Application of High-Pressure Processing for Quality and Stability of Smoked Fish Fillets and Shrimp, and Optimization of Shrimp Peelability

By

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ABSTRACT

Seafood possesses significant nutritional value, which is attributed to its protein content and omega-3 polyunsaturated fatty acids, albeit it is highly perishable. Microbiological and biochemical changes restrict the shelf life of unprocessed fish products to a mere few days even when stored under refrigerated conditions. Various processing and packaging methods, including refrigeration, freezing, modified atmosphere packaging, and natural preservatives, have been investigated to address the need for shelf-life extension of marine foods. This study was aimed at evaluating the influence of high-pressure processing (HPP) on the quality and stability of hot-smoked trout and Arctic Char *(Salvelinus alpinus*) fillets during a 21-day refrigerated storage at 4^{o}C. In addition, the effect of HPP was also evaluated and optimized for the peeling of Black Tiger shrimp, and its stability was evaluated during refrigerated storage. The optimum HPP condition was found through an experimental design created using the response surface methodology (RSM).

In the first objective, smoked fish fillets were deskinned, cut into uniformly sized pieces, and vacuum sealed into High density polyethylene (HDPE) pouches, following which they were high pressure (HP) treated at 150 and 350 MPa for 10 to 30 min. The samples were then stored at 4^{o}C and evaluated on days 1, 7, 14, and 21 for texture, color, and microbial growth. For all treated samples, the firmness, tenderness, and lightness values were significantly superior ($p<0.05$) as compared with the untreated samples, and microbial growth was observed only on Day 21 for the treated samples (150 MPa for 30 min and 350 MPa for 10 min) while the untreated samples had growth from Day 7. This clearly indicated that HP treatment had better control over quality parameters as well as microbial growth, thereby extending the refrigerated shelf life.

For the second objective, which involved the storage of shrimp, a response surface methodology was used with two critical factors, pressure and time, through a central composite design. Seven response variables were evaluated: Hardness, gumminess, L*, b*, hue, ΔE, and microbial count. The 13 trials included five central points to evaluate the model accuracy and reliability. It was observed that the lightness, yellowness, hardness, and gumminess values increased with treatment severity and maintained higher values during storage while the redness value decreased. The processing conditions were optimized for each quality parameter on storage on

Days 1,7,14 and 21, with a notion that samples to be consumed on Day 1 of storage can be treated at milder conditions, in a way that it will be microbiologically safe with an added advantage of quality retention, whereas the samples to be stored from day 7 onwards would need a higher pressure or holding time to keep the microbial growth under control. Hence, processing conditions for day 1 were found adequate at 300 MPa with a holding time of 10 min. However, a pressure of 300 MPa for 30 min was required to keep the bacterial growth lower after storage for 7 to 21 days.

In the third objective, HPP was evaluated as an alternative to conventional peeling and for refrigerated storage of Black Tiger shrimp. A custom-developed method was used to quantify the peeling force in real-time during the controlled rotational peeling of shrimp. Results showed that an optimized HP treatment at 200 MPa for 3.4 min was sufficient to peel the shrimp, as it gave the highest peelability (lower peeling force). Higher pressures could also be used for peeling, but they would likely cause more protein denaturation, meat sticking to the peel, and color changes.

Overall, in this study, the effectiveness and efficiency of HPP for enhancing the quality and refrigerated storage stability of selected seafood were demonstrated, and HPP was highlighted as a flexible non-thermal alternative for processing the selected seafood products.

Keywords: High-Pressure Processing, Response Surface Methodology, Peelability, Mesophiles, Psychrophiles.

RÉSUMÉ

Les fruits de mer possèdent une valeur nutritionnelle importante attribuée à leur teneur en protéines et en acides gras polyinsaturés oméga-3, bien qu'ils soient hautement périssables. Les changements microbiologiques et biochimiques limitent la durée de conservation des produits de la pêche non transformés à quelques jours seulement, même lorsqu'ils sont stockés dans des conditions réfrigérées. Diverses méthodes de transformation et d'emballage, notamment la réfrigération, la congélation, l'emballage sous atmosphère modifiée et les conservateurs naturels, ont été étudiées pour répondre au besoin de prolongation de la durée de conservation des aliments marins. Cette étude visait à évaluer l'influence du traitement à haute pression (HPP) sur la qualité et la stabilité des filets de truite et d'omble chevalier (Salvelinus alpinus) fumés à chaud au cours d'un stockage réfrigéré de 21 jours à 4 °C. De plus, l'effet du HPP a également été évalué et optimisé pour le décorticage des crevettes Black Tiger, et sa stabilité a été évaluée pendant le stockage réfrigéré. Les conditions HPP ont été optimisées grâce à des conceptions expérimentales impliquant une méthodologie de surface de réponse (RSM).

Dans le premier objectif, les filets de poisson fumé ont été décortiqués, coupés en morceaux de taille uniforme et scellés sous vide dans des sachets en PEHD, après quoi ils ont été traités HP à 150 et 350 MPa pendant 10 à 30 min. Les échantillons ont ensuite été conservés à 4 °C et évalués aux jours 1, 7, 14 et 21 pour leur texture, leur couleur et leur croissance microbienne. Pour tous les échantillons traités, les valeurs de fermeté, de tendreté et de légèreté étaient significativement supérieures ($p<0,05$) par rapport aux échantillons non traités, et une croissance microbienne n'a été observée qu'au jour 21 pour les échantillons traités (150 MPa pendant 30 min et 350 MPa pendant 10 min) tandis que les échantillons non traités présentaient une croissance à partir du jour 7. Cela indiquait clairement que le traitement HP avait un meilleur contrôle sur les paramètres de qualité ainsi que sur croissance microbienne, prolongeant ainsi la durée de conservation au réfrigérateur.

Pour le deuxième objectif, qui concernait le stockage des crevettes, une méthodologie de surface de réponse a été utilisée avec deux facteurs critiques, la pression et le temps, via un plan composite central. Sept variables de réponse ont été évaluées : dureté, caractère gommeux, L*, b*, teinte, ΔE et nombre microbien. Les 13 essais comprenaient cinq points centraux pour évaluer l'exactitude et la fiabilité du modèle. Il a été observé que les valeurs de légèreté, de jaunissement, de dureté et de caractère gommeux augmentaient avec la sévérité du traitement et maintenaient des valeurs plus élevées pendant le stockage tandis que la valeur de rougeur diminuait. Les conditions de traitement ont été optimisées pour chaque paramètre de qualité lors du stockage les jours 1, 7, 14 et 21, avec l'idée que les échantillons à consommer le jour 1 du stockage peuvent être traités dans des conditions plus douces, de manière à être

microbiologiquement sûrs avec un avantage supplémentaire en termes de avantage de la rétention de qualité, alors que les échantillons à conserver à partir du jour 7 nécessiteraient une pression ou un temps de maintien plus élevé pour maintenir la croissance microbienne sous contrôle. Par conséquent, les conditions de traitement pour le jour 1 se sont révélées adéquates à 300 MPa avec un temps de maintien de 10 min. Cependant, une pression de 300 MPa pendant 30 minutes était nécessaire pour maintenir la croissance bactérienne à un niveau inférieur après un stockage de 7 à 21 jours.

Dans le troisième objectif, le HPP a été évalué comme alternative au décorticage conventionnel et pour le stockage réfrigéré des crevettes Black Tiger. Une méthode développée sur mesure a été utilisée pour quantifier la force de pelage en temps réel lors du pelage en rotation contrôlé des crevettes autour d'une aiguille. Les résultats ont montré qu'un traitement HP optimisé à 200 MPa pendant 3,4 minutes était suffisant pour décortiquer les crevettes car il offrait la plus grande capacité de pelage (force de pelage inférieure). Des pressions plus élevées pourraient également être utilisées pour le pelage, mais elles provoqueraient probablement davantage de dénaturation des protéines, une viande collant à la peau et des changements de couleur.

Dans l'ensemble, dans cette étude, l'efficacité et l'efficience du HPP pour améliorer la qualité et la stabilité du stockage réfrigéré des fruits de mer sélectionnés ont été démontrées, et le HPP a été souligné comme une alternative non thermique flexible pour la transformation des produits de la mer sélectionnés.

Mots clés : Traitement haute pression, méthodologie de surface de réponse, capacité de pelage, mésophiles, psychrophiles.

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CONTRIBUTION OF AUTHORS

Miss Sneha George is the MSc Candidate at McGill University. She is enrolled in the Department of Food Science and Agricultural Chemistry, where she is doing her thesis under the supervision of Dr. Hosahalli S Ramaswamy. She did an elaborate literature review from the time she started the course, formulated the research protocol with the help of her supervisor, Research Associate and lab mates, conducted experiments, gathered and analyzed the data, and presented the results for seminars and posters and drafted the thesis for publication.

Dr. Hosahalli Ramaswamy is the supervisor under whose guidance the studies were carried out. The MSc candidate had guidance throughout the research with planning the experiment, suggesting scientific solutions to problems, providing financial support, reviewing results, giving feedback by editing the power points, poster and thesis.

Dr. Ali T. Taherian is the research associate in the laboratory who guided the MSc candidate regarding the proper usage of all the equipment, helping with any technical difficulties, reviewing results, providing transportation for purchasing samples, giving feedback about how to sort and analyze data, as well as giving preliminary editing for poster, slides and thesis.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this thesis has been presented as a poster at a conference.

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CHAPTER 1

INTRODUCTION

Seafood plays a vital role in billions of individuals' well-being across advanced and emerging nations. The demand for seafood is increasing and is anticipated to surge further, with the global population on the rise. Seafood is a rich source of complete proteins, including essential amino acids for building cellular proteins. A 100 g serving of shellfish or cooked fish provides around 25 g of protein, making it a healthier option compared to other animal-derived foods. Seafood is low in cholesterol and total fat, with a total fat content of less than 2%. It is a distinctive source of long-chain omega 3 polyunsaturated fatty acids, such as DHA and EPA. Fish also contain B-complex vitamins, with certain species ranking among the highest sources. Some fish fillets have significant amounts of vitamin D. Additionally, seafood can contain minerals like zinc, iodine, iron, and trace minerals, such as zinc in eastern oysters (Liu & Ralston, 2021).

The task of harnessing the considerable potential of seafood involves sustainable production, enticing seafood products that will meet customer expectations that require freshness, in the absence of additives. This entails maintaining the nutritional value of seafood while guaranteeing a sufficient level of food safety all across the supply chain. Because of its great sensitivity and perishability, seafood poses difficulties for conventional processing and preservation methods including sterilization and thermal pasteurization, sometimes too harsh to me*et al*l required standards (Puértolas & Lavilla, 2020).

Certain seafood commodities are naturally riskier than others owing to many factors, including the environment from which they come, their mode of feeding, their harvesting season, and method of preparation and served. Crustaceans, fish and mollusks can obtain pathogens from several sources (Laein, 2018).

Following capture, the fish may be stored for durations varying from a few hours to many weeks in melting ice, cold brine, or refrigerated saltwater at -2 °C. Insufficient circulation of chilled brine may lead to localized anaerobic proliferation of some microbes, resulting in deterioration and the generation of off odors. Refrigerated brines that are reused may include elevated levels of psychotropic spoilage bacteria, and their reutilization will exacerbate the cross-contamination of other fish with these germs. To prevent the deterioration of fish stored

on board for extended period, freezing facilities at -18 °C are increasingly utilized (Ashie *et al*., 1996).

Processing technologies play an important role in preservation of seafood, serving multiple roles that enhance the overall safety and quality of the product (Kulawik *et al*., 2023). High-pressure processing (HPP), a distinctive non-thermal food processing method, has been widely employed for food pasteurization to extend shelf life while maintaining critical attributes such as natural color, flavor, and nutrients (Ramaswamy & Shao, 2010). Unlike conventional heat processing methods that may result in significant nutrient loss, HPP preserves the nutritional quality of marine items (Humaid *et al*., 2020). HPP has been effective in reducing the microbial load in seafood products, including pathogenic bacteria such as *Listeria monocytogenes* and *Vibrio parahaemolyticus*, commonly associated with seafood-borne (Roobab *et al*., 2022). This effectively extends the shelf life and safety of the product. Besides its antimicrobial effects, HPP also inactivates enzymes that are responsible for food spoilage, further aiding in shelf life extension (Chen *et al*., 2022). HPP technology has also been used as an alternative method for the shucking of oyster, where HPP treated oyster improved the yield up to 25% immediately after treatment (Puértolas *et al*., 2023). Furthermore, minimal studies have been conducted on optimizing processing parameters to prolong the shelf life of shrimp under refrigeration, as well as on alternative applications of HPP, such as enhancing shrimp peeling.

The general objectives of this thesis research were:

1. To study the effect of HP treatment on the refrigerated shelf life and quality attributes of hot-smoked trout and Arctic Char
2. To optimize the HP processing conditions to extend refrigerated shelf life and evaluate quality parameters of shrimp using RSM
3. To optimize the HP processing conditions to improve the peelability of shrimp (Black Tiger) (*Penaeus monodon*)

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CHAPTER 2

LITERATURE REVIEW

2.1. Fish Spoilage

The microbial composition of seafood products is affected by factors such as temperature and water pollution levels (Sheng & Wang, 2021). The deterioration of fresh fish can occur rapidly once caught, particularly in the warm temperatures of tropical regions where rigor mortis sets in within 12 hours. It leads to the stiffening of fish muscles shortly after death. The degradation of most fish species is attributed to factors such as lipases and digestive enzymes, microbial spoilage, and oxidation from surface bacteria (Ghaly *et al*., 2010). During the spoilage process, various components undergo breakdown, leading to the formation of new compounds. These compounds are responsible for the alterations that take place in the color, texture, and flavor of the commodity (Tavares *et al*., 2021).

Changes in composition that occur as fish undergo spoilage encompass processes such as lipid oxidation and protein degradation, leading to the unfortunate loss of other valuable molecules. To devise an optimum preservation technique for these value-added products in active forms, it is essential to dig into a comprehensive understanding of the complex mechanism responsible for their degradation, contributing to the overall enhancement of food quality and safety in the fisheries industry (Ghaly *et al*., 2010). The fish industry is confronted with significant apprehension over supply losses of marketable fish, primarily attributed to spoilage. This concern is underscored by the interplay of three fundamental mechanisms: enzymatic autolysis, oxidation, and microbial degradation (Nie *et al*., 2022).

The determination of spoilage in food products, particularly in the context of meat and fish, relies on various factors such as maximum acceptable microbial level and the occurrence of unmistakable off-odor and off-flavor (Tavares *et al*., 2021). This identification is intricately linked to several factors, including the initial population and types of contaminating microorganisms, the progression of their growth, as well as the concurrent processes of lipid oxidation and autolytic enzymatic reactions.

Meat and fish serve as highly conductive environments for microbial activity due to their inherent physico-chemical characteristics. The combination of factors such as pH level, aw (activity water) and high moisture content creates an environment that is exceptionally supportive of the growth and proliferation of a diverse array of microorganisms. This microbial

activity, in turn, plays a pivotal role in the spoilage process (Gokoglu & Yerlikaya, 2015; Remenant *et al*., 2015)

2.2. Enzymatic autolytic spoilage

Following the immediate post-slaughter phase, fish undergoes intricate biochemical transformations driven by the activity of autolytic enzymes within its flesh. Notably, the autolytic enzymes in seafood exhibit heightened activity, causing a more rapid onset of autolysis compared to terrestrial animals. This accelerated autolysis is attributed to the fact that the pH in seafoods does not typically decline to the levels observed in terrestrial muscle tissues (Geng *et al*., 2023). Consequently, the shelf life of fish can be notably curtailed by the action of these enzymes, even in the absence of dominant spoilage microorganisms (Kontominas *et al*., 2021). The provision of refrigeration or freezing, however, serves to decelerate the pace of autolysis in fish (Romulo, 2021) Specific classes of lipases and proteases play pivotal roles in the postmortem degradation of fish muscle and other tissues during processing and storage, leading to various changes in the sensory attributes of fish (Roobab *et al*., 2022). The primary autolytic change initiated is the enzymatic breakdown of adenosine 5′ -triphosphate (ATP) and its associated products, followed by the activation of numerous other proteolytic enzymes (Nie *et al*., 2022). These changes predominantly impact textural quality, accompanied by the production of formaldehyde and hypoxanthine. Digestive enzymes contribute to extensive autolysis, leading to the rupture of the belly wall, meat softening, and the drainage of blood water, which contains both oil and protein. The proteolytic enzymes found in the viscera and muscle of the fish after catch significantly contribute to postmortem degradation observed in fish muscle and fish products during subsequent storage and processing (Ghaly *et al*., 2010) .

2.3. Oxidative spoilage

Oxidative spoilage has several detrimental impacts on the quality of fish, leading to degradation of texture, flavor, and color. This deterioration is accompanied by a simultaneous decline in the nutritional attributes of the preserved fish (Amit *et al*., 2017). Pelagic fish, including mackerel and herring, characterized by a high content of oil and fat in their flesh tissues, are particularly prone to lipid oxidation, which is the primary cause of deterioration and spoilage. Various oxidative processes, such as photo-oxidation, auto-oxidation, thermal oxidation, and enzymatic oxidation, can contribute to degradation. The auto oxidation of lipids, involving their spontaneous

reaction with atmospheric oxygen, is the most commonly observed oxidative degradation in fish (Hassoun & Çoban, 2017).

Lipid oxidation typically results from the reaction of oxygen with the double bonds present in fatty acids. Consequently, fish tissues with a high concentration of unsaturated lipids become highly prone to rapid degradation and peroxidation. The rate of lipid oxidation, along with its degree of saturation, is influenced by factors such as temperature, presence of salt brine, metal ions, and temperature (Domínguez *et al*., 2019).Increased protein denaturation, the breakdown of endogenous antioxidant systems, and a parallel decrease in nutritional value resulting from the loss of fat-soluble vitamins and other components all directly relate to the formation of lipid oxidation products (Nie *et al*., 2022).

With fish, lipid oxidation can take place through enzymatic or non-enzymatic processes. The enzymatic breakdown of fats by lipases is known as lipolysis. leading to the deterioration of fat. In this process, lipases cleave glycerides, resulting in the formation of free fatty acids. These free fatty acids are accountable for the degradation of oil quality and the development of a common off-flavor known as rancidity (Ghaly *et al*., 2010).

Non-enzymatic oxidation is induced by compounds including hemoglobin, myoglobin, and cytochrome catalysis, resulting in the production of hydroperoxides. The fatty acids generated through the hydrolysis of fish lipids interact with myofibrillar and sarcoplasmic proteins, causing denaturation (Bernardo *et al*., 2020).

2.4. Microbial spoilage

Microbial spoilage stands as the primary factor responsible for the deterioration of the quality of fresh or minimally preserved fish, leading to a significant loss of marketable fish, reaching up to 25-30% in severe cases (Nie *et al*., 2022). The high concentration of non-protein low molecular weight nitrogenous compounds, specifically NPN, and the low acidity (pH<6) of fish flesh create favorable conditions for the growth of spoilage bacteria. As these bacteria proliferate, they generate metabolic by-products, and the accumulation of these by-products results in organoleptic rejection (Boziaris & Parlapani, 2017) .

The initial microbiota of seafood at the beginning of product shelf life comprises both exogenous and indigenous components. Indigenous microbiota refers to the natural bacterial populations present in the skin, digestive tract, and gills, closely linked to the waters in which the fish live.

The exogenous microbiota arises from the contamination of the product by microbes from the terrestrial environment, personnel during product handling, and food contact surfaces (Borda *et al*., 2017). Throughout the processing conditions, which utilize techniques such as salting, smoking, acidification, thermal treatments, and during storage, in which temperature and atmosphere are factors, only a portion of the initial microbiota persists. This subset grows faster than the rest of the microorganisms and becomes the dominant spoilage microbiota. The specific fraction of the dominant microbiota, possessing the ability to cause spoilage (by causing off odors qualitatively) and spoilage activity (quantitative metabolite production), is referred to as Specific Spoilage Organisms (SSOs) (Boziaris & Parlapani, 2017).

2.5. Preservation of Seafood

Due to the perishable nature of fish, it is mandatory to undergo processing and preservation. Preservation aims to extend the shelf life of fish and fish products. This involves storing surplus fish when they are abundant, ensuring they can be consumed as if fresh during periods of shortage or when it needs to be transported for a very long distance. Preservation serves two purposes: (1) to maintain the original freshness and properties of fish, and (2) to induce changes in the original properties to create new products. The main objective of these two cases is to prevent spoilage, particularly by microorganisms. Several preservation methods have been developed, each offering different durations of shelf life. The selection of a preservation method is influenced by factors such as its properties, storage facilities, availability of energy, and associated costs. In certain cases, different methods might need to be combined (Yu *et al*., 2019).

2.6. Smoking

Diverse preservation techniques are employed to mitigate postharvest losses and deterioration. Methods include drying, salting, fermenting, salting, and smoking. Smoking refers to the exposure of fish to the direct or indirect effects of smoke resulting from the incomplete combustion of specific types of wood utilized as fuel. The smoking of foodstuffs significantly enhances organoleptic properties, increases moisture loss, and diminishes microbial burden (Chakroborty & Chakraborty, 2017). Smoked fish products are abundant in vital amino acids, minerals, fat-soluble vitamins, and unsaturated fatty acids, rendering them nutritious, ready-to-eat options. Smoking can inhibit microbial growth in food goods and prolong the prevention of

oxidation (Fuentes *et al*., 2008). Smoking technology preserves food's physicochemical properties, such as pH, Thio-barbituric acid reactive substances (TBARS), and fatty acid composition, enhancing sensory quality and prolonging shelf life. The spoilage and bactericidal action of smoke are due to heating, salting, drying, and deposition of polyphenolic compounds on fish, which have bactericidal properties that inhibit fungal proliferation and virus activity.(Huang *et al*., 2019; Ayofemi & Adeyeye, 2019).

2.7. Cooling

Fresh fish is commonly transported and sold on flaked ice, maintaining a temperature of approximately 0°C. While refrigeration or cooling helps preserve freshness, it does not eliminate bacteria, prevent the biological deterioration process, or halt enzymatic activity entirely, but they are merely slowed down (Yu *et al*., 2019). Conversely, psychotropic bacteria thrive around 0°C and can rapidly destroy food, even at lower temperatures. The growth rate of *Shewanella putrefciens* at 0°C is less than one-tenth of its optimal growth temperature (Wright *et al*., 2016). Fish and shellfish from colder water that contain *S. putrefaciens* may deteriorate quickly than those from warm waters. To ensure the desired shelf life, consistent refrigeration is essential throughout the entire storage and transportation (Alice *et al*., 2020). On fishing vessels, besides conventional ice flakes, there is a growing preference for chilled seawater, which can be created by adding ice to saltwater, and mechanically refrigerated seawater (RSW). These methods have proven effective in preventing fish and shrimp from spoiling faster than regular ice. This improvement is attributed to the use of chilled water, creating more favorable anaerobic conditions. However, if partial deterioration occurs, the destroyed organisms may proliferate through the load, posing a significant drawback to this cooling method. Furthermore, ice slurries cause less physical damage due to their spherical particles compared to flakes. Different additives, such as natural antioxidants and organic acid combinations, are being added to the ice slurries to improve their effectiveness (Rumape *et al*., 2022).

2.8. Freezing

Freezing has traditionally been considered a viable method for preserving various kinds of food over extended periods. However, freezing can have a profound impact on the structural and chemical characteristics of muscle, including an increase in the concentration of free fatty acids (FFA) and lipid oxidation products. While freezing reduces enzymatic and microbial activity,

preserving taste and nutritional qualities more efficiently than cold storage, the formation of ice crystals during freezing is a critical juncture. Larger ice crystals pose a greater risk of membrane rupture as well as textural damage, leading to heightened oxidation. Therefore, during freezing, the generation of ice crystals is crucial to prevent increased oxidation and textural degradation upon thawing. Faster and more homogenous freezing processes result in smaller and more uniform ice crystals (Rumape *et al*., 2022).

2.9. Modified Atmosphere Packaging

Fish and shellfish are prone to quicker spoilage in our country's high ambient temperature due to postmortem autolysis and microbial growth, primarily driven by bacterial action (Ikape, 2018). Common methods to slow down biochemical and microbial spoilage during distribution and marketing of freshly caught fish involve the use of crushed block ice as well as mechanical refrigeration, providing a shelf life ranging from 2 to 14 days. However, the melting of ice may compromise quality and contaminate the fish, thereby accelerating fish spoilage (Lougovois & Kyrana, 2014).

Modified Atmosphere Packaging (MAP) is a non-thermal method of food preservation utilizing nitrogen (N_2), Oxygen (O_2), and Carbon dioxide (CO_2). N_2 primarily acts as a filter to prevent the pack from collapsing. Although N_2 is an inert gas without antimicrobial effects, it creates an anoxic atmosphere effective against anaerobic and aerotolerant *Lactobacillus*. O_2 inhibits the growth of anaerobic bacteria and has fungistatic and bacteriostatic properties. N_2 is an alternative to vacuum packaging, replacing O_2 to slow down oxidative rancidity and inhibit the growth of aerobic microorganisms (Hauzoukim *et al*., 2020).

The effective inhibition of bacterial growth during refrigerated storage for packaged fresh fishery products is achieved by employing advanced barrier films along with CO_2 containing MAP. To maintain the red color, elevated levels of oxygen are applied to red meat and fish varieties like tunas and yellowtails, assisting in minimizing and delaying browning caused by metmyoglobin formation. Within modified atmosphere packages, oxygen also hinders the conversion of trimethylamineoxide (TMAO) to trimethylamine (TMA) in fresh fish, thereby preventing the characteristic fishy odor linked to spoiled fish (Hauzoukim *et al*., 2020).

2.10. Irradiation

The antibacterial property of ionizing radiation helps in extending the shelf life of food products. Irradiation method damages microbial DNA, disrupting their regular metabolic functions. Even at low concentrations, such as 1.5 -2.5 kGy irradiation, it is highly effective in eliminating bacteria such as *Escherichia coli*, *Salmonella, Pseudomonas,* and preventing the formation of toxins from *Clostridium botulinum* spores. However, irradiation generates hydroxyl radicals, which cause increased oxidation in seafood products. Microorganisms in seafood can be eliminated in high doses, like 50 kGy, but it will affect the texture and taste. The World Health Organization (WHO) has set a 10 kGy upper limit for food processing, restricting its use due to potential changes in quality that may not be desirable (Rumape *et al*., 2022).

2.11. High pressure processing

Current consumer trends in the food sector favor minimally processed, fresh-like like and additive-free foods (Singh & Ramaswamy, 2013). Seafood is a highly sensitive and perishable food product, so conventional preserving and processing technologies such as thermal sterilization and pasteurization are mostly too aggressive to me*et al*l these requirements. Hence, a solution to address this challenge involves the advancement and implementation of innovative non-thermal processing technologies like high pressure processing (HPP). These methods aim to generate or preserve sensory and nutritional qualities, while reducing adverse alterations that are associated with traditional processing and preservation, and ensuring a suitable shelf life (Puértolas & Lavilla, 2020).

The majority of high-pressure studies on fish and seafood products have been conducted at lower pressures to control vegetative pathogens and spoilage bacteria, followed by storage under refrigerated conditions, or to achieve other goals such as enhancing sensory properties and facilitating processes like freezing and thawing (Ramaswamy & Shao, 2010). This approach effectively preserves key food quality aspects, including appearance, texture, nutritional quality, and taste. This technique is proven to be equally effective for the preservation of both high-moisture solid foods and liquids. Although it is lethal for microorganisms, this treatment does not damage covalent bonds, thereby preserving the chemistry and quality of food. Consequently, the application of HPP minimizes the need for thermal treatments and chemical preservatives in food processing (Khaliq *et al*., 2021).

2.11.1 Works carried out using HPP

High-pressure processing is a novel technique for processing food and has garnered significant interest in recent years (Ramaswamy, 2013). Extensive research has been carried out on various products with the application of HPP, such as pectin methyl esterase (PME) inactivation in apple juice (Riahi & Ramaswamy, 2003), HP kinetics of three common spoilage organisms such as *Zygosaccharomyces bailii*, *Pichia membranaefaciens* and *mesenteroides* in mango juice (Hiremath & Ramaswamy, 2012) Extraction of lycopene from tomato paste waste (Xi, 2006) Comparison of HP treatment and hot dip treatment for dyeing of poplar wood (Yu *et al*., 2019) Quality improvement of Indian cottage cheese (paneer) (Kapoor *et al*., 2021), study on low temperature HP treatment for the reduction of *E. Coli* in milk (Li *et al*., 2020) Study of *clostridium sporogenes* -spore destruction kinetics in milk under elevated pressure and temperature conditions (Shao & Ramaswamy, 2011). These diverse applications make HPP a cutting-edge and versatile application.

2.11.2. Governing Principles

2.11.2.1. Isostatic principle

The primary factor to consider when employing high pressure is the isostatic principle, which presumes that the application of pressure acts uniformly in all directions. A true hydrostatic condition, considered independent of time and space, is achieved when a fluid transmits pressure throughout the food sample. In high-pressure applications, the pressure applied and its effects are uniformly and rapidly dispersed within the food, irrespective of the sample size and shape. This distinctive attribute has facilitated the creation of processes that have achieved successful commercialization (Balasubramaniam *et al*., 2015a).

2.11.2.2. Le Chatelier's principle

According to this principle, any phenomenon involving phase transition, alteration in molecular configuration, or chemical reaction that results in a decrease in volume is intensified by pressure. If the extensive variable, pressure, changes, then the equilibrium shifts in a direction that tends to minimize the change in the corresponding intensive variable, volume. Consequently, the pressure directs the system towards achieving the lowest possible volume (Balasubramaniam & Farkas, 2008).

2.11.2.3. Principle of molecular ordering

The principle of microscopic ordering elucidates the influence of temperature and pressure on molecular structure and chemical processes. At a steady temperature, increased pressure enhances the molecular ordering, indicating opposing effects of temperature and pressure on chemical reactions (Oladimeji *et al*., 2024 **;** Baldelli *et al*., 2024)**.**

2.11.2. Equipment

HPP systems come in both batch and semi-continuous equipment lines. The prevalent choice for manufacturing equipment is high-strength steel alloy due to its corrosion resistance and high fracture toughness. Figure 2.1 shows a schematic diagram of an HPP unit that generally has the following components:

- a thick-walled cylinder that serves as the pressure vessel
- Two end closures to encase the cylindrical pressure vessel
- yoke, a structure for restraining end closures during pressurization
- high-pressure pump and intensifier for achieving target pressures
- process control and instrumentation
- a handling system designed for loading as well as removing the product (Khaliq *et al*., 2021).

