

**Effect of High Pressure Processing on Refrigerated Storage Stability of Fresh and
Smoked Salmon Fillets**

By

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ABSTRACT

Salmon is a highly popular seafood for several consumers, and it has remained an attractive choice because of its health benefits and high omega-3 fatty acid content. Due to the high lipid content, high moisture, and high nutrient profile, it can undergo rapid deterioration without adequate processing or improper handling. Therefore, processing of salmon is important to make it easily accessible, to make sure it remains in compliance with food safety and that it provides increased nutrition to the consumers. Food industries usually adopt many well recognized processing methods including thermal processing, drying, freezing, baking, fermentation and/or using chemical preservatives; however, consumers prefer for salmon that has undergone the least processing and for it to be free from chemical additives. Furthermore, the popular thermal and dehydration methods can also reduce the commercial and nutritional value by damaging the vitamins, polyunsaturated fatty acids, or other flavor compounds. This study was carried out to evaluate the effect of high pressure processing (HPP) on fresh and smoked salmon fillets during refrigerated storage (4 °C) of 21 days. The study was performed on both fresh and hot smoked salmon samples to evaluate their effect on refrigerated storage stability. For the fresh market salmon samples, three high pressure (HP) treatment levels were applied at 150, 250 and 350 MPa with holding times of 10, 20, 30 min, and their quality was evaluated across refrigerated storage (4 °C) for up to 21 days. Samples were analyzed for texture (tenderness and firmness), color (lightness, redness, chroma and hue), protease and microbial growth with the tests being performed on day 1, 7, 14 and 21. The control samples were the fresh salmon that were not HP treated.

For in case of the industry supplied hot smoked salmon, HP treatment was carried out only at 350 MPa for three different holding times of 10, 20, 30 min. Quality evaluation was

performed similar to fresh salmon for texture, color and microbial growth for similar quality evaluation days. The control here were the smoked salmon not treated with pressure.

For all HP treated samples, it was observed that there was an increase in firmness and tenderness in texture, increase in lightness and hue, decrease in redness and chroma as color indicators. The protease activity and microbial growth were also better controlled for the HP treated samples across the storage. Furthermore, the loss in textural and color properties were slower in HP treated samples across refrigerated storage. And this loss reduced with increased pressure level and holding times. Better control was observed in samples treated at higher pressure levels for longer holding times. On comparing the fresh and smoked salmon treated with HP, the smoked salmon showed even better preservation for texture, color and microbial stability properties than fresh samples possibly derived by the synergy between smoke curing and HP treatment.

RÉSUMÉ

Le saumon est un fruit de mer très populaire auprès de nombreux consommateurs, et il reste un choix attrayant en raison de ses bienfaits pour la santé et de sa teneur élevée en acides gras oméga-3. En raison de sa teneur élevée en lipides, de son humidité élevée et de son profil nutritionnel élevé, il peut subir une détérioration rapide sans un traitement adéquat ou une mauvaise manipulation. Par conséquent, la transformation du saumon est importante pour le rendre facilement accessible, pour garantir qu'il reste conforme à la sécurité alimentaire et qu'il apporte une nutrition accrue aux consommateurs. Les industries alimentaires adoptent généralement de nombreuses méthodes de transformation bien reconnues, notamment le traitement thermique, le séchage, la congélation, la cuisson, la fermentation et/ou l'utilisation de conservateurs chimiques ; cependant, les consommateurs préfèrent que le saumon ait subi le moins de transformation et qu'il soit exempt d'additifs chimiques. En outre, les méthodes thermiques et de déshydratation populaires peuvent également réduire la valeur commerciale et nutritionnelle en endommageant les vitamines, les acides gras polyinsaturés ou d'autres composés aromatiques. Cette étude a été réalisée pour évaluer l'effet du traitement à haute pression (HPP) sur les filets de saumon frais et fumé pendant un stockage réfrigéré (4 °C) de 21 jours. L'étude a été réalisée sur des échantillons de saumon frais et fumé à chaud afin d'évaluer leur effet sur la stabilité du stockage réfrigéré. Pour les échantillons de saumon du marché frais, trois niveaux de traitement haute pression (HP) ont été appliqués à 150, 250 et 350 MPa avec des temps de maintien de 10, 20, 30 min, et leur qualité a été évaluée dans un stockage réfrigéré (4 °C) pendant une durée allant jusqu'à 21 jours. Les échantillons ont été analysés pour la texture (tendresse et fermeté), la couleur (légèreté, rougeur, saturation et teinte), la protéase et la

croissance microbienne, les tests étant effectués les jours 1, 7, 14 et 21. Les échantillons témoins étaient le saumon frais qui a été pas traité HP.

Dans le cas du saumon fumé à chaud fourni par l'industrie, le traitement HP a été effectué uniquement à 350 MPa pendant trois temps de maintien différents de 10, 20, 30 min. L'évaluation de la qualité a été réalisée de manière similaire à celle du saumon frais pour la texture, la couleur et la croissance microbienne pendant des jours d'évaluation de la qualité similaires. Le contrôle ici était le saumon fumé non traité sous pression.

Pour tous les échantillons traités par HP, il a été observé une augmentation de la fermeté et de la tendreté de la texture, une augmentation de la luminosité et de la teinte, une diminution de la rougeur et de la saturation en tant qu'indicateurs de couleur. L'activité protéase et la croissance microbienne étaient également mieux contrôlées pour les échantillons traités par HP dans l'ensemble du stockage. De plus, la perte des propriétés de texture et de couleur était plus lente dans les échantillons traités par HP lors du stockage réfrigéré. Et cette perte diminue avec l'augmentation du niveau de pression et des temps de maintien. Un meilleur contrôle a été observé dans les échantillons traités à des niveaux de pression plus élevés pendant des temps de maintien plus longs. En comparant le saumon frais et fumé traité avec HP, le saumon fumé a montré une conservation encore meilleure des propriétés de texture, de couleur et de stabilité microbienne que les échantillons frais éventuellement dérivés de la synergie entre le fumage et le traitement HP.

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CONTRIBUTION OF AUTHORS

Miss Greeshma Cyriac Vettickathadathil is the M.Sc. candidate at McGill University. She is enrolled in the Department of Food Science and Agricultural Chemistry where she is under the supervision of Dr. Hosahalli S. Ramaswamy. She conducted the experiments, gathered, and analyzed the data, and presented the results under the guidance of her supervisor. She drafted the thesis, poster and manuscripts for scientific conferences and publications.

Dr. Hosahalli S. Ramaswamy is the supervisor under whose guidance the research was carried forward. He assisted the M.Sc. candidate throughout the research by planning the experiment, providing the equipment, financial assistance, reviewing results, and with the final editing of the thesis. His help has also been extended towards the editing of the posters for conferences and manuscripts for publications.

Dr Ali R. Taherian is the research Associate working in the laboratory used by the M.Sc. candidate. He has helped the candidate carry out the experiment, and provided technical support to the use of equipment, and with the editing of presentations, posters, and manuscript.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this thesis has been presented/ submitted as a poster at the following scientific conferences:

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CHAPTER 1

Introduction

Processing of food is an area of high importance as it helps in making available foods that are safe to consume, convenient, easily accessible, diversified, and provides nutritional benefits to the consumers (Fellows, 2022). Conventionally, food processing techniques focus on the use of thermal and other treatment methods or chemical preservatives to achieve this. But lately in consideration of consumer preference for the availability of food products that have undergone the least processing and contains the least chemicals, with an extended storage, the demand for new processing methods is of high importance (Bhargava et al., 2021).

In recent years, a variety of techniques that do not use heat or chemicals have gained popularity due to their ability to produce minimally processed food. Some examples include, pulsed electric field (PEF), cold plasma treatment, irradiation, ultraviolet (UV) light treatment or ultrasound (US) treatment. High pressure processing (HPP) is one such promising and growing non-thermal technology that does not use heat or chemicals, but instead uses pressure to achieve food processing needs. It is also commonly known by other names such as high hydrostatic pressure processing or ultra-high-pressure processing (Jadhav et al., 2021).

HPP holds potential for the reduction of pathogenic organisms in food and at the same time it presents it as naturally as they are to the consumers. Furthermore, HPP can also serve as an alternative to the commonly employed thermal treatment methods such as pasteurization or sterilization by ensuring microbiological decontamination and maintenance of food freshness in addition to increasing the shelf-life of the products while most importantly, maintaining the natural attributes of the food product (Nabi et al., 2021).

In recent years HPP has become a major part of the food industry for various specific applications or for reconditioning. It has been incorporated for the use of several products such as fruits and vegetables, dairy, meat products, fruit juices and even in the seafood industry. Generally, various non-thermal treatment can be used for inactivating microorganisms and achieving extended storage, however HPP has gained wide importance in the seafood industry because of its popularity with food safety and ready-to-eat products (Riahi & Ramaswamy, 2003).

