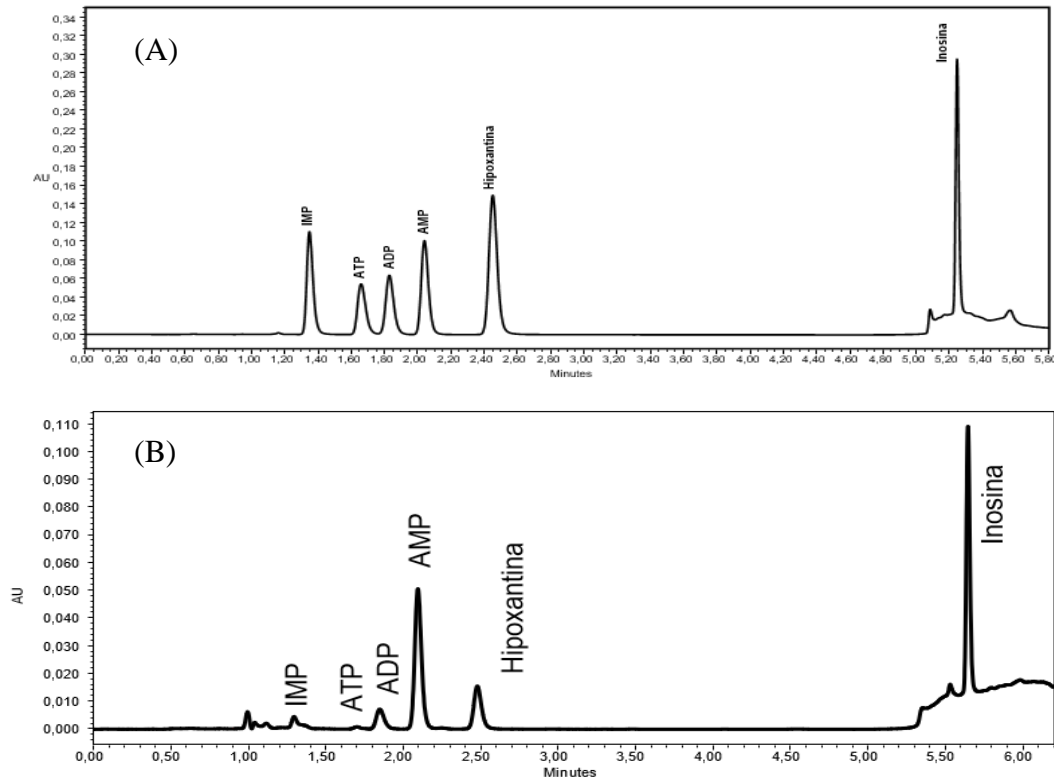
Figura 1. Cromatograma da mistura de padrões (A) e do extrato da amostra controle no primeniro dia de estocagem (B).

Os valores de ATP foram 0,09 (±0,01), 0,46 (±0.04), 0,08 (0,00) µmol/g para os tratamentos controle, 300MPa/2,5min e 400/5min respectivamente no dia 0 de estocagem. O nível desse componente declinou rapidamente nos três tratamentos nos 3 primeiros dias de estocagem à níveis abaixo do limite de detecção. Este resultado é consistente com um relatos anteriores, em que a conversão de ATP para AMP foi quase concluída em um dia e presume-se que seja um processo totalmente autolítico (JONES, 1965; ALASALVAR *et al.*, 2002; LI *et al.*, 2017).

A Figura 2 mostra as médias dos produtos de degradação do ATP (ADP, AMP, IMP, HxR e Hx) e também valor K para as amostras controle, 300MPa/2,5 min e 400MPa/5min armazenadas a 4°C por 17dias. Neste estudo, o ADP foi detectado (Figura 2a), e não está de acordo com estudos realizados com a mesma espécie (*N. nodosus*) por PACHECO-AGUILAR *et al.* (2008) e sugere que a vieira *N. nodosus* possa apresentar duas vias, uma que envolve a formação de inosina monofosfato e outra que considera uma sequência de desfosforilação até adenosina. De acordo com a Figura 2a é possível observar que a APH promoveu um aumento significativo (P<0,05) no valor do ADP no tempo 0 de estocagem. No entanto, a partir do dia 3

até o último de estocagem os tratamentos pressurizados apresentaram valores inferiores ao controle.