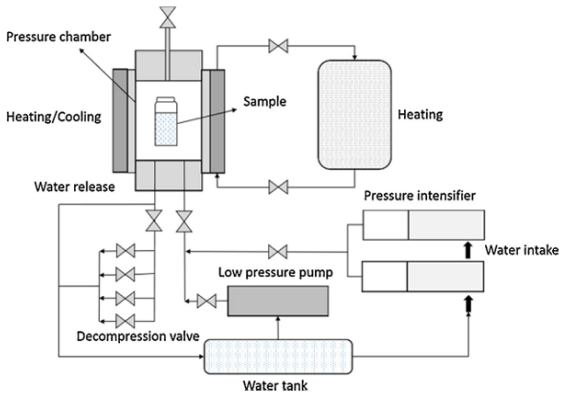

Figure 2.1. Schematic diagram of a HPP unit

(Rong *et al*., 2018)

Batch processing is employed for solid and liquid foods, while for viscous foods, a semi-continuous process is preferred. In operational terms, the HPP batch process is similar to retort systems in thermal processing. In HPP, food is vacuum-packed and then loaded into a basket, which is subsequently positioned in a pressure vessel containing pressure-transmitting fluid (Balasubramaniam & Farkas, 2008). HPP necessitates the pre-packaging of food components, and the selected packaging material must have adequate flexibility to withstand a compression of at least 15%. Only plastic-type packaging materials, such as polyethylene bags, are adequate for fulfilling this criterion. Moreover, HPP identifies processing limitations in low moisture commodities, including powders, pastes, and flours (Gokul *et al*., 2023).

The pressure vessel employs an end closure to confine the fluid, with the yoke structure gliding over the closed vessel to retain pressure on both the top and bottom closures. An intensifier and pump work in tandem to compress the pressure-transmitting fluid, achieving the desired pressure. Pressure is sustained for the required duration and released upon completion of the process. The product is unloaded from the vessel after the treatment. Typically, a pressure holding time of less than 10 minutes is adequate for producing a commercially viable product (Khaliq *et al*., 2021).

2.11.3. Pressure transmitting fluid

The central element of the equipment relies on a fluid that uniformly transmits the pressure throughout the product. Water is the most frequently utilized pressure-transmitting fluid on an industrial scale. However, for laboratory scale and pilot plant setups, pressure transmitting fluids such as castor oil, aqueous glycol, sodium benzoate solution, and silicon solution are employed (Heinz & Knorr, 2001). These alternatives safeguard low-cost steel equipment against corrosion and damage caused by friction. The selection of the pressure transmitting fluid takes into account various factors, including the pressure endurance of seals, heat compression, changes in viscosity of fluid under pressure, and anti-corrosion properties of materials. Moreover, the fluid composition, thermal characteristics, and fluid-to-sample ratio are very crucial in determining the food's thermal behavior under pressure. The compression heating properties of the pressure transmitting fluid impact the kinetics of microorganisms and their spores, directly dependent on the compressibility values and heat of compression (Aganovic *et al*., 2021).

2.11.4. Effect of HPP on seafood

2.11.4.1. Effect on protein

Le Chatelier's principle governs the impact of HPP on proteins, resulting in irreversible structural and functional alterations due to the breaking of covalent bonds. The intermolecular bonds formed due to this cause the protein's secondary, tertiary, and quaternary structure to stabilize, resulting in the product's stabilization and the destruction of microbes without altering their sensory qualities (Basak & Ramaswamy, 1998).

Depending on the treatment parameters, such as pressure level, processing time, and temperature, as well as the physicochemical characteristics of food, HPP mainly affects the tertiary and quaternary structure of proteins. Notably, the primary and secondary structure of protein remains unaffected by the HPP process. Pressures lower than 150 MPa can influence the quaternary structure of proteins, given that weak non-covalent bonds are responsible for maintaining this structure. Pressure exceeding 200 MPa impacts the tertiary structure, while a pressure range of 300-700 MPa is necessary to induce alterations in the secondary structure and to induce denaturation. The proteins responsible for the main functional properties of raw meat, texture and color are the water soluble sarcoplasmic proteins, salt soluble myofibrillar proteins and stroma present in fish muscle which can be modified by pressure treatment (Guyon *et al*., 2016) . In accordance with Le Chatelier's principle, a decrease of up to 1% in protein volume can occur during HPP (Ucak & Toepfl, 2017).

2.11.4.2. Effect on lipid oxidation

The primary factor leading to deterioration in foods rich in unsaturated fatty acids, particularly seafood, is lipid oxidation. Seafood is abundant in long-chain polyunsaturated fatty acids like EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). However, Poly unsaturated fatty acids (PUFA) are highly susceptible to oxidation, resulting in undesirable odors, flavors, and a decline in sensory quality. The presence of biological and inorganic catalysts, such as enzymes and metal ions, strongly enhances the lipid oxidation (Medina-Meza *et al*., 2014). Fish are very prone to lipid oxidation due to the presence of haeme pigments like myoglobin and hemoglobin, as well as metallic ions like copper and iron present in trace amounts (Ucak & Toepfl, 2017). The denatured ferric form of myoglobin, induced by pressurization, contributes to

catalyzing the lipid oxidative reaction. This could potentially be a reason why pressure might promote lipid oxidation (Ikeuchi, 2011).

According to studies, HPP induces lipid oxidation in meat and seafood. Pressure less than 300 MPa has minimal effect on lipid oxidation, while pressures exceeding this value (300–400 MPa) are essential for inducing oxidation. Several attributes, such as fiber type, pressure level, process time , chemical composition, fat profile, and pre-processes, among others can alter the impact of HPP on lipid oxidation (Truong *et al*., 2015).

A heightened sensitivity to HPP is observed in older animals due to the accumulation of pro-oxidants. Additionally, minced meat is more susceptible to HPP due to its exposure to oxygen. The evaluation of rancidity in fish is facilitated by the Thiobarbituric acid reactive substances (TBARS) test, which measures secondary oxidation products and oxidation levels. High levels of polyunsaturated fatty acids in crustacean tissue membranes make them more vulnerable to lipid oxidation, as the processing may adversely affect these membranes. In contrast, lipid oxidation in smoked fish exhibits greater stability when it is exposed to HPP (Oliveira *et al*., 2017).

2.11.4.3. Effect on microorganisms

The seafood industry demands enhanced safety and extended shelf life for its products, making high pressure a prominent method for the inactivation of most microorganisms. HPP has the potential to inactivate enzymes and destroy spoilage bacteria that affect the food quality adversely. The nature and source of enzymes determine the magnitude of pressure required to inactivate the enzymes (Riahi & Ramaswamy, 2003) **.** HPP has emerged as an effective and gentle pasteurization technique, ensuring the production of high-quality and microbiologically safe foods. The underlying mechanism of microorganism inactivation under HPP primarily involves the destruction of cell walls and membranes, as well as the denaturation of enzymes and proteins associated with cell membrane metabolism or DNA replication/ transcription (Sehrawat *et al*., 2021).

Microbial inactivation is, in general, affected by biological species, cell morphology and status, surrounding matrix, detection method, and instrument, etc. Microbial resistance to HHP inactivation can, in general, be listed in order with bacterial spores being the most resistant, followed by gram-positive bacteria, gram-negative bacteria, and lastly, yeasts and molds (Daryaei *et al*., 2016). HPP disrupts the internal organization of cells, leading to altered

distributions of DNA and Ribosomes. Pressures ranging from 300 to 600 MPa at ambient temperatures have been recommended for the inactivation of many vegetative bacteria and fungi. Multiple HPP cycles can be applied for spore inactivation, inducing germination under mild conditions. While vegetative bacteria, yeasts, and molds can be inactivated by pressure application between 300 and 600 MPa, ascospores of molds can withstand pressures exceeding 600 MPa. It is also important to note that HPP treatment may not always completely inactivate microorganisms but may injure a proportion of the population. The recovery of injured cells depends on subsequent conditions. Generally, HPP ensures a 3-5 log unit reduction in vegetative microorganisms, and the response of microorganisms to high pressure is influenced by factors such as pressure level, pH, temperature, time, water activity, food composition, and the type of microorganisms (Campus, 2010).

2.11.4.4. Effect on color

Color measurement is a critical sensory characteristic for fish products, significantly impacting product acceptability. Spoilage can lead to changes in color due to the degradation of blood pigments. One potential adverse effect of HPP on the sensory attributes of fish is its cooked appearance, which is based on the increase in the whiteness and opaqueness of the fish muscle (Oliveira *et al*., 2017; Truong *et al*., 2015). The color change in fish due to low myoglobin content occurs as a result of myofibrillar and sarcoplasmic protein denaturation, which occurs at pressures above 150-300 MPa (Puértolas & Lavilla, 2020).

The CIELAB system is commonly utilized to analyze the color of food, as it is a simple and standard method. This method is based on the calculation of three coordinates that define a color in three-dimensional space: L* (lightness), a* (red-green; + a* for redness, − a* for greenness), and b* (yellow-blue; + b* for yellowness, − b* for blueness)(Granato & Masson, 2010).

The HPP-induced changes in color are predominantly reflected in the increase of the L* value, which represents the lightness of the product. For example, de Alba *et al*., (2019) observed an increase of L* from 53.60 to 72.73 after treating mackerel fillets at 500 MPa for 5 mins. The impact of HPP on a* (redness) appears to be highly dependent on the fish species and treatment conditions. For instance, a* values tend to decrease in tuna, fresh cod, and mackerel following HPP, increase in salmon, and remain more or less stable in tilapia and turbot (Suemitsu & Cristianini, 2019).

2.11.4.5. Effect on texture

High pressure can influence molecular interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic bonds, as well as protein conformation. This can result in protein aggregation, gelation, or denaturation (Campus, 2010). Hardness, cohesiveness, springiness, gumminess, chewiness, and adhesiveness are the most investigated characteristics in measuring texture profile (Truong *et al*., 2015; Ucak & Toepfl, 2017).

2.11.4.6. Effect on vegetative microorganisms

One of the primary applications of HPP in the food industry is the eradication of vegetative microorganisms for food preservation. The response of yeasts, molds, and vegetative bacteria to pressure varies, depending on factors such as species, strain, substrate, and processing temperatures (Patterson, 2005). The viability of vegetative microorganisms may be compromised by structural changes in the cell membrane and inactivation of the enzymes involved in microorganism metabolism. Protein and lipid membranes are among the most significant targets of pressure in vegetative cells. It is noteworthy that different microorganisms exhibit varying degrees of resistance to HPP treatment (Heinz & Knorr, 2001). Generally, gram-positive bacteria demonstrate more resistance to heat and pressure compared to gram-negative bacteria, potentially attributable to the complexity of the cell membrane of the latter. Additionally, cocci show more resistance than rod-shaped bacteria (Shigehisa *et al*., 1991).

2.11.4.7 Effect on bacterial spores

Bacterial spores pose a significant threat to food safety due to their robust resistance to both physical and chemical hurdles. The presence of spores in a food matrix can result in post-pasteurization re-contamination, following germination and colonization. To address this concern, the food industry has employed chemical and thermal inactivation strategies. Although these processes are effective in ensuring safety, they often have deleterious effects on the organoleptic and nutritional properties of food. High pressure has been proposed as a promising alternative for sterilization to mitigate such drawbacks (Heinz & Knorr, 2001).

2.12. Applications of HPP in seafood

2.12.1. High pressure assisted thawing and freezing

The methodology and technique used to thaw and freeze foods play a crucial role in preserving the overall quality of frozen foods (Zhu *et al*., 2004)**.** Pressure treatment at 210 MPa causes

depression of the freezing point of water from 0◦C to −21◦C. When the pressure is reversed to ambient conditions, this phase change phenomenon is reversible. Foods that are high in moisture content find this property useful in rapidly freezing and thawing. Due to intense nucleation and intracellular ice crystal formation, several commercial freezing processes can cause mechanical damage to frozen food (Zhu *et al*., 2003). Pressure-assisted freezing (PAF) cools the sample to its phase transition temperature at the applied pressure. Super-cooling the product at a rapid ice-nucleation rate freezes the product under pressure. This method preserves food and biological material microstructure. Pressure Assisted Thawing (PAT) thaws food under constant pressure, and can reduce thawing time and drip loss (Balasubramaniam *et al*., 2015).

2.12.2. Extraction of Astaxanthin

Astaxanthin, a reddish-orange carotenoid pigment, boosts the immune system. It possesses 100 times the antioxidant capacity of vitamin E and 40 times that of beta carotene in neutralizing singlet oxygen quenching potential. Astaxanthin which can be extracted from shrimp carapace, reduces the manufacturing costs. High yield of high-quality astaxanthin can be extracted from Black tiger shrimp's carapace using HPP with less extraction time than chemical methods. This highlights HPPs capacity to boost astaxanthin yield and quality from shrimp waste, suggesting that it can replace chemical extraction methods (Irna *et al*., 2018).

2.12.3. Shucking of seafood

Shucking of oysters, being the main application in oyster processing, is based on the spontaneous opening of its shell caused by the denaturation of the adductor muscle. HP treatment of oysters results in a significant increase in yield as well as a better appearance of the oyster, with no muscle tissues remaining on the shell (Puértolas *et al*., 2023). An optimized HPP treatment allows 100% shucking of the oyster, with the added benefit of reduced microbial population and minimum changes in sensory quality. Shucking oysters at 300 MPa gave a yield of 15.32g/100g compared to 12.25g/100g by the untreated samples (Cruz-Romero *et al*., 2008). This was assumed to be caused due to increased water retention that makes the oyster voluminous and complete removal of the meat from the shell. In a study conducted by He *et al*., (2002), it was found that moisture uptake happens only when oysters are in direct contact with water during HPP. Similarly, HPP was an effective processing technique for shucking of bay scallop, with significantly higher yield compared to manual shucking, where pressure had a significant effect

on scallops compared to holding time. HPP has been recorded to reduce the growth of both aerobic psychrophiles and coliform bacteria in a study conducted by, where they observed that 200 MPa for 3 min could obtain 100% separation of muscles (Yi *et al*., 2013). HPP was also applied to evaluate the shucking of crayfish, where HP treatment resulted in tightened muscle tissue, partial protein denaturation, increased hardness, and reduced springiness. The optimal condition obtained was 200 MPa for 5 min for shucking and retaining high-quality meat (Shao *et al*., 2018)**.**

2.12.4. HP assisted peeling of shrimp

Peelability in shrimp refers to the ease with which the shell can be removed. Peelability is said to be poor if there are excessive shell residues clinging to shrimp meat after peeling. Although hand peeling ensures less shell residue with better meat integrity, it can cause food safety issues due to potential cross-contamination, and can be labor-intensive and time-consuming (Thi *et al*., 2018). The seafood industry has used cooking as a conventional method to increase peelability by breaking down connective tissues. The epidermis connects the shell to the muscle, making peeling difficult (Grant *et al*., 1980). A study by Yang *et al*., (2020) described the effect of HP in significantly improving peelability with better tail integrity, minimal color changes, and reduced drip loss in whiteleg shrimp (*Peneaus vannamei*). They recorded that pressure treatment at 200 MPa for 3 min was adequate for enhancing peeling. However, they reached the conclusion by monitoring the manual peeling time, which can vary from person to person, and is not reliable. Therefore, there is a need to evaluate the peeling efficiency of HP-treated shrimp mechanically by measuring the force required to separate the shell and muscle.

2.12.5. HP treatment for raw consumption and extension of refrigerated storage life

Raw fish intake has expanded rapidly in recent years, even in parts of the world where it is not a traditional practice, due to changes in food taste and the adoption of foreign culinary traditions. Asian specialties like sushi and sashimi are growing more popular throughout Europe. Moreover, minimally processed seafood, such as smoked fish, is in great demand. This has resulted in an escalated rise in microbiological concerns as well. Above all, seafood is generally highly perishable and has a short refrigerated shelf life, and in order for raw seafood to be widely accepted and safely available, it is important to apply a non-thermal processing technique such as HPP that limits the microbial load (Cristina *et al*., 2023). The rationale for employing HPP for

seafood is based on its capacity to inactivate pathogenic and spoilage microorganisms as well as microbial enzymes, hence extending shelf life (Alba *et al*., 2019).

There have been studies on shrimp subjected to HP treatment and analysis of several parameters like color, texture, changes in TVBN, TVC etc, on chilled storage as well as optimization of processing conditions to achieve maximum bacterial inactivation with minimal changes in quality attributes (Chen *et al*., 2022; Kaur & Rao, 2016), but there aren't any works done on optimization for each storage day, which is a very crucial aspect both from a manufacturer's as well as a customer's point of view.

2.13. Advantages of high-pressure processing

- The application of high pressure in this process is independent of the size and shape of the food.
- High pressure operates independently of time and mass, acting instantaneously and consequently reducing processing time.
- It avoids breaking covalent bonds, preventing the development of unfamiliar flavors in the products and preserving their natural taste.
- It can be applied at room temperature, thus decreasing the amount of thermal energy during conventional food processing.
- This process holds the potential to minimize or eliminate the use of chemical preservatives.
- Since high-pressure processing employs the Isostatic principle (pressure uniform throughout the food), the food is preserved evenly throughout without any portion escaping the treatment.
- Processing is possible with packaging material for this technology.
- The process is eco-friendly as it solely necessitates electric energy and produces no waste products (Naveena & Nagaraju, 2020).

2.14. Limitations of HPP

- Products of HPP need to be stored and transported under refrigerated conditions, as HPP alone is insufficient to limit the activity of pathogenic spores, for example, *C. botulinum*.

- The packaging material must be flexible enough to undergo a compression of at least 15% a criterion that is met exclusively by plastic-type packaging.
- HPP also encounters challenges when dealing with foods that have low moisture content, such as powdery commodities.
- Pressure-resistant entities like food enzymes and bacterial spores demand exceedingly high pressure levels for effective inactivation (Khaliq *et al*., 2021; Naveena & Nagaraju, 2020).
- HPP costs 7 times more than thermal processing. HPP is less cost-effective than traditional methods due to capital, labor, and utility expenditures (Sampedro *et al*., 2014).
- HPP equipment is expensive and requires considerable initial investment for installation. Maintaining and training workers to operate the equipment also adds to the total expense, making it difficult for small- to medium-sized food manufacturers to adopt them (Sharma *et al*., 2024)

2.15. Experimental design and Implementation

Experimental design, as a component of scientific inquiry, is a prevalent and extensively utilized research methodology. The response surface methodology is being used due to its multipurpose application in research work, where a representative minimum experimental runs give optimal trends using analysis of variance (ANOVA) (Shinde & Ramaswamy, 2021). The objective is to determine the influence of an independent variable on a dependent variable. Dependent variables are measured and/or observed during the experiment, whereas independent variables are established by the researcher before the experiment begins (Bell, 2009). Response surface methodology (RSM) is a significant aspect of experimental design. It comprises a set of statistical and mathematical methodologies employed for the development, enhancement, and optimization of processes (Kleijnen, 2015). In RSM, models are developed based on data from experimental design, elucidating the relationship between factors (independent variables) and responses (dependent variables). These correlation models are employed to assess the impact of factors and interactions on responses and process optimization. The results are typically shown by a 3D plot or a 2D contour plot. The application of the response surface approach as an optimization tool necessitates several stages, including the selection of components, experimental design, appropriate model selection, assessment of model adequacy, graphical

representation of the model, and ultimately, optimization to achieve optimal conditions (Jasem *et al*., 2021).

2.16. Applications of RSM in food industry

In the food industry, RSM has been used in clarification, metabolite production, formulation, microencapsulation, enzymatic hydrolysis, and product improvement, in thermal treatments such as cooking, non-thermal treatments such as HPP, cold plasma, and packaging, due to their properties and potential applications (Bazaria & Kumar, 2018; Botinestean *et al*., 2020; Liu *et al*., 2024; Saranya & Kundukulanagara, 2025; Tirado-kulieva *et al*., 2021)

Conclusions

High-pressure processing is acknowledged as an effective non-thermal processing method that can deactivate microorganisms and endogenous enzymes, resulting in greater shelf life while maintaining freshness and nutritional quality of seafood. This process can minimize or eliminate the use of chemical preservatives, which is one of the greatest concerns of the customers. HP treatment presents a possibility for microbiological decontamination; nonetheless, it is incapable of eradicating every pathogen in food, which is why HPP needs to be combined with other technologies to make use of its full potential. The high equipment cost is one of the reasons for small and medium-scale companies not adopting this technology, as they struggle to justify the cost of the equipment.

The gaps that exist in high-pressure processing of seafood encompass the absence of optimized pressure-time combinations across various species, such as trout and shrimp, as each species responds differently to identical pressure and holding time combinations. Additionally, there is a necessity to elucidate the alterations in macromolecules such as proteins and carbohydrates that lead to color and texture changes, as well as to investigate the interaction between pressure and time, and possibly temperature and product-related compositional variations, which is pivotal in the applications of HPP in general. Some of these are addressed in this study as related to their influence on quality and refrigerated storage stability of trout and shrimp.

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Connecting Statement to Chapter 3

In the previous chapter, a detailed review on enzymatic and microbiolgy related to fish spoilage, methods of preservation to extend the shelf life and quality retention was provided including some knowledge gaps with respect to application of high pressure processing (HPP) for seafood preservation. In this chapter, the effectiveness of HPP on hot smoked trout and arctic char (AC), during refrigerated storage will be highlighted. The pressure levels chosen are based on a previous study conducted in our lab in raw and smoked salmon. The effects of pressure and holding time on trout and artcic char as well as salmonid species are with respect to its color, texture and microbial quality during a refrigerated storage of 21 days.

A manuscript is prepared for publication in a refereed journal (to be selected):

George, S., Taherian, A and Ramaswamy, H.S. 2025. Evaluation of high pressure treatment for enhancing quality retention and refrigetated shelf life extension of smoked trout and arctic char.

CHAPTER 3
EFFECT OF HIGH-PRESSURE TREATMENT ON REFRIGERATED STORAGE STABILITY AND QUALITY OF HOT-SMOKED ARCTIC CHAR AND TROUT FILLETS

Abstract

High Pressure Processing (HPP) is a widely used non-thermal, additive-free preservation method for perishable foods like meat and fish. It is advantageous in preserving freshness as well as nutritional profile through inactivation of pathogenic microorganisms, modification of enzymatic activity, and reduction in loss of desirable compounds. The method is especially relevant and highly significant in the case of fresh fish and seafood with high perishability and short shelf life. Moreover, HPP has been beneficial in retaining organoleptic properties such as odor, flavor, color, liquid loss, and texture change during storage. Alongside microbiological characteristics, it is essential to monitor changes in these characteristics throughout storage. The primary aim of this study was to examine the quality and refrigerated storage stability of hot-smoked Arctic Char and Trout fillets subjected to high-pressure treatment. Uniform-sized smoked fillets were prepared, vacuum-packed into high-density polyethylene (HDPE) pouches, and subjected to HP treatments at 350 MPa for 10,20,30 min for both trout and Arctic char fillets and 150 MPa and 250 MPa for Arctic char for 30 min (subsequent study). The treated products and the control (untreated) were stored at 4°C for 21 days. These samples were evaluated on days 1, 7, 14, and 21 for quality parameters like texture and color, as well as microbial growth. As a result, the applied pressure treatment resulted in a notable improvement in textural properties like firmness and tenderness, an increase in L* (lightness), and a decrease in a* (redness). It was also noted that the retention of textural parameters was higher in the treated samples, depending on the intensity and duration of treatment. The effect of HP treatment on the reduction of microbial growth was significant ($p<0.05$), and it was hypothesized that HP treatment can be applied for enhancing and/or retaining quality, as well as extending the shelf life of smoked fillets.

Keywords: High Pressure Processing, smoked fillets, texture, color, microbial growth

3.1. Introduction

Fresh fish is a high-quality commodity of significant economic value. It is typically marketed

vacuum-sealed and maintained under refrigeration without additional processing. Seafood is recognized for its high perishability, possessing a shelf life of a maximum of 14 days for fresh or thawed products. After 7 days of cold storage, the product deteriorates in quality and is often sold at a discounted price or discarded/disposed of (Castrica *et al*., 2021). Moreover, the quality of fresh fish depends on the species, with different biological and microbiological compositions as well as storage conditions (Teshome *et al*., 2022). The consumer demand for nutritious fresh foods that are free from additives, minimally processed, and with extended shelf-life has paved the way for the development of non-thermal technologies such as HPP (Lisboa *et al*., 2024).

High-pressure processing (HPP) is a non-thermal food preservation technique employed for highly perishable items like meat and fish, aimed at prolonging shelf life without the use of additives. The application of HPP inactivates harmful germs, modifies enzymatic activity, and reduces the loss of beneficial nutrients, so preserving the freshness and nutritional content of food. The shelf-life of fresh fish and shellfish is limited; therefore, utilizing processing methods to prevent rotting and prolong shelf-life would be advantageous (Sehrawat *et al*., 2021).

High-pressure processing, along with other non-thermal preservation techniques, has achieved significant acceptability and has been utilized for the commercial processing of many food products in developed nations (Huang *et al*., 2017). It has been reported to ensure both microbial safety and shelf-life stability for various meat and seafood products effectively. HPP exclusively influences the pressure-sensitive non-covalent bonds of molecules, while low molecular weight compounds with covalent bonds are generally unaffected by pressure. Components responsible for color characteristics, volatile and non-volatile compounds, as well as bioactive compounds, are included in the latter category, except when associated with proteins, like in red meat, colored fish, etc., facilitating the retention of fresh-like qualities in meals by HPP [(Tewari *et al*. (2017) (Huang *et al*. (2019)].

HPP is governed by three basic principles. First, is Le Chatelier's principle, which states that any process in equilibrium (conformational change, phase transition, chemical reaction), which is accompanied by a decrease in volume, can be enhanced by pressure (Kumar *et al*., 2018), resulting in decreased volume of HP-treated products. The second concept is the isostatic principle, which states that during compression, pressure is uniformly transferred from all directions, regardless of the object's geometry, allowing it to maintain its shape upon decompression (Pascal principle). This unique property has been responsible for the

development of HPP into commercial success. The third principle, microscopic ordering, states that increasing pressure at a constant temperature enhances molecular alignment and kinetic energy (Sehrawat *et al*., 2021; Yordanov & Angelova, 2010). HPP is gaining popularity in the food industry in the Asia/Pacific region and has been in commercial practice in the United States for the last 15 years. HPP technology is predicted to grow in the coming years, with the global food market reaching 55 billion US dollars by 2025 (Huang *et al*., 2017).

The potential health benefits of HPP also include retention of freshness of food products, minimizing the use of chemical preservatives, and lowering the destruction of natural components in fruits and vegetables (Pottier *et al*., 2017). HPP food products have a higher content of resistant starch and a lower glycemic index, facilitating reduced calorie intake, leading to better control of blood glucose levels (Xia *et al*., 2017). This enhances the clean label concept that is the primary consumer appeal of HPP technology. Apart from inactivation of microorganisms, HPP technology has been applied in areas like cellular metabolic pathways with biosynthesis of useful metabolites, particularly γ-aminobutyric acid (GABA) in brown rice (Poojary *et al*., 2017). HPP has also been applied in reducing the level of sodium and chloride content in processed foods, thus minimizing the risk of cardiovascular diseases (Rodrigues *et al*., 2016). Extraction and enhancement of functional components from food processing waste have also been made possible through the application of HPP.

HPP has wide applications in the food industry, including seafoods, such as oysters, lobster, crab, mussels, clams, and scallops, owing to enhanced procedural efficiency, higher productivity, and efficient usage of manpower. More importantly, the risk of recontamination in HPP products is totally eliminated through the post-package processing (Ghafoor *et al*., 2020). Therefore, this comparative study was carried out to investigate the effect of HP treatment on the quality and storage stability of hot-smoked trout fillets and Arctic char.

3.2 Materials and methods

3.2.1. Sample Preparation

Hot-smoked trout fillet was purchased from a local supermarket and then immediately transported to the lab under ice storage conditions. Hot-smoked Arctic char was obtained from a local supplier. They were kept frozen at -18°C until use. The required number of fillets was thawed overnight, the day before the experiment at 4-7°C. The dorsal muscular part was cut carefully, avoiding the thin sides, so that there was uniformity in weight and size. The fillets

were deskinned and cut into small pieces of 20 mm x 20 mm x 15 mm using a clean knife. Following this, the samples were sealed into HDPE pouches, removing the air by rolling the pouches and using a vacuum sealer prior to HP treatment. It was also ensured that the vacuum sealing was done only for a few seconds, just enough to remove the air and not to exert suction force onto the sample. This step also safeguarded the vacuum-packed samples not to float inside the HP unit vessel. No chemicals or additives were added prior to the treatment. Each sealed HDPE pouch consisted of about 30-45 g of smoked fillets. Apart from control, the vacuum-packed samples were then subjected to HP treatment for different pressure-time combinations. The pressure transmitting medium was water with 2% food-grade mineral oil added for lubrication. Immediately after HP treatment, the selected number of treated and untreated samples were analyzed for quality characteristics, and the remaining samples were stored under refrigerated conditions (4°C) for a systematic assessment of microbial growth and quality degradation after 7, 14, and 21 days.

Trout belong mainly to two genera: *Oncorhynchus* and *Salvenlinus*. The genus *Oncorhynchus* includes the salmon as well as several trout species, while the genus *Salvelinus* contains several trout species that may be regarded as chars. Members of the two genera are chiefly distinguished by differences in body coloring, the shape of the vomer bone in the roof of the mouth, and the teeth. The Arctic char (*S. alpinus*) of North America and Europe inhabits the Arctic and adjacent oceans and enters rivers and lakes to breed. Some populations are restricted to freshwater lakes, which they colonized in glacial times. In this study, both trout and char have been used as they were of interest to the local processing industry.

3.2.2. High pressure processing equipment

The high-pressure processing equipment used (AE 400 MPa - Isostatic Press, Autoclave engineering, Columbus, Ohio) consisted of a vessel chamber, fluid reservoir, and valves for controlling the pressure transmission. The pump supplied pressure to the water, which acted as the pressure-transmitting medium to the samples under treatment. The first valve is closed to move the water inside the chamber. Once the pressure is brought up to the specific pressure level, it is held for the required holding time. After the required time of processing, the pressure is released by opening the pressure release, safety, and pressure shut-down valves.

3.2.3 High pressure treatment

Some preliminary experiments were conducted using hot-smoked trout, where the fillets were subjected to 350 MPa for 10, 20, and 30 min. Later, the trout samples were also conducted with hot-smoked Arctic char (AC), as there weren't a lot of studies conducted on it. As a result, the same treatment was applied to AC was well. It was observed that 30 min treatment had the most quality retention, but at the same time, it had adversely affected the color of the samples. As a result, fillets were subjected to HP treatment of 150 MPa, 250 MPa for 30 min to study the effect of lower pressures on various quality parameters and shelf-life extension. After HP treatment, the samples were stored at a refrigerated temperature (4°C). The untreated and pressure-treated samples were tested on Days 1, 7, 14, and 21 for microbial and quality attributes.

3.2.4. Texture measurement

A TA.XT plus texture analyzer (Texture Technologies, New York, USA) was used for analyzing the effect of HP treatment on its textural parameters. A multi-wired probe, developed in our laboratory, was used to carry out experiments. This probe was 70 mm in diameter and equipped with 10 wires of 0.25 mm in thickness and 6 mm apart. The base was a stainless-steel circular model of 60 mm diameter. The samples of size 20 x 20 x 15 mm were placed on the base and compress-cut by 80% of their height. The compression cutting force and area of the work were defined as firmness and tenderness, respectively. These values for firmness and tenderness were obtained directly from the Exponent software (Texture Technologies, New York, USA).