Human diet includes a good portion of seafoods which are a major source of proteins, omega-3 fatty acids, iron, calcium, and various other minerals (Khalili and Sampels, 2018). As due to the presence of high-water activity and active enzymes in seafoods, it is highly susceptible for spoilage and towards the development of undesirable odor or rancid flavors upon storage (Abedi-Firoozjah et al., 2023). Traditional methods by which seafood is preserved by industries include freezing, chilling, drying, salting, smoking, drying, fermentation, canning, all of which aims towards reducing the water activity, microbial growth, and enzymatic changes (Singla and Sit, 2021). However, these methods result in certain changes to the seafood products. For instance, freezing results in causing protein denaturation, ice crystals disrupt the cellular structure that affects the texture, color, and other sensory properties, thereby preventing the use of the original product as a raw material for further products such as fish cakes or minced fish (Zhang et al., 2023). During cold storage, due to the presence of high levels of unsaturated fatty acids in fish, they become rancid (Ding et al., 2020). To overcome this, the use of popular chemical preservatives such as formaldehyde, nitrites, sulphites, or synthetic antioxidants are used (Sen, 2021). Overall, these traditional methods result in additional processing for quality that comes with an increased storage cost. The incorporation of HPP in seafood preservation has

shown improvement in terms of enhancing its microbiological, physiochemical, shelf-life and sensory qualities (Roobab et al., 2022). This is because of the destabilization of the enzymes that helps in controlling the microbiological flora, bursting of microbial cells, reducing drip loss, enhanced water holding capacity, and denaturation of proteins, that helps with extending the shelf-life (Puértolas and Lavilla, 2020). Thus, the use of HPP in the seafood industry helps achieve products that are natural, healthier and of higher nutritional quality. There is only limited amount of published information of seafood processing using HP treatment for preservation purposes although much of the earlier work has been focussed on shucking of oysters, gel formation in surimi type of products etc.

The general objectives are:

1. Evaluation of high pressure treatment for better retention of the quality and refrigerated stability of fresh salmon fillet.
2. Evaluation of the quality and refrigerated storage stability of HP treated smoked salmon fillets and compare them with that of HP treated fresh salmon.

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CHAPTER 2

Review of Literature

2.1. High pressure processing

The innovative and non-thermal method of high-pressure processing (HPP) paves way for industries to replace the use of harmful chemical preservatives and conventional heat treatment methods. It brings forth the idea of fresh foods that are subjected to being minimally processed (Galanakis, 2021). The resulting pressure treatment of seafood helps in inducing altered biochemical reactions, changes in cell membrane and genetic mechanisms, extended shelf life, inactivate pathogenic and spoilage microorganisms, all of which occurs with the availability of nutritional food that is not seriously affected in terms of its color, texture, quality, or other sensory parameters (Levy et al., 2021).

2.2. Evolution of HPP

Literature survey indicates that a steady increase in research related to high pressure processing was carried out during the last twenty years, even though the use of pressure for food applications has been researched since the nineteenth century (Singla and Sit, 2021). The first studies related to the use of HP treatment for seafood was carried out successfully by the pioneers Ohshima et al. (Ohshima et al., 1993) and Lanier (Lanier, 1998). Later during the early 2000s, the effect of HPP on color and texture other quality parameters of fish was studied (Matser et al., 2000). By the year 2005, HPP was most popular for its effect on controlling the pathogenic microbial bacteria in fish thereby ensuring better food quality and stability. In the following years, the physiochemical changes related to subjecting seafood products to HPP was studied in addition to its extended shelf life (Roobab et al., 2022). Recent reviews indicate how HPP can be used to reduce seafood allergens and improve its digestibility. This was carried out by the modification of the binding site or the epitopes of the immunoglobulins to

enhance binding capacity (Zhang et al., 2019).

Other approaches throughout the years also include use of HPP (600 MPa for 10 min) to inhibit oxidation of cholesterol in minced mackerel. HP treatments (450 and 600 MPa for 1–5 min) that holds capability of replacing steam precooking especially in canned tuna industry (Jiranuntakul et al., 2018); HPP (300 MPa at 20 °C for 15 min) for biofilms to prolong the shelf life of smoked sardines (Günlü et al., 2014). Low pressure HPP treatment of hilsa fillets at a pressure of 200 MPa at 30 °C for 10 min helped in reducing the microbiological count by 2 log units in addition to improving the firmness (Chouhan et al., 2015). Studies have also been carried out towards the incorporation of phytochemicals or plant extracts to minimize the oxidation within this food group (Roobab et al., 2022). Food products subjected to the HPP was first introduced in the market in 1993 by food companies operating in Japan. Presently, several products in the market have been commercialized by the HPP technology that includes beverages, meat products, vegetables and seafood commodities. In addition to the pathogenic and quality aspects, work has been carried out regarding the scope of HPP to modify the ingredients in food, thus paving way towards food with novel functionalities (Huang et al., 2020).

Several works carried out in the area of HPP in seafoods included the use of pressure for studying endogenous enzymes of pressurized fish that resulted in having enhanced storage quality (Ashie et al., 1997), improved physio-chemical properties of HP treated tuna with reduced histamine development across refrigerated storage (Zare and Ramaswamy, 2004), HP assisted thawing in salmon, which suggested that HP thawing had an improved rate of thawing in comparison to conventional thawing (Li, 2024), reduced drip loss was observed in HP thawed salmon (Zhu et al., 2004), HPP in Atlantic salmon also resulted in improved structure

stability of myofibrillar protein (Li, 2024), pressure-shift freezing in carp resulted in reduced TBA content along with reduced drip loss (Sequeira-Munoz et al., 2005).

2.3. How HPP works

For the HPP application, water acts as the media for transmitting pressure to the seafood product. The product is vacuum packed or sealed and any packaging material capable of flexibility can be used. It is placed into a chamber, thereby subjecting the sample to pressure treatment (Levy et al., 2021). Generally, HP treatment can be carried out in two methods which consists of the batch method and the other being the semi-continuous method. In most cases, industries use the batch method which can be employed for both moist solid and liquid food products. The equipment consists of conveyors for loading and unloading of baskets that carries the products; vessel where the products are processed by high pressure; plugs and wedges to close the vessel; yoke to withstand the pressure produced; intensifier pumps for pressure generation and a system for monitoring and controlling the pressure and temperature. A major part of the equipment is the pressure chamber and the intensifier pumps that helps with generating pressure (Nabi et al., 2021).

The seafood products are introduced into the vessel or termed as loading of the products after which the vessel gets aligned with the yoke. Packing of the food sample before loading must be taken into consideration due to the decrease in volume by about 10-20% due to pasteurization and the following return to nearly the original volume once the pressure is removed. Low pressure water is then pumped into the vessel which then is closed by the plugs and wedges. The high-pressure intensifiers then begin pushing more water into the vessel. A few pressure transmitting media other than water include castor oil, ethanol, glycol, and sodium benzoate. Each of these intensifiers consists of a piston that is pushed by the hydraulic

oil. Considering that the area of the piston is much larger than the plunger, and because of this section difference the pressure of the fluid gets intensified inside the vessel (Huang et al., 2017). This pressure is then held for a few minutes during which the destruction of microorganisms, inactivation of enzymes and some modification of food quality takes place mostly retaining the food freshness without thermal application. Finally, the pressure is released, and depressurization occurs, with further steps involving the opening of the vessel. Unloading of the product takes place and thus reaches the consumers in a non-thermally treated manner. The throughput of the equipment is based on the cycle time and the loading factor (Ramaswamy and Shao, 2010). Cycle time is the total time required for pasteurization, holding of the pressure and the following de-pasteurization. Loading factor is the percentage of the vessel volume that gets used for holding the sample and the shape of the package. As the process takes place, with the increase in pressure, a significant rise in temperature occurs because of adiabatic heating due to the fluid compression (Zhu et al., 2004). Some popular suppliers of HP processing equipment include Avure Technologies from USA, NC Hyperbaric from Spain and UHDE based in Germany, Kobelco in Japan, Stansted in UK, Bao Tao Kefa in China etc. Vessel configurations of both horizontal and vertical models are available in the market presently. Most commercial HPP equipment have a processing pressure limit of 700 MPa, with research equipment having the ability to go up to 1400 MPa (Nabi, 2021).