MENDES *et al.* (2001) relataram que o AMP é um dos nucleotídeos dominantes de moluscos e crustáceos e que sua acumulação é o resultado de uma atividade altamente reduzida ou inexistente da enzima AMP deaminase. Este relato está de acordo com a baixa concentração de IMP encontrada neste estudo (Figura 2c). A concentração inicial de AMP (Figura 2b) obtida na amostra controle está acima do valor encontrado por PACHECO-AGUILAR *et al.* (2008) para a mesma espécie armazenada em gelo (2,8µmol/g), e se assemelhou ao valor encontrado por OCAÑO-HIGUERA *et al.* (2006) em vieiras Catarina (4,6µmol/g). No entanto, o conteúdo do AMP da amostra controle declinou bruscamente a partir do terceiro dia de estocagem.

A APH a 400MPa/5min aumentou significativamente (P<0,05) a concentração de AMP. E tanto o nível 300MPa/2,5min como 400MPa/5min mantiveram concentrações de AMP maiores que o controle durante toda a estocagem refrigerada. O teor de IMP no musculo adutor de vieiras tratadas por APH apresentou aumento significativo (P<0,05). As pressões 300MPa/2,5min e 400MPa/5min promoveram um leve aumento no conteúdo do IMP durante toda a estocagem. No entanto, o aumento do conteúdo de IMP pode ser consequência da ativação enzimática . Portanto, mais experimentos devem ser realizados para estudar o efeito da APH nas atividades de várias enzimas-chave. Segundo HUIJUAN *et al.* (2018) em estudo sobre o efeito da APH no conteúdo de IMP em carne suína verificaram que o nível de pressão de 300MPa/10min promoveu o aumento da adenosina monofosfato deaminase (AMPD) e redução da atividades das fosfatases acida (ACP) e alcalina (ALP). A AMPD é a enzima responsável pela desaminação do AMP para formar IMP e as fosfatases acida e alcalinas são enzimas que fazem com que o IMP se degrade em hipoxantina (HxR). Dessa forma, a APH atuou na manutenção do IMP que responsável pelo sabor umami em pescados.

O acúmulo de Hx reflete a fase inicial de deterioração autolítica, bem como a deterioração bacteriana e, portanto, é um importante parâmetro de qualidade para diferentes espécies de pescado, como observado no presente estudo e relatado em estudos anteriores. No presente estudo, os valores de HxR na amostra controle aumentou significativamente com o tempo de estocagem (P<0,05). A APH promoveu o aumento significativo (P<0,05) da concentração de HxR, tanto a 300MPa/2,5min como em 400MPa/5min, e também durante a estocagem refrigerada, conforme Figura 2d e 2e. O que contradiz a atuação da APH em fosfatases acida e alcalina. Dessa forma, um estudo aprofundado sobre os efeitos da APH nessas enzimas especificamente nessa matriz seria interessante. O acúmulo de Hx em peixes e moluscos é aparentemente responsável pela perda progressiva de um sabor desejável, resultando em um sabor amargo. A Hx pode ser formada pela quebra autolítica de nucleotídeos, mas também pode ser formada por bactérias, incluindo *Pseudomonas spp.*, *S. putrefaciens* e *P. phosphoreum* (HUSS, 1995).