Different texture parameters were evaluated from the force deformation curves as follows:

$$\text{Firmness} = \frac{Maximum\ force}{Maximum\ deformation} \quad (1)$$

$$\text{Tenderness} = \frac{force/cross-sectional\ area}{deformation/intial\ length} \quad (2)$$

Retention % of firmness/tenderness

$$= (100\text{-loss }\%) = 100 - \frac{(Value\ of\ day\ 1 - value\ of\ day\ 7,14,21)}{Value\ of\ day\ 1} \times 100 \quad (3)$$

3.2.5. Color measurement

Color was measured using a Minolta Tristimulus Colorimeter (Minolta Crop, Ramsey, NJ, USA). The color parameters L*, a*, b*, hue angle and delta E (ΔE) were assessed using the software Spectra Magic (Minolta Crop, Ramsey, NJ, USA) connected to the colorimeter. Uniformly shaped samples were subjected to color assessment using the colorimeter to measure the lightness, redness, yellowness, hue, and ΔE of the control of the pressure-treated samples and the untreated control. To study the change in color of HPP fish samples, CIELAB system was used. Color is expressed as a three-dimensional diagram with L* planes (brightness scale from 0 (black) to 100 (white), a* (scale from -a (green) to +a (red)), and b* (scale from -b (blue) to +b (yellow) (Bharatbhai & Kavilakath, 2024). The lightness was analyzed as L*, redness as a*, yellowness as b*, hue angle, and finally Delta E as ΔE. Measurement was taken 10 times for each sample, after which the average value was considered. The parameters were derived as shown below:

$$\text{Retention \% of a* = (100-loss \%) = 100 - } \frac{(Value\ of\ day\ 1 - value\ of\ day\ 7/14/21)}{Value\ of\ day\ 1} \times 100 \quad (4)$$

$$\text{Hue angle} = \tan^{-1}\frac{b}{a} \quad (5)$$

$$Delta\ E = SQRT\left((\Delta L)^2 + (\Delta A)^2 + (\Delta B)^2\right) \quad (6)$$

where L* is lightness, a* is redness, and b* is yellowness of the samples.

3.2.6. Microbial analysis

The Aerobic Plate Count (APC) method was used for the enumeration of aerobic bacteria. For APC, a 1/10 dilution was prepared by adding 5 g of the sample to a stomacher bag with a filter, followed by the addition of 45 mL 0.85% sterile saline. The sample bag was smashed for 120 s in the stomacher, and the filtrate was used to do a serial dilution. 0.1 mL of the filtrate was transferred to 0.9 mL of 0.85% NaCl solution. 0.1 mL of each dilution was plated into a Tryptic Soy Agar (TSA) plate and spread using an L-shaped spreader. Enumeration was done after incubation of the Petri plates at 35 °C for 48 h. Colony of culture was calculated as log10 colony-forming units (CFU) per g. Results were taken in triplicate, and the mean value was considered.

3.3 Results and Discussion

3.4. Hot smoked trout

The effects of HPP on Trout were first evaluated at 350 MPa for time intervals of 10, 20, and 30 minutes. The smoking treatment, which involves the application of volatile compounds produced from the thermal decomposition of wood onto meat or fish, provides unique flavor, color, and aroma to food while enhancing preservation through its dehydrating, antibacterial, and antioxidant properties (Adeyeye, 2019). In this study, the effect of HP treatment on textural parameters (firmness and tenderness), color (L*, a*, b*, Hue and ΔE) as well as microbial quality on days 1,7,14 and 21 was evaluated.

3.4.1 Effect of HPP on texture parameters

Textural and structural measurements are crucial factors in assessing the freshness quality of fish, as texture significantly affects consumer acceptability (Liu *et al*., 2019). The storage temperature during handling and processing substantially influences fish texture, leading to prevalent defects such as muscle softening and gaping, resulting from both pre- and postmortem treatments (Singh & Singh, 2020). These issues are chiefly associated with alterations in chemical composition and the deterioration of muscle proteins. Textural properties are typically evaluated via descriptive sensory or instrumental analysis Chen and Opara (2013), highlighting the significance of texture measurement to guarantee superior quality and consumer satisfaction. In this study, the multi-wired probe was used to derive the firmness and tenderness of the fish samples studied. Firmness indicates the maximum force the sample can withstand (Cheng *et al*., 2014), whereas tenderness refers to the softness a sample achieves under high pressure.

3.4.1.1 Effect of HPP on firmness

Firmness is a critical texture parameter for assessing food freshness. The firmness test is typically conducted to assess the quality of food products (Gokul *et al*., 2023). Moreover, firmness aids in assessing the quality of a sample throughout its shelf-life, as foods exhibiting greater resistance to deformation signify a higher degree of firmness (Hyldig & Nielsen, 2001). The results of the study on the effect of HPP on firmness are shown in Figure 3.1. Treated samples showed a significantly higher firmness compared to untreated samples throughout storage. In contrast, untreated samples showed a steady decline in firmness. This indicated that the untreated samples had less resistance to texture degradation, which can be achieved even by

short treatment time, such as 10 min, which is significantly different from the untreated sample throughout the storage. On Day 1, the firmness of 20 min and 30 min treated samples did not differ significantly ($p>0.05$), although the untreated and 10 min treated samples were different from each other. From Day 7 until Day 21, it can be noted that the 10 min and 20 min treated samples were not significantly different, although their values were reducing at a steady rate. However, all treated samples retained higher firmness compared to untreated samples. As the samples progressed to the end of storage on day 21, it can be observed that samples treated for 30 min had the highest firmness values, suggesting that a longer holding time can have a positive effect on providing better structural resilience. Tsironi and Taoukis (2023) reported comparable findings, indicating that HP treatment of European sea bass fillets, at pressures exceeding 200 MPa, increased hardness values. Specifically, hardness nearly doubled for fillets subjected to 400 MPa compared to untreated or those treated at 100 MPa. It was reported by Kumar *et al*., (2019) that the increase in hardness is associated with the inactivation of proteolytic enzymes in HP-treated (at pressures over 200 MPa) fish samples, resulting in denser structures.

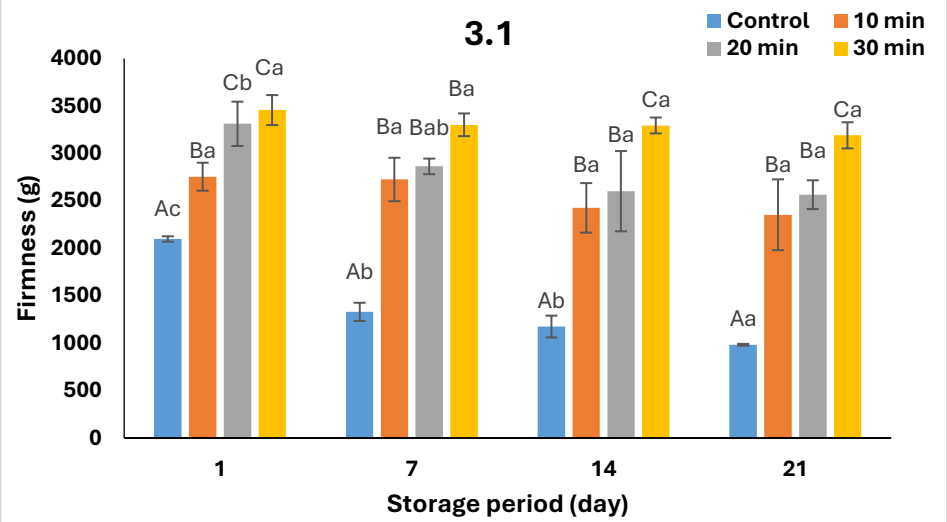


Figure 3.1. *Effect of HP treatment on firmness of treated hot smoked trout as a function of treatment time (min) and refrigerated storage period (days) at 350 MPa. Values are the mean of 4 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p<0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p<0.05$).*

A method used by Vettickathadathil *et al*., (2024) was applied in this study, where the firmness loss on days 7, 14, and 21 was calculated with respect to Day 1, from which the firmness retention percentage was calculated. It can be observed from Table 3.1 that the retention % is significantly higher for all the treated samples compared to the untreated samples. The 30 min treatment ensured excellent firmness retention throughout time, with only a slight reduction by Day 21. The 10 min treatment has significant initial retention, although it exhibits a more pronounced drop by Day 21 in contrast to the 30-minute treatment. The 20 min treatment has intermediate retention levels, demonstrating a moderate decline in firmness over time. Untreated samples demonstrated the most significant reduction in firmness, losing around 50% of their firmness, highlighting the importance of HP treatment in maintaining texture.

Table 3.1. Retention % for firmness of hot-smoked trout sample treated at 350 MPa

Treatment duration (min)	Day 7	Day 14	Day 21
0	63.4	56.0	46.8
10	98.9	88.1	85.4
20	86.5	78.6	77.5
30	95.5	95.3	92.3

Overall, the results of this study indicated that HPP markedly enhanced the firmness of treated samples relative to untreated samples throughout the storage duration. The untreated samples showed a consistent reduction in firmness, indicating their vulnerability to texture deterioration. The treated samples consistently retained greater firmness, with even brief treatment durations (10 min) demonstrating efficacy throughout the storage period. No significant differences were noted between the 10 and 20 min treatments on Day 1; however, by Day 21, the samples subjected to 30 min treatments exhibited the greatest firmness retention. This trend indicates that prolonged treatment durations enhanced the structural integrity and resilience of the samples, rendering HPP an effective method for maintaining texture during storage.

3.4.1.2 Tenderness of hot smoked trout

Tenderness is an essential quality characteristic in assessing the freshness and market worth of fish. It is affected by multiple factors, including species, age, size, and nutritional condition of the fish. Postmortem alterations, including glycolysis and rigor mortis, considerably influence texture (Hyldig & Nielsen, 2001). Instrumental techniques, such as shear force measurements, are frequently employed to evaluate tenderness, yielding objective data on the textural characteristics of fish muscle (Komolka *et al*., 2020). It is the area under the force deformation curve when the sample is compressed, calculating the compression energy (Taherian *et al*., 2019).

Figure 3.2 illustrates the effect of HPP at 350 MPa for 10, 20, and 30 min on the tenderness of hot smoked trout analyzed over a period of 21 days, with untreated samples serving as control (0 min). The results highlight the effect of processing time as well as the effect of refrigerated storage. The results obtained clearly showed that HPP significantly increased the tenderness of samples, with longer holding times yielding higher values. The untreated sample was significantly ($p<0.05$) different from the treated samples at all times throughout the storage. It can be seen from the results that the trend for tenderness was similar to the trend obtained for firmness of hot smoked trout. On days 1 and 7, it can be observed that there are no significant ($p>0.05$) differences between 10 and 20 min treatments, whereas, as storage period extended to days 14 and 21, all the treatments were significantly different from each other, indicated by the alphabets A, B, C and D. The 30 min treated sample had the highest tenderness values across all time points, indicating the effect of longer holding time on enhancing textural properties.

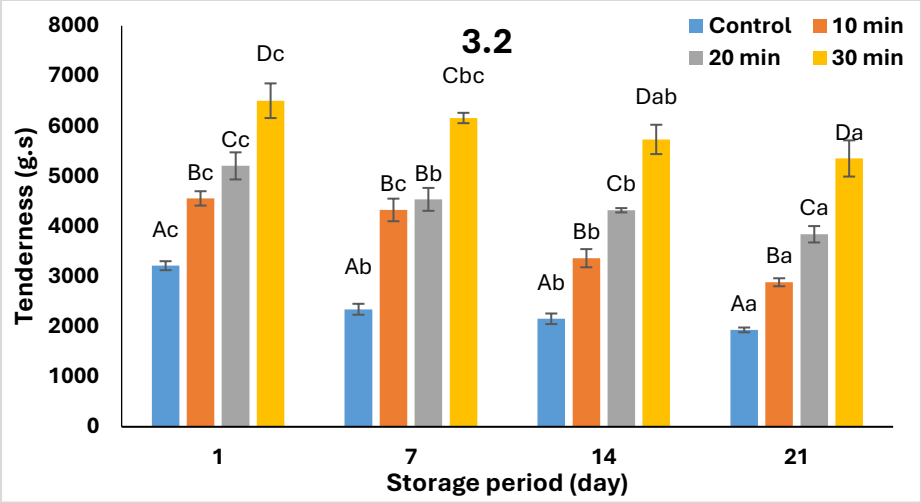


Figure 3.2. *Effect of HP treatment on tenderness of treated hot smoked trout as a function of treatment time (min) and 4 °C storage time (days) at 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).*

Table 3.2 shows the tenderness retention percentage of untreated, and HP-treated hot-smoked trout. A trend similar to firmness retention was observed here also. The 30 min sample demonstrated the highest retention throughout the storage, indicating that better retention of textural tenderness can be obtained by longer holding time. This is a remarkable 36.8% improvement over the untreated sample, demonstrating the superiority of extended HP treatment in maintaining tenderness. When comparing the 10 min treated samples to untreated samples, the retention of tenderness revealed that the two were relatively close in performance, especially as storage progressed, highlighting a diminishing advantage of the 10 min treatment over time.

Table 3.2. Retention % for tenderness of hot smoked trout sample treated at 350 MPa

Treatment Duration (min)	Day 7	Day 14	Day 21
0	72.9	67.0	60.1
10	95.0	77.8	66.7
20	87.2	83.0	73.8
30	94.7	88.1	82.3

Shen *et al*. (2021) observed that postmortem changes of collagen solubility might be responsible for the rapid softening at the early stages of grass carp fillets during chilled storage. Accordingly, the disintegration of collagen in connective tissues might have caused the muscle segments to loosen, leading to a decrease in shear force.

Overall, HPP at 350 MPa markedly enhanced the tenderness of hot smoked trout, with extended processing durations further improving textural attributes. Untreated samples exhibited consistently diminished tenderness, whereas the 30 min treatment demonstrated the highest value over the 21-day storage period. Textural trends for tenderness paralleled those of firmness, highlighting the efficacy of HPP in quality preservation.

3.4.2 Effect on color

Color is the primary attribute evaluated by customers to ascertain the quality of food products, especially the freshness of perishable commodities like seafood. Pigmented fish with attractive colors is often sold at a premium price in markets (Lin *et al*., 2024). This is clearly evident in case of popular salmon with a deep red, orange or pink color of the skin and flesh (Lerfall & Birkeland, 2014). It is a widely recognized fact that HPP negatively impacts fish coloration, resulting in increased whiteness and opaqueness of the muscle, so imparting a cooked appearance (Oliveira *et al*., 2017; Truong *et al*., 2015). The altered appearance has been due to changes in muscle hydration status, changes in pigmentation, a changed profile of sarcoplasmic and myofibrillar proteins, and oxidative changes in protein and lipids (Oliveira *et al*., 2017).

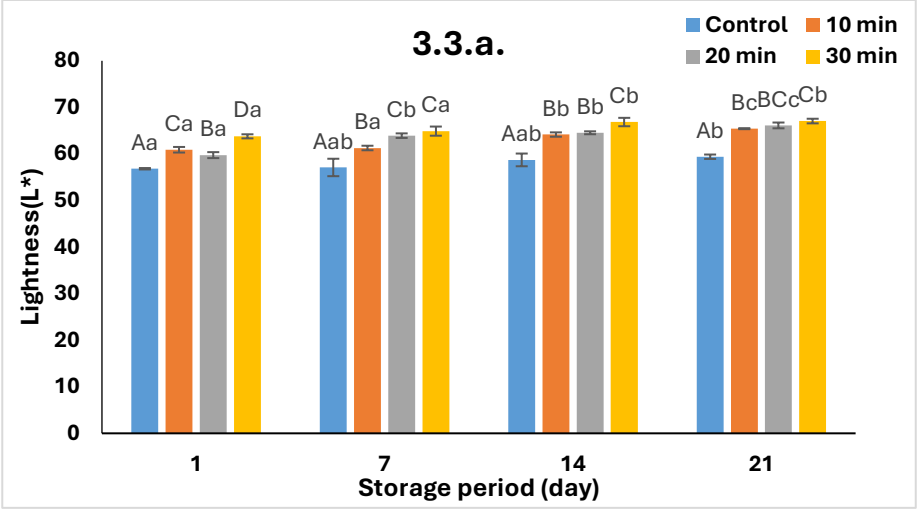
3.4.2.1 Effect on L* and b*

Figure 3.3a illustrates the variation in L* values (lightness) of hot-smoked trout subjected to high-pressure processing at 350 MPa for 10, 20, and 30 min, measured over a 21-day storage period. On Day 14, while the untreated sample maintained consistent values, significant differences emerged among the treated samples. While the samples treated for 20 and 30 min showed no significant ($p>0.05$) differences on day 7, the 30 min and 10 min treated samples showed an increase in the L* value, resulting in the 10 min and 20 min samples not exhibiting any significant differences on day 14. On Day 21, the untreated sample continued to have the lowest L* value, indicating a darker appearance. The 30 min treatment consistently showed the lightest appearance while the 10 and 20 min samples showed no statistical difference. These

findings demonstrate the progressive effect of holding time and storage duration on the lightness of HP treated trout.

Figure 3.3b shows the variations in the b* values of hot-smoked trout processed under high-pressure processing 350 MPa for durations of 10, 20, and 30 min throughout a 21-day storage period. The results demonstrated a trend analogous to the L* values, with all the samples exhibiting a consistent increase in b* values during storage. On Day 1, the untreated and 10 min treated samples had similar b* values, indicating that a short time may not have caused a significant change in b* value. However, the 20 min and 30 min treated samples remained significantly ($p<0.05$) different from each other. By Day 7, an interesting observation emerged: b* values for all four samples became statistically similar. The untreated, 10 min, and 20 min samples exhibited significant increases in yellowness, aligning with the 30 min treatment, which showed minimal changes over this period. As storage continued through Days 14 and 21, the 30 min treated sample maintained a consistently higher b* value compared to the other treatments, indicating its distinctive color stability and enhanced yellowness. In contrast, the untreated, 10 min, and 20 min samples showed smaller and gradual changes that resulted in their b* values converging and becoming closely aligned by the end of storage.

Similar results were obtained in a study conducted by Chouhan *et al*., (2015) on Hilsa fillets where L* and b* values increased with a* reducing as a result of storage after HP treatment at 250 MPa and 350 MPa for a time ranging between 5 min and 25 min. The increased value of b* was correlated with the increased thiobarbituric acid (TBA) values, which is a measure of lipid oxidation. The alterations in color induced by HPP are primarily indicated by an elevation in the L* value, which correlates with the product's lightness. Furthermore, a study conducted by Alba *et al*.,(2019) noted an increase in L* from 53.60 to 72.73 following treatment at 500 MPa for 5 minutes in mackerel fillets. These results can be explained by the conclusions obtained from the study by Oliveira *et al*., (2017) that the low myoglobin content in fish muscles causes color changes from pressurization, primarily due to the denaturation of proteins such as myosin and actin, giving effects like those of cooking.



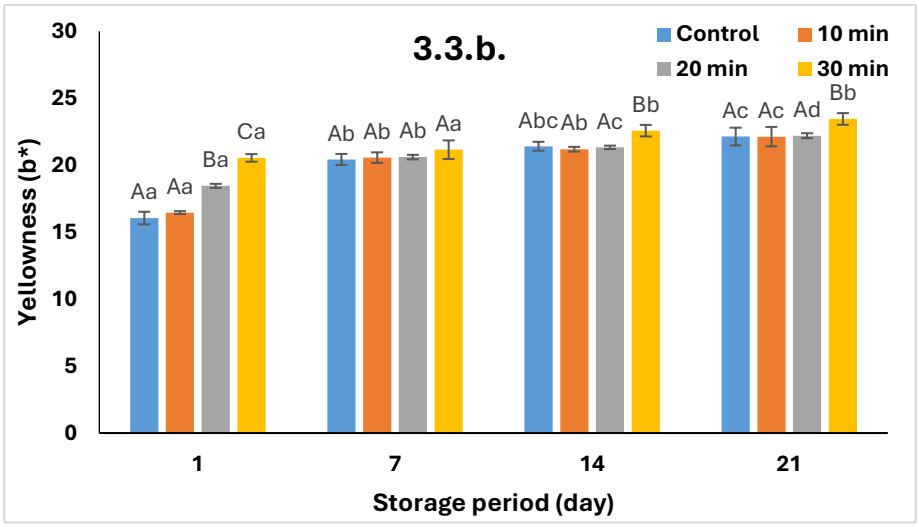


Figure 3.3. *Effect of HP treatment on* ***(a) lightness (L*)*** *and* ***(b) yellowness (b*)*** *of treated hot smoked trout as a function of treatment time (min) and 4 °C storage time (days) at 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).*

Overall, the results demonstrate that HPP has a significant effect on the color parameters of hot-smoked trout, particularly the lightness and yellowness values, throughout the storage period. Samples treated with HPP consistently exhibited elevated values relative to untreated controls, indicating increased lightness and yellowness, likely resulting from structural alterations and lipid oxidation caused by high-pressure processing. The untreated sample exhibited the darkest coloration during the entire storage period, although its lightness showed a slight increase over

time. The differences in values across treatment durations of 10, 20, and 30 min decreased by day 21, suggesting that holding time has a more significant impact in the early stages of the storage period. The observations highlight the influence of HPP parameters and storage conditions on the visual quality and color stability of hot-smoked trout, demonstrating HPP's potential to alter and maintain seafood characteristics during prolonged storage periods.

3.4.2.2 Effect on a*

The effect of HPP and storage on the redness (a*) of hot-smoked trout is shown in Figure 3.4. On Day 1, the untreated sample (0 min) displayed the highest a* value relative to all treated samples. Among the treated samples, the 30 min treatment had the lowest a* value, followed by the 20 min and 10 min treatments, suggesting that HPP treatment reduces the initial intensity of redness. On Day 7, all the samples except 30 min treated sample showed a significant ($p<0.05$) decrease in a* values, indicating rapid pigment degradation, probably resulting from oxidative or enzymatic reactions without sufficient protective influence from HPP. On Day 14, there were no significant ($p>0.05$) differences between untreated, 10 min, and 20 min treatments, whereas 30 min treatment continued to show the lowest redness value. By the end of the storage period, it was noticed that the untreated and 10 min treated samples were similar, whereas there were no statistical differences between 20 min and 30 min treatments either.

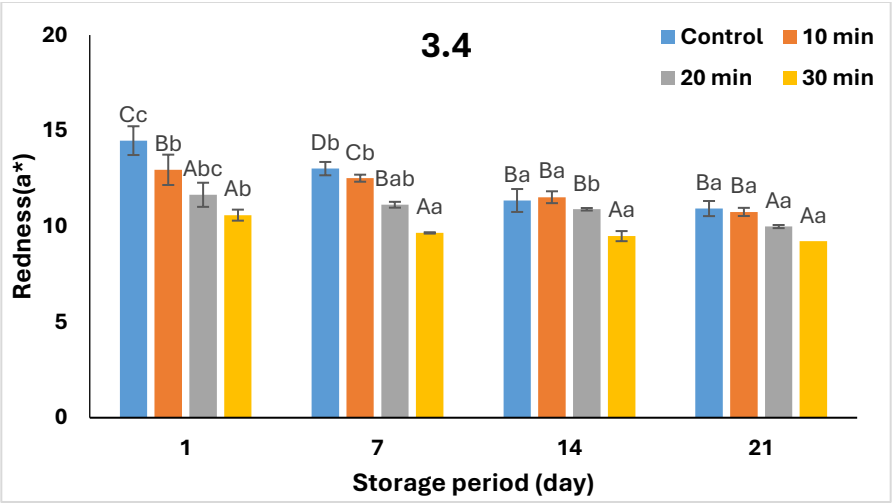


***Figure 3.4**. Effect of HP treatment on redness (a*) of treated hot smoked trout as a function of treatment time (min) and 4 °C storage time (days) at 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p <0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p <0.05$).*

Similar results were obtained by Ramirez-Suarez & Morrissey, (2006) who studied the effect of HPP and storage on the shelf life of albacore tuna minced muscles, and observed an increase in L* value, but a decrease in a* value upon storage. These changes were probably due to denaturation of globin protein or release of heme protein as stated by (Cheftel & Culioli , 1997). A 21-day storage study on smoked salmon conducted by Vettickathadathil *et al*., (2024) reported analogous findings, indicating that the untreated sample exhibited a significantly higher redness value. Conversely, the sample subjected to a 30-min treatment, which displayed the least redness, demonstrated the highest retention of a* at the conclusion of the storage period compared to the other treatments. It was concluded from their study that HPP treatment significantly increased firmness and tenderness, adversely affecting the Myoglobin (Mb) protein content. Reduction in the level of Mb, which is also responsible for redness, leads to lower a* values. The texture results, which increased significantly even after 10 minutes of HP treatment, suggest that changes in myofibrillar proteins of smoked salmon may have contributed to these changes. Table 3.3 shows the retention % of redness in hot-smoked trout as influenced by storage and treatment time. Unlike the textural parameters, initial redness is observed to have a greater impact with increasing holding time. Lower treatment times (10 min & 20 min) showed a notably higher retention of a* until day 14, compared to 30 min treatment, presumably due to the reduced impact on protein denaturation. The untreated sample, as anticipated, exhibited a more rapid decline in redness after storage, despite recording the greatest redness values on all days.

Table 3.3. Retention % for redness (a*) of hot smoked trout sample

Treatment duration (min)	Day 7	Day 14	Day 21
0	89.9	78.4	75.5
10	96.6	92.1	85.9
20	95.5	93.5	85.8
30	91.2	89.6	87.1

Overall, it was observed from Figure 3.4 that the untreated samples showed the highest redness value, which was justified by their appearance in the early days of storage. However, the redness reduced significantly for all samples, including treated and untreated samples. The 30 min treated sample consistently showed lower values, but the highest retention out of all samples. These results can be attributed to the fact that astaxanthin responsible for giving the orange/red color in trout, is pressure sensitive. It has been hypothesized that muscle proteins decompose, leading to weakening of the protein-astaxanthin bond, especially α-actinin and actin (Ojagh *et al*., 2011), adversely affecting the stability of the pigment , as evident from the blanching of salmonids subjected to HPP (Lerfall & Birkeland, 2014). From the results of our present study on texture, it was observed that tenderness and firmness reduced upon storage, which may have been caused by protein denaturation. Hence, the results obtained for loss of texture during storage can be linked with loss of redness (a*) after HPP.

3.4.2.3 Effect on hue

Hue angle, a critical parameter in color science, represents the specific shade of a color as perceived by the human eye. It is calculated from the chromaticity coordinates a* and b* in the CIE L*a*b* color space, which quantify the red-green and yellow-blue components of color, respectively. It is used to define the relationship between the redness and the yellowness of the fillet, and a hue angle of 0◦ corresponds to red and 90◦ for yellow (McLellan *et al*., 2007).

In the present study, illustrated in Table 3.4, the b* value was higher for treated samples, and it progressively increased across storage for all samples. This clearly resulted in increased hue values as well. It can be observed that the shift in hue angle was greater for the untreated sample from 47 to 63, whereas there was only a difference of 5 units for the sample treated at 350 MPa for 30 min. However, it was noted that all the samples experienced a shift from red to a shade of yellow by the combined effect of pressure treatment and refrigerated storage. Several studies indicate a decrease in a* (loss of red) and an increase in b* (gain of yellow), with variations observed depending on species and pressurization parameters. It can also be observed that the hue value of HP treated sample at 30 min on day 1 is almost the same as the untreated sample on day 21, indicating the effect of high pressure and holding time on altering the color of trout starting from the initial stage of storage.

Table 3.4. Effect of HPP on hue of hot smoked trout

Storage duration	Control	10	20	30
Day 1	48.0 ± 2.29 ^{Aa}	51.8 ± 1.73 ^{Ba}	57.8 ± 1.42 ^{Ca}	62.7 ± 0.64 ^{Da}
Day 7	57.5 ± 1.11 ^{Ab}	58.7 ± 0.80 ^{Ab}	61.6 ± 0.31 $_{Bb}$	65.5 ± 1.01 ^{Cb}
Day 14	62.1 ± 1.47 ^{Ac}	61.5 ± 0.66 ^{Ac}	63.0 ± 0.04 ^{Ab}	67.2 ± 0.19 ^{Bc}
Day 21	63.7 ± 1.47 ^{Ac}	64.1 ± 0.58 ^{ABd}	65.8 ± 0.17 ^{Bc}	68.0 ± 0.83 ^{Cc}

Values are the mean ± standard deviation (n=4) samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

3.4.2.4 Effect on ΔE

The difference between two colors in the three-dimensional L*a*b* color space is known as Delta E (ΔE) (Yeole *et al*., 2018). The calculation was based on the L*, a*, and b* values acquired on Day 1 and Day 21, as shown in Table 3.5. The results indicate a more significant color change between Day 1 and Day 21 of storage in the 10 min and 20 min treated samples, with only a marginal difference between them and the untreated samples. Nonetheless, the samples subjected to treatment at 350 MPa for 30 min exhibited minimal alterations in color owing to superior retention. It was observed from the redness and lightness results that HP-treated samples showed significant changes compared to the untreated samples on all days of storage. However, the treated samples, especially the 30 min treated sample, did not undergo significant changes in their total color when comparing between days, thereby giving a smaller value of ΔE.

Table 3.5. ΔE of HP treated and untreated samples between Day 1 and Day 21

Control	10	20	30
7.53 ± 1.28B	7.60 ± 0.85B	7.60 ± 0.19B	4.64 ± 0.53A

Values are the mean ± standard deviation (n=4) samples during 21 days of storage. For each evaluation point (treatment time), different uppercase letters indicate significant differences among treatment times ($p < 0.05$).

3.4.3. Effect of HPP on microbial growth

The qualitative sensory attributes and shelf-life of smoked fish are influenced by factors such as microbial growth, processing conditions, post-process handling of the product, and storage temperature (Hagos, 2021). Salting and dehydration that is consequent to smoking reduces the water activity (a_w), below 0.86, so that growth of bacteria and mold is inhibited for a period of one or two weeks, as has been reported (Løvdal, 2015). It was in this context that the present study was carried out, in order to extend the shelf life beyond 3 weeks in refrigerated conditions.

As shown in Table 3.6, microbial growth was detected only on Day 21 in the 10 min treated sample, while untreated samples exhibited growth from Day 7, reaching 5.36 Log CFU/g on Day 21. The untreated sample, the smoked trout, already possesses some antimicrobial property that is believed to be due to the combined effects of heating, drying, salting, and also as a result of deposition of polyphenolic constituents like formaldehyde and acetic acid, as reported earlier (Adeyeye, 2019).This explains why the untreated sample itself had a shelf life of 7 days.