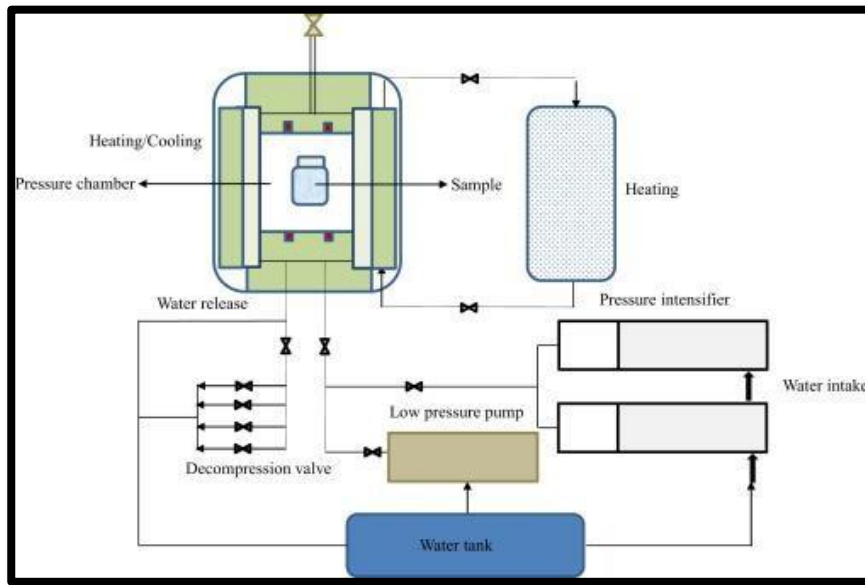


Fig.2.1. Schematic diagram of vertical high pressure processing equipment.

(Levy et al., 2021)

2.4. Governing principles of HPP

There involve two scientific principles based upon which the HP treatment is carried out in food samples. The primary one is based on Le Chatelier's principle or is also called as the equilibrium law that is based on thermodynamics. It states that when a system that is at equilibrium is disturbed, it behaves in a manner so as to minimize the disturbance caused, and that is by moving in a direction with an attempt towards reducing the change as it returns to equilibrium. In industries this helps in studying the effect pressure and temperature will have towards the equilibrium position. This means that in HP, phenomenon which are associated with a decrease in reaction volume are enhanced by the effect of pressure, but those accompanied with increase in volume are inhibited (Evrendilek, 2023). Here, based on the principle, due to varied pressure and temperature in a system, a corresponding shift in equilibrium is seen through the reduction in volume. Hence, pressure is favorable towards reactions that are involved with the decrease in volume. Here pressure transmission is not dependent of the mass or time thus

resulting in faster treatment time and further up scaling of the technology into commercial applications (Barba et al., 2020).

The second principle is based on the isostatic rule which conveys that when the sample is under pressure, the pressure is uniformly and instantly distributed within the sample. This is in considering that the sample is in direct contact with the pressure transmitting medium or sealed hermetically in a packaging material that is flexible in nature (Abera, 2019). This is because when there is a certain level of pressure applied, the fluid that is referred to as hydrostatic can transfer this pressure without any friction. Here when the pressure is applied, the distance between the food molecules gets altered and as a result, the volume of the material reduces without shape alteration. As compared to electrostatic, hydrogen bonding or hydrophobic interactions; the covalent bonds less affected because their working distance are not affected by this pressure. And this acts as the major reason for the ineffectiveness of prevention of activities for functional groups of food. It also indicates as to how nonporous foods are not affected macroscopically by applied pressure. Overall, it is noticed that the transfer of pressure is isostatic and quasi-instantaneous in nature. As compared to thermal processing the processing by pressure is independent of sample size, nor the geometry or size of the equipment (Naveena and Nagaraju, 2020).

2.5. Existing work carried out using HPP

Several multifaced work has been carried out in the area of HPP for the improved nutritional and safety aspect of food along with their enhanced storage life. A wide array of food products ranging from dairy, meat, seafood, beverages, fruits and vegetables, have been researched on using HPP in addition to other areas of food research such as packaging design, innovative and improved products, and most popularly increased food safety. Improved shelf-life

of milk by pressure destruction of *L. monocytogenes* (Mussa and Ramaswamy., 1999), high-pressure high-temperature spore destruction of *Clostridium botulinum* in milk (Shao, et al., 2022) HP induced destruction of *Clostridium sporogenes* in milk at quasi-elevated temperatures (Ramaswamy, et al., 2010), increased storage moduli of HP treated yogurts (Ramaswamy, 2015), impregnation of ascorbic acid into apple cubes through HPP for nutritional fortification and reduced browning (Vatankhah, and Ramaswamy, 2019), reduced retrogradation of HP treated lentil slurry (Ahmed et al., 2009), lower gelatinisation of HP treated Basmati rice samples (Ahmed, et al., 2007), limited protein structural changes in soybean after HP treatment (Alvarez et.al., 2008), texture improvement of HPP treated pork (Singh and Ramaswamy, 2012), high pressure induced destruction of effective inactivation of avidin in eggs (Singh et al., 2015), improved rheological characteristics of egg components (Singh et al., 2015), reduced oxidation and texture degradation of HP treated tuna (Zare and Ramaswamy, 2004), HPP used for the destruction kinetics of *E.coli* (O157:H7) and *L.monocytogenes* in mackerel fish slurry (Ramaswamy, H. S.,et al., 2008) improved storage of mango juices through the utilisation of HP destruction kinetics (Hiremath and Ramaswamy, 2005), improved microbiological stability of orange juice (Basak and Ramaswamy, 2001), HP induced inactivation of pectin in apple juice (Riahi and Ramaswamy, 2003), improved functional properties of aquafaba (Alsalman and Ramaswamy, 2020), are some amongst many work carried out using HPP in food products.

Apart from food processing, HPP offers benefits for several other areas too. Thermal properties of polyactides for the development of food packaging materials was studied using HPP (Ahmed, et al.,). HPP was used for dyeing of wood where better intensity and uniform dyeing was achieved through HPP in comparison to conventional hot dip method (Yu, Y et al.,

2019, product homogenisation purpose in cosmetic industry (Dumay, et al., 2013), high-pressure steaming of cellulose fabrics which indicated improved shrinkage and fabric hand (Ohshima, 2003), high-pressure and high-temperature graphene inlaying of fabric for anti-static and anti-ultraviolet properties (Zhang, 2019), biotechnological application for improved homogenisation of high-pressure homogeniser (Shirgaonkar, 1998), are some amongst non-food processing applications of HPP.

2.6. Effect of HPP on seafood

2.6.1. HPP induced changes in proteins

The changes induced by the application of HPP on seafood proteins is vital towards understanding several sensory attributes that contribute towards the color, texture or even juiciness of the seafood product under study. A governing principle towards the use of this cold pasteurization technique for industry level applications is based on this change in structure of the protein which ultimately is based on the law of equilibrium. This equilibrium phenomenon change is because of the volume reduction that is mediated by pressure during the HP treatment. The pressure applied or even the compressibility of proteins causes an effect towards the primary, secondary, tertiary, or quaternary structures of proteins by stabilizing, denaturing or sometimes not affecting these structures (Dehnad et al., 2023). In case of the tertiary and quaternary structures, they are associated with weak electrostatic and hydrophobic interactions which can be easily altered by the HP treatment at around 50 MPa for quaternary structures and 200 MPa for affecting tertiary structures. Denaturation of the protein secondary structures occurs at around 100-300 MPa (Lanier, 1998). Hence based on these changes of the protein structures, concepts such as gelatinization, denaturation, aggregation, disassociation, and unfolding can be brought upon by HPP.

In case of seafood, fish muscles are mainly composed of proteins which comprises of

myofibrils, sarcoplasm and stroma or collagen. Within the total crude proteins of these fishes, myofibrils is present in a percentage of 40-60, sarcoplasm about 30% and stroma about 10-20%. Amongst these, sarcoplasm is the most resistant to pressure thus denaturing at about 400 MPa and myofibrils require a pressure of about 100-200 MPa. Some recent applications of altering seafood proteins includes them being processed in to gelling agents or fish pastes, as these proteins are sensitive to pressure and labile to denaturing (Puértolas & Lavilla, 2020). Further studies are also being conducted on specific fish protein also taking into consideration their species, chemical composition or even their processing technique (Kristinsson and Bosco, 2000).

2.6.2. HPP induced changes in lipids

Seafood products are very susceptible to unfavorable changes during storage as a result of high lipid content present in them. These spoilage changes are because of oxidation of fatty acids and reflect as changes in color and flavor which subsequently affects the sensory characters. High presence of polyunsaturated fatty acids (PUFA) that includes eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, metal ions and catalysts accelerate this oxidation process during the storage of these seafood products (Li et al., 2021). The effects of HPP on lipid oxidation has been studied by taking into consideration the secondary lipid oxidation products generated that includes thiobarbituric acid-reactive substances TBARS or the hexanal content. In addition to this, the lipid oxidation content can be measured by analyzing the free fatty acid content or the antioxidant enzyme activities (Bolumar et al., 2021). It has been found that the HP treatment in seafood results in the acceleration of the process of lipid oxidation but in analyzing the results of cooked products after chilled storage it has been found that the results have been same. Hence it is considered for HPP to accelerate the oxidation of lipids but not

increase the overall oxidation of the seafood product. A pressure treatment of 200 MPa was observed to induce the accelerated formation of TBARS in salmon and pressure of 100-600 MPa induced increased TBARS in case of prawns (Cropotova et al., 2020). Altogether, the use of several antioxidant compounds such as sage, rosemary, oregano, gelatin-based edible films, chitosan or egg whites have been used to reduce rancidity of seafood during storage. Methods involving vacuum packing or even modified atmosphere packing helps achieve the same result of reduced lipid oxidation during the products storage (Huang and Ahn, 2019).