Um dos índices mais amplamente empregados para avaliar o frescor é o valor K, que é definido como a razão entre a soma de Ino e Hx e a soma de todos os produtos de degradação de ATP (SAITO, 1959). As alterações no valor de K dos músculos adutor de vieira são mostradas na Figura 1f. Os valores iniciais de K das amostras controle e pressurizadas a 300MPa/2,5min e 400MPa/5min foram 52,18%, 44,56% e 24,42%, respectivamente. O valor de K inicial foi muito alto indicando teoricamente que este pescado já apresentava perda do frescor. O que não condiz com a avaliação sensorial subjetiva realizada no momento das análises. Sabe-se que a avaliação sensorial/olfativa do pescado é o melhor atributo para indicar a sua qualidade (DORE, 1991; AARAAS *et al.*, 2004). No entanto, é possível verificar que a aplicação da APH promoveu a redução significativa (p>0,05) desse parâmetro. O que nos dá um indício de que a tecnologia é capaz de auxiliar no frescor de vieiras refrigeradas nas condições do estudo.
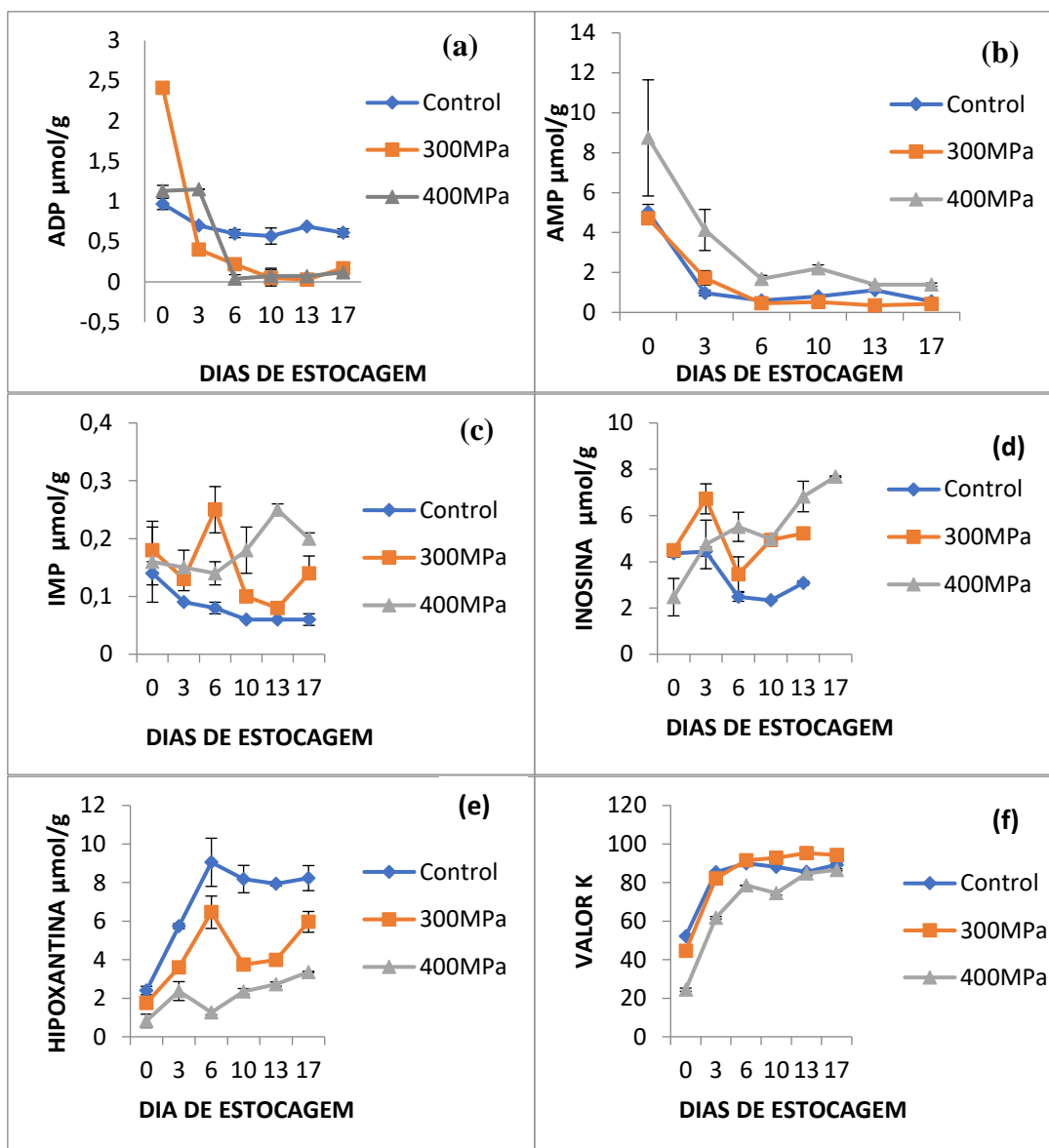
Figura 2. Alterações nos teores de (a) ADP, (b) AMP, (c) IMP, (d) HxR, (e) Hx e (f) Valor K de músculo adutor de vieira durante o armazenamento de 4°C por 17 dias.

3.3   Medição do pH

A mudança no pH dos frutos do mar é geralmente um bom índice para avaliação da qualidade, e é um dos métodos de controle físico de qualidade mais frequentemente utilizados para produtos do mar. Em geral, o pH de um alimento é determinado principalmente por grupos carboxila e amino livres em compostos de baixo peso molecular e, em menor grau, em macromoléculas celulares (proteínas, ácidos nucléicos, polissacarídeos) (CRUZ-ROMERO *et al*., 2007). E pode ser afetado pelas mudanças nas concentrações de íons hidrogênio e hidroxila livres devido a mudanças no balanço de oxidação-redução do alimento pela atividade de microrganismos ou enzimas (VARLIK *et al.*, 2000). E de fato, é comumente relacionado ao acúmulo de ácido lático e octopina, em moluscos, gerado pelo glicogênio em condição anóxica e/ou ao acúmulo de substâncias básicas, como amônia e trimetilamina, derivadas principalmente da ação de bactérias alcalinizantes (ARU *et al.*, 2016). Conforme relatado na literatura, bivalves frescos e de boa qualidade exibem valores de pH variando de 6,0 a 7,0 (CRUZ-ROMERO *et al.*, 2004; KHAN *et al.*, 2005; CRUZ-ROMERO *et al.*, 2007).