Table 3.6. Effect of HPP on microbial growth expressed as logarithmic CFU/g when treated at 350 MPa

Storage duration	Control	10	20	30
Day 1	ND	ND	ND	ND
Day 7	1.44 ± 1.24^{Aab}	ND	ND	ND
Day 14	2.20 ± 0.17^{Bb}	ND	ND	ND
Day 21	5.36 ± 0.02^{Cc}	4.31 ± 0.15^{Bb}	ND	ND

Values are the mean ± standard deviation (n=4) samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

A recent study by Vettickathadathil *et al*. (2024) compared raw and smoked salmon subjected to HPP at 350 MPa for 30 min, concluded that raw salmon exhibited an APC of 5.74 log CFU/g, while smoked salmon showed no growth until day 14, reaching 4.69 log CFU/g by day 21. In

contrast, untreated samples of raw and smoked salmon exceeded 7.5 log CFU/g and 5.77 log CFU/g, respectively, by Day 21.

A study revealed an overall enhancement in shelf life of cold smoked salmon fillets treated at 250 MPa, 3 °C for 5 min and at 250 MPa, 25 °C for 10 min, subsequently stored at 2 °C. Control samples were deemed suitable for a duration of 6 weeks, whereas HPP-treated samples were acceptable for 8 weeks, hence extending the shelf-life by 2 weeks (Erkan *et al*., 2022). Seafood undergoes post-harvest degradation, triggered by a set of enzymes that decompose macromolecules such as proteins, glycogen, and nucleic acids into small molecular substances, ideal substrates for microbial action (Kim *et al*., 2017).

High pressure treatment has been shown to significantly impact the functionalities and cellular structure of microbial cells. The effects of the HPP technique are presumed to result from concurrent influences on membrane permeability, cellular morphology, modified biochemical reactions, and genetic mechanisms (Yordanov & Angelova, 2010). HP treatment has been reported to extend the shelf life of food products up to 120 days, depending upon the selection of process parameters (pressure, temperature, and holding time) and product characteristics (acidity, water activity, and composition) (Janahar & Balasubramaniam, 2022). It can be concluded from the present study that trout fillets, even after smoking, were susceptible to microbial growth to some extent. However, HP treatment with holding time 20 to 30 min resulted in extending the shelf life up to 21 days or even more.

3.5. Arctic char

A comparable treatment of 350 MPa for durations of 10, 20, and 30 minutes was applied to hot-smoked Arctic Char *(Salvelinus alpinus)*, a distinct salmonid species. This was compared to treatments at reduced pressures, specifically 150 MPa and 250 MPa for 30 min, to evaluate both the time effect at a constant pressure and the pressure effect at a fixed duration. A different experimental design was used to test the effect of pressure treatment times at 350 MPa, and the effect of pressure levels at a constant holding time of 30 min.

3.5.1 Effect of HPP on texture

3.5.1.1 Effect on firmness

In meat and seafood, firmness, an essential textural characteristic, is determined by connective tissue, mainly collagen, and myofibrils composed of proteins (actin and myosin) (Chouhan *et al*., 2015). Firmness is crucial in raw and smoked seafood products, as soft flesh can result in quality degradation in processing industries, such as salmon, and diminished consumer acceptability (Yagiz *et al*., 2009). It is intrinsically linked to hardness, which is a crucial textural attribute that greatly influences consumer acceptance, especially in ready-to-eat products (Zhu *et al*., 2011).

The results obtained for firmness after treating the samples at 350 MPa for 10, 20, and 30 min (AC350), and for 30 min at 150 MPa, 250 MPa, and 350 MPa (AC30) are illustrated in Figures 3.5 (a) and 3.5 (b), respectively. From Figure 3.5 (a), it is clear that the untreated sample had the lowest firmness and showed a steady decline, with significant differences ($p<0.05$) across the storage days (indicated by lowercase letters). The treated samples, however, had higher firmness than the control and demonstrated a gradual decline. On Day 1, it can be observed that the firmness increased significantly with treatment time for all samples, with the 30 min treatment having the highest firmness, and 10 min and 20 min having comparable firmness throughout storage (indicated by uppercase letters).

From Figure 3.5 (b) it is clear that the control and 150 MPa were statistically similar, mostly throughout the storage, indicating that low pressures such as 150 MPa, may not have altered the firmness of samples compared with the 250 MPa and 350 MPa, which were also processed for 30 min. Higher pressures resulted in higher values of firmness with a very slow decline of textural properties during the storage (desirable).

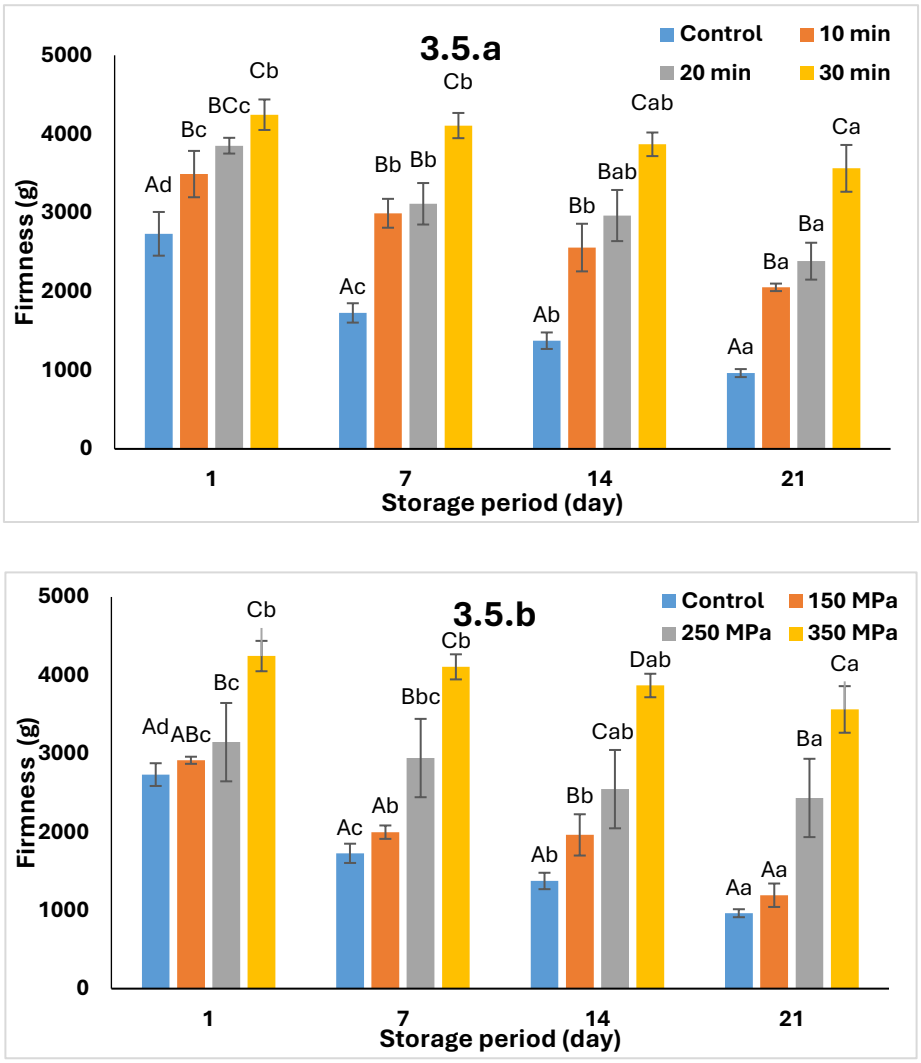


Figure 3.5. *Effect of HP treatment on firmness (%) of treated hot smoked AC as a function of treatment time (min) and 4 °C storage time (days) at* ***(a)350 MPa*** *and* ***(b) 30 min****. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).*

Table 3.7 and Table 3.8 show the retention percentage of AC350 treated for different times and AC30 retention percentage at different pressure levels treated for 30 min calculated for each day with respect to Day 1, respectively. Firmness retention declined progressively over time, but the rate of decline was strongly pressure-dependent, with higher pressures (250 MPa & 350 MPa) showing significantly lower loss. 350 MPa treatment showed the least decline, maintaining a 96.8% on Day 7 and still retaining 83.9% on Day 21, reflecting the best stabilization effect,

whereas the untreated sample dropped sharply from 63.2% (Day 7) to 35.2% (Day 21), indicating significant ($p<0.05$) degradation occurring without pressure treatment. Similar results were obtained for the retention percentage of AC350 shown in Table 3.6, where higher holding times retained better firmness by the end of storage. The control sample lost its firmness rapidly, resulting in a loss percentage of around 65%. This may be due to enzymatic activity, which could otherwise be slowed down by the effects of HP treatment.

Table 3.7. Retention (%) for firmness of hot smoked AC sample treated at 350 MPa

Treatment time (min)	Day 7	Day 14	Day 21
0	63.2	50.3	35.3
10	85.7	73.3	58.8
20	80.8	77.0	61.9
30	96.8	91.2	84.0

HPP has been the subject of extensive research regarding its impact on fish texture and protein structure. Matser *et al*., (2006) examined the effects of HPP on the texture of cod (*Gadus morhua*) following frozen storage for a duration of six months. The findings indicated a significant ($p<0.05$) increase in hardness at pressures of 200 MPa and 400 MPa, whereas treatment at 100 MPa exhibited no notable effect relative to non-pressurized samples. Zhu *et al*., (2011) emphasized the significance of intrinsic structural components in fillet firmness, showing a strong correlation between breaking stress, indicative of firmness, and the abundance of collagen in fish. Bolumar *et al*., (2021) investigated the molecular mechanisms involved in texture modifications, demonstrating that HPP-induced protein oxidation and increased endopeptidase activity lead to the degradation of myofibrils and the release of soluble proteins at pressures exceeding 200 MPa. Higher pressure levels induce protein unfolding, aggregation, and agglomeration, leading to significant modifications in texture and enhanced functional properties of the proteins.

Table 3.8. Retention (%) for firmness of hot smoked AC sample treated for 30 min

Treatment pressure (MPa)	Day 7	Day 14	Day 21
0	63.2	50.3	35.3
150	68.5	67.3	40.9
250	93.6	80.9	77.4
350	96.8	91.2	84.0

Tsai *et al*., (2022) investigated albacore tuna exposed to pressures reaching 600 MPa, concluding that the denaturation and aggregation of myofibrillar proteins are responsible for the increased hardness of fish flesh under these conditions. These findings highlight the complex relationship between pressure levels, protein structure, and the textural properties of HP processed fish products.

Protein oxidation and increased endopeptidase activity due to HPP influence myofibrillar proteins and their functional characteristics. Pressure over 200 MPa degrades the myofibrils and releases soluble proteins. Increased pressure induces protein unfolding, agglomeration, and aggregation, leading to textural alteration and enhanced functional characteristics (Bolumar *et al*., 2015). In a recent study by Tsai *et al*., (2022) indicated that pressurizing albacore tuna to 600 MPa may be associated with the denaturation and aggregation of myofibrillar protein, which could contribute to the increased hardness of the fish flesh under high pressure.

Overall, the sample treated for 30 min remained the firmest throughout the storage, although all the samples, including treated and untreated, lost their firmness to varying degrees. In both AC350 and AC30, it was observed that the untreated sample showed the steepest decline at every checkpoint of storage. However, the firmness of the untreated sample remained significantly different from every other treatment in AC350, whereas in AC30, there is a closer link between the untreated and 150 MPa on all days (indicated by the uppercase letters), except Day 14. This shows that pressure as low as 150 MPa may not alter the firmness of smoked AC fillets to a great

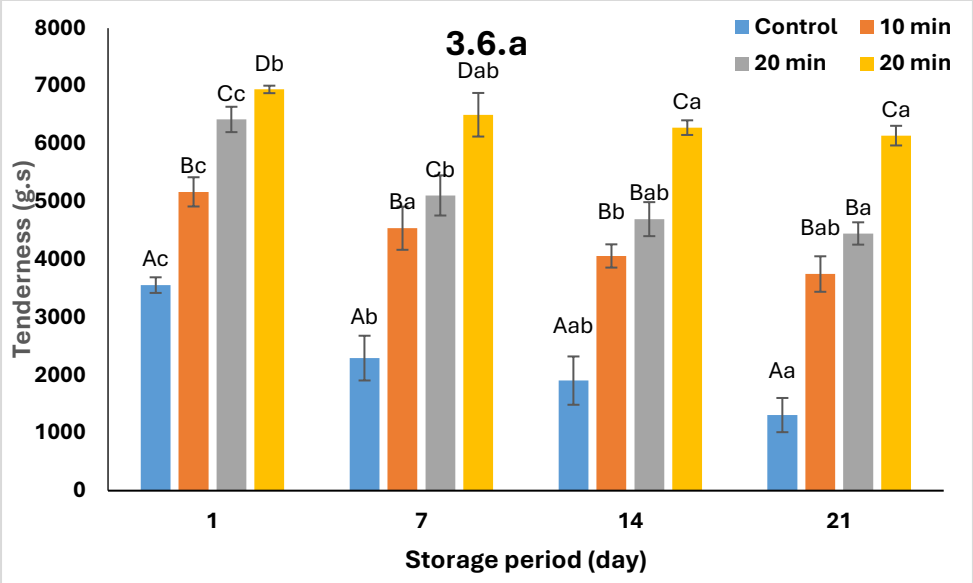
extent. In AC350, the 10 min and 20 min treatments are statistically similar after Day 1, following which they decline, but in a similar fashion.

3.5.1.2 Effect on tenderness

The impact of HP treatment and storage on tenderness is shown in Figure 3.6, analogous to the format employed for firmness. Figure 3.6 illustrates the effect on tenderness via bar graphs segmented into two subplots, (a) and (b). This denotes treatment effects at pressures of 150, 250 and 350 MPa for 30 min (AC30), in addition to time effects (10-30 min) at 350 MPa. The figure illustrates the impact of treatment duration and storage time, encompassing HP treatment durations of 10 to 30 min and storage intervals of up to 21 days at 4 °C. Error bars are displayed for each data point, and statistical significance is denoted with letter codes.

Figure 3.6a illustrates the time effects at a constant pressure of 350 MPa on the tenderness values. It was interesting to note that the tenderness of all AC350 samples exhibited significant differences until Day 7, after which no statistical differences ($p>0.05$) were observed between the samples treated for 10 min and those treated for 20 min (indicated by uppercase letters). The 30 min treated sample showed significantly ($p<0.05$) higher firmness value throughout storage, as observed in firmness.

Figure 3.6b indicates the pressure effects at a constant time of 30 min. Notably, each treatment was statistically different at all storage intervals, as denoted by uppercase letters. A consistent pattern emerges, indicating that the greatest tenderness correlates with the longest holding time.



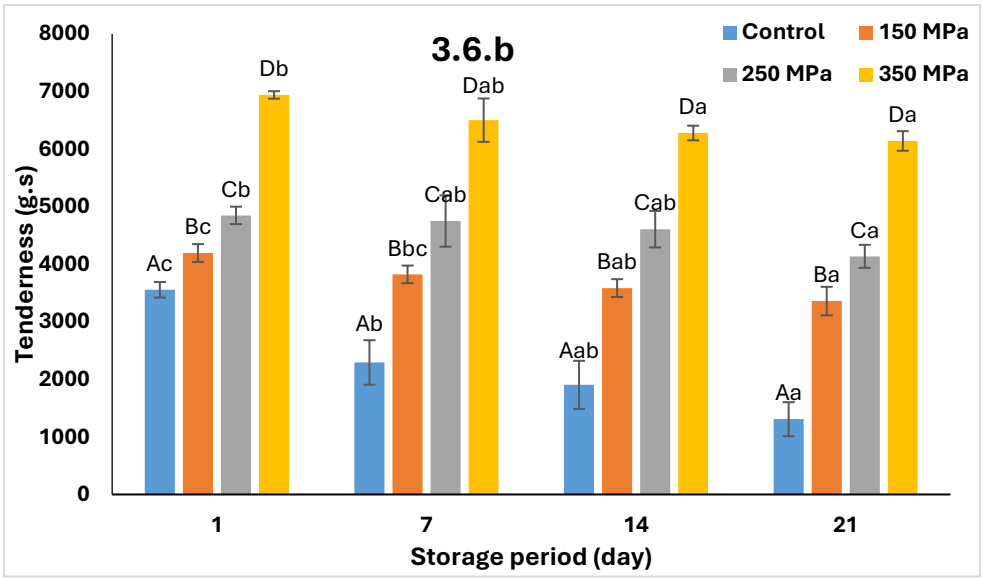


Figure 3.6. *Effect of HP treatment on tenderness (%) of treated hot smoked AC as a function of treatment time (min) and 4 °C storage time (days) at* ***(a)350 MPa*** *and* ***(b) 30 min****. For each evaluation day, different uppercase letters indicate significant differences among treatment times (p <0.05) and lowercase letters indicate significant differences of treatments across evaluation days (p <0.05).*

The tenderness retention percentage is illustrated in Table 3.9 and 3.10 for AC350 and AC30, respectively. On Day 7, it was observed that the sample treated at 150 MPa for 30 min exhibited superior texture retention compared to the sample treated at 350 MPa for 10 min, indicating that

a longer holding time at lower pressure is more effective than higher pressure with shorter holding time. A moderate pressure of 250 MPa for 30 min exhibited the highest retention until Day 14 among all treatments, with a 10% decrease in retention rate by Day 21, showing a marginal difference of 3% compared to the 350 MPa treatment for 30 min.

Table 3.9. Retention (%) for tenderness of hot smoked AC sample treated at 350 MPa

Treatment time (min)	Day 7	Day 14	Day 21
0	64.5	53.5	36.8
10	87.9	78.6	72.5
20	79.5	73.1	69.3
30	93.7	90.5	88.5

Table 3.10. Retention (%) for tenderness of hot smoked AC sample treated for 30 min

Treatment pressure (MPa)	Day 7	Day 14	Day 21
0	64.4	53.5	36.8
150	91.2	85.5	80.1
250	98.0	95.1	85.3
350	93.7	90.5	88.5

Smoking the fish at controlled humidity creates a tough surface while keeping the flesh moist and tender. Water loss, fat diffusion, structural and connective tissue protein denaturation, and proteolysis cause textural change in hot-smoked fish. The sarcolemma and myocommata connective tissue gelatinize completely during hot smoking (Sikorski & Kolodziejska, 2002). Wu *et al*., (2014) showed the importance of collagen deterioration on the texture of seafood. Post-mortem, fish muscle softens quickly due to the breakdown of the extracellular matrix

(ECM), particularly collagen. This is driven by proteolytic enzymes like calpains, cathepsins, and matrix metalloproteinases (MMPs), which degrade collagen and other structural proteins. This enzymatic activity results in a loss of muscle integrity and soft texture during cold storage. High-pressure processing can mitigate this degradation by inactivating these enzymes. By preserving collagen and maintaining muscle structure, HPP helps to retain and extend shelf life while ensuring better texture stability during storage.

3.5.2 Effect on color

Color is the primary attribute of food that affects purchase intention. For fish, appearance is crucial for consumers as it signifies the product's freshness. Appearance is a primary criterion consumers utilize to assess the quality of food products. The color of a product can frequently indicate its pigment content, which is often a measure of quality. Quality is not a singular, clearly defined attribute; rather, it encompasses numerous properties or characteristics (Pathare *et al*., 2013).

3.5.2.1 Effect on lightness (L*) and yellowness (b*)

Samples subjected to 350 MPa (AC350) for varying durations (10, 20, 30 min) exhibited significant effects on both b* and L* values, as seen in Figure 3.7a and Figure 3.7b. On Day 1, all treated samples exhibited statistically comparable L* values, signifying negligible initial variations attributable to holding time. Extended storage, however, resulted in longer holding times (20 and 30 min), preserving superior stability of L*, with minimal alterations compared to the untreated and 10 min treated samples, which exhibited considerable variability. For b*, extended holding durations exhibited enhanced stability, with 20 and 30 min treatments showing minimal alterations throughout the storage period, while untreated and 10 min treated samples exhibited greater variability and diminished overall stability.

The impact of HPP for 30 min (AC30) at different pressures (150 MPa, 250 MPa, 350 MPa) on Arctic char (shown in Figure 3.8a and b) exhibited significant ($p<0.05$) variations in yellowness (b*) and Lightness (L*). On Day 1, the untreated, 150 MPa, and 250 MPa samples exhibited similar L* values, whereas the 350 MPa sample demonstrated a markedly elevated lightness. Over time, all samples demonstrated increases in L*, but by Day 21, the untreated, 150 MPa, and 250 MPa samples were statistically similar ($p>0.05$) and retained a brighter appearance, whereas

the 350 MPa sample remained the lightest of all. For b*, the 350 MPa treatment exhibited notable increases in yellowness relative to lower pressures. Although all treatments demonstrated an increase in b* during storage, the 150 MPa sample exhibited the greatest stability, followed by the 350 MPa sample, which revealed significant differences only after day 14. The results indicate that increased pressures or extended holding durations can improve color stability in Arctic char during storage.

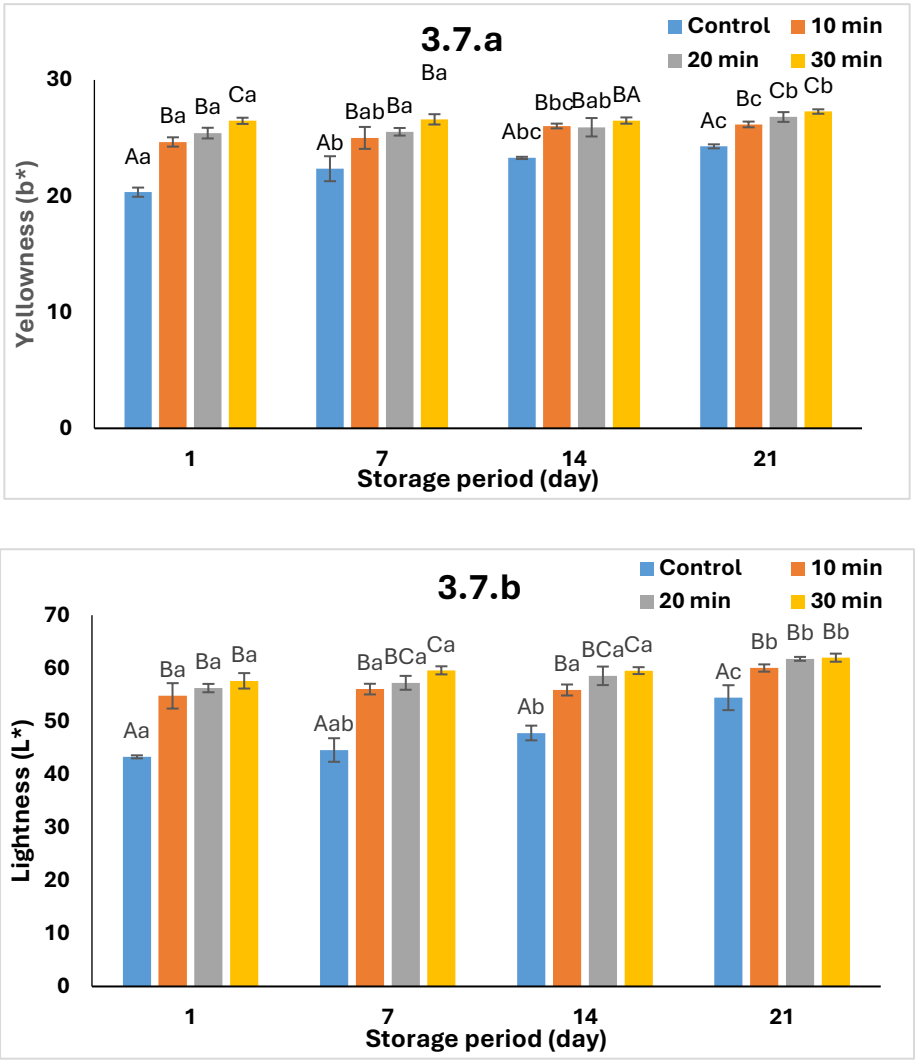
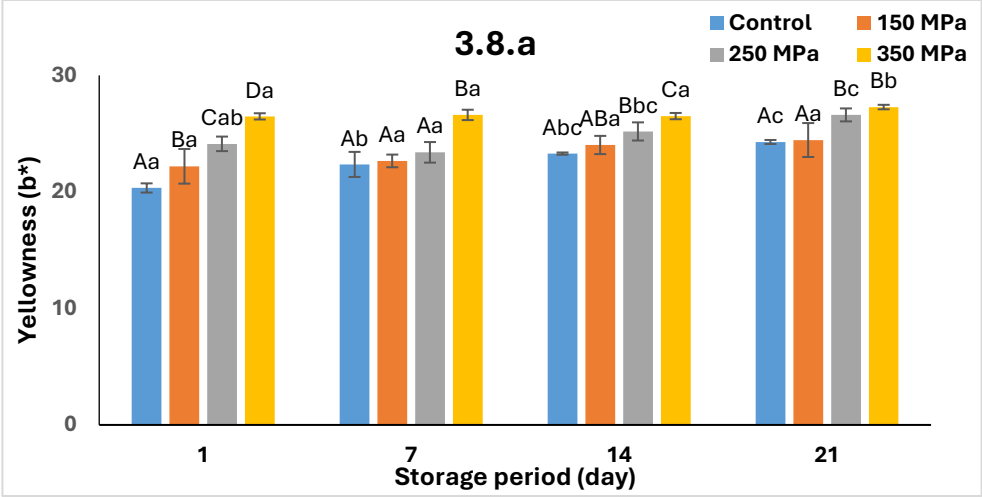


Figure 3.7. *Effect of HP treatment on* ***(a) yellowness (b*)*** *and* ***(b) lightness (L*)*** *of treated hot smoked AC as a function of treatment time (min) and 4 °C storage time (days) at 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).*



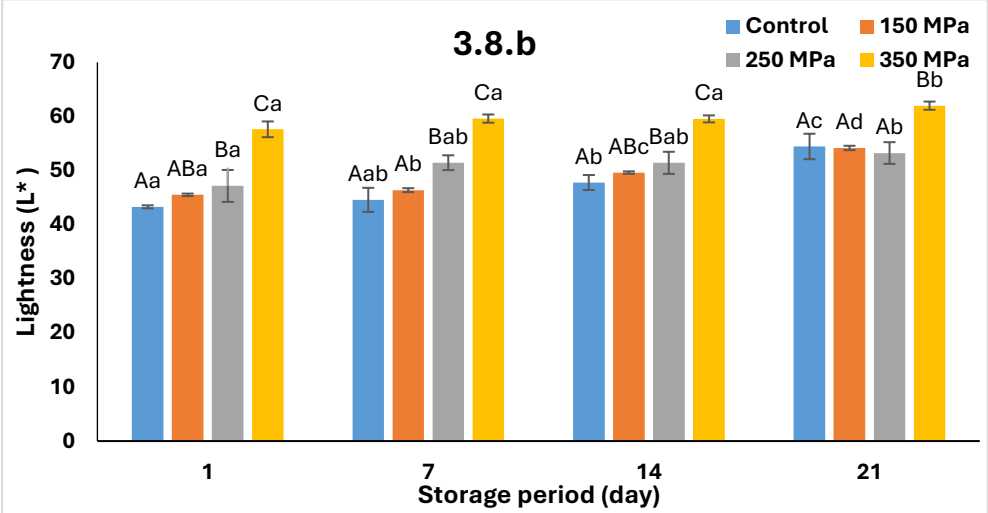


Figure 3.8. *Effect of HP treatment on* ***(a) yellowness (b*)*** *and* ***(b) lightness (L*)*** *of treated hot smoked AC as a function of treatment time (min) and 4 °C storage time (days) for 30 min. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).*

The lightness value (L*) was reported to have decreased in a study conducted by Tsai *et al.* (2022) on yellowfin tuna where the L* value (lightness) of the control group rose from 28.9 to 56.9 in the 600 MPa group as pressure increased ($p < 0.05$), whereas the b* value showed a slight upward trend from -4.29 in control to -1.50 in 600 MPa group. The heightened lightness was assumed to have resulted from reduced pigment activity and protein denaturation, which alter the surface properties of the sample, enhancing light reflection and yielding a white appearance. Similar results were observed in Atlantic Salmon dark muscle, where the highest-

pressure level (300 MPa) resulted in a cooked appearance, increasing 32% in L* value and decreasing 13% in a* value for dark muscle at the end of the storage period. This was reportedly attributed to protein denaturation, resulting in a lighter appearance (Yagiz *et al*., 2009).

3.5.2.2 Effect on a*

The redness (a*) value results as influenced by pressure and is presented in a similar format in Figure 3.9 described for L* values. As compared to L* values, the effect of pressure on a* was reversed. The samples treated had a lower a* value as compared to the control and as the storage time increased, the a* decreased further for all samples.

Figure 3.9a shows the effect on the redness of samples subjected to 350 MPa for 10, 20 and 30 min (AC350). The results were similar to the AC30, where the untreated sample had a significantly ($p<0.05$) higher redness value than the treated ones, whereas the treated ones remained more or less similar throughout storage, with 30 min having a more cooked appearance than the rest. However, by the end of storage on Day 21, there aren't any significant ($p>0.05$) differences between the treated ones (indicated by the uppercase letters).

The effect of HP treatment for 30 min at different pressures (AC30) on smoked AC is shown in Figure 3.9b. With the application of HP, a significant decrease in a* was observed for all treatment times and on all days. During storage, it was further observed that the a* significantly decreased for all samples. Untreated samples (0 MPa) consistently had the highest a* values, indicating a stronger red color component. The redness for untreated samples declined slightly as storage progressed but remains more pronounced compared to treated samples. By the end of storage, differences in redness diminished among treatments, particularly between the 150, 250, and 350 MPa samples.

The retention percentage was calculated for both AC350 and AC30 as shown in Tables 3.11 and 3.12). Unlike the case of textural parameters, the retention of redness was higher in the higher-pressure treatments. The percentage of retention for the 150 MPa and 250 MPa samples was surprisingly lower than the untreated sample, although the difference was marginal. The 350 MPa treatments showed better retention throughout storage, suggesting that redness may be influenced negatively initially by higher pressure, but it can aid in stabilizing the loss occurring during storage.