2.6.3. Effect of HPP in microbiology

A major effect induced by the application of HPP to seafood is the HPP equipment's capability to inactivate the microbial organisms, thereby reducing the presence of these pathogenic organisms in food. With the subjection of the seafood products at pressure around 100 MPa, there occurs protein denaturation of the microbes and at pressure levels around 200 MPa their cell structure and membrane get affected (Rode and Rotabakk, 2021). Pressure treatment above 300 MPa results in irreversible enzymatic and protein changes and eventually leads to the inactivation of these organisms. In consideration of bacteria and molds possessing a higher resistance towards HPP, it is important to properly store the products that are processed at below room temperature levels for the purpose of controlling their growth. For increased food safety and food quality, high pressure sterilization treatment can be accomplished by techniques such as High Pressure Thermal Sterilization (HPTS) or Pressure Assisted Thermal Sterilization (PATs) as opposed to conventional retort techniques (Sehrawat et al., 2021).

The major parameters that govern microbial inactivation in seafood products include pressure, temperature and the holding time. For instance, when pressure is increased from 275 MPa to

310 MPa with the simultaneous increase in holding time from 2 to 6 min, there occurs the inactivation of bacteria by 4 log reduction within day 10 of refrigerated storage in minced tuna samples (Ramirez-Suarez & Morrissey, 2006). Generally, it has also been established that Gram-negative bacterium present in seafood samples are more susceptible to the HP treatment as compared to Gram-positive sample, due to greater resistance of Gram-positive. As a result, the presence of more Gram-positive bacteria is seen in pressure treated seafood samples (Karim et al., 2011). The most sensitive bacteria to HPP include the Gram-negative bacteria *Pseudomonas* and H₂S producing bacteria such as *Shewanella putrefaciens* (Kontominas et al., 2021)

In general HP treatment with pressure around 250-300 MPa helps with the inactivation of some spoilage bacteria while pressure treatment below 250 MPa is futile for the same reduction of these unfavorable microbial organisms. For instance, HPP treated samples such as salmon, cod and mackerel were found to possess fewer log reduction of aerobic bacteria in comparison to untreated samples (Aganovic et al., 2021).

HPP also holds potential for inactivating viruses in seafood such as shellfish. As due to the structural diversity of viruses, they possess a wide range of resistance to the pressure processing. For the control of virus such as norovirus in oysters, treatment with pressure at 400 MPa for 5 min at 5 °C inactivates the virus for up to 4 log cycles (Leon et al., 2011). Here HPP denatures the capsid protein of viruses and thus prevents the binding to the host cell. Other property such as the media that surrounds the sample and the temperature also affects this inactivation by pressure (DiCaprio et al., 2019).

2.6.4. Effect of HPP on color

A major sensory parameter that helps in determining the quality of the seafood is the visual aspect of color. It acts as a sensory indicator amongst consumers during purchase of the product for determining the freshness of the seafood product. The treatment of seafood products with HPP results in having a resemblance to cooked seafood, with the production of a certain degree of whiteness or opaque appearance. This happens as a result of myoglobin denaturation in fish muscles and the further protein denaturation of myofibrillar and sarcoplasm proteins at pressure levels greater than 150-300 MPa. Apart from these protein changes, other factors such as muscle hydration status, pigments and lipid oxidation changes can also contribute towards this color change in seafood muscles. Furthermore, the influence of time is also a key parameter for this color change. This was observed by taking into study tilapia fillets and subjecting them to pressure treatment of 200 MPa for 1 and 3 min. Clear modification of fish color was noted at higher treatment times (Suemitsu & Cristianini, 2019). In addition to this, the fish species also contribute towards the change in color, for instance, pressure treatment of 200 MPa for hake did not induce color changes whereas for turbot it displayed a cooked appearance, for the same pressure processing treatment (Puértolas & Lavilla, 2020).

The CIELAB system is used for the study of color changes in seafood for research or industrial purposes. CIELAB is a 3-Dimensional color space that helps accurately quantify color based on three color values. Here numerical values help to determine change in color, and this is done by using an equation $L^*a^*b^*$. Here L^* indicates the lightness parameter based on a scale of 0-100; a^* negative indicates green, a^* positive indicates red; b^* negative indicates blue, b^* positive indicates yellow. When the seafood products are treated by HP, they indicate an increase of L^* value and therefore conveys the increase in color lightness of fish.

Upon HPP treatment, for certain fish species the L^* value increases as in mackerel, tuna, tilapia, hake, salmon and cod. As compared to a^* and b^* , it is L^* that is considered more important when analyzing this seafood quality parameter. For some species the redness or a^* value decreases as in tuna, fresh cod and mackerel; and the b^* value increases in species such as tilapia, tuna, cod and salmon (Puértolas & Lavilla, 2020).

In case of oysters, an increased L^* or opaque appearance was observed for HP treatment of 300 MPa at 20 °C for 10 min and at about 100 MPa the redness value decreased. For pressurized prawns at 100 MPa the color began to whiten, where results were also dependent on species variability. Polyphenoloxidase (PPO) causes blackening in prawns as a result of oxidation of phenols to quinones and is considered to be a poor organoleptic quality and this therefore is controlled by the addition of sulfites. HP treatment between 300 to 400 MPa carried out at a temperature less than 10 °C for about 10 min has shown to reduce about 80% PPO activity in prawns (Duranton et al., 2014).

2.6.5. Effect of HPP on texture

Textural changes in seafood by high pressure processing are mainly associated with its denaturation effects on proteins. The application of pressure induces hardness and higher the pressure, more will be this hardness. Hence because of pressure application, processes including the disassociation of oligomers, precipitation, denaturation, unfolding and gelatinization take place, all of which affects the texture of the product. Seafood products are very fragile and hence require the need of having cohesion or firmness for their consumption. Another factor that contributes towards textural changes is the water loss that occurs as a result of protein denaturation. Fragmentation of myofibril structures and the reduction of sarcomere length can also induce textural changes in the seafood muscle. These textural changes can be

efficiently characterized by studying parameters such as chewiness, springiness or hardness. Gentle pressurization of 100 MPa for 2-5 min on mackerel fillets was reported not to affect these parameters, but intense pressurization of about 500 MPa for the same time was reported to cause a major change (de Alba et al., 2019). And hence the increased springiness of the fish muscle could be as a result of the increased hardness and the formation of hydrogen bonded network (de Oliveira et al., 2017). Study on adhesiveness was evaluated on albacore tuna where pressure treatment at 50-150 MPa showed no difference between treated and control samples, 200-250 MPa treatment resulted in increased adhesiveness, and range of 300-500 MPa resulted in vanishing of the progressively increased adhesiveness. The loss of myosin fiber could be the reason for increased adhesiveness at mid pressures and for decreased adhesiveness at high pressure could be because of unfolding of actin and sarcoplasmic proteins and the along with the formation of networks which are hydrogen-bonded (Cartagena et al., 2019).

2.6.6. HPP induced enzymatic changes

Post-mortem biochemical changes that occur in seafood is important for analysis of fish quality. Proteolytic activity that occurs in post-mortem fish results in the deterioration of myofibers in seafood. In terms of fish quality management, proteolytic degeneration causes an overall negative effect. A major enzyme that causes post-mortem proteolysis is calpain, which is a main proteinase group that causes hydrolysis of myofibrillar proteins. This enzyme activity therefore affects the texture of the seafood product. Seabass fish sample treated at 100 MPa showed results that were similar to untreated fish sample. Samples treated at 250 MPa for 15-30 minutes and pressure treatment at 400 MPa for 5 to 30 min resulted in the inactivation of this enzyme (Olsen et al., 2023). This decrease in the calpain activity with higher pressure and

increased holding time can be because of the probable disassociation of the subunits of the proteinase enzyme. As a result of the inhibition of this enzyme the resulting post-mortem degradation of the fish sample can be reduced resulting in longer shelf-life because of increased hardness of seafood (Olsen et al., 2023). Cathepsins are also a group of proteases that cause the softening of tissue because of lysosomal degradation that takes place during post-mortem. Cathepsin B activity in Seabass treated at the pressure of 100 MPa for 0 to 30 min did not show any significant difference as compared to untreated samples (Singh & Singh, 2020). With an increase in pressure to about 250 MPa for 5 min, there was a proportional increase in the enzyme activity. However, pressure processing at 400 MPa for 5 min was reported to result in a decrease in the cathepsin B enzyme. The possible reason for this increase in enzyme activity at 250 MPa could be because of the disruption of lysosomes which subsequently leads to the interaction of the enzyme and substrate therefore resulting in the softening of fish muscle (Teixeira et al., 2013). In general, the effect of HPP on enzymes depends on several factors such as the enzyme itself, the media, pressure and temperature, which overall results in changes such as the texture and flavor of the seafood product (Munshi et al., 2021).