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Alterações no pH do músculo adutor de vieiras armazenadas durante 21 dias são mostradas na Tabela 3. O pH dos músculos adutores de vieiras controle foi de 6,29, concordando com PACHECO-AGUILAR *et al.* (2008) e BELTRÁN-LUGO *et al.* (2006) em estudos com a mesma espécie. O pH das amostras 300MPa/2,5 min e 400MPa/5 min apresentaram aumento significativo (p≤0,05) em comparação com controle, porém os tratamentos 300MPa/2,5min e 400MPa/5min não diferiram significativamente entre eles. Autores relatam um aumento no valor do pH em ostras após tratamento por APH (CRUZ-ROMERO *et al.*, 2004; CRUZ-ROMERO *et al.*, 2007) e abalones (BRIONES *et al.*, 2010) e sugerem que tal incremento no valor de pH possa ser atribuída a mudanças conformacionais em proteínas musculares associadas à sua desnaturação, devido à maior ou menor exposição de grupos de aminoácidos ácidos e básicos (TEIXEIRA *et al.*, 2014).

Durante a estocagem, amostras controle do dia 0 até o dia 10 apresentaram um declínio no valor de pH, variando de 6,29 (dia 0) a 6,01 (dia 10), e depois voltou a subir chegando a 6,44 no dia 21, porém sem efeito estatístico significativo (p≥0,05). Para amostras pressurizadas (300MPa/2,5 e 400MPa/5min) os valor do pH não apresentaram efeito claro. No entanto, pode-se observar uma tendência a aumentar conforme o decorrer dos dias de estocagem, mas ambos sem diferença significativa entre os dias e entre os tratamentos (p≥0,05).

Tabela 3. Alterações nos valores de pH, N-BVT e TBRS nos músculos adutores pressurizados e controle antes no armazenamento refrigerado a 4°C.

Atributos de qualidade	Tratamentos	Tempo de estocagem (dias)						
		0	3	6	10	13	17	21
pH	Controle	$6.29 \pm (0.07)^{Ba}$	$6.21 \pm (0.05)^{Ba}$	$6.12 \pm (0.04)^{Ba}$	$6.01 \pm (0.04)^{Ba}$	$6.30 \pm (0.18)^{Ba}$	$6.15 \pm (0.13)^{Ba}$	$6.44 \pm (0.03)^{Ba}$
	300MPa	$6.77 \pm (0.05)^{Aa}$	$6.64 \pm (0.04)^{Aa}$	$6.62 \pm (0.08)^{Aa}$	$6.66 \pm (0.02)^{Aa}$	$6.60 \pm (0.01)^{Aa}$	$6.69 \pm (0.03)^{Aa}$	$6.56 \pm (0.08)^{Aa}$
	400MPa	$6.60 \pm (0.09)^{Aa}$	$6.76 \pm (0.11)^{Aa}$	$6.66 \pm (0.03)^{Aa}$	$6.87 \pm (0.01)^{Aa}$	$6.61 \pm (0.03)^{Aa}$	$6.74 \pm (0.01)^{Aa}$	$6.67 \pm (0.04)^{Aa}$
N-BVT	Controle	$10.40 \pm (0.63)^{Ad}$	$13.07 \pm (0.60)^{Acd}$	$15.75 \pm (0.51)^{Acd}$	$17.17 \pm (0.95)^{Abc}$	$21.58 \pm (1.08)^{Ab}$	$27.88 \pm (0.79)^{Aa}$	$31.50 \pm (1.45)^{Aa}$
	300MPa	$10.08 \pm (0.00)^{Bd}$	$11.97 \pm (0.73)^{Bcd}$	$11.97 \pm (0.73)^{Bcd}$	$12.44 \pm (0.79)^{Bbc}$	$14.18 \pm (1.21)^{Bb}$	$17.17 \pm (1.40)^{Ba}$	$17.64 \pm (1.45)^{Bb}$
	400MPa	$10.40 \pm (0.63)^{Bd}$	$10.24 \pm (0.32)^{Bcd}$	$10.08 \pm (0.00)^{Bcd}$	$11.18 \pm (0.79)^{Bbc}$	$13.40 \pm (0.60)^{Bb}$	$15.75 \pm (0.73)^{Ba}$	$17.01 \pm (0.73)^{Ba}$
TBARS	Controle	$0.026 \pm (0.00)^{Be}$	$0.035 \pm (0.00)^{Bd}$	$0.070 \pm (0.00)^{Bcd}$	$0.084 \pm (0.01)^{Bbcd}$	$0.114 \pm (0.01)^{Bbc}$	$0.129 \pm (0.01)^{Bb}$	$0.327 \pm (0.02)^{Ba}$
	300MPa	$0.051 \pm (0.00)^{Ae}$	$0.062 \pm (0.00)^{Ad}$	$0.092 \pm (0.00)^{Acd}$	$0.120 \pm (0.00)^{Abcd}$	$0.158 \pm (0.00)^{Abc}$	$0.158 \pm (0.00)^{Ab}$	$0.620 \pm (0.01)^{Aa}$
	400MPa	$0.055 \pm (0.00)^{Ae}$	$0.064 \pm (0.00)^{Ad}$	$0.094 \pm (0.01)^{Acd}$	$0.124 \pm (0.01)^{Abcd}$	$0.160 \pm (0.01)^{Abc}$	$0.166 \pm (0.01)^{Ab}$	$0.636 \pm (0.05)^{Aa}$