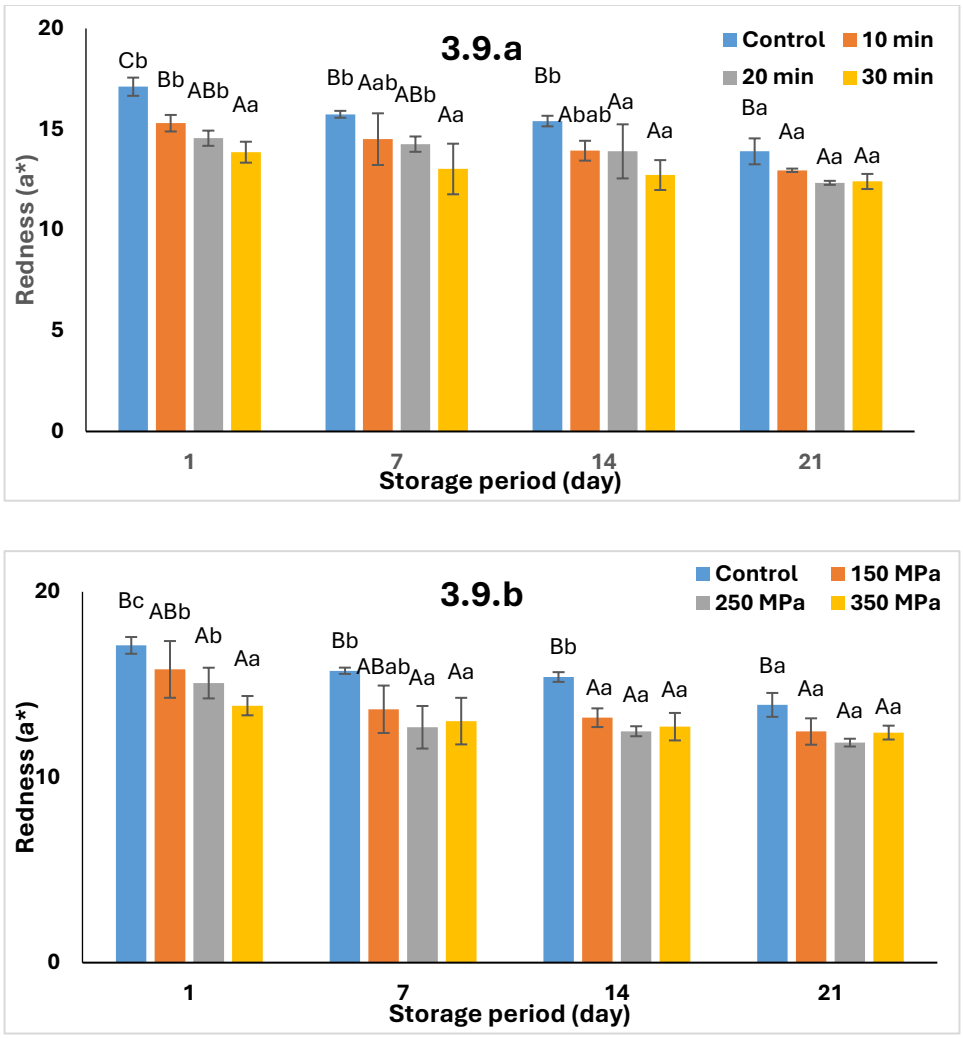


Figure 3.9. *Effect of HP treatment on redness (a*) of treated hot smoked AC as a function of treatment time (min) and 4 °C storage time (days) at* ***(a) 350 MPa*** *and* ***(b)30 min*** *. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$)*

Table 3.11. Retention (%) for redness (a*) of hot smoked AC treated at 350 MPa

Treatment time (min)	Day 7	Day 14	Day 21
0	92.0	90.0	81.2
10	94.8	91.1	84.7
20	98.0	95.5	84.8
30	94.0	91.8	89.5

Table 3.12. Retention (%) for redness (a*) of hot smoked AC treated for 30 min

Treatment pressure (MPa)	Day 7	Day 14	Day 21
0	92.0	90.0	81.3
150	86.4	83.5	78.8
250	84.2	82.8	78.7
350	94.0	91.8	89.5

Overall, the a* values, used as an index of visual redness, was observed to have decreased to a small extent initially in the treated samples. The untreated samples with higher redness, however, had less retention during storage, suggesting that HPP can stabilize the changes associated with redness. This aligned with other studies conducted on different species of fish such as Tuna, where they observed only minor changes in the a-value, whereas the other factors, attributes like L* and b*, changed significantly at pressures beyond 150 MPa- 200 MPa, resulting in a slightly cooked appearance (Matser *et al*., 2006).

3.5.2.3 Effect on hue

Hue angle (h*), regarded as the qualitative characteristic of color, is the criterion by which colors have been conventionally categorized as reddish, greenish, etc., and it serves to delineate the variation of a certain hue in relation to gray of equivalent lightness. This feature pertains to the variations in absorbance across distinct wavelengths. A greater hue angle indicates a reduced yellow characteristic in the tests. An angle of 0° or 360° signifies a red hue, whereas angles of 90°, 180°, and 270° denote yellow, green, and blue hues, respectively (Pathare *et al*., 2013).

Table 3.13 and Table 3.14 illustrate the effect of HP treatment on hue angle, affected by HP treatment in combination with refrigerated storage at 4°C for 21 days. The hue angle was calculated using the same equation used in the previous chapter. As hue angle is a factor of both a* and b*, with b* in the numerator, the hue angle showed an increasing trend with increasing storage, higher holding time as well as higher magnitude of pressure. The untreated sample had a

hue angle of 50, suggesting a reddish orange appearance. The samples treated, however, showed a higher value suggesting a shift towards yellow, which can be correlated to the b* value.

Table 3.13. Effect of HPP on hue of hot smoked AC treated at 350 MPa

Day	Control	10 min	20 min	30 min
1	49.9 ± 0.86^{Aa}	58.2 ± 1.00^{Ba}	60.2 ± 1.00^{Ba}	62.4 ± 1.14^{Ca}
7	54.8 ± 1.33^{Ab}	60.6 ± 2.76^{Bab}	60.8 ± 0.71^{Ba}	64.0 ± 2.31^{Bab}
14	56.5 ± 0.37^{Ab}	61.5 ± 1.10^{Bab}	61.8 ± 2.32^{Ba}	64.4 ± 1.38^{Bab}
21	60.2 ± 1.32^{Ac}	63.4 ± 0.81^{Bb}	65.3 ± 0.47^{Cb}	65.5 ± 0.69^{Cb}

Table 3.14. Effect of HPP on hue of hot smoked AC treated for 30 minutes

Day	Control	150 MPa	250 MPa	350 MPa
1	51.2 ± 0.79^{Aa}	54.6 ± 1.44^{Aba}	58.0 ± 1.69^{Ba}	62.4 ± 2.87^{Ca}
7	56.9 ± 2.72^{Aab}	58.9 ± 2.85^{Aab}	61.5 ± 2.43^{Abb}	66.1 ± 1.69^{Bab}
14	58.0 ± 2.15^{Aab}	61.2 ± 1.46^{Ab}	63.6 ± 0.79^{Bbc}	67.0 ± 1.06^{Cb}
21	62.0 ± 5.82^{Ab}	62.9 ± 2.82^{Abb}	66.0 ± 0.81^{ABc}	69.0 ± 0.98^{Bb}

3.5.2.4 Effect on ΔE

The difference between two colors in the three-dimensional L*a*b* color space is referred to as ΔE (Yeole *et al*., 2018). It was calculated with respect to the L*, a* and b* values obtained on Day 1 and Day 21 as shown in Tables 3.15 and 3.16. The results obtained suggest that there was a greater color change between Day 1 and Day 21 of storage in the untreated samples, compared to the treated samples, with 350 MPa for 30 min having the least changes in color due to better retention.

Variations in perceivable color can be analytically categorized as very distinct ($\Delta E>3$), distinct ($1.5<\Delta E<3$) and small difference ($1.5<\Delta E$) (Adekunte *et al*., 2010). Overall, we can conclude that smoked AC fillets undergo major color changes during refrigerated storage, however, it can be slowed down by subjecting the samples to HP treatment, which minimizes these changes occurring due to enzyme activity, microbial growth etc.

Table 3.15. Effect of HPP on ΔE of hot smoked AC treated at 350 MPa

Control	10	20	30
12.3 ± 1.86B	6.0 ± 1.80A	6.2 ± 0.40A	4.8 ± 0.87A

Table 3.16. Effect of HPP on ΔE of hot smoked AC treated for 30 min

Control	150	250	350
12.3 ± 1.86B	5.35 ± 0.86A	7.64 ± 3.74AB	4.80 ± 0.87A

3.5.3 Effect on microbial growth

Fish is highly perishable and deteriorates rapidly unless different preservative methods, like smoking, are employed. Smoking reduces spoilage and pathogenic bacterial activities due to the integrated effect of heating, drying, salting, and also as a result of the deposition of polyphenolic compounds (Hagos, 2021). Although it has all these qualities, it can still facilitate bacterial growth two or three weeks after smoking. The present study was aimed at extending the shelf life, at least by 21 days, under refrigerated conditions, and the presence of aerobic mesophiles was evaluated through the Total Plate Count method (Aerobic plate count, APC).

The growth of aerobic mesophiles, as estimated by total plate count for the untreated smoked AC and the pressure treated samples are illustrated in Table 3.17 for samples treated at 350 MPa at varying times (AC350) and Table 3.18 shows the results of APC on samples treated for 30 min to study pressure effects at constant time (AC30). Tables 3.17 and 3.18 represent the growth of AP as logarithmic (CFU/g) of all samples across 21 days of 4°C refrigerated storage. It was observed that HP treatment had a detrimental effect on microbial growth throughout the storage. It was observed that the only treated sample that supported microbial growth was 150 MPa treated for 30 min, on the 21st day of storage. All the other treatments did not show any sign of growth throughout the storage study, expressed as ND (not detected). However, the untreated smoked AC started showing microbial growth, day 7 onwards, clearly indicating the effectiveness of HP treatment.

Table 3.17. Effect of HPP on microbial growth of hot smoked AC treated at 350 MPa and different holding times

Day	Control	10 min	20 min	30 min
1	ND	ND	ND	ND
7	1.44 ± 1.24^{Aab}	ND	ND	ND
14	2.20 ± 0.17^{Bb}	ND	ND	ND
21	5.28 ± 0.04^{Bc}	ND	ND	ND

Table 3.18. Effect of HPP on microbial growth of hot smoked AC treated for 30 min and different pressures

Day	Control	150 MPa	250 MPs	350 MPa
1	ND	ND	ND	ND
7	1.44 ± 1.24^{Aab}	ND	ND	ND
14	2.20 ± 0.17^{Bb}	ND	ND	ND
21	5.28 ± 0.42^{Cc}	3.44 ± 0.06^{Bb}	ND	ND

Based on the above results, it was concluded that HP treatment delayed the onset of bacterial growth of fish, especially smoked fillets stored at refrigerated conditions. This observation is consistent with the findings by Montiel *et al*., (2012) on pressurized smoked cod treated at 400, 500, and 600 MPa for 5 and 10 min, respectively, and observed that elevated pressure markedly decreased the microbial count in smoked cod and inhibited microbial proliferation in fish meat during cold storage (5°C). Therefore, it was suggested that smoked cod should be pressurized at 400 MPa for 10 min or 500 MPa for 5 min for prolonging storage life. Similarly, a study by Tsai *et al*., (2022) revealed that tuna samples when stored at 4°C following high-pressure processing at 200, 300, 400 MPa, and 500 and 600 MPa, could extend shelf life by 3, 6, 9 and 15 days, respectively, based on the day in which it exceeded the standard limit of 6.47 log CFU/g.

Conclusions

This study investigated the effects of HPP on shelf-life stability of hot smoked fillets of trout and arctic char by evaluating their physicomechanical and microbial quality on Day 1, 7, 14 and 21 of storage. The smoked trout and AC were subjected to HP treatment at 350 MPa for 10, 20 and 30 min and it was observed that AC showed higher values for firmness and tenderness throughout storage and that both HP treated AC and trout had higher retention of texture compared to the untreated samples. However, when it comes to comparison of texture retention between species, they showed similarity on Day 7, but hot smoked trout showed notably higher retention rates until Day 21. The Lightness of the trout samples was significantly higher than AC, owing to its cooked appearance. The lightness and yellowness of all the samples increased and the redness decreased throughout storage. However, the loss of these attributes was slower across 21 days of storage compared to the control. AC treated at 150 MPa and 250 MPa for 30 min showed lower textural values than 350 MPa and the 150 MPa treatment did not show significant differences with the control in terms of color and texture at certain points of storage. This suggested that low pressure at high holding time may not impact the quality of the fillets to a greater degree. This result can be correlated to microbial growth as well. The 150 MPa samples started showing APC starting on day 21, whereas the other pressure-treated samples did not show any signs of growth, indicating that higher pressure at higher holding time can effectively improve the shelf-life stability of refrigerated smoked fillets.

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CONNECTING STATEMENT TO CHAPTER 4

The previous chapter focused on the use of HPP on hot smoked trout and AC to prolong the refrigerated shelf life of fillets. The use of three pressures and three holding times gave a conclusion that fillets treated at a pressure of 350 MPa gave a higher retention of both color and texture. However, microbial growth could be retarded even with lower pressure treatment. The this chapter aims at studying and optimizing processing conditions for evaluating the quality and storage stability of shrimp at refrigerated conditions. For this purpose, response surface methodology (RSM) was used and an experimental design consisting of 13 runs with axial points at 100 MPa and 300 MPa pressure, and holding times 10 min and 30 min was obtained. This study sought to optimize processing conditions for each checkpoint of refrigerated storage on days 1, 7, 14, and 21, with the understanding that there would be minimal or reduced microbial growth on the processing day. Consequently, the optimized conditions may involve mild treatments that do not induce other quality alterations, such as changes in texture or color, as observed in the preceding chapter. From a customer's standpoint, if one intends to purchase shrimp for storage over 14 days, a more intensive treatment is required compared to shrimp intended for immediate consumption on Day one.

CHAPTER 4

EFFECT OF HIGH-PRESSURE PROCESSING ON QUALITY AND REFRIGERATED STORAGE STABILITY OF SHRIMP

ABSTRACT

Crustaceans constitute the predominant seafood sector, and shrimps and prawns are the second most exported species. Nonetheless, mechanical damage to fish during catching, together with microbial deteriorations and safety-related issues in post-harvest handling, icing, packaging, processing, storage, and distribution can cause losses of billions of tons every year for fish producers, processors, and distributors. An optimum HP treatment that can extend the shelf life of shrimp was obtained through response surface methodology (RSM). A central composite design (CCD) was used to assess the impact of two principal factors: pressure (MPa) and time (min) on ten measured response variables, specifically total aerobic psychrophiles (Log CFU/g), hardness (g), gumminess, springiness (%) L*, a*, b*, hue, chroma and ΔE. The experimental design comprised 13 trials with 5 central points to guarantee the precision and dependability of the response surface model. The shrimp samples were peeled by hand, vacuum sealed, and subjected to HP treatments at ambient conditions. These samples were stored at 4°C and evaluated on days 1, 7, 14, and 21. The applied pressure treatment resulted in an enhancement of textural properties such as hardness and gumminess, which may have also caused to elevate lightness and a decrease of redness of the samples. It was observed that the more severe HP treatments (higher pressure and longer holding time) resulted in reducing the microbial growth, thereby extending the shelf life to more than 21 days in refrigerated conditions.

Keywords: RSM, CCD, texture, HP treatment, psychrophiles

4.1. Introduction

High-pressure processing (HPP) is particularly suitable for seafood that is commonly consumed raw or minimally cooked. This non-thermal technology yields fresh-tasting, minimally processed foods with the same level of food safety as heat pasteurization (Hsu *et al*., 2014). The adoption of this technology reportedly increases shelf-life, with minimum loss of quality. Furthermore, it does not result in undesirable alterations associated with thermal processing,

whilst maintaining the nutritional value of the food (Zhao *et al*., 2019). However, the extremely perishable seafood products are to be properly processed and preserved to ensure quality and safety. Quality deterioration of seafood products include changes in fish odor, flavor, texture, protein degradation, lipid oxidation etc., leading to short shelf life, and poor consumer acceptance (Bonfim *et al*., 2024).

Employing traditional methods of preservation, such as freezing, packaging, chemical treatments etc. are some ways to prevent microbial or enzymatic spoilage, rancidity, or lipid oxidation. Some of the traditional preservation strategies, though easy to carry out, may help to suppress proteolytic reactions and spoilage effectively, but also result in affecting the product quality. It is in this context that advanced technologies such as modified atmosphere packaging, active packaging, edible coatings, high-pressure preservation, etc. hold significance in ensuring the quality of the seafood (Peng *et al*., 2022). Moreover, adopting the appropriate methodology of food preservation holds tremendous significance in preventing food wastage and hence has always been given high priority, as the issue is of great concern, affecting the quality of life of millions of ordinary people across the globe (Rabiepour *et al*., 2024).

Historically, shrimps have been one of the extensively traded seafood products, with the major producers being Asia and Latin America, and China as an important emerging market (FAO, 2020). Fish and other seafood products are commodities of high commercial value, owing to their good-quality proteins, unsaturated fatty acids, minerals, and vitamins. These highly essential and beneficial dietary factors for human nutrition are well appreciated globally. Polyunsaturated fatty acids (PUFAs), especially omega-3 and omega-6 fatty acids, help lower blood pressure and plasma triglyceride levels, prevent atherosclerosis, thrombosis, and coronary heart disease, and alleviate autoimmune disorders and arrhythmias (Peng *et al*., 2022).

Shrimps possess a semitransparent body, compressed from side to side, a flexible abdomen terminating into a fanlike tail, long whip-like antennae, and appendages modified for swimming in shallow and deep waters - both freshwater and marine (Chen *et al*., 2022b). Like other marine foods, shrimp also deteriorates easily, causing degradation of color, flavor, and texture due to enzymatic activities and microbial action, facilitated by high-water content (Wu *et al*., 2021).

Quality of shrimp products fluctuates significantly due to internal and external factors such as drip loss, protein degradation, softening, discoloration, and decomposition of nucleotides (Yu *et al*., 2020). Moreover, quorum sensing and metabiosis, temperature, nature of habitat, season of harvest, storage conditions, and methods of processing also influence shrimp quality in terms of nutrition and commerce (Calliauw *et al*., 2016). In addition to the above factors, monovalent copper ions (Cu+) present in dead shrimp are oxidized to bivalent copper ions (Cu2+), inducing tyrosinase to produce melanosis to give body darkness, and other metabolic changes that raise serious food safety concerns (Gonçalves & de Oliveira, 2016).

During the development of a product or process, a proper experimental design plays a vital role. This is true in the case of food products, including seafood like shrimp. To develop a superior shrimp product, high nutritional value, safety standards, consumer acceptance, and commercial viability, which are critical parameters of an ideal product formation, are to be optimized.

Optimization is extensively used to find the best combination of parameters, among all the available possibilities, resulting in ideal product formation (Reji & Kumar, 2022). An excellent experimental design requires a strong understanding of the system under study. Response surface methodology (RSM), a mathematical and statistical technique for designing experiments, is used to optimize a response that is influenced by several independent variables. RSM was employed for its versatile application in research, where representative minimal experimental runs yield ideal trends through analysis of variance (ANOVA). A fundamental characteristic of RSM is to derive a statistically significant model for each response based on process factors. (Shinde & Ramaswamy, 2021). Several researchers have used response surface methodology for optimizing process parameters and developed a regression equation to establish a response (Shinde & Ramaswamy, 2023; Meng *et al*., 2021; Alsalman & Ramaswamy, 2021; George & Ravishankar, 2022). A second order polynomial regression equation as shown in equation below is used to predict the response by considering the input parameters.

$$S = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i=1}^{k} a_{ii} x_i^2 + \sum_{j=1 j \neq i}^{k} a_{ij} x_i x_j \qquad (1)$$

where S is a response, a_0 is the average response; ai, aii and aij are response coefficients. The second, third, and fourth terms denote linear, higher order and interaction effects, respectively (Chelladurai *et al*., 2020). Therefore, RSM is widely used in situations where multiple input variables (independent or dependent factors) influence the performance measure of a process (Kleijnen, 2015).

Therefore, the objective of this chapter is to optimize processing parameters, pressure (MPa) and time (min), with an aim of minimizing microbial growth on each checkpoint of storage, while studying the effect of HP treatment on other quality parameters such as texture and color during refrigerated storage.

4.2. Materials and methods

4.2.1. Sample Preparation

Frozen raw shrimp were received from the seafood industry and transferred to the lab under frozen storage conditions. The samples were thawed overnight under refrigeration. On the day of treatment, the thawed raw shrimp was peeled by hand, removing its skin, head, and legs. Afterwards, it was packed and vacuum sealed in a high-density polyethylene (HDPE) pouch. The air inside these pouches was carefully removed before sealing to prevent the sample bags from floating inside the HPP unit chamber. Each sample bag contained 4 shrimp weighing approximately 40 ± 5g intended for quality tests, with each treatment having 4 separate bags for analysis on Days 1, 7, 14, and 21.

For microbiological tests, the samples were thawed similarly but were packed aseptically in a laminar air flow hood to prevent any cross-contamination. 5 g of sample was weighed in a disinfected weighing balance, transferred to a sterile pouch, and sealed aseptically. There were four pouches of 5 g for each treatment, intended for testing on Days 1, 7, 14, and 21. After HP treatment, the samples were all transferred under ice conditions and stored in a refrigerator at 4°C.

4.2.2. Experimental design

An RSM was employed to evaluate the influence of independent variables: pressure (MPa) and holding time (min) on the textural properties, color changes, and bacterial growth in shrimp. The experiments were designed according to the central composite rotatable design (CCRD) using

Design Expert version 10.1.1 software (Stat-Ease, USA). The experimental design illustrated in Table 4.1 includes five levels for each of two independent variables, with a total of 13 experiments consisting of four 2 x 2 corner points, four extended points (±α) and 5 replications at the center point. This type of central composite design is the most commonly used response surface designed experiment. The pressure time combination for the treatments was chosen based on the literature.

Table 4.1. Experimental design of the factors pressure (MPa) and time (min) for storage study of shrimp.

PRESSURE (MPa)	TIME (min)
58.57	20
100	10
100	30
200	5.8
200	20
200	20
200	20
200	20
200	20
200	34.14
300	10
300	30
341.42	20

4.2.3. Texture profile analysis

The texture profile of the samples was evaluated using a texture analyzer (TA. XT-Plus, Texture Technologies Corp., NJ., USA) equipped with a 50 mm cylindrical probe. The whole shrimp was compressed up to 40% of its original height at a test speed of 2 mm/s. The parameters such as

hardness (g), gumminess, and springiness (%) were then calculated using Texture Exponent 32 software connected to a texture analyzer.

4.2.4. Color measurement

The color of the shrimps was measured with a Minolta Tristimulus Colorimeter (Minolta Crop, Ramsey, NJ, USA). The data for color was generated from the Spectra Magic software (Minolta Crop, Ramsey, NJ, USA), as was previously described in Chapter 3.

4.2.5. Microbial analysis

Total Aerobic Psychrophiles were determined using direct plate count. The microbial enumeration was done as described in Chapter 3 with the exception of incubation time and temperature. In this case, the TSA plates were incubated at 4°C for 7 days, and 10 days if no growth was observed on Day 7.

4.3. Results and Discussions

4.3.1 Response surface methodology

The output response results from the CCRD design of experiments for all days of storage is summarized in Table 4.2-4.5. The tables detail the selected RSM models for each response based on their statistical significance as well as lack of fit values. The cubic model was selected for all the responses, and the lack of fit was also tested. A non-significant lack of fit explains that the selected model can be used to predict the responses using the predicted equations (Table 4.2 - 4.5) for each response (Hardness, L* etc.) on day 1, 7, 14 and 21, as a function of the input variables with 95% confidence.

4.3.2. Effect of HPP on microbial growth

Crustaceans such as prawns and shrimps are nutrient-dense with high protein content. However, owing to high-water activity and neutral pH, they exhibit high susceptibility to spoilage by microorganisms that are present as normal microbiota (Parlapani, 2021). Chilling is the most common method of storing seafood for a short duration. Psychrophiles are typically characterized as organisms that exhibit optimal growth below a certain temperature, usually 20°C or 12 to 18°C, and sometimes as low as 5 to 10 °C (Mol *et al*., 2007).

The response surface plots showing the effects of pressure and time on the growth of aerobic psychrophiles (AP) are presented in Figure 4.1. The model chosen was cubic due to its high R^2 value of 0.93 and it was observed by the ANOVA that the lack of fit of the model was significant ($p<0.05$), with a p value of 0.0005. Pressure has a significant effect until Day 14 of storage meaning that the increase in pressure resulted in reduced microbial growth. The effect of time was not significant on most days of storage, meaning increasing or decreasing time did not show a significant effect on keeping the microbial growth under control.

Table 4.2. Predicting equations and combined ANOVA results for CCRD responses on Day 1

Sl No	Responses	Equation	Lack of fit	R^2
1	Color (L)	58.69 + 4.25 *A + 0.042* B -2.44 *AB - 2.97*A^2 - 0.14 * B^2 - 0.053 * A^2B + 2.67 *AB^2	0.0733	0.983
2	Color (a)	7.58 + 0.43* A -0.8* B + 0.53* AB-1.94* A^2 + 1.06* B^2 + 0.56* A^2B + 0.9* AB^2	0.1601	0.552
3	Color (b)	9.91+ 0.45* A+ 1.68* B-1.5* AB+0.26* A^2-1.06* B^2-3.02* A^2B + 1.87* AB^2	0.2903	0.758
4	Chroma	12.65+ 0.59* A+ 0.4* B-0.97* AB-0.99* A^2 - 0.034* B^2-1.66*A^2B+2.13* AB^2	0.0481	0.550
5	Color (Hue)	52.95-0.93* A+7.29* B-7.14* AB+7.57* A^2 - 6.43* B^2-8.46* A^2B+0.88* AB^2	0.5445	0.701
6	Hardness (g)	1647.23+ 552.66*A+9.77* B-33.93* AB+33.73* A^2-51.31* B^2-39.6* A^2B-190.87* AB^2	0.0015	0.963
7	Springiness (%)	53.56+6.47*A+0.83* B-0.63* AB-1.48* A^2+ 2.94*B^2+ 0.7* A^2B-12.1*AB^2	0.0036	0.924
8	Gumminess	769.44+274.69*A+54.92* B-31.02* AB-33.47* A^2-25.32* B^2-65.19* A^2B+ 44.71* AB^2	0.0013	0.939
9	Log CFU/g	3.23-1.6*A-0.26*B-0.005*AB-0.62*A^2-0.049*B^2-0.26*A^2B-0.66*AB^2	0.0003	0.931

where A is pressure (MPa), B is time (min).

Table 4.3. Predicting equations and combined ANOVA results for CCRD responses on Day 7

Sl No	Responses	Equation	Lack of fit	R^2
1	Color (L)	60.25+3.53* A+0.41* B-1.32* AB-1.67* A^2-1.27* B^2-1.11*A^2B+2.66* AB^2	0.8635	0.9485
2	Color (a)	5.42+0.39* A+0.24* B-8.23E-03* AB+0.025* A^2+1.29* B^2+0.37* A^2B+0.49* AB^2	0.0736	0.7026
3	Color (b)	12.37-0.51* A-1.06* B+0.83* AB+0.42* A^2+0.35* B^2+1.75* A^2B+3.65* AB^2	0.0601	0.6765
4	Chroma	13.52-0.32* A-0.78* B+0.72* AB +0.38*A^2+0.91*B^2+1.7*A^2B+3.49* AB^2	0.0462	0.6453
5	Color (Hue)	66.31-2.02* A-2.56* B+1.9* AB+0.17* A^2-3.95* B^2+0.98*A^2B+4.94* AB^2	0.8255	0.8473
6	Hardness (g)	2447.85+636.93*A-31.47* B+123.86* AB-80.23* A^2-79.48* B^2+329.31* A^2B-26.3* AB^2	0.0874	0.9459
7	Springiness (%)	39.83+2.69* A-1.02* B-0.86* AB-1*A^2+0.66* B^2+2.43* A^2B-9.59*AB^2	0.0159	0.7983
8	Gumminess	966.19+314.11*A+8.49* B-81.78* AB-81.99* A^2-51.44* B^2-66.3* A^2B+0.96* AB^2	0.0779	0.9220
9	Log CFU/g	3.84-2.24* A-0.062* B-0.92*AB-0.11* A^2+0.28* B^2 -1.7*A^2B+0.46* AB^2	<0.0001	0.9674
10	Delta E	5.27-1.12*A-2.93*B+1.56*AB+0.080*A^2+0.30* B^2+3.88*A^2B+2.17*AB^2	0.5553	0.7992

where A is pressure (MPa), B is time (min).

Table 4.4. Predicting equations and combined ANOVA results for CCRD responses on Day 14

Sl No	Responses	Equation	Lack of fit	R^2
1	Color (L)	61.35+3.62* A-0.011* B-0.8* AB-2.23* A^2-0.19* B^2-0.028*A^2B+3.33*AB^2	0.7077	0.9590
2	Color (a)	5.18-0.17*A-0.6*B+0.4*AB-0.13*A^2+0.05* B^2+0.48* A^2B+0.72*AB^2	0.0748	0.5362
3	Color (b)	13.4+0.99*A+0.86*B+0.012*AB+0.77*A^2 +1.16*B^2-0.65*A^2B+3.52*AB^2	0.0155	0.7954
4	Chroma	14.37+0.89* A+0.63* B+0.16* AB+0.69* A^2+1.13* B^2-0.5*A^2B+3.54* AB^2	0.0164	0.7711
5	Color (Hue)	68.87+1.56* A+2.63* B-1.91* AB+1.08* A^2+0.93* B^2-1.38*A^2B+2.47* AB^2	0.2878	0.8965
6	Hardness (g)	2648.3+685.31*A+100.72*B+234.81*AB-146.67*$A^2$5.8*B^2+266.67*A^2B+7.58*AB^2	0.5273	0.8500
7	Springiness (%)	34.24+1.79*A-1.71*B+0.084*AB-0.34* A^2+1.09*B^2+1.95*A^2B-6.53*AB^2	0.0005	0.7842
8	Gumminess	1109.03+355.27*A+1.79*B-99.65*AB-102.76*A^2-79.3*B^2-69.73*A^2B-25.84* AB^2	0.0603	0.9171
9	Log CFU/g	5.26-1.02*A-0.36*B -0.46*AB +0.41*A^2+0.27*B^2-0.38*A^2B+0.074* AB^2	0.0008	0.9763
10	Delta E	5.87-0.068*A-0.78*B+2.11*AB+0.073* A^2+1.75*B^2+2.01*A^2B+2.02*AB^2	0.0996	0.6857

Where A is pressure (MPa), B is time (min).