2.7. Applications of HPP

2.7.1. Shellfish shucking and meat extraction

The application of HPP of shellfish such as crustaceans and mollusks have gained popularity in seafood industry over the last two decades. In fact, from a more general aspect, it was the shucking of oysters using HP treatment that first commercially reached the markets. As a result of the success, there has been an increase in companies across the world adopting this technique, along with the attempt towards trying other shellfish such as clams and mussels

for HP treatment or even extraction of meat from crustaceans such as lobsters.

Mollusks are generally bivalve organisms which require the need of opening of shells to extract their delicate meat inside. Generally, this is done manually but HPP has become a revolutionary treatment technique in this sector that allows for the pressure to induce shucking of mollusks and at the same time not affecting the taste or texture of the product. Mollusks have abductor muscles that help towards closing of the valves of the organism tightly when required. HP treatment to such valve organisms helps in protein denaturation of this muscle and therefore enables in the opening of the shells spontaneously. In case of oysters, treatment at about 240 MPa for about 2 min helps in this muscle detachment and a pressure range of 310 MPa that is followed by immediate release of pressure helps in shucking of shells at an efficiency of 100%. This therefore helps in increasing the overall yield as a result of the easier extraction and absence of manual shucking (Puértolas & Lavilla, 2020). Shucking can also be done using seawater which helps towards inducing the salt into the meat. This method helps in increasing the flavor of the shellfish without affecting its quality.

Shellfish are also greatly associated with the presence of pathogenic organisms especially *Vibrio*. The use of HP treatment helps in inactivation of this bacteria and help in sanitation of the product. Intense treatment can also result in provoking changes in color or texture of the product and therefore optimization of the right treatment to achieve best result is important. Pressure treatments between 200 to 500 MPa for a time period of 1 to 2 min are enough for performing shucking in mollusks. For commercial purposes pressure between 200 and 350 MPa are usually used (for pacific oyster's 240-275 MPa was used). For oysters, around each mollusk, a plastic band is placed before treatment in order to keep them closed during HPP. This is because generally in oysters, the presence of a shell acts as an indicator of

freshness as it indirectly conveys the presence of a live oyster. Hence, placing of the band helps in providing minimum pressure so as that the shucking can be performed without loss of the internal fluid. Hence, this method of incorporation of HPP for commercial oyster processing helps in increasing yield at reduced cost as labor and the time-consuming process of manual shell removal is eliminated (Tian and Liu, 2023).

The usual practice of thermal processing of crab in industries at about 90 °C for about 20 min, results in cooking of the flesh of the crab. This allows for the formation of primary flavor compounds and thereby results in the inactivation of pathogenic activity in crab meat. However, these extreme conditions result in the loss of moisture in addition to the extraction yield. For conducting processing by these industries, incorporation of HPP in the pressure range of 100 to 300 MPa, contributes towards the improved properties of the proteins, increase water holding capacity and higher extraction yield. This is because of the partial denaturation of the myofibrillar proteins and other such reduced protein conformational changes. There is ease associated with the removal of crab shell by using HP treatment at 345 MPa, which is in addition supported by quality characters such as increased juiciness and springiness, maintenance of the natural sweetness and an increase in the freshness appearance (Martínez et al., 2017). There has been a good acceptance of these HP treated crabs that have an increased shelf-life of up to three weeks. An effective way of increasing their shelf-life is to combine HP treatment with super chilling.

2.7.2. Gelling of seafood

Proteins are functional group of components that can form gels. This functional property of proteins has especially been put into use in the seafood industry, due to the increase in demand of comminuted seafood products and maximization of raw materials. The

gelation of proteins that takes place in fish is because of the aggregation of the protein myosin. During the settling process of gelation, the development of a three-dimensional structure through the linkage amongst the tail molecules via hydrophobic interactions takes place and this linkage continues across the subsequent process of gel formation. This gelling property of fish proteins can be utilized to produce fish paste or surimi products. Conventionally, fish gelation is done through thermal treatments which however results in the washing away of the sarcoplasmic proteins during surimi synthesis. With an aim towards utilizing this washed away protein, high pressure processing at a pressure of 300 MPa was carried out after initially coagulating the protein before pressure treatment (Hwang et al., 2007). The resulting gel obtained exhibited a much springier texture than thermally treated gel, which thereby indicates that mechanisms involved with thermal and pressure treatments for gelation are different (Larrea-Wachtendorff et al., 2022). For instance, gels obtained from sardine pressure-induced process were glossier and had a reduced presence of bubbles as compared to the thermally treated method.

Generally, thermal gelation occurs because of protein unfolding which gives rise to a three-dimensional structure that is stabilized by hydrogen bonding and hydrophobic interactions; and pressure induced gelation occurs as a result of disruption of hydrophobic interactions which cause protein unfolding. On applying pressure during HPP, the formation of disulphide bonds occurs due to the reduction in space between sulfhydryl groups and during depressurization, hydrophobic interactions and hydrogen bonding occurs. In addition, it was also noted that pressure induce gels consisted of myosin strands that were engaged in head-to-head associations as compared to thermally induced gels which consisted of tail-to-tail linkages. To improve the quality of gel stabilizing agents such as addition of 8-12% of sorbitol

helped in reducing aggregation of myofibrillar protein for pressure treatment of 600 MPa for 5 min at a temperature of 4 °C. Hydrocolloids such as carrageenan are also employed as gelling agents or thickening agents. Overall pressure induced gelling of fish, avails the benefits of a gel having higher quality and better texture than thermally induced fish gels (Ribeiro et al., 2018).

2.7.3. Surimi processing using HPP

Surimi refers to the minced flesh of fish that is converted into a gel like paste form so as to mostly resemble imitation seafood, for instance to popularly mimic crab meat. It is able to mimic the same texture and color of the imitated product and can also be commercialized into various shapes, sizes or textures. Being a traditional food of Japan, surimi was considered to be the ground fish paste. At present, this minced fish paste only serves as a starting material which is subjected to washing, deboning and then incorporated with other flavoring compounds (Chen et al., 2020). Hence, minced fish paste, and surimi are two different concepts. Other than the popular crab meat, fish species such as Alaskan pollock, red hake or Pacific whiting are some popular species being used for surimi processing in Northern America. In this multi-step procedure, parts of the fish such as its head and guts are initially removed, including the fish bones. It is then subjected to washing with a large quantity of water, so as to separate the fish muscle from the considered waste materials. The separated muscle is then minced, after which it is passed through a mesh, with the aim towards removing the cartilage, blood, skin and other undesirable parts. This results in a minced paste that is very stable and has the capability of yielding higher potential. The product is then again subjected to washing where the number of washing and the volume differs based on the species, facility capacity etc. But this washing is generally done in a ratio of between 1:5 and 1:10. After

washing, the products mainly consists of myofibrillar proteins, with reduced amount of blood, fat or other undesirable materials, which subsequently helps towards increasing shelf-life (Adegoke and Tahergorabi, 2021). The later steps include the addition of cryoprotectants to stabilize the proteins, after which it is packed and then frozen. A major parameter to consider while preparation of surimi, is the generation of a gel that is colorless, odorless and have the capability to produce maximum gelling (Lu et al., 2021)

The incorporation of HPP for surimi processing helps towards retaining the sarcoplasmic proteins. In general, during the step of washing, this protein gets washed away as due to the absence of a gel formation while heating. But HP treatment of these proteins at 300 MPa helps to coagulate these proteins and thereby allow the subsequent formation of surimi products. The gel that is thus produced is considered to have a springier texture in comparison to the thermally produced ones (Chen et al., 2020). This high pressure induced surimi also had a higher water holding capacity and cohesiveness in comparison to thermally induced gels which had a lower cohesiveness and increased hardness.

2.7.4. Fish cakes using HPP

High pressure processing can be successfully employed to produce fish cakes. Pressure treatment between 200 MPa to 300 MPa helps in the processing of fish belonging to the species of fatty fish or even lean fish. This application of pressure before freezing preservation can help towards the control of microbial growth. In addition, it helps in reducing lipid oxidation which thereby inactivates prooxidative endogenous enzymes. The application of pressure treatment to fish cakes helps in protein denaturation and as a result helps in making them softer and lighter, thereby contributing towards enhancing the sensory parameters. For fish cakes prepared from minced hammock and mackerel, their texture

indicated a reduced hardness and cohesiveness in comparison to untreated fish samples. This can be because of the degradation of proteins from the pressure induced which results in degradation of myosin and actin. This protein denaturation can also be result from the action of some proteolytic enzymes such as cathepsins B or D (Cropotova et al., 2020). Hence, the tenderization of pressure treated fish can be because of high pressure as well as the proteolytic enzymes.