Médias com letras maiúsculas iguais na mesma coluna não diferem significativamente entre si (p <0,05) pelo Teste de Tukey
Médias com letras minúsculas iguais na mesma linha não diferem significativamente entre si (p <0,05) pelo Teste de Tukey
Tratamentos: Controle (sem pressão); 300Mpa por 2,5 minutos e 400Mpa por 5minutos
N-BVT: mg de N-BVT/100g de amostra; TBARS: mg de malonaldeído/kg de amostra

A legislação brasileira, através do regulamento da inspeção industrial e sanitária de produtos de origem animal (RIISPOA, Art. 211 inciso III) regulamenta um limite de 6,85 para moluscos classificado como fresco (MAPA, 2017). Pode-se verificar que a maioria dos valores, independente do tratamento com APH ou controle, apresentaram valores inferiores ao estabelecido pela legislação. No entanto, além da medição do pH faz-se necessário a realização de outras análises para poder verificar o estado de frescor ou mesmo o início da deterioração do pescado, uma vez que o pH sofre oscilações durante a estocagem refrigerada e é variável entre as diferentes amostras (OGAWA e MAIA, 1999).

3.4   Nitrogênio das Bases Voláteis Totais (N-BVT)

O N-BVT tem sido relatado como indicador de deterioração de peixes e moluscos (CHENG *et al.*, 2014), incluindo as medições de trimetilamina, dimetilamina (produzida por bactérias deteriorantes), dimetilamina (produzida por enzimas autolíticas durante o armazenamento refrigerado), amônia (produzida pela desaminação de aminoácidos e catabólitos de nucleotídeos) e outros compostos, que vêm do degradação de proteínas e compostos nitrogenados não proteicos por enzimas endógenas (LI *et al.*, 2013; RONG *et al.*, 2018). Neste estudo, a quantidade de N-BVT no músculo adutor aumentou com o tempo de estocagem (Tabela 3). O conteúdo inicial de N-BVT no dia 0 foi de 10,40 ± 0,63 mg/g, valor abaixo ao relatado por RUIZ-CAPILLAS *et al.* (2001) no músculo adutor do rei vieira (*Pecten maximus*), com 11 mg/100 g e para o músculo adutor de vieira Catarina, com 13,5 mg/100g (OCAÑO-HIGUERA *et al.*, 2006). O N-BVT aumentou linearmente com o tempo em todos os tratamentos até um valor final de 31,50 ± 1,45 mg/100g para amostra controle e 17,64 ± 1,45 e 17,01 ± 1,45 mg/100g para os tratamentos 300Mpa/2,5min e 400Mpa/5min, respectivamente no dia 21. Níveis semelhantes foram reportados por BINDU *et al.* (2013) em camarão branco e ERKAN e ÜRETENER (2010) em dourada (*Sparus aurata).*

O limite de aceitabilidade do valor de N-BVT, pela legislação brasileira, é de 30 mg N/100g de tecido muscular (MAPA, 2017). De acordo com os valores (Tabela 3) pode-se observar que somente a amostra controle no dia 21 excedeu esse limite. E que a APH reduziu os níveis de N-BVT, em comparação com o controle, de forma que as amostras pressurizadas a 300MPa/2,5min no dia 17 de estocagem (17,17 ± 1,40) e 400Mpa/5min no dia 21 de estocagem (17,01± 0,73) tiveram valores equivalentes ao 10° dia de estocagem da amostra controle (17,17± 0,95).