Table 4.5. Predicting equations and combined ANOVA results for CCRD responses on Day 21

Sl No	Responses	Equation	Lack of fit	R^2
1	Color (L)	$62.15+4.4*A-0.1*B+0.88*AB-2.12*A^2-0.54*B^2+0.75*A^2B+0.5*AB^2$	0.3565	0.9538
2	Color (a)	$5.81+0.091*A+0.76*B+0.26*AB-0.55*A^2-1.05*B^2-0.3*A^2B-0.43*AB^2$	0.5859	0.2531
3	Color (b)	$16.15+0.92*A+1.26*B-1.17*AB+0.57*A^2+0.96*B^2+0.14*A^2B+2.34*AB^2$	0.1002	0.7055
4	Chroma	$17.4+0.91*A+1.39*B-1.08*AB+0.27*A^2+0.54*B^2+0.039*A^2B+2.19*AB^2$	0.0417	0.7074
5	Color (Hue)	$70.12+0.48*A-1.36*B-2.19*AB+2.18*A^2+3.96*B^2+1.61*A^2B+3.45*AB^2$	0.9245	0.3223
6	Hardness (g)	$2937.95+719.52*A+202.33*B+297.97*AB-260.09*A^2-39.58*B^2+232.97*A^2B-81.37*AB^2$	0.0006	0.9866
7	Springiness (%)	$30.37+0.39*A-0.56*B-2.61*AB-0.49*A^2+0.15*B^2+3.07*A^2B-2.09*AB^2$	0.0003	0.7184
8	Gumminess	$1337.61+421.85*A+24.24*B-106.24*AB-176.6*A^2-136.39*B^2-83.43*A^2B-52.16*AB^2$	0.0028	0.9363
9	Log CFU/g	$6.99-0.75*A-0.16*B-0.39*AB+0.41*A^2-0.14*B^2-0.52*A^2B-7.09E-03*AB^2$	0.0478	0.7104
10	Delta E	$8.75+0.45*A-1.18*B+1.13*AB+0.074*A^2+1.92*B^2+2.81*A^2B+0.15*AB^2$	0.4078	0.6051

Where A is pressure (MPa), B is time (min).

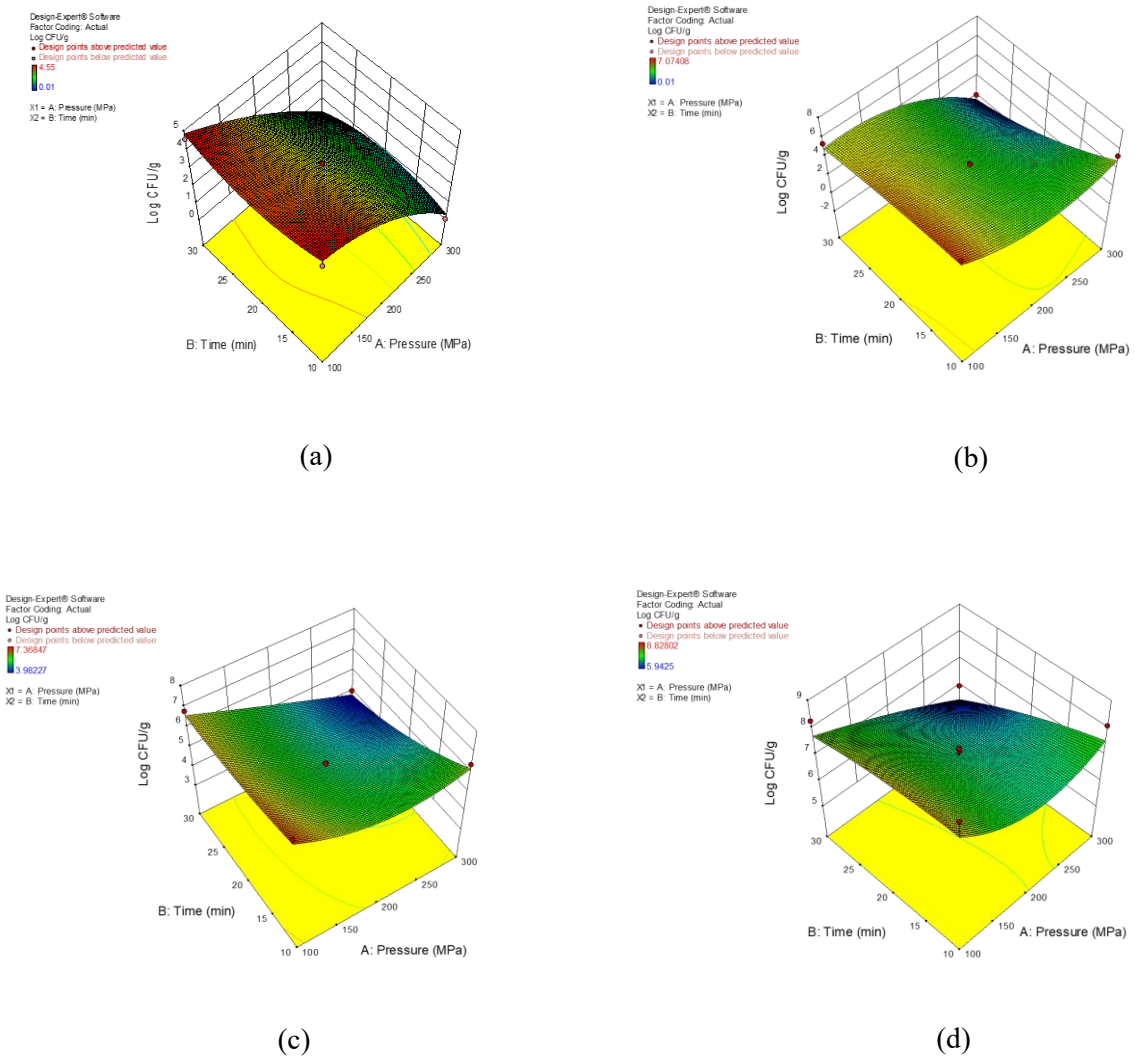


Figure 4.1. *Response surface plots for growth of aerobic psychrophiles affected by pressure and temperature on* ***(a) day 1, (b) day 7, (c) day 14*** *and* ***(d) day 21.***

From the table of regression coefficients shown in Tables 4.2 - 4.5, it is seen that only pressure and the quadratic term of pressure significantly ($P<0.05$) affected the growth of aerobic psychrophiles on Day 1. The AP count in the untreated shrimp sample was 5.39 log CFU/g, whereas the samples treated above 300 MPa for 10, 20, and 30 min did not show any growth on Day 1. As storage proceeded to Day 21, the untreated sample showed a count of 10 log CFU/g, whereas the highest count obtained on a treated sample was 8.82 log CFU/g in shrimps treated at 100 MPa for 10 min, both of which exceeded the acceptable limit of microbial growth. However,

the samples treated above 300 MPa and 200 MPa for more than 30 min showed the lowest counts of 5.9 log CFU/g. This proved that at higher pressure and holding time, the bacterial growth of shrimp stored at refrigerated conditions can be delayed.

Similar results were reported by Ucak *et al*., (2018) who worked with herring fillets inoculated with *Morganella psychrotolerans* and pressurized for 100, 200,300, and 500 MPa. These authors noted that the bacterial population of control, 100, 200 and 300 MPa demonstrated an exponential growth throughout the storage, surpassing 7 CFU/g on the 12th day, which was deemed an acceptable limit for fish species. There were no significant ($p > 0.05$) differences between the control and 100 MPa pressure-treated group during the storage. The application of 500 MPa showed a significantly ($p < 0.05$) inhibitory effect on the growth of total psychrophilic bacteria until 17 days, which indicated enhancement of shelf life.

Overall, HPP has proven to significantly retard the growth rate of psychrophilic bacteria, with a superior effect by pressure than by time. Bacterial growth was present even in the HP-treated samples with a short holding time and less intense pressure treatment, whereas higher pressure and longer holding time kept the bacterial growth below the acceptable limit even on Day 21. This highlighted the importance of processing seafood at higher pressure for a longer time to extend shelf life.

4.3.3. Effect on texture

The texture and structure of fish muscle are critical indicators of freshness quality, influenced by various attributes such as hardness, cohesiveness, springiness, chewiness, resilience, and adhesiveness, as well as the internal cross-linking of connective tissue and the detachment of fibers (Cheng *et al*., 2014).

Texture is one of the sensory attributes that consumers utilize to assess food acceptability and purchasing decisions. This attribute is very intricate including the use of different senses (e.g., vision, hearing, and touch) are used simultaneously to evaluate diverse textural properties, such as hardness, cohesiveness, adhesiveness, gumminess, and springiness (Day & Golding, 2018).

An important breakthrough in the field of sensory analysis, instrumental TPA is popularly used in the food processing industry as well as the scientific community (Rahman *et al*., 2021).TPA, being an objective method, the parameters defined it are correlated with the sensory textural parameters. The response surface plot was used to analyze the effect of HPP on textural parameters such as hardness (g), springiness (%), and gumminess.

Pressure was identified to be a significant factor ($p<0.05$) for all the textural parameters such as hardness, springiness, and gumminess. Pressure had a significant effect on all textural parameters throughout storage, indicating that an increase in pressure would result in higher hardness, springiness, and gumminess on all days of storage. Time had a positive effect on texture on day 1, however, the effect was not consistent throughout refrigerated storage.

4.3.4. Effect on gumminess and hardness

According to Nishinari *et al*., (2019) Hardness can be defined as the force required to make a certain level of deformation of the sample and corresponds to the maximum force acquired by the first uniaxial compression cycle ("first bite") (Bernardo *et al*., 2022). Hardness, the most crucial textural attribute in seafood, is described as a property contingent upon the connective tissue composed of collagen and the myofibrils (myosin and actin) (Kaur *et al*., 2015).

The effect of HPP on hardness and gumminess of samples throughout storage is illustrated in Figure 4.2 & Figure 4.3. The values of hardness ranged from 1023 to 2586 g on Day 1 of storage, with the highest and lowest values shown by samples treated at 341 MPa for 20 min and 58.57 MPa for 20 min, respectively. The results of gumminess were analogous to hardness. Both parameters showed an increase in value throughout storage.

Both pressure and time had a significant ($p<0.05$) effect on hardness and with a greater effect by pressure, followed by a smaller effect by holding time, as shown by the equations in Tables 4.2-4.5. As storage proceeded to Day 7, the highest value of hardness was obtained by samples treated at 300 MPa for 30 min, whereas the lowest values were maintained by the same treatment on all days of storage, with slight increase in values between days. Pressure continued to have a superior effect compared to holding time throughout storage, as indicated by the coefficients of pressure (A) and time (B) in the table. The interaction effects (AB, A^2B and AB^2) suggest that extreme pressure and long holding time may have diminishing or high negative effects on the

hardness on day 1, whereas on Day 21, the interaction effects suggest that the highest hardness occurs at a balance of moderate to high pressure and time, but excessive time can reduce the benefits of high pressure.

In a study conducted by Kaur *et al*., (2015) the HP-treated sample exhibited considerably greater hardness values ($p<0.05$) in comparison to untreated samples. Among the HP-treated samples, those subjected to 435MPa exhibited markedly increased hardness values. Hardness readings increased by 12.18%, 18.15%, and 59.92% in samples subjected to pressures of 100, 270, and 435 MPa, respectively, immediately following pressurization, in comparison to the untreated sample. The observed result was associated with an increase in hardness attributed to myofibrillar protein denaturation and aggregation of myosin molecules (Yamamoto *et al*., 1994).

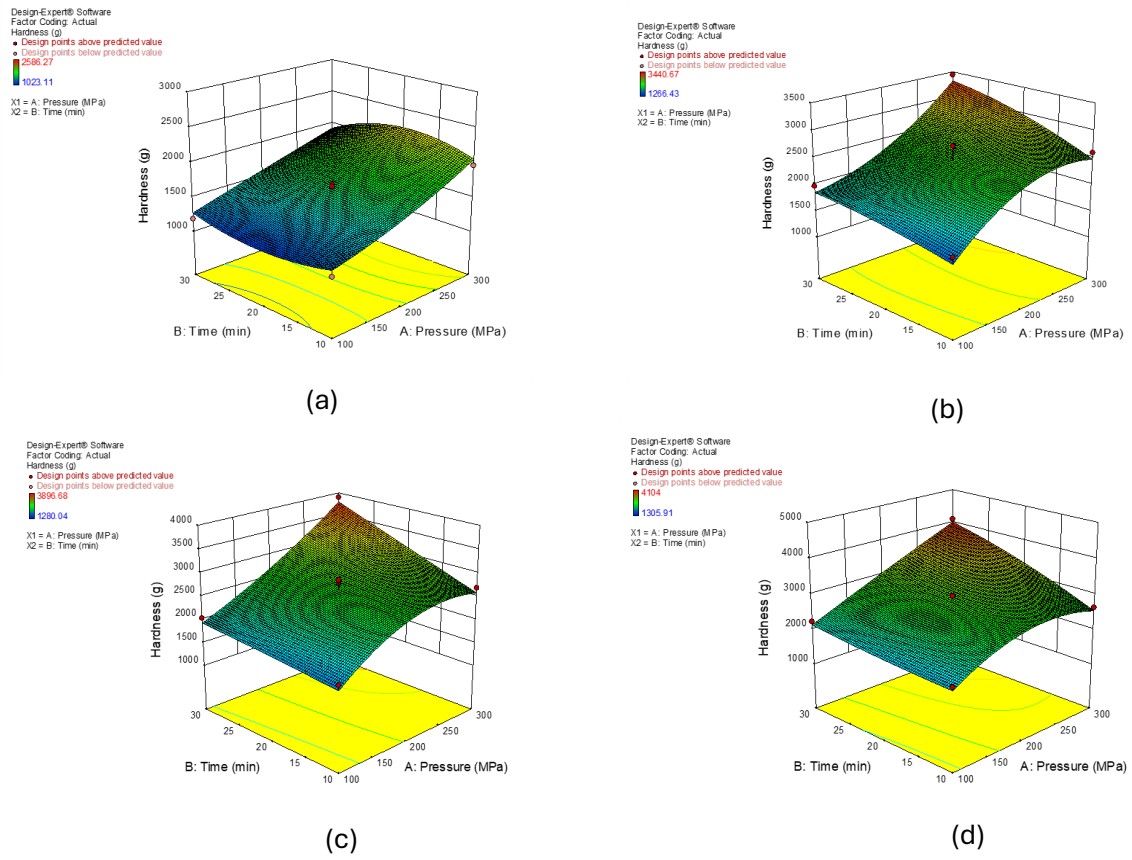


Figure 4.2. *Response surface plots for hardness of shrimp affected by pressure and temperature on* ***(a) Day 1, (b) Day 7, (c) Day 14*** *and* ***(d) Day 21****.*

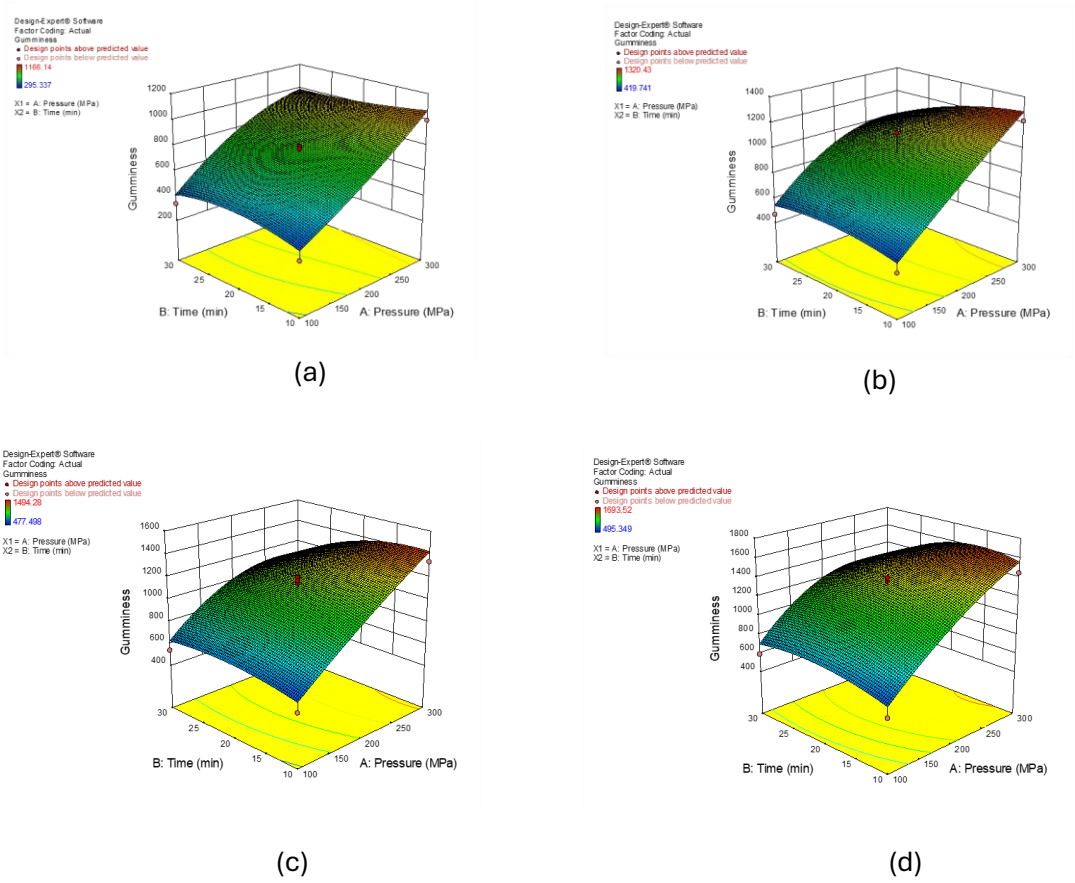


Figure 4.3. *Response surface plots for gumminess of shrimp samples affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21.***

4.3.5 Effect on springiness

The next textural parameter measured was springiness. Springiness, first characterized as elasticity, quantifies the degree of recovery following the initial compression, reflecting the restoration that occurs during the interval between the end of the first bite and the commencement of the second bite. Springiness indicates the disruption of the sample's natural structure due to initial compression (Rahman *et al*., 2021).

The effect of HPP on the springiness of shrimp from the day of processing until Day 21 is depicted in Figure 4.4. Pressure had a positive effect on the springiness on all days of storage, however, time showed a positive effect on Day 1 and then a diminishing effect until the end of storage. For springiness, the cubic model was chosen due to its high R^2 value of 0.9240,

however, it shows significant lack of fit ($p<0.05$) suggesting that the model does not explain the relationship between pressure, time, and springiness. This is seen throughout storage pressure and time had comparatively less effect on springiness indicated by the coefficients of A and B. Pressure had a weak positive effect throughout storage whereas time had a positive effect on storage immediately after processing, after which it showed a negative effect until Day 21, Nonetheless, the linear influence of both pressure and time did not markedly impact springiness; instead, the interaction effect AB^2 greatly influenced it during storage, as illustrated in Tables 4.2-4.5.

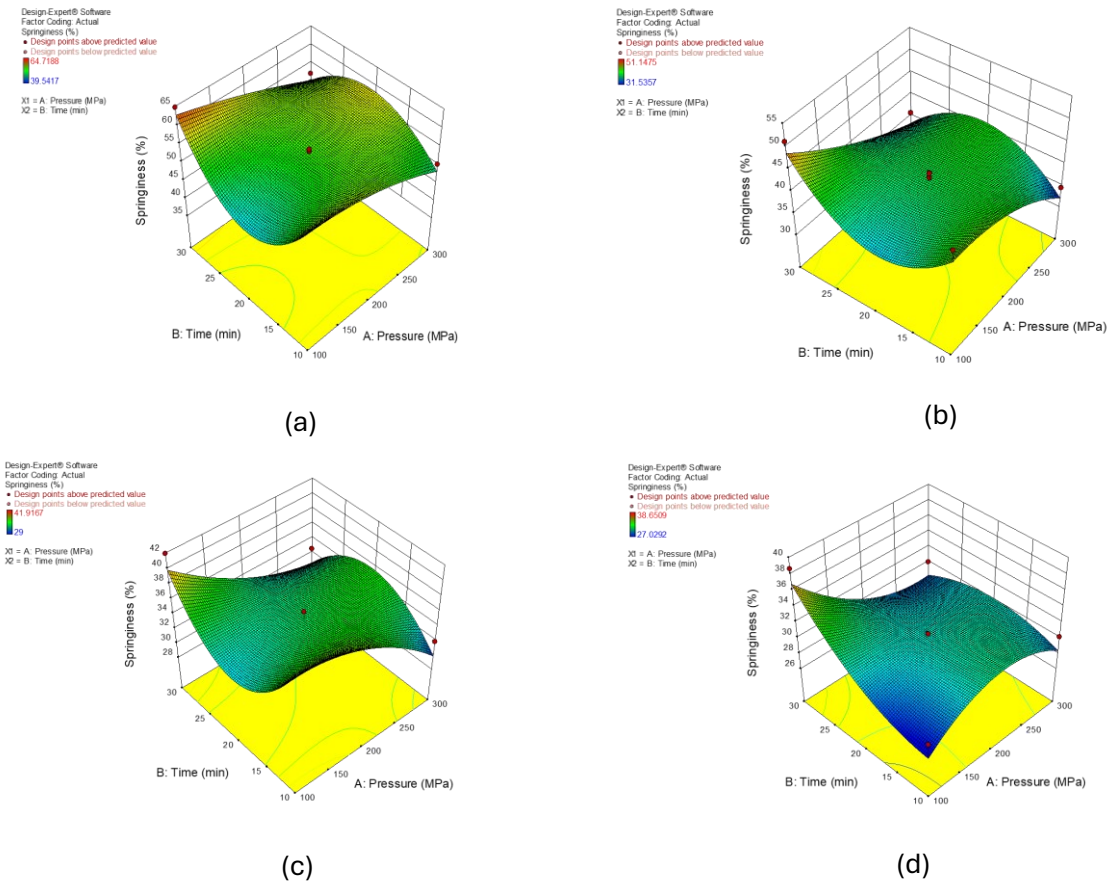


Figure 4.4. *Response surface plots for springiness of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21.***

Chen *et al*., (2022a) conducted a study on black tiger shrimp during chilled storage, revealing significant changes in texture. The texture of the shrimps increased with treatments of 100, 300, and 500 MPa, while resilience, cohesiveness, adhesiveness, and springiness decreased. The hardness of the shrimps increased by 198, 238, 252, and 153%, respectively. The elasticity of the control and 100 MPa-treated groups diminished by 160 and 155%, respectively. To summarize, HPP may enhance myofibril breakdown by altering the natural conformation and liberating cathepsin from lysozyme (Kemp *et al*., 2010). Simultaneously, by altering the water phase in the muscle and suppressing proteases, HPP kept the shrimp's texture properties stable during refrigeration.

4.3.6. Effect on color

In seafood and meat products, color and appearance directly influence value and consumer acceptance. Such products are often selected based on visual assessment of the color of their muscles (Clydesdale, 1991). Evaluation of product quality of seafood and other foods is increasingly used through instrumental measurements (Chouhan *et al*., 2015).

The natural brown–black appearance of raw shrimps is reported to be due to non-covalent interaction between blue, crustacyanin-type astaxanthin protein complex and orange-red, uncompleted astaxanthin or astaxanthin esters (Li *et al*., 2016). Astaxanthin, a carotenoid, appears to be the primary pigment responsible for color in crustaceans, whereas the highly reactive astaxanthin pigment is stabilized by protein crustacyanin, which binds to the pigment forming a carotenoprotein complex, thereby maintaining its tertiary and quaternary structure (Ertl *et al*., 2013).

Figure 4.5 illustrates the effect HP treatment on the lightness of samples on different days of storage**.** The lightness of samples was significantly affected by the linear (A) and quadratic terms (A^2) of pressure throughout storage, indicating that an increase in pressure increased the lightness of the sample. The holding time had no significant ($p>0.05$) impact on the lightness of the sample; however, the interaction between time and pressure (AB) considerably influenced the lightness ($p<0.05$). This may indicate that time did not contribute to increasing the lightness of samples and may have helped in retaining some of its existing color.

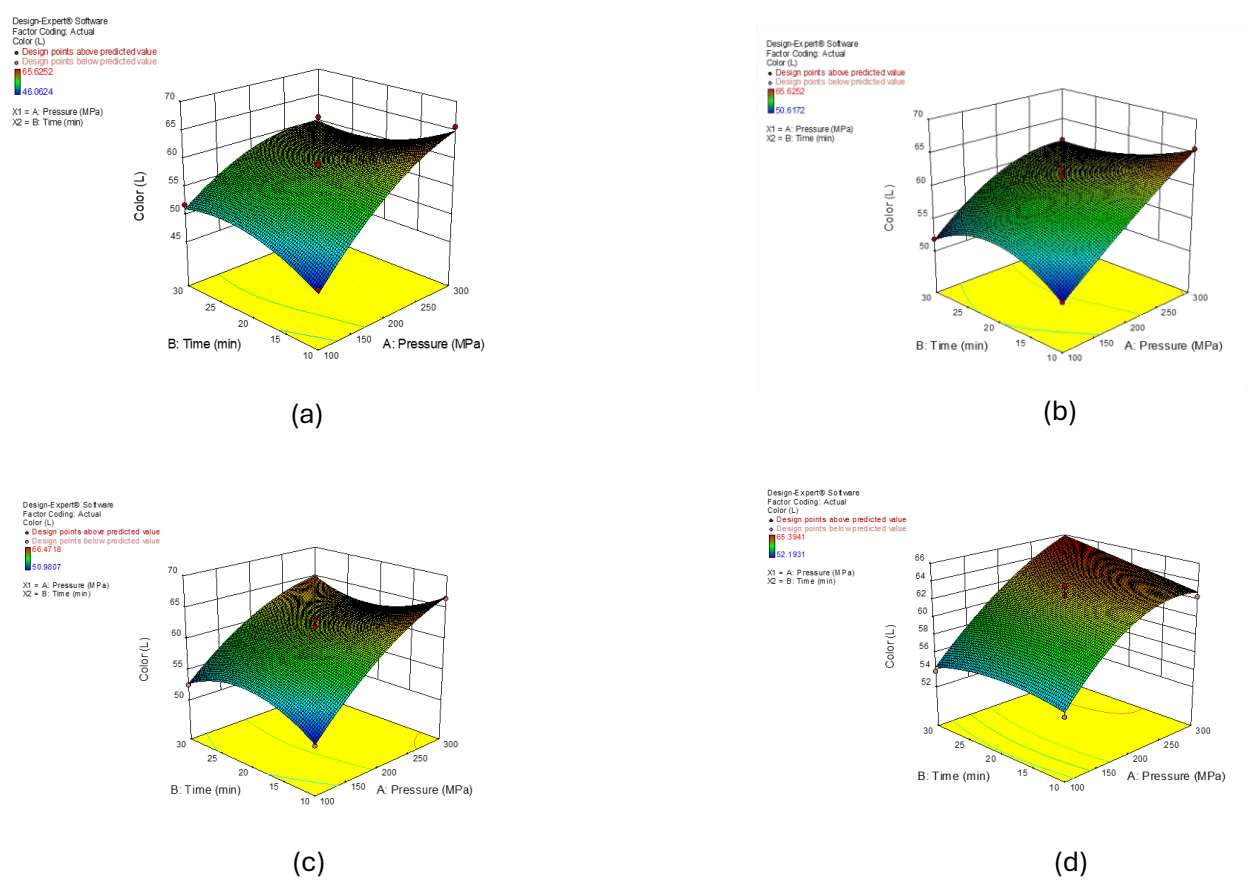


Figure 4.5. *Response surface plots for lightness (L*) of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21***

Figure 4.6 depicts the changes in redness (a*) associated with HP treatment and refrigerated storage. The redness was observed to decrease with storage, with very less effect by both pressure and time. The cubic model was chosen, however, their low R^2 values suggested low predictive power, meaning a very large percentage of variability in the data was not explained by the model. The yellowness (b*) of the samples increased on refrigerated storage, and it can be seen from Table 4.2 - Table 4.5 that, unlike lightness and redness, time had a slightly stronger effect (indicated by the coefficient of B), however, both variables did not have a significant effect on the yellowness of the samples throughout the refrigerated storage. Figure 4.7 depicts that yellowness of samples was higher in samples treated at higher pressures and longer holding times, indicated by the red shade in the response surface plot.

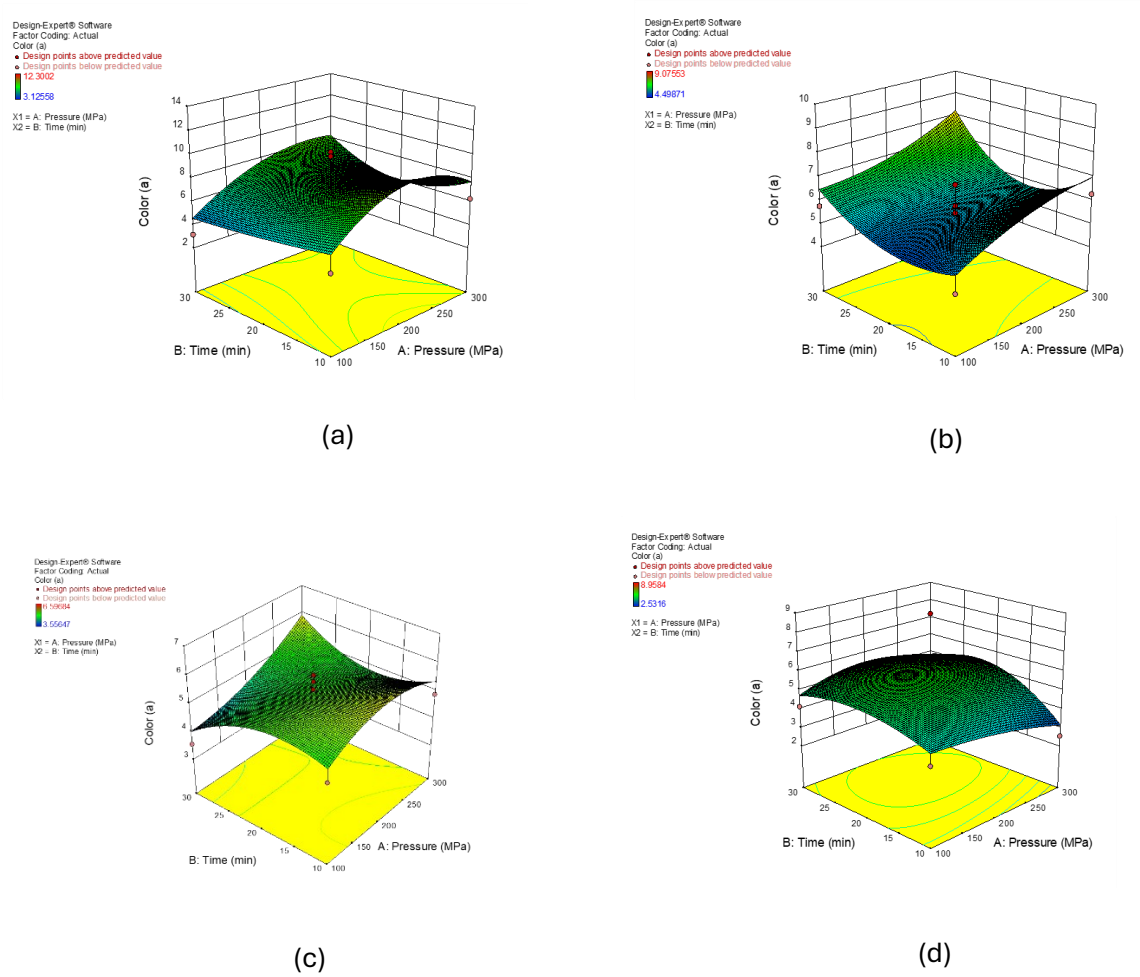


Figure 4.6. *Response surface plots for redness (a*) of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21***

Chroma (C*), the quantitative attribute of colorfulness, determines the extent of hue variation in comparison to a grey color with the same lightness. The chroma value is directly proportional to the color intensity of samples, as perceived by humans. Hue angle (h*), is the qualitative attribute of color, upon which colors are traditionally defined as reddish, greenish, etc. It is used to define certain colors with reference to grey color with the same lightness, based on absorbance at different wavelengths (Granato & Masson, 2010).