2.7.5. Pressure assisted thawing of fish muscles

General thawing practices of frozen seafood can be replaced by Pressure Assisted Thawing (PAT) for the purpose of achieving thawing faster. Here the heat flux rate is increased by increasing the temperature difference between the heat source and the phase-change temperature or melting temperature (Zhu et al., 2004). As a result of pressure application at 210 MPa, the phase-change temperature of the pressure mediating fluid, which is mostly water, is shifted to a lower temperature. This happens as due to the increase in pressure from atmospheric pressure to a higher-pressure range. As a result of this, the temperature gap is widened, and this reduces the time taken for thawing. PAT was applied to fish samples such as cod, haddock, redfish, Atlantic salmon and sea bass. This resulted in a reduced thawing time as for instance, PAT time taken in case of cod, haddock and redfish at pressure of 200 MPa at a temperature of 13 °C was 50% less than the time taken for thawing performed in a water bath at a temperature of 15 °C (Tironi et al., 2007).

In case of carp samples, PAT resulted in fish muscles that were of superior quality and improved elasticity and breaking stress, in comparison to water treated thawing at 15-17 °C. Furthermore, this treatment using PAT also resulted in sarcoplasm between the myofibril and the membrane structure had good preservation. In comparison to thawing at a water bath at a

temperature of 10 °C, the use of PAT resulted in decreased drip loss. Apart from this, PAT applied at 200 MPa at 10 °C for about 60 min for several fish samples such as salmon, rainbow trout, cod, whiting, resulted in a decrease in Total Viable Counts (TVC) as compared to conventional thawing at 15°C (Schubring et al., 2003).

With the application of PAT, there could be the possible loss in transparency because of increased lightness. This was observed in samples such as carp, Atlantic salmon, cod, and rainbow trout. In some sample species, protein denaturation also occurred, indicating that low pressure application must be conducted for the purpose of enhanced quality of fish muscle (Cropotova et al.2020).

2.7.6. Pressure shift freezing of fish muscles

With the aim towards achieving ultra-rapid and uniform supercooling in seafood samples, the technique of pressure shift freezing (PSF) using HPP can be conducted. Here with the widening of temperature difference between the fish muscles and phase-change temperature, PSF can be achieved. In this technique, the fish muscles are cooled under a pressure of 200 MPa, to a temperature of -18 °C which is just above the freezing temperature of -21 °C. Then the pressure is quickly released, or depressurization is done suddenly to induce formation of ice crystals. As a result of this rapid depressurization, the temperature gap is widened, thus resulting in supercooling (Truong et al., 2015).

In comparing PSF to conventional freezing method, the advantage of well-maintained fish fibers is noticed. This is because of formation of intracellular ice crystals that are homogeneously distributed as in case of fish samples such as PSF salmon, PSF turbot and PSF seabass. The reason as to better results in PSF fish samples in comparison to traditional freezing is because of the formation of large ice crystal because of cell dehydration in the

conventional method. This is because, the nucleation and growth of ice crystals depend on the rate of heat removal. Therefore, because of slow nucleation, large extracellular are formed that causes shrinkage or even deformation of muscle fibers (Cheng et al., 2021).

PSF can also contribute to reduced drip loss when compared to conventional freezing. For instance, upon comparing PSF turbot to air blast freezing technique, after a storage period of 45 day at -20 °C, a significant reduction in drip loss from PSF treated salmon fish was observed (Zhu et al., 2004). In comparison of PSF sea brass to air blast freezing, it was found that water holding capacity and protein denaturation was significantly better by using the former method for analysis done after a period of 5 months storage at a temperature of -15 °C (Jia et al., 2021).

2.7.7. Extension of storage life

The very initial proposal towards the use of HPP of food samples is ideally due to their ability of extending the shelf-life of products because of protein denaturation and microbial inactivation. And the incorporation of this technique brings forth the possibility of minimally processed food having important sensory changes. Seafood such as fish and shellfish are mainly associated with the presence of gram-negative bacteria that are sensitive to pressure. Due to this, the employment of HPP as a processing technique result in the decontamination of these bacteria which is confirmed by the presence of gram-positive bacteria such as Lactic Acid Bacteria (LAB) after the pressure treatment. LAB in addition helps with the inhibition of other pathogenic organisms. HPP also helps with inactivation of enzymes that can cause food spoilage, thereby increasing the shelf life or seafood products (Lee et al., 2021). Other studies include reduced growth of *E.coli* with increased pressure in apple juice (Ramaswamy et al., 2003), higher rates of destruction using HPP in milk (Mussa and Ramaswamy, 1997),

improved storage of textural properties of selected fruits and vegetables using HPP (Basak and Ramaswamy, 1998), increased storage of HP treated mango pulp along with improved consistency (Ahmed et al., 2005), all of which increase storage life of the pressurized products.

In case of seafood, HP treatments between 250 to 600 MPa is aimed towards microbial decontamination, induces change in terms of color of the seafood product and thereby gives the appearance of a cooked product as due to the increase in opaqueness. As a result of this, HPP is employed as an alternative for fish species such as cod or hake where the color change is less evident. In case of seashells, in addition to applications such as extraction of meat from crustaceans or shucking of mollusks, HPP treatment also helps increase the shelf-life through the applied pressure treatment (Puértolas & Lavilla, 2020).

2.7.8. Ready to eat products

The global trend of consuming prepared meals has increased over the years. The concept of Ready-To-Eat (RTE) meals have become extremely popular in case of seafood as it is advantageous to those who wish to consume nutritious food without the need for incorporating much time into the preparatory process which is laborious. Properly optimized HP treatment at low or medium temperature helps with the production of minimally processed RTE seafood products. Furthermore, these products are also controlled in terms of harmful pathogens such as *Salmonella spp.* or *Listeria monocytogenes* (Gill and Ramaswamy, 2008). In the seafood industry, HPP treated thick slices of salmon, hake or tuna are commercially available that is enabled with the ease of quick grilling at home. HPP incorporated sous-vide RTE products are also available commercially in markets for instance sous-vide RTE seabream sample that have been subjected to a treatment of 300 MPa to 600 MPa for 5 min at a temperature of 5 °C (Espinosa et al., 2015). In such studies, microbial count was reduced in

comparison to control and certain enhanced textural attributes were also associated with the product (Puértolas & Lavilla, 2020). RTE seafood products such as fish patties and minced fish products are also presently available in market. In general, HPP treated RTE products offer the benefit of certain increase in sensory characters in addition to microbial decontamination with minimum processing.

2.8. Future possibilities

To completely utilize and take advantage of the opportunities offered by HP treatment, some gaps requires to be filled. For instance, with the use of HPP for microbial decontamination, it is not possible to eliminate the presence of all microbial contaminants in food. Some of these disease-causing organisms, remerge upon depressurization even during pressure treatments at 450 MPa. As a result, the HPP needs to be combined with other treatment practices such as heating or freezing. Another gap is the high cost that is associated with the pressure treatment equipment. Therefore, some industries only use equipment of a specific pressure range and at times this requires the need to compensate for the lack in pressure ability because of which HPP is conjugated with other treatment methods (Ohshima et al., 1993). Based on the effect of HPP on fish muscles, concepts such as the development of a kinetic model that helps towards better understanding of microbial and enzymatic deactivation; HPP effect on packaging of fish muscles; texturization of fish paste that is induced by HPP for development of new products such as fish sausage; combined treatment of fish muscle with other products such as HP treatment of tuna in sunflower oil; HP induced production of very fine surimi; enhancement of flavor of shellfish such as oysters; inhibition of undesirable enzymatic activity that results in deterioration of food quality are some features that can be studied further for increased opportunities.

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Connecting Text to Chapter 3

In this chapter, the effectiveness of high pressure processing (HPP) on fresh salmon obtained from a supermarket, during refrigerated storage was studied. Three pressure levels and three holding times were carefully selected to ensure that the HPP application does not cause major color changes in the salmon, as can be expected at treatment pressure higher than 300 MPa. The basis for selecting HPP of salmon has been detailed in Chapters 1 and 2, and research objectives have been laid down. The salmon was selected for quality evaluation during refrigerated storage of 3 weeks at 4 °C and the quality analyses were performed weekly.

CHAPTER 3

Effect of High-Pressure Treatment on Refrigerated Storage Stability and Quality of Fresh Salmon Fillet

3.1. Abstract

High pressure processing (HPP) is a nonthermal method that has gained importance for producing minimally processed products. HPP has been adopted for the highly shelf-life sensitive seafood products to improve the storage stability and retain the quality characteristics. The main aim of this study was to investigate the quality and storage stability of fresh Atlantic salmon (*Salmo salar*) achieved using HP treatment. Similar size fresh Atlantic salmon pieces were prepared, vacuum packed into high-density polyethylene (HDPE) pouches and subjected to HP treatments at 150, 250 and 350 MPa with holding times of 10, 20 and 30 min. The treated products and control (without treatment) were stored at 4°C for up to 21 days. The control and treated samples were evaluated every week for microbial growth, texture, color, and protease activity. Applied pressure treatment demonstrated an immediate increase in textural properties such as firmness and tenderness which resulted in some loss in color parameters. As a result of storage, the lightness (L^* value) increased, the redness (a^* value) decreased and lower percentage of texture loss was associated with HP treated samples depending upon the severity and duration of HP treatment. The reduction in microbial spoilage was achieved and, overall, it was demonstrated that HP treatment can slow down the quality deterioration rate and hence extend the refrigerated shelf life of salmon.