3.5   Oxidação Lipídica: Substâncias reativas ao ácido tiobarbitúrico (TBARS)

O índice TBARS é uma medida do teor de malonaldeído, um dos produtos de degradação de hidroperóxidos lipídicos, formado durante o processo de oxidação de ácidos graxos poli-insaturados com o oxigênio (DE AZEVEDO GOMES *et al.*, 2003). Os valores de TBARS encontrados nas amostras de músculo de vieira estão apresentadas na tabela 3. A APH promoveu o aumento do teor de malonaldeído em detrimento da amostra controle. Os valores obtidos das amostras 300MPa/2,5min e 400MPa/5min diferiram significativamente do controle (P<0,05) mas não apresentaram diferença significativa entre eles no dia 0 de armazenamento. Durante o armazenamento refrigerado, todos os tratamentos apresentaram crescimento linear nos níveis de malonaldeído, tendo as amostras pressurizadas (300MPa e 400MPa) apresentado valores bem maiores no último dia de armazenamento (21° dia). O valor de TBARS de 2,0 mg / kg é normalmente considerado como o nível de deterioração, acima do qual os produtos da pesca desenvolverão odor e gosto desagradáveis para consumo humano (RONG *et al.*, 2018). Os valores de TBARS dos tratamentos controle e com APH não excederam 2,0 mg / kg durante o período de armazenamento.

Alguns autores relatam aumento do teor de malonaldeído após o tratamento por APH. RONG *et al.* (2018) em estudo com ostras, verificou que a pressão de 300MPa/2 min promoveu o aumento do valor de TBARS em comparação com amostras controle de imediato e durante a estocagem refrigerada. LAKSHMANAN *et al.* (2005) relataram um aumento apreciável nos níveis de TBARS durante o armazenamento refrigerado de salmão defumado frio (300 MPa) pressurizado. Camarões-branco foram tratados por APH em níveis de 100, 270, 435 e 600 MPa por 5 min e foi relatado um acréscimo significativo nos níveis de TBARS no período de armazenamento (BINDU *et al.*, 2013).

Em produtos derivados do peixe, a auto oxidação lipídica parece ocorrer em baixas pressões, já que um ligeiro aumento no valor de TBARS é observado desde 200 MPa em filés de pregado , mas nenhuma hidrólise de triglicerídeos é revelada (CHEVALIER *et al.*, 2001), sugerindo que o tratamento por pressão aplicado não afetou os mecanismos de hidrólise. Ocorre que o tratamento com APH pode promover a liberação em íons metálicos, como ferro e cobre, que aceleram a oxidação lipídica (RONG *et al.*, 2018). Assim, a oxidação lipídica não se deve ao efeito direto da pressão sobre os lipídios, mas sim à ação combinada de oxigênio e catalisador como íons metálicos, proteínas ou enzimas (MEDINA-MEZA *et al.*, 2014).

4. CONCLUSÃO

Os resultados indicam que quanto maior o nível de pressão e tempo, maior a vida de prateleira do músculo adutor de vieiras do aspecto microbiológico (mésofilos, pscicrotróficos). .... De acordo com os dados físico-químicos (pH, N-BVT e TBARS) não houve diferenças estatística entre os tratamentos 300MPa/2,5min e 400MPa/5min. No entanto, o presente estudo sugere um nível de 17mg N-BVT/100g de amostra como um limite aceitável para a espécie em estudo. Os nucleotídeos resultantes da degradação do ATP são parâmetros relevantes para a determinação do frescor durante a estocagem de vieiras. A APH foi capaz de aumentar a concentração de IMP no músculo durante a estocagem, componente responsável pelo sabor umami em pescado. Embora os valores obtidos de hipoxantina estejam acima dos relatados pela literatura, a informação mais relevante aponta para a redução da hipoxantina pelo nível de pressão de 400MPa/5min. Nesse sentido, é necessário aprofundar os estudos em relação as enzimas que participam da degradação bioquímica do ATP nessa matriz assim como o efeito da APH sobre esses componentes.

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