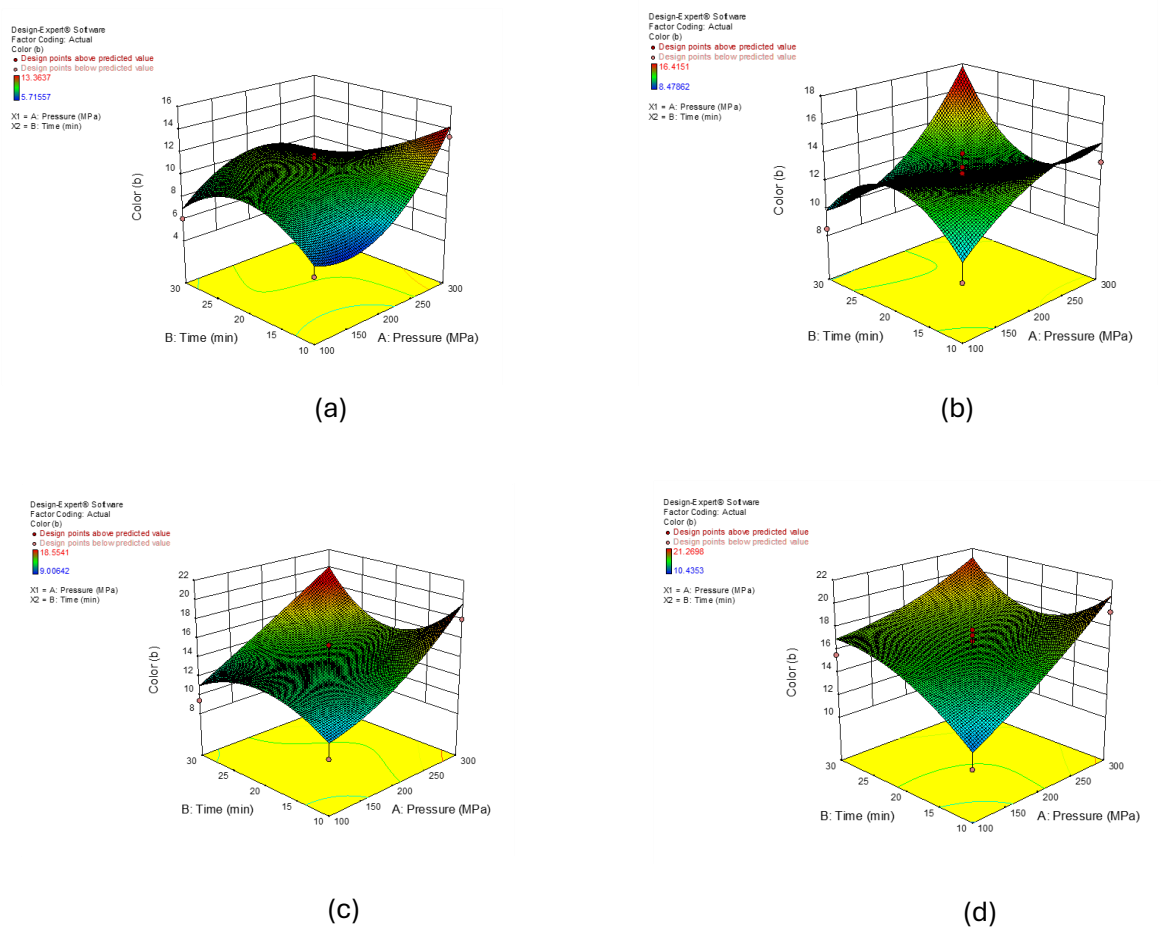


Figure 4.7. *Response surface plots for yellowness (b*) of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21.***

The changes in chroma were analyzed as shown in Figure 4.8, and the values ranged from an average of 12 to 17 from Day 1 to Day 21, which means that the samples color slightly more vivid during storage. The hue angle was also calculated for each sample, and the effect of pressure and time, as well as storage, can be observed in the 3D plots shown in Figure 4.9. The hue angle of the treated samples ranged from an average value of 53.6 to 73.9, indicating that the samples shifted from an orange-red hue to a yellow-brown shade. From Tables 4.2-4.5, it can be noted that both hue and chroma were not significantly ($p>0.05$) affected by pressure and time, mostly throughout refrigerated storage. The effect of processing parameters on total color changes of treated shrimps is depicted in Figure 4.10**.** ΔE was calculated for Days 7, 14, and 21

with respect to its Day 1 value, to understand the total change in color as storage proceeds from the day of processing to each checkpoint. ΔE values increased with storage and were higher in samples treated at higher pressures for longer time, indicating that the color of samples was affected significantly owing to increasing lightness and decreasing redness as discussed before. This distinctively demonstrated the effect of pressure and holding time on color changes.

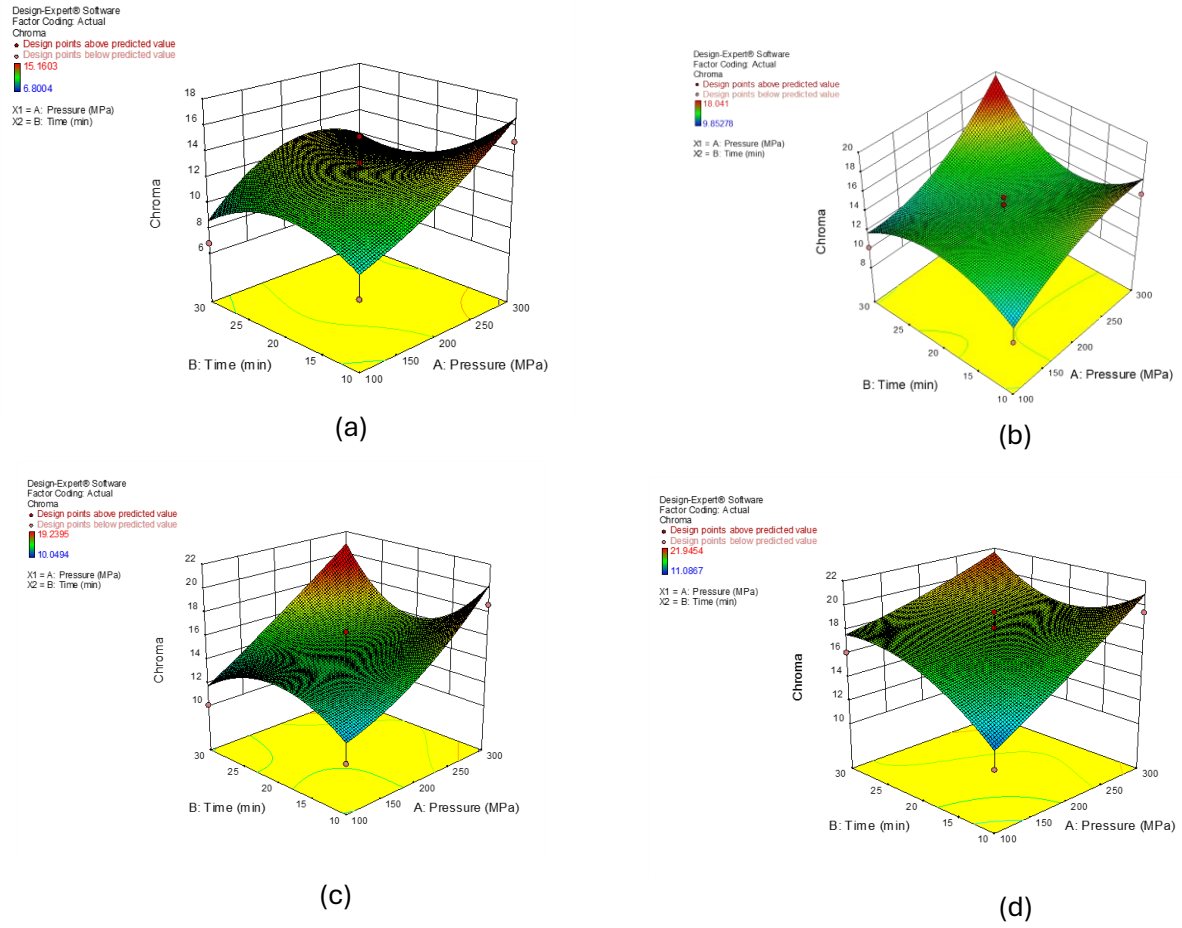


Figure 4.8. *Response surface plots for chroma of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21***

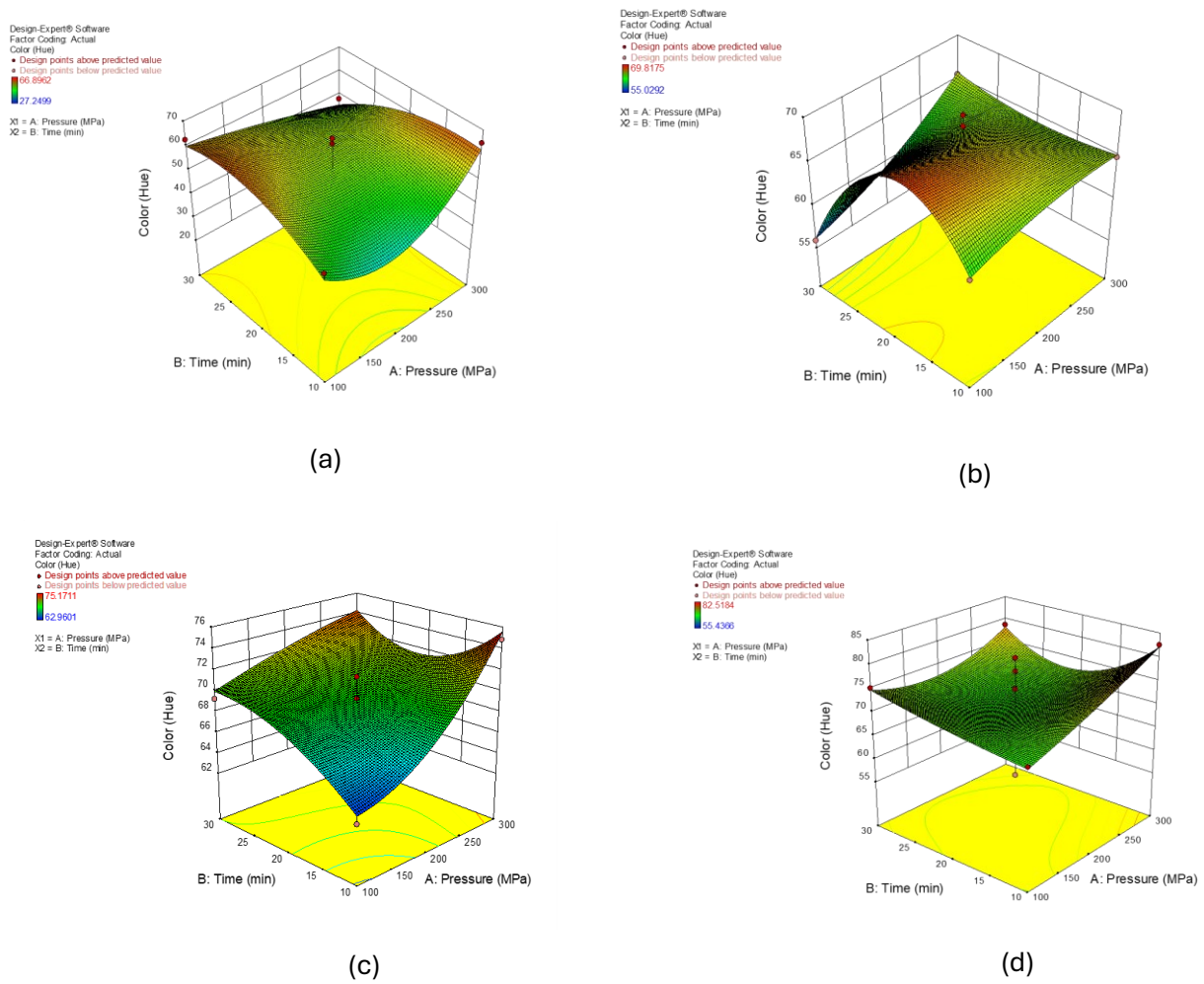
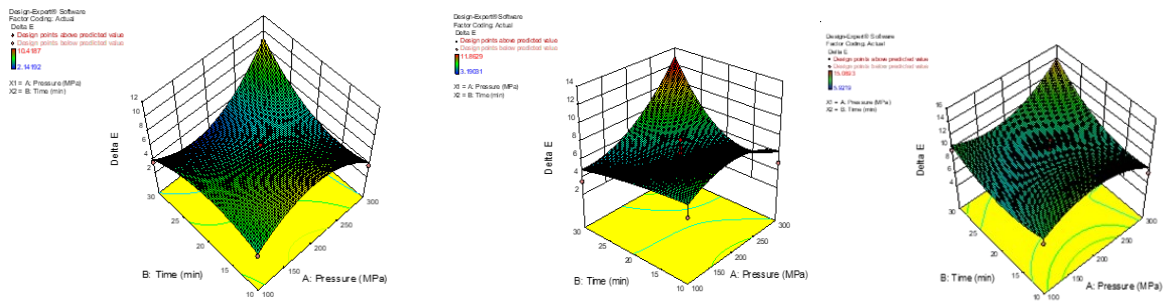


Figure 4.9. *Response surface plots for hue angle of shrimp affected by pressure and temperature on* ***(a) Day 1 (b) Day 7 (c) Day 14*** *and* ***(d) Day 21.***



(a) (b) (c)

Figure 4.10. *Response surface plots for Delta E of shrimp affected by pressure and temperature on* ***(a) Day 7 (b) Day 14*** *and* ***(c) Day 21.***

The findings of the present study were in agreement with Cruz-Romero *et al*., (2008) who reported that both a* and b* values underwent significant alterations after HPP treatment (≥260 MPa) and storage at 2ºC, and the change in a* and b* values was attributed to crude lipid oxidation and denaturation of the myofibrillar and sarcoplasmic proteins.

A proposed explanation by Cruz-Romero *et al*., (2008) stated that the reason for the color alteration is lipid oxidation resulting from the degradation of the primary carotenoid pigment in shrimp, astaxanthin, which leads to the release of Cu and Fe ions from the muscle tissue. Whereas Sequeira-Munoz *et al*., (2006) proposed that pressure-induced coagulation of sarcoplasmic and myofibrillar proteins accounted for the alterations in the color values of the samples.

Li *et al*., (2016) studied the effect of HP treatment on the color of shrimp samples. Compared to the slightly translucent appearance of raw shrimps, HPP-treated shrimps became opaque and whiter. When the pressure and holding time increased, *L** exhibited a significant increase ($p < 0.05$), the brightness increase for the HHP treatments was attributed to the depletion of active pigments and coagulation of protein (Yi *et al*., 2013). It was also presumed that the coagulation of protein changed the sample surface properties, which increased light reflection, creating a whitened color (Kruk *et al*., 2011). There are other studies where higher pressure and holding time gave a whiter appearance or opacity to flesh (Murchie *et al*., (2005); López-Caballero *et al*. (2000).

4.4 Optimization and validation

The factors, pressure and time were optimized to minimize the growth of aerobic psychrophiles throughout storage. Rather than applying a single optimized condition for the entire storage period, optimization was conducted for each day individually to identify conditions that would effectively reduce microbial growth on that specific day. Although a treatment designed to achieve minimal microbial growth by day 21 would likely be effective on earlier days as well, it could lead to significant deterioration in quality parameters such as color and texture. To prevent such undesirable changes, milder processing conditions were considered, balancing microbial proliferation with the retention of high product quality. This approach ensured that microbial growth remained controlled while maintaining better structural and sensory attributes over time. An optimal condition of 300 MPa for 10 min was obtained for day 1 to achieve minimum microbial growth, whereas by day 7, a pressure of 295.69 MPa was held for 28.6 min to meet the requirements. For further storage, a higher pressure and holding time of 300 MPa for 30 min was obtained for both days 14 and 21.

Based on the above criteria, the predicted optimal conditions leading to high desirability function on days 1,7,14 and 21 is shown in Tables 4.6-4.9. Further, the samples were processed under these conditions, and the values obtained were compared to the predicted values obtained from the software, verifying that the optimized HP conditions aid in achieving reduced microbial growth in refrigerated storage of shrimp.

Table 4.6. Predictive model validation for Day 1 at 300 MPa/10 min

Responses	Predicted value	Experimental value
Log CFU/g	0.295	0.21
Hardness	2055	2049
Gumminess	1071	1070
Springiness	48.5	47.4
L*	65.0	65.0
a*	7.7	7.7
b*	14.3	14.1
Chroma	16.3	16.1
Hue	62.3	61.5

Table 4.7. Predictive model validation for Day 7 at 295.69MPa/28.6min

Responses	Predicted value	Experimental value
Log CFU/g	0.295	0.01
Hardness	3241	3200
Gumminess	1042	1034
Springiness	35.5	35.0
L*	61.4	60.1
a*	7.6	7.6
b*	16.3	16.2
Chroma	18.0	17.9
Hue	65.2	64.92318787
Delta E	8.5	5.2

Table 4.8. Predictive model validation for Day 14 at 300 MPa/30 min

Responses	Predicted value	Experimental value
Log CFU/g	3.789	3.71
Hardness	3790	3786
Gumminess	1088.80	1091
Springiness	30.6	31.1
L*	65.0	65.0
a*	5.9	6.0
b*	20.1	20.1
Chroma	20.9	20.9
Hue	74.3	73.4
Delta E	13.0	6.2

Table 4.9. Predictive model validation for Day 21 at 300 MPa/30 min

Responses	Predicted value	Experimental value
Log CFU/g	5.4	5.4
Hardness	4010	4007
Gumminess	1229	1223
Springiness	28.2	29.0
L*	65.9	65.2
a*	4.6	4.5
b*	21.2	21.2
Chroma	21.7	21.6
Hue	78.3	78.0
Delta E	14.1	7.7

Conclusions

This study investigated the use of RSM in optimizing pressure-time conditions that can provide reduced microbial growth in shrimp samples stored at refrigeration storage for 21 days by optimizing conditions that suit different stages of storage. It was observed that the optimized conditions for day 1 was 300 MPa for 10 min, whereas the other days required higher intensity treatments such as 295.69 MPa for 28.6 min on day 7 and 300 MPa for 30 min on day 14 and 21 to minimize microbial growth.

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CONNECTING TEXT TO CHAPTER 5

The previous chapter explored the use of HPP in extending the shelf life of shrimp and optimizing processing conditions to minimize microbial growth throughout the refrigerated storage. We observed that higher pressure and holding time are required for extending the shelf life of shrimp. In the 5th chapter, we are exploring another application of HPP with Black tiger shrimp, to evaluate the peelability/ease of peeling the shell, after subjecting it to HP treatment. For this purpose, we optimized processing conditions with lower pressures than what we used for storage, with the axial points ranging between 100 MPa and 200 MPa, as our primary objective is to minimize the peeling force, and then study the changes in colour and texture associated with the HP treatment**.**

CHAPTER 5

EVALUATION OF PEELABILITY OF BLACK TIGER SHRIMP TREATED WITH HIGH-PRESSURE PROCESSING

Abstract

High-pressure processing (HPP) is a widely used non-thermal food processing technology that has acquired significance as it preserves fresh-like quality and extends the shelf life of food products. In shrimp processing, traditional maturation methods involve ice storage to facilitate easier peeling, but these practices often result in quality deterioration, including changes in color, texture, and flavor. This study evaluates the use of HPP to improve peeling efficiency in black tiger shrimp by analyzing the peeling force required to separate the shell from the meat. An optimum level of pressure and time required to facilitate the peeling was achieved through a response surface methodology (RSM) experimental design. A central composite design (CCD) was utilized to assess the impacts of two principal factors: pressure level (MPa) and pressure treatment time (min) on seven measured response variables, specifically the peeling force (g), hardness (g), gumminess, L*, b*, hue, and ΔE. The experimental design comprised 13 trials with 5 central points to assess the precision and dependability of the response surface model. The results showed that a moderate value of 150 MPa for 4 min gave the highest peelability (lower peeling force). Further, lower pressures and times were not effective enough to facilitate meat-shell separation, and higher pressures, like 220 MPa for 4 min, may have caused protein denaturation, resulting in an even tighter meat-shell attachment. Low efficiency during mechanical processing or manual peeling and impairment to the shrimp meat integrity are caused due to tight shell-muscle adhesion. Consequently, prior to peeling, it is desirable to have a means to detach the shell-muscle adhesion of shrimp, and this can be facilitated using HP treatment, with minimal change in color and texture.

Keywords: Response surface methodology (RSM), peelability, peeling force, shrimp, Black Tiger

5.1 Introduction

Crustacean species are the most predominant seafood sector, with shrimps and prawns ranking as second most exported species. Shrimp and its derivatives are extensively consumed in multiple

ways and are witnessing increasing demand in industrialized countries (Sharif *et al*., 2023). Global crustacean production reached approximately 12 million tonnes in 2021, with high-value shrimp and prawn species contributing 60.9% of this total production. Among penaeids, the Black Tiger shrimp (*Penaeus monodon*) is one of the largest species and the second most produced crustacean after white leg shrimp (*Litopenaeus vannamei*), with nearly 99% of its culture occurring in Asia (Shrimp Culture) (Bondad-reantaso *et al*., 2012). Deep-sea shrimp are valued for their elevated levels of omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), rendering them a rich source of essential fatty acids, minerals, amino acids, and proteins, with neutral effects on cholesterol levels (Bondad-Reantaso *et al*., 2012). Additionally, shrimp exoskeletons, composed primarily of chitin (15–40%), calcium carbonate (20–50%), and protein (20–40%), hold economic significance, with desiccated exoskeletons valued high with great commercial revenues [Yang & Chen (2015), Sharif *et al*., (2023)].

Shrimps are highly regarded as nutritious and flavorful seafood, and are commercialized in various forms, including peeled and ready-to-eat products. Hand peeling is considered the gold standard for ensuring optimal quality; however, this labor-intensive method is not preferred by the industry due to high costs (Thi *et al*., 2018). Mechanical peeling offers a faster and more cost-effective alternative but often results in poor-quality products due to the tight adhesion of muscle to the shell. To mitigate this issue, a pretreatment or maturation process is commonly implemented to weaken the shell-muscle bond before mechanical peeling (Dang *et al*., 2018). Typically, ice storage for 2–7 days is used for maturation, but prolonged storage can cause quality deterioration, leading to undesirable changes in color, flavor, and texture (Gringer *et al*., 2018).

Peelability in shrimp refers to the ease of removing the shell. Poor peelability is indicated by excessive shell residues clinging to the shrimp meat after mechanical peeling. While hand peeling ensures better meat integrity and less shell residue, it is labor-intensive, time-consuming, and raises food safety concerns due to potential contamination (Thi *et al*., 2018). In the seafood industry, cooking has long been employed as a conventional method to enhance peelability by breaking down connective tissues. The continuous attachment of the shell to the muscle via the epidermis complicates the peeling process. The connective tissue of the attachment is primarily

composed of a collagen-like protein that differs from vertebrate collagen in molecular weight and amino acid composition (Grant *et al*., 1980).

Various other methods have been used to facilitate the peeling of shrimps. Enzymatic treatment as a pre-treatment prior to peeling has been applied in food industry for long time. This method involves using a mixture of enzymes, typically proteases, carbohydrases, and cellulases, to degrade the proteins and polysaccharides that connect the shrimp meat to the shell (Fehmerling, 1970). A study by Dang *et al*., (2018) suggested that enzymatic maturation efficiently enhanced the peelability of shrimps. The determinants influencing the peelability of the enzyme-matured shrimps were the enzyme type, concentration of enzyme and duration of maturation, while pH alterations had no impact. However, there is a risk of unexpected hydrolysis of shrimp meat during treatment, which can lead to yield and product quality loss. It's necessary to inactivate both external and internal enzymes in the processed shrimps to prevent deterioration during storage (Thi *et al*., 2018). In the shrimp industry, microwave treatment is also employed for thawing shrimp, as opposed to the traditional process of thawing under running water. The peeling yield of shrimp thawed in a microwave was 1.7% lower than that of shrimp thawed in water. The microwave approach utilized less water for thawing and yielded greater protein retention throughout the subsequent cooking step (Bezanson *et al*., 1973). A major drawback of microwave processing is the potential for uneven heating, which can lead to some areas being overcooked while others remain undercooked. Uneven heating can also lead to the survival of some microorganisms in local spots, posing a microbiological risk (Gundavarapu *et al*., 1995). Ultrasound uses high-frequency sonic waves to modify food properties. Low-frequency, high-power ultrasound works through cavitation. Ultrasound can widen the intercellular spaces in tissue and disrupt cell-to-cell adhesions (Awad *et al*., 2012). This disruption could facilitate the separation of the shell from the meat. Although no studies have investigated the direct use of ultrasound for shrimp peeling, the sources suggest its potential (Thi *et al*., 2018). An alternative method for deshelling shellfish involves high-pressure processing (HPP). The principle behind HPP aligns with Le Chatelier's principle, which states that reactions and phase transitions involving volume reduction are favored under increased pressure (Bharatbhai & Kavilakath, 2024)

HPP mainly disrupts non-covalent interactions (hydrogen, ionic, and hydrophobic bonds) while having minimal impact on covalent bonds. Although low-molecular-weight compounds, such as flavor compounds, remain largely unaffected, high-molecular-weight molecules, particularly proteins, can undergo structural modifications under pressure (Baldelli *et al*., 2024). Pressures below 150 MPa can cause dissociation of oligomeric proteins, while pressures above 150–200 MPa induce significant tertiary structure alterations, resulting in protein denaturation (Siddiqui & Khan, 2024). Similar effects of HPP on shell detachment have been reported in Scallop (Yi *et al*., 2013), Crayfish (Shao *et al*., 2018) and Oyster (Puértolas *et al*., 2023).

These findings show that HPP leads to muscle detachment due to pressure-induced denaturation of connective tissues and proteins holds great promise in enhancing shrimp peelability, making the process more cost-effective and commercially viable. Hence, the objective of this study was to explore the evaluation of HPP for facilitating easy peelability of shrimp and to optimize pressure and treatment levels, identifying the most desirable peeling conditions requiring the least peeling force.

5.2 Materials and methodology

5.2.1 Sample preparation

Fresh raw Black Tiger shrimps (*Peneaus monodon*) were purchased from a local market and transferred to the laboratory under iced conditions. Each shrimp was tied using a cotton thread to prevent the sample from getting disfigured. They were then transferred into HDPE pouches and sealed using a vacuum sealer to remove the air. This may be an important step to avoid the floating of sample bags inside the HPP chamber that contains water for transmitting pressure. Each treatment had two pouches with 6 whole shrimps weighing approximately 60 g for peelability and 60 g for color and texture evaluation. The HP treatment was given in a cold isostatic press (AE 400 MPa - Isostatic Press, Autoclave engineering, Columbus, Ohio) with 13 different pressure-time treatment combinations. Control sample pouches, without the HP treatment, were prepared and stored in ice conditions until the treatments were done for other samples. All the treated and control samples were then transferred to the lab in an ice box and analyzed for peelability (peeling force), color (L*, chroma, hue, delta E), and texture (hardness and gumminess) on the same day.

5.2.2 Experimental design

RSM was used to study the effect of independent variables: pressure (MPa) and holding time (min) on the peelability, textural properties, and color changes in shrimp. The experiments were designed according to the central composite rotatable design (CCRD) using Design Expert version 10.1.1 (Stat-Ease, USA). The experimental design illustrated in Table 5.1 is similar to Chapter 4, but with less intense pressure and holding time. The design includes five levels each of two independent variables, with a total of 13 experiments consisting of four 2 x 2 corner points, four extended points (±α) and 5 replications at the center point. The pressure time combination for the treatments was chosen based on the literature.

Table 5.1. Experimental design obtained using CCRD

PRESSURE (MPa)	TIME (min)
79.29	4
100	2
100	6
150	1.17
150	4
150	4
150	4
150	4
150	4
150	6.83
200	2
200	6
220.71	4

5.2.3 Peelability

The setup for evaluating shrimp peelability was constructed using a wooden stand with a base and two parallel vertical boards positioned on opposite sides to provide structural support, **as**

shown in Figure 5.1**.** A stainless-steel needle with a diameter of 0.8 mm was securely mounted horizontally between the boards, serving as an axis for the shrimp to rotate during testing. This aided in keeping the shrimp in a steady position, so as to facilitate peeling from one end of its shell to the other, separated at the lower midsection by a thin membrane. A commercially available paper clip was modified and fixed to a probe attached to the Texture Analyzer XT Plus (Stable Micro Systems Ltd, UK). The shrimp's midsection was pierced with the needle, ensuring that the needle passed through one side and exited through the other. The probe was lowered until it made contact with the shrimp, and one side of the shrimp was manually clamped between the teeth of the paper clip. During testing, the probe moved upward at a constant speed of 5 mm/s, applying force to peel the shrimp starting from the clamped end. The rotational motion of the shrimp around the needle facilitated controlled peeling, and the texture analyzer recorded the peeling force in real time, quantifying the peelability of the shrimp.

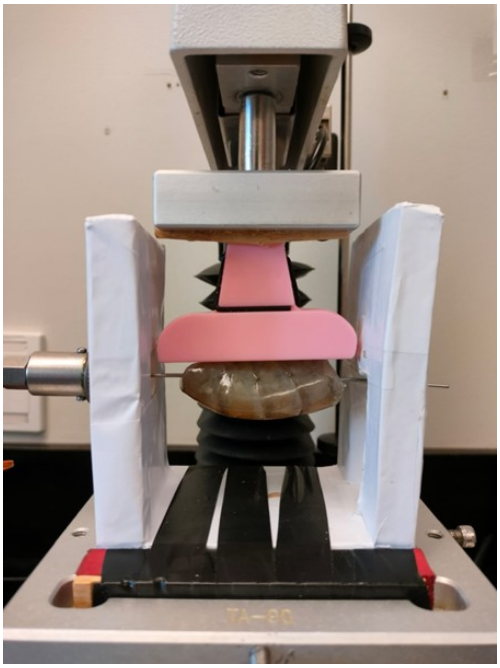


Figure 5.1. Setup for evaluating peelability of Black Tiger shrimp

5.2.4 Texture profile analysis (TPA)

The texture profile of test samples was evaluated using a texture an analyzer (Model: TA.XT-2, make: Stable Micro Systems, UK) equipped with a 25 kg load cell capacity. The whole shrimp was compressed by 40% of its original height using a cylindrical probe of 50 mm diameter with a test speed of 2 mm/s. From the TPA curves so obtained, the following textural parameters were calculated: hardness (g) and gumminess calculated as hardness × cohesiveness.