KEYWORDS: High pressure processing, fresh Atlantic salmon, texture, color, protease, microbial

3.2. Introduction

Seafood is a rich source of proteins, fats, vitamins, and minerals, and remains a healthy choice for several consumers. In the area of human nutrition, the omega-3 polyunsaturated fatty acids (PUFA), proteins and other nutrients obtained from seafood consumption offer health benefits (Govzman et al., 2021). Studies have shown that the consumption of seafood has been related to improved blood flow, cardiovascular advantages, and various other health benefits (Liu and Ralston, 2021). However, the high perishability of seafood makes it a challenge for consumers and processors alike even under refrigerated storage and achieving improved refrigerated storage stability is an advantage.

Generally, seafood has a shelf-life of about 14 days under refrigerated storage before it starts undergoing deterioration in quality (Kontominas et al., 2021). It is highly degradable due to its high-water content, oxidation compounds, autolytic enzymes, and fast microbial activity. The oxidation of lipids and myoglobin are considered as the primary components for causing rancidity and spoilage changes in seafood (Geng et al., 2023). Hence processing of seafood is considered important for the extension of its shelf-life.

Conventionally in the food industry, various processing techniques are focused for preservation of seafood, which are often based on the use of chemicals, ice/refrigeration, freezing, dehydration or thermal treatment methods for achieving the extension of product shelf-life (Safwa et al., 2023). While these methods provide different levels of shelf-life protection, they also have an impact on the quality parameters such as color, texture, weight loss, enzyme/oxidative rancidity etc. as well as economical aspects. Furthermore, for clean labels and in consideration of consumers' preference for the availability of products that have undergone minimal processing, the development of alternative and innovative methods have been in much focus over the last decades (Lopes et al., 2023).

Many minimal and nonthermal processing techniques have evolved in the past decade with a primary focus on short-term shelf-life extension with higher quality retention in products (Iturralde-García et al., 2022). Among several approaches used for such a purpose like, high pressure processing (HPP), pulsed electric field (PEF), pulsed light (PL), ozone, plasma, plasma water etc, HPP has been a very promising and growing nonthermal processing technique that helps to extend the shelf-life and preserve the quality of food. In recent years, HPP has become a major part of the food industry for specific technical purposes, several applications or even for reconditioning (Allai et al., 2023). The HPP technique uses water as a pressure-transmitting medium and helps achieve microbial destruction and enzyme inactivation with minimal changes induced to the food. HP treatment has also been stated to have resulted in reduced weight loss in comparison to untreated products and its use has been widely recognised in the food industry in light of extension of shelf life of food products (Cartagena et al., 2019). Therefore, HPP can serve as an alternative with regard to the commonly employed thermal treatment methods such as pasteurisation or sterilisation, and other chemical methods.

HPP helps achieve the extension of products by working under two major principles, isostatic and Le Chatelier's principle. Under the isostatic principle, when the product is under pressure, the pressure is evenly and instantly distributed within the sample. As a result, uniform processing of the sample occurs using water as a pressure transmitting medium (Khaliq et al., 2021). This uniform distribution of pressure treatment across the sample is considered as the greatest advantage for HPP as compared to other techniques as it allows for products to be uniformly processed, resulting in reduced industrial cost economics (Huang et al., 2020). This application of HP also creates an enormous amount of mechanical stress resulting in changes such as inactivation of enzymes and microorganisms. Le Chatelier's principle is associated with

pressure influence on the associated volume. According to Le Chatelier's principle all chemical reactions are associated with a volume change of activation. Some reactions are accelerated by the negative volume changes and others that result in positive volume changes are retarded by HP. This results from the knowledge that higher pressure results naturally in volume shrinkage. Further, when the food is subjected to pressure, the system works in a way to counter the pressure changes. As a result, the food shrinks and reduces in volume therefore causing the contraction of cell components. Once the pressure is released, the food comes to its original state and as a result, the bursting of microbial cell membrane and other endogenous protein and enzymatic changes occur (Sehrawat et al., 2021). As the loss of freshness in seafood is primarily associated with microbial activity and growth as well as enzymatic changes, the HP treatment helps to reduce the microbiological and enzyme activity as well as minimise their growth during storage thereby prolonging the refrigerated storage life. It has also been known that higher pressure conditions could result in greater changes in quality parameters of food products (Singh et al., 2022).

HPP has been widely applied in the food industry, with the majority of these studies related to food types such as juices, dairy products, fermented foods, meat products and seafoods. Seafoods are extremely perishable and are classified as highly deterrable food products that pose a high health risk if consumed raw or undercooked (Bozianis et al., 2021). The consumer health can be affected as a result of the proliferation of bacteria, lipid oxidation and various other factors that can affect human health. Technologies that help reduce these microbial groups, extend storage, and maintain the quality of seafoods is important towards the seafood industry. HPP has gained wide recognition as a technique that helps control the bacterial growth and maintain the nutritional profile of the food. In recent years, several studies have been carried

out in the area of HPP in the seafood industry. Inactivation of bacterial spores in mackerel and haddock, improved color and textural properties in albacore tuna, reducing the oxidative enzymes that promote lipid oxidation in Mahi Mahi fish, alteration of protein structure for better gelation properties in blue crab, shucking of shellfish which helped in denaturation of proteins of abductor muscles for increased ease in extraction of bivalve muscle, improved gelling in surimi application for springier texture and improved retention of sarcoplasmic proteins that do not get washed away as a result of protein coagulation, preparation of RTE fish balls, pressure assisted thawing and pressure assisted freezing in sea bass are some examples of the use of HPP in the seafood industry (Ramirez-Suarez et al., 2006, Cropotova et al., 2020; Yagiz, Y. et al., 2007; Martínez-Maldonado et al., 2020; Puértolas & Lavilla, 2020; Chen et al., 2022; Luo et al., 2021; Tironi et al., 2010).

In the seafood industry, the investigation of HPP in salmon has led to several positive resulting studies. Some of them include, the inactivation of microbial growth, decreased acid phosphatase activity, reduced susceptibility to oxidation of fatty acids, improved physiochemical parameters of texture and color, improved water holding capacity, and RTE salmon products, which are some amongst several other beneficial studies conducted in salmon. (Gudbjornsdottir et al., 2010; Rode and Hovda, 2016; Yagiz et al., 2009; Arnaud et al., 2018; Christensen et al., 2017). The use of HPP as a combination technique with other food processing techniques has also been studied such as combining HPP with pulsed electric field or HPP combined with CO₂ or with ultrasound treatment in salmon (Pérez-Won et al., 2021).

However, further study in the area of seafood sector is required for diversifying applications, achieving improved storage and for further understanding of market trends (Khaliq et al., 2021). Moreover, particularly in fish, the results vary depending on the processing

equipment, holding times, pressure range, temperature, and storage time (Fellows 2022; EFSA BIOHAZ Panel, 2022). Therefore, this comparative study was conducted to investigate the effect of high-pressure (HP) treatment on the quality and storage stability of fresh salmon fillets.

3.3. Materials and Methods

3.3.1. Sample preparation

Fresh Atlantic salmon fillet was purchased from a local supermarket and then immediately transported to the lab under ice storage conditions. The upper dorsal side of the fish was selected for preparing the samples. The fillets were deskinning 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 insert suction force on to the sample. This step also safeguarded the vacuum-packed samples to not float inside the HP unit vessel. No chemicals or additives were added prior to the treatment. Each sealed HDPE pouch consisted about 30-45 g of salmon sample. With the exception of control, the vacuum-packed samples were then subjected to HP treatment at various pressure levels, ranging between 150 to 350 MPa and different holding times using a full factorial design. The hydrostatic fluid used for the pressure treatment 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 condition (4 °C) for a systematic assessment of microbial growth and quality degradation after 7, 14 and 21 days.

3.3.2. 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 media to the samples under treatment. The first valve was closed to move the water inside the chamber. Once the pressure was brought up to the specific pressure level, it was held for the required holding time. The induced pressure resulted in reduction of volume as an influence of compression. After the required time of processing, the pressure was released by opening the pressure release, safety, and pressure shut-down valves. During the pressure release step, the samples returned to its original volume.

3.3.3. Experimental design

JMP software (JMP 4.3 SAS Institute Inc., Cary, NC, USA) was used to design the experimental model. A full factorial design $2^3 = 9$ runs as in Table 3.1. was designed for running the experiment. The two variables in this study included time and pressure which were studied at three pressure levels and three holding times using the model below. The experimental analysis was carried out in triplicates.