5.2.5 Color measurement

The color measurement on the shrimp samples were made using a Minolta Tristimulus Colorimeter (Minolta Crop, Ramsey, NJ, USA). The data for color was generated from the Spectra Magic software (Minolta Crop, Ramsey, NJ, USA) similar to those detailed in Chapters 3 and 4.

5.3 Results and discussions

5.3.1 Response surface methodology

One of the essential parts of RSM is to obtain a statistically significant model for each response as a function of process variables ANOVA. Table 5.2 details the selected RSM model for each response based on its statistical significance as well as lack of fit values.

The cubic model was selected for all the responses due to its high R^2 values. The lack of fit values for each chosen model was not significant ($p>0.05$), which makes the models significant. A non-significant lack of fit indicates that the chosen model can accurately predict the responses using the predicted equations shown in Table 5.2 for each response, as a function of input variables with >95% confidence.

5.3.2 Evaluation of peelablity

In this study, response surface methodology (RSM) was employed to evaluate the effects of HPP on shrimp peelability, with pressure and time as primary factors. The response surface plot illustrated in Figure 5.2 was generated to visualize the relationship between these factors and peeling force. Different models, including linear, quadratic and cubic, were assessed to determine the best fit for the experimental data. Among the models tested, the cubic model exhibited the highest R^2 value, indicating the best fit for the experimental data, followed by the

quadratic model with R^2 value of 0.69. A higher R^2 value suggests that the cubic model effectively captured the variability in the response and provided more accurate predictions compared to the other models. The lack of fit test assessed how well the selected model explained variations in shrimp peelability.

Table 5.2. Predicting equations and compiled ANOVA results for the responses

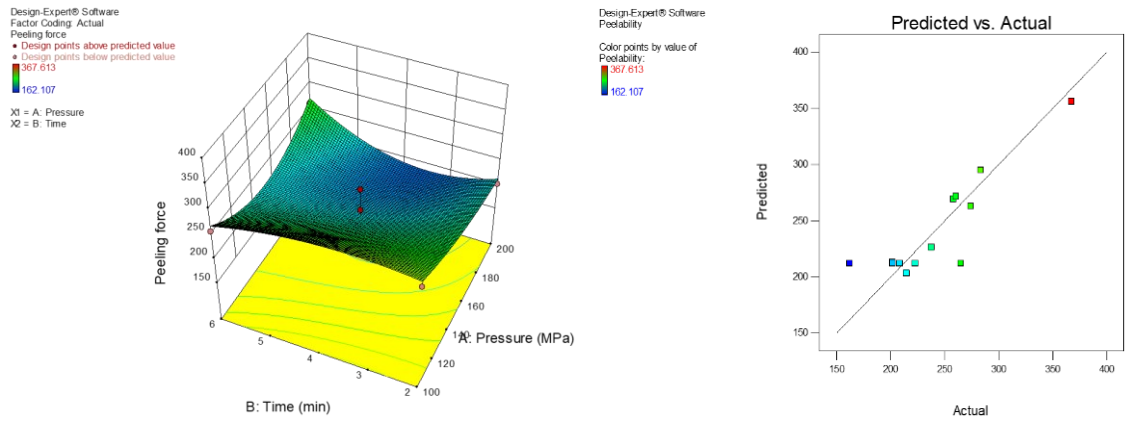
Sl No	Responses	Equation	Lack of fit	R^2
1	Peelability	212-54.06*A-12.97*B+21.08*AB+33.92* A^2 +16.34*B^2+21.23*A^2B+34.12*AB^2+0*A^3+0* B^3	0.4378	0.7920
2	Hardness (g)	1278.62+306.84*A+172.06*B+64.44* AB +48.25*A^2-126.19*B^2-40.95*A^2B-220.9*AB^2+ 0*A^3+0* B^3	0.3045	0.9182
3	Gumminess	791.27 +232.27*A+106.74*B+75.75* AB +53.89*A^2-79.32*B^2-19.45*A^2B-167.33*AB^2+0* A^3+0* B^3	0.2221	0.9130
4	Color (L)	32.07+3.09*A+2.14*B-0.044*AB-0.045* A^2+0.064*B^2+0.34*A^2B-0.48*AB^2+0*A^3+0* B^3	0.1933	0.9617
5	Color (Hue)	108.38-4.69*A+0.2*B+1.11* AB-0.23* A^2-0.57* B^2-1.16* A^2B+2.98* AB^2+0*A^3+0*B^3	0.6006	0.7756
6	Chroma	8.65+0.14* A+0.21* B-1.61*AB-1.3*A^2-0.19* B^2-0.73* A^2B-0.24* AB^2+0* A^3+0* B^3	0.6145	0.8511
7	Delta E	9.88+3.09*A+2.14*B+0.27*AB-0.031*A^2+ 0.13*B^2+5.84E-03*A^2B-0.75*AB^2+0*A^3+0* B^3	0.0826	0.9464

where A is pressure (MPa), B is time (min).

The response surface plot revealed that the lowest peeling force was observed for samples treated at 150 MPa for 4 min, whereas the highest was observed for 79.2 MPa for 4 min. The first treatment facilitated the ease of separation by interacting with the cellular components, while the second one, with a lower severity, was insufficient to create an effect. This highlighted that a certain critical pressure is required for lower peeling force when samples were treated at the same time. It was also observed that upon increasing pressure further up to 220 MPa, the peeling force increased, making it harder to take the shell off. These higher-pressure treatments had more serious interactions with the cellular components and possibly strengthened adhesion of the tissue to cling to the shell. As noted earlier, the peeling force was higher for lower-pressure

treatment as well (lower than the optimum level), presumably due to insufficient effect of pressure on the protein structure that separates the shell from its body. These findings suggest that an optimal combination of pressure and time is necessary to maximize peelability while maintaining shrimp integrity.

From Table 5.2, it can be noted that pressure had a superior effect on peelability, indicated by the larger coefficient of A (pressure), as compared to time. However, both factors had a negative coefficient, suggesting that peeling force increased as pressure and time reduced. The interaction effects, however, have a positive coefficient indicating that peeling force increased with increasing pressure and time severity, and it became even more difficult to peel on further increasing the factors. However, only pressure has a significant ($P<0.05$) effect on peelability. whereas time (B) or any of the interactions does not have a significant effect ($P>0.05$). The lack of fit of the model was not significant, indicating that the model explained the relationship between the factors and response adequately.



(a) (b)

Figure 5.2. (a) Response surface plots for effect of pressure and time on peeling force of black tiger shrimp *and* ***(b) Actual vs predicted values of peeling force as affected by pressure and time.*** *The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or corelation.*

A study was carried out by Yang *et al*., (2020) where the manual shrimp shelling was monitored after HP treatment. It was reported that HP treatment at 200 MPa -300 MPa for 3-5 min made shelling easier and that the treated samples took only 1/3rd of the time taken by untreated samples. However, this result could vary from one individual to another depending on their

speed and expertise. Another study compared the effect of ohmic heating and HP treatment on peelability of shrimp. They recorded that the optimum condition for peeling was 100 MPa for 3 min at 5°C, and pressures above 350 MPa reduced peelability. This was attributed to the stabilization of cuticular and epidermal collagen that generated new collagen-like structures by new linkages, which strengthened the muscle-shell connection and reduced the peelability (Dang *et al*., 2018). This aligns with the results obtained in our study that pressures that are too high may have formed new bonds or strengthened existing ones to keep the epidermis close to the shell, thereby lowering peelability. Muscle attachment in shrimp has three layers: the exoskeleton, epidermis, and muscle. The epidermis is linked to muscle by significant interdigitation. Only actin filaments merge into the muscle component of this junction. The epidermis is connected to the shell with muscular attachment fibers, known as intracuticular fibers (Talbot *et al*., 1972). HPP can cause denaturation of muscle proteins, such as actin and myosin, along with connective tissue. This denaturation diminishes the link between the muscle and the shell.HPP-induced denaturation is anticipated to affect the actin filaments associated with this attachment (Aubourg, 2018).

5.3.3 Effect on texture

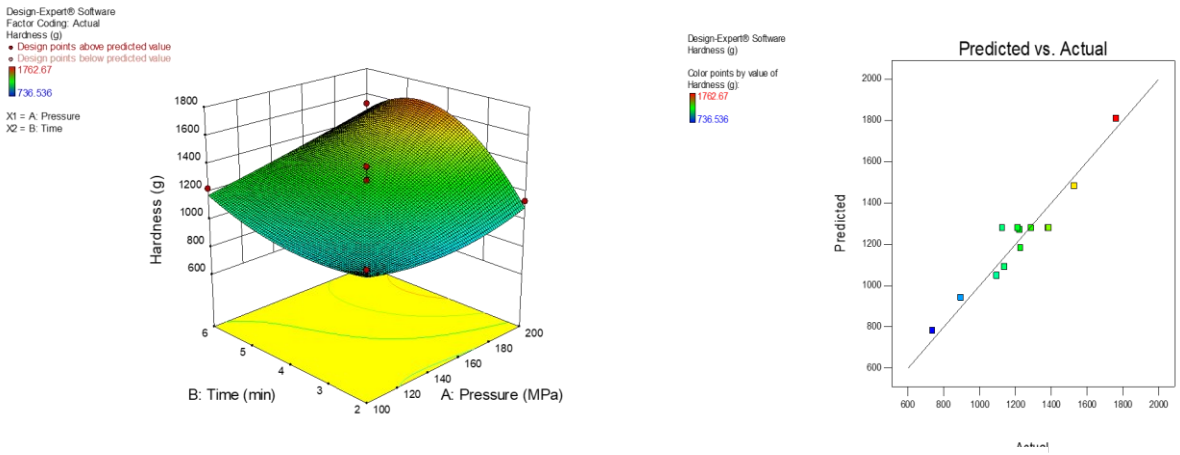
Texture is one of the attributes used by consumers to assess the food quality (Day & Golding, 2018). Texture profile analysis (TPA) is one of the common methods for measuring fish texture (Bland *et al*., 2018). Response surface plot was used to see the effect of HPP on various textural parameters. Texture Profile Analysis (TPA) was performed to evaluate the Hardness (g) and gumminess of shrimp samples.

Hardness was the most critical textural attribute in meat or seafood that determines its marketability (Kaur & Rao, 2016). The effect of pressure and holding time on samples followed a direct relationship, as shown in Figure 5.3. That means the higher the holding time and pressure, the higher the hardness of the samples, indicated by the red color in the plot, whereas a shade of blue indicates low hardness corresponding to low pressure and less holding time. The hardness of the sample was predominantly affected by pressure rather than time, indicated by the coefficients of A and B. From Table 5.2, it can be observed that both pressure and time had a significant effect on hardness. The highest value of hardness was observed for the samples treated at 220 MPa for 4 min, whereas a short processing time, such as 150 MPa for 1.1 min

produced the lowest value. Different models were tested to find the best fit, and the cubic model with a high R^2 value of 0.9182 was selected, and the lack of fit was not significant ($p>0.05$), demonstrating that the response surface equations sufficiently described the data. From the positive coefficients of the terms AB, it can be concluded that pressure and time synergistically influence shrimp muscle hardness. However, this effect was not significant. It is of importance to note the difference in hardness of samples treated for improving peelability compared to the samples treated at higher pressure and longer holding time to improve refrigerated shelf life, as discussed in Chapter 4. When HP treatment was applied to extend shelf life using higher intensity treatments ranging between 100 MPa and 300 MPa for 10 and 30 min, the hardness values ranged from 1023 to 2586 on day 1, whereas for achieving peelability, milder treatments showed minimal changes, as shown in Figure 5.3. This clearly shows the effect of different intensities of pressure treatment on the textural properties, as well as the importance of subjecting samples to treatments, depending on the product requirements.

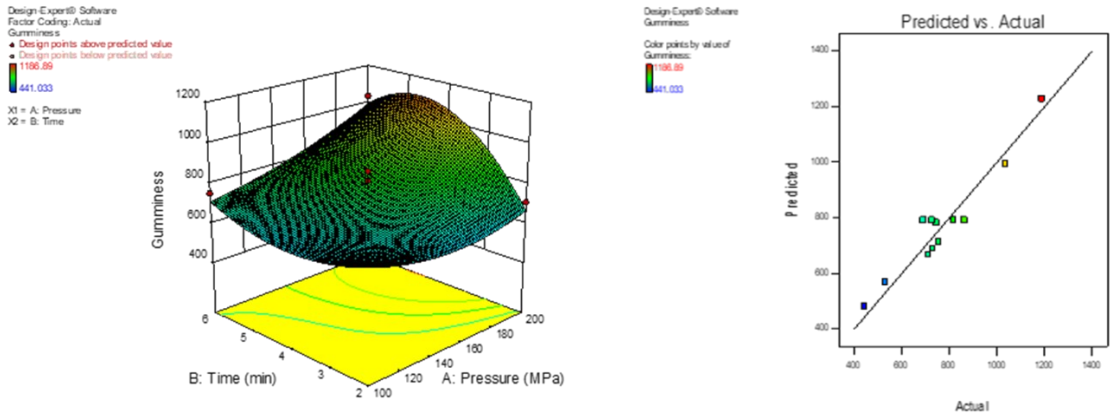
A study conducted by Kaur *et al*., (2016) on Black Tiger shrimp gave a hardness that was 2.38 times and 7.26 times better for the samples treated for 300 MPa for 3 min and 600 MPa for 15 min compared to the control. This increased hardness may be attributed to partial unfolding and denaturation of monomeric structures, protein aggregation and gelation, and dissociation of proteins from their oligomeric structures following HPP (Kaur *et al*., 2013).

Finally, gumminess was assessed. It is defined as the product of hardness and cohesiveness. Gumminess quantifies the force required to break down particles for ingestion; therefore, increased hardness correlates with elevated gumminess. The response plot obtained is illustrated in Figure 5.4. The results were analogous to those of hardness, as gumminess is the product of hardness and cohesiveness. Both pressure and time had a positive coefficient, with pressure having a higher value, resulting in an increase in gumminess. The cubic model was chosen, as it gave a higher R^2 value of 0.91. Unlike hardness, only pressure had a significant effect, along with the interaction effect with the quadratic term of B (AB^2). Previous studies about the effect of HPP on gumminess were analogous to results obtained for hardness, as gumminess is a multiple of hardness (Pal *et al*., 2016; Yagiz *et al*., 2009).



(a) (b)

Figure 5.3. (a) Response surface plots for effect of pressure and time on hardness of Black Tiger shrimp *and* ***(b) Actual vs predicted values of hardness as affected by pressure and time****. The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or corelation pattern of the response.*



(a) (b)

Figure 5.4. (a) Response surface plots for effect of pressure and time on gumminess of Black Tiger shrimp *and* ***(b) actual vs predicted values of gumminess as affected by pressure and time.*** *The scatter represents the models predictive performance and the distribution of point suggests the deviation or correlation pattern.*

Overall, both textural parameters of the samples were observed to have increased upon increasing pressure and holding time. This increase in texture may have contributed to the peelability of the shrimp at moderate pressure and time conditions.

5.3.4 Effect on color

The effect of HP treatment on the color of the samples was also evaluated. Changes with respect to L*, chroma, hue and ΔE are illustrated in several figures below. Lightness (L*) is the key parameter to tracking changes associated with HP treatment in black tiger shrimp due to its grey color in the raw state. "L*" represents the measure of degree of lightness, values range from 0 to 100, where 0 = pure black and 100 = pure white (Hasan *et al*., 2022). As observed in Figure 5.5, the lightness of samples ranged from 37.60 to 27.15 between samples, where 200 MPa held for 6 min gave the highest reading, and a lower pressure, such as 79.30 MPa for 4 min, gave the lowest lightness. Unlike other parameters, all the models gave a similar high R^2 value, with Cubic continuing to give the highest value of 0.96. The Lightness of samples was significantly affected by the linear effects of pressure, time, as well as some quadratic effects of time, as shown in Table 5.2. Hence, we can say that time had a superior effect over pressure in altering the lightness of shrimp samples.

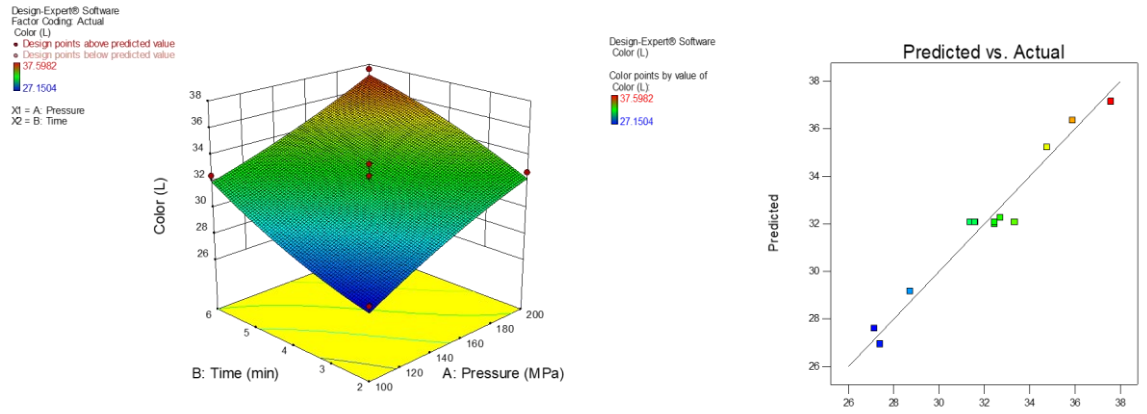
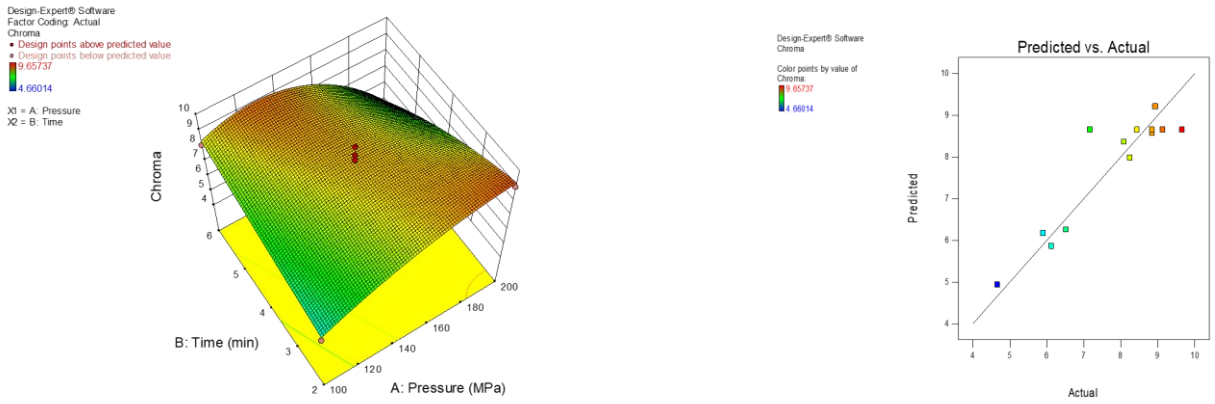


Figure 5.5. (a) Response surface plots for effect of pressure and time on lightness (L*) of black tiger shrimp *and* ***(b) actual vs predicted values of lightness (L*) as affected by pressure and time.*** *The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or corelation pattern of the response.*

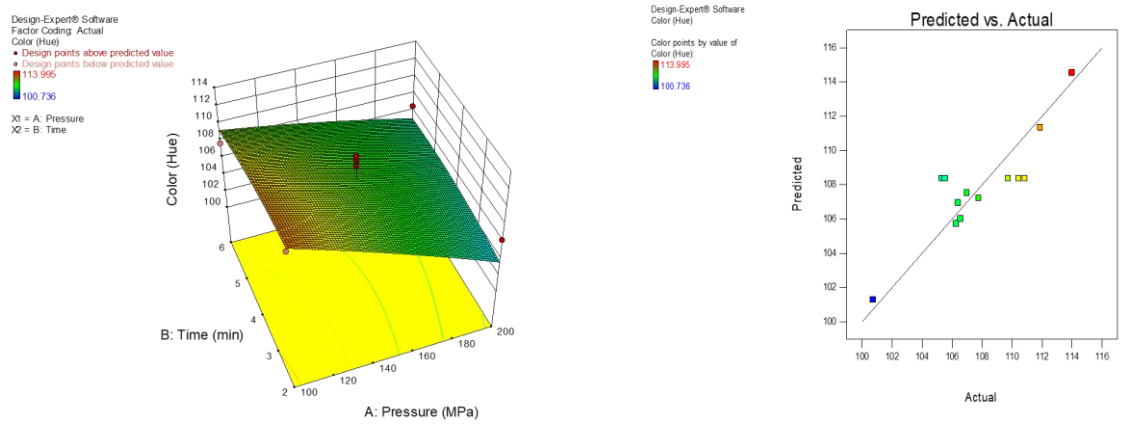
Chroma was evaluated, and the results are illustrated in Figure 5.6. The values ranged from 9.66 to 4.66, where the higher value was obtained from samples treated for 150 MPa for 4 min and the lower value from samples treated for 200 MPa for 6 min. The cubic model had the highest R^2 value of 0.85, and the quadratic model had a comparable value of 0.8083. The polynomial equation showed that pressure and time had a weak positive effect on the chroma of the sample. Chroma of the sample was not affected by the individual terms of pressure and time; however, the interaction effect, AB, and quadratic effect of pressure affected the chroma of samples significantly ($P<0.05$).



(a) (b)

Figure 5.6. (a) Response surface plots for effect of pressure and time on chroma of black tiger shrimp *and* ***(b) actual vs predicted values of chroma as affected by pressure and time.*** *The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or corelation pattern of the response.*

The effect of HPP on hue value is shown in Figure 5.7. The value ranged from 101 in samples treated for 4 min at 220 MPa, to 114 in samples treated for 79.2 MPa for 4 min. Hue was calculated for each sample with respect to its b* and a* values. It can be seen from Table 5.2 that pressure had a higher effect, with a negative coefficient, and time had a weak positive effect on the hue angle of samples. Out of all the terms, only pressure had a significant ($p<0.05$) effect on hue angle of the sample. Lack-of-fit value for regression equation is not significant ($p=0.60$). Non-significant lack-of-fit is good and indicates that the model equation adequately predicts the change in hue under any combination of values of the variable.



(a) (b)

Figure 5.7. (a) Response surface plots for effect of pressure and time on hue of black tiger shrimp *and* ***(b) actual vs predicted values of hue as affected by pressure and time.*** *The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or corelation pattern of the response.*

ΔE indicates the total color change associated with the sample, obtained from the L*, a*, b* values of the treated and untreated samples. The 3D plot shown in Figure 5.8 shows that the values of ΔE values ranged from 4.8 to 15.4. The smaller the value of ΔE, the smaller the deviation in color changes with respect to the untreated sample. The least difference in color was observed in the lowest pressure-time combination, such as 79.2 MPa for 4 min, followed by 100 MPa for 2 min, and then 150 MPa for 1.17 min, whereas the highest color difference was observed in the 200 MPa sample treated for 6 min. From Table 5.2, it can be seen that both pressure and time significantly affected the delta E. All the models had high R^2 values, with the highest being Cubic with 0.95, followed by linear with 0.93 and quadratic with 0.94. The R^2 value (0.94), which quantifies the proportion of variance around the mean, explained by the model indicated that only 6% of the variation for the response remains unexplained by the model and demonstrating a satisfactory fit (Kaur & Rao, 2016).

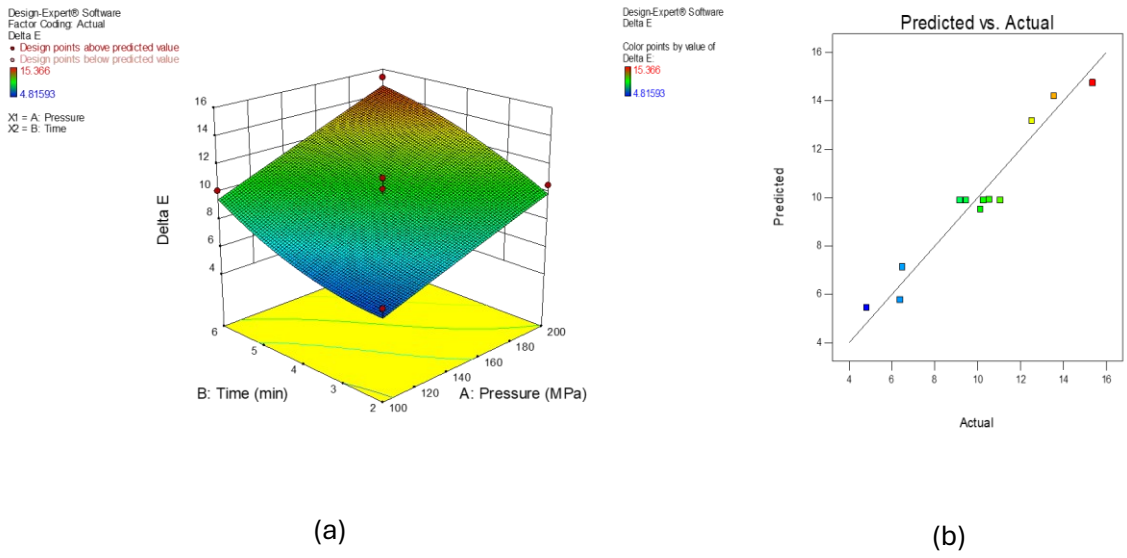


Figure 5.8. (a) Response surface plots for effect of pressure and time on delta E (ΔE) of black tiger shrimp *and* ***(b) actual vs predicted values of delta E (ΔE) as affected by pressure and time.*** *The scatter represents the models predictive perfomance and the distribution of point suggests the deviation or correlation pattern of the response.*

Overall, the aim was to evaluate the changes in color of the samples when they were subjected to HP treatment to enhance peelability. It was also observed that pressure played a comparatively bigger role for altering the color, such as an increase in lightness, changes in hue, and ΔE. As the samples were not intended for storage, lower pressure and holding time were applied, and it did not cause major changes visually, other than the natural variability that comes with biological samples.

5.4 Optimization and validation

The optimization function of Design Expert software was employed to obtain the optimized condition for peeling of shrimp samples. The optimal conditions were established to minimize peeling force, keeping all the other responses in the experimental range. Amongst various optimization conditions, the solution that obtained higher desirability (0.865) was chosen to validate the process, as indicated in Table 5.3. The variable constraints, such as pressure and time, were kept “in range” to optimize the process. The responses hardness, gumminess,

Lightness, chroma, hue and ΔE were kept as 'none' , as our primary focus is to minimize the peeling force and observe the effect of the optimized conditions (pressure and time) on other parameters, including color and texture. The obtained conditions were validated for each response, and the experimental values obtained are given in Table 5.3. The optimized pressure and time were 200 MPa and 3.4 min to achieve a peeling force of 187.589 g. The other responses also recorded values close to the predicted value.

Table 5.3. Predictive model validation at 200 MPa/3.4 min

Responses	Predicted value	Experimental value
Peeling force	186	181
Hardness	1450	1459
Gumminess	937	943
L*	342	31.5
Chroma	8.1	8.0
Hue	105	104
ΔE	11.9	-

Conclusions

This chapter focuses on a less explored application of HPP of Black Tiger shrimp (*Penaeus monodon*) in facilitating peeling of its shell. This study involved the application of high pressure to raw fresh shrimp at reduced pressure and holding time, and then evaluating the effect of pressure and time on quality attributes like texture and color. From the polynomial equations obtained, it was found that pressure had a superior effect on all the tested responses, such as peeling force, color, and texture of the shrimp. However, the coefficient was negative for peeling force as well as hue angle, indicating that peeling force and hue decreased with increasing pressure. However, the effect of time was very weak and not significant ($P>0.05$). The textural parameters, hardness and gumminess, showed an increasing value with increasing pressure and holding time, possibly due to changes in myofibrillar protein structure. The optimized pressure and time were 200 MPa for 3.4 min, which gave a low peeling force (high peelability). This

study concluded that HP treatment can effectively reduce the peeling force by detaching the shell from the shrimp muscle, that also causes improvement in texture, and causing slight changes in color However, this treatment will not lead to extension of refrigerated shelf life, and must be considered as an additional step to facilitate peeling before subjecting the shrimp to higher intensity treatments as seen in chapter 4.

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CHAPTER 6

GENERAL CONCLUSION

This study explored the application of high-pressure processing in seafood preservation and processing, focusing on texture, color, microbial stability and peeling efficiency across different seafood species. For the first objective, HPP at 350 MPa for 10, 20 and 30 min improved texture retention in both smoked trout and AC, with AC maintaining better firmness, while trout exhibited better retention after Day 7. Initial color changes occurred evidently, but the HP treated samples showed higher retention over refrigerated storage compared to the control sample. Lower pressures like 150 MPa and 250 MPa had minimal impact on AC quality, with microbial growth appearing in 150 MPa samples by Day 21, whereas the higher pressures effectively extended shelf life.

The second objective utilized RSM to optimize processing conditions to extend shelf life of shrimp for different checkpoints of storage such as day 1, 7, 14 and 21. It was observed that treating shrimp at 300 MPa for 10 min was sufficient to minimize microbial growth. The low intensity treatment not only destroyed microorganisms but also resulted in better visual appearance. However, the optimized conditions for day 7, 14 and 21 resulted in higher pressure and holding time to minimize microbial growth, altering the texture and color, highlighting the balance between quality and preservation.

Finally, for the third objective, HPP was tested as an alternative to ice storage for peeling black tiger shrimp. Pressure significantly reduced peeling force and hue, while hardness and gumminess increased. The optimal conditions obtained was 200 MPa for 3.4 min , minimizing peelability and preserving quality.

HPP proved effective in improving seafood quality and shelf life. It enhanced texture and microbial stability in smoked fillets, optimized shrimp preservation and facilitated easier peeling. Future research could further refine HP parameters for better industrial applications.

RECOMMENDATIONS FOR FUTURE RESEARCH

Growing awareness about the detrimental effects of processed foods, including the presence of artificial additives, preservatives and loss of nutrients due to thermal treatments, has led to the increasing demand for healthier and more natural alternatives. This shift in preference presents a great opportunity for HPP, as it can retain quality parameters, while maintaining microbial safety. This calls for meeting the challenges caused by HPP. Based on our research and many other studies, one of the crucial aspects that needs to be addressed is the change in color when treatments above 300 MPa is applied. This limits its application in meat and seafood for commercial purposes. Studies must be conducted on how the loss of color can be prevented from a molecular level. Combining HPP with other techniques may aid in this.

Furthermore, when considering a broader perspective in terms of potential applications, this approach could also significantly contribute to advancements in the plant-based food industry. The consumption of plant-based foods is on the rise due to consumer interest in sustainable and healthy alternatives for meat or animal origin foods. However, there exists a barrier to incorporate them into novel food formulations, due to limited knowledge about plant proteins, compared to animal proteins. HPP may become useful in unlocking its full potential in improving its texture, digestibility, shelf life etc.. By addressing these issues, HPP may help in advancing the plant-based food industry, offering healthier, cleaner and a much more sustainable protein option.

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