Table 3.1. Factorial design of (2 factors and 3 levels)

Runs	Pressure (MPa)	Time(mins)
1.	150	10
2.	150	30
3.	350	10
4.	350	30
5.	150	20
6.	350	20
7.	250	10
8.	250	30
9.	250	20

3.3.4. High pressure treatment

For this study, three pressure levels, 150, 250, and 350 MPa were opted. Each pressure level had samples treated for three holding times of 10, 20 and 30 min. After HP treatment, the samples were stored under refrigerated temperature (4 °C). The untreated and pressure treated samples were tested on day 1, 7, 14 and 21 for microbial and quality attributes.

3.3.5. Texture measurement

A TA. XT plus texture analyser (Texture Technologies Corp, New York, USA) was used to perform the texture analysis. For the preliminary analysis, various probes were used to find the one appropriate for more consistency and accuracy of test results. Probes such as the cylindrical, Warner-Bratzler, puncture, and grated probe were used for the preliminary analysis. Finally, a multiple wired probe, developed in our laboratory, was used to carry out the 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).

Table 3.2. Preliminary data of force (g) used

Probes used	Day 1	Day 7	Day 14	Day 21
Cylindrical probe	579.6	212.8	207.4	144.3
Warner-Bratzler Puncture probe	46.4	52.5	37.4	33.5
Grated	1029.5	1774.7	935.1	880.2

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

$$\text{Firmness} = \frac{\text{maximum force}}{\text{maximum deformation}} = \frac{MF}{MD} \quad (1)$$

$$\text{Tenderness} = \frac{\text{force/cross sectional area}}{\text{deformation/initial length}} = \frac{F/Ao}{\Delta L/L} \quad (2)$$

Retention % of firmness = 100-loss %

$$= 100 - \left(\frac{\text{sample for each storage day} - \text{sample with zero storage}}{\text{sample with zero storage}} \times 100 \right) \quad (3)$$

Retention % of tenderness = 100-loss %

$$= 100 - \left(\frac{\text{sample for each storage day} - \text{sample with zero storage}}{\text{sample with zero storage}} \times 100 \right) \quad (4)$$

$$\text{Relative firmness} = \frac{\text{Treated sample}}{\text{Untreated sample}} \quad (5)$$

$$\text{Relative tenderness} = \frac{\text{Treated sample}}{\text{Untreated sample}} \quad (6)$$

3.3.6. Color measurement

Color measurement was done using a Minolta Tristimulus Colorimeter (Minolta Crop, Ramsey, NJ, USA). The color parameters L*, a*, C*, and hue angle were assessed using the software Spectra Magic (Minolta Crop, Ramsey, NJ, USA) connected to the colorimeter. Uniform shaped samples were subjected to color assessment using the colorimeter to measure the lightness, redness, chroma and hue of the control of the pressure treated samples and untreated control. The lightness was analysed as L*, redness as a*, C* for chroma and finally the

hue angle. Measurement was taken 10 times for each sample after which the average value was considered.

The parameters were derived as shown below:

% Retention of L^ = 100-loss %*

$$= 100 - \left(\frac{\text{sample value for each storage day} - \text{sample with zero storage}}{\text{sample with zero storage}} \times 100 \right) \quad (7)$$

% Retention of a^ = 100-loss %*

$$= 100 - \left(\frac{\text{sample value for each storage day} - \text{sample with zero storage}}{\text{sample with zero storage}} \times 100 \right) \quad (8)$$

$$\text{Relative } L^* = \frac{\text{Treated sample}}{\text{Untreated sample}} \quad (9)$$

$$\text{Relative } a^* = \frac{\text{Treated sample}}{\text{Untreated control}} \quad (10)$$

3.3.7. Protease Analysis

The enzyme protease activity was measured spectrophotometrically as a modification to Wang and Taylor method (Zara and Ramaswamy, 2004). The crude extract was prepared by homogenising 15 g of salmon with 100 mL of 0.2 M sodium phosphate buffer, for 2-3 min. Every 20 s, the sample was placed in an ice bath to prevent enzyme destruction. The homogenate was then centrifuged at 12100 g for 30 min. This supernatant was considered as the crude extract for protease analysis. For protease activity measurement, 3 mL of 0.5% casein was added as substrate to 1mL of the extract. The mixture was then incubated at 45 °C for 30 min. After the incubation, 3.0 mL 5% (w/v) trichloroacetic acid (TCA) was added to the mixture to stop the reaction and precipitate proteins. The mixture was left to stand at room temperature for 1 h and then the precipitate was removed from the supernatant by filtration through Whatman No.1 filter

paper. The absorbance (A) of the supernatant was measured at 280 nm. Control sample was run by adding the crude enzyme after the TCA solution was added (Zara and Ramaswamy, 2004).

3.3.8. Microbial Analysis

Estimation of Aerobic Plate Count (APC) was used for enumeration of aerobic bacteria. For APC, a 1/10 dilution was performed by adding 5 g of the sample to a centrifuge tube containing 45 mL 0.85% sterile saline. The tube was then homogenised at 1200 rpm for 2 min. From the supernatant, serial dilution was performed up to 10^{-4} . Respectively, 0.1 mL from the tubes was also plated into Tryptic Soy Agar (TSA) plate and spread using a spreader. Enumeration was done after incubation of the petri plates at 35 °C for 48 h. Colony of culture was calculated as $\log_{10}$ colony forming units (CFU) per g. Results were taken in triplicates and the mean value was considered (Tsai et al., 2022).

3.3.9. Statistical Analysis

The analysis was done in triplicates for all tests. The data were subjected to a one-way analysis of variance ($\alpha=0.05$) using the IBM SPSS Statistics Version 21 software (IBM Corporation, USA). For the significant differences observed, mean treatments were compared using Tukey's test. Interaction effects were studied by subjecting the data to a two-way and three-way analysis of variance ($\alpha=0.05$). Effect response plots were generated as well.

3.4. Results and Discussion

3.4.1. Effect of HPP on texture parameters

Texture is a determinant character that helps in studying the quality of samples across the storage time. In this study, the multi-wired probe was used to derive the firmness and tenderness of fish samples. Firmness represents the deformation the sample can withstand, and tenderness defines how soft a sample is when acted by force.

3.4.1.1. Effect of HP treatment on firmness

Texture assessment of processed food helps in addressing the palatability and mouthfeel of a product and also sheds light on consumer satisfaction (Bernardo et al., 2022). Firmness in case of solid foods is a key parameter for analysing the mouthfeel of samples. It indicates the deformation or compression that a sample can withstand, when applied with force (Xie, et al. 2023). Furthermore, firmness helps in understanding the quality of a sample during its shelf-life, as foods having a higher resistance to this deformation indicate a higher value of firmness (Liu et al., 2019).

The results for firmness of treated and untreated salmon are illustrated in Figures 3.1.a-c for 150, 250 and 350 MPa. From Figure 3.1.a, it was observed that all treated samples had a higher firmness than the control, with firmness increasing with HP treatment time. It was observed on day 1, that at least 20 min was required to achieve a significant ($p < 0.05$) increase in firmness for 150 MPa treated samples. From day 7 onwards, a significant difference in firmness was observed for all treatment times, with 30 min treated samples resulting in the highest firmness significantly ($p < 0.05$) for all days. On day 7 there was no significant difference between 10 and 20 min treatments; however, all treatments times were significantly ($p < 0.05$) different from each other from day 14 onwards. Across storage it was observed that the firmness value decreased with storage time for all samples significantly.

Figure 3.1.b shows the effect of HP treatment at 250 MPa on the texture of treated samples were observed. For all days, the samples had significantly higher firmness than the control. In comparison to 150 MPa, on day 1 even the 10 min treated samples showed a significant difference, indicating that even 10 min can induce an increase in firmness significantly. However, there was no significant difference between the treatments on the first

day. From day 7 onwards, only the 30 min treated samples were significantly higher than 10 and 20 min, both of which did not show significant difference between the two treatment times even up to day 21. Across storage, for the 10 min treated sample there was no significant difference between day 14 and 21, whereas for 20 and 30 min treated samples there was no significant difference between day 7 and 14.

Figure 3.1.c shows the effect of HPP for 350 MPa treated fresh salmon. For all days, the treatments had significantly higher firmness than the control. For day 1, all treatments were significantly different from the other, with higher treatments having higher firmness value significantly. Furthermore, except for day 7, where 10 and 20 min treatment were not significantly different from each other, all other days had treatments which had significantly higher firmness. Across storage, there was a significant decrease in firmness for all samples, except for 10 min treated samples on day 7 and 14, and 30 min treated samples on all days, which showed no significant difference.

Overall, it was observed from Figure 3.1 that HP treatment induced an increase in firmness significantly ($p < 0.05$). For 150 MPa, at least a 20 min treatment was required to achieve this increase in firmness whereas, for 250 and 350 MPa, 10 min treatment itself could induce a significant increase. However, on day 1, for 250 MPa the three treatments times were not significantly different ($p > 0.05$) from each other whereas for 350 MPa, all the treatments gave significantly higher firmness. Across storage for day 7, 14 and 21, all treatments for all pressure levels studied indicated having a significantly higher firmness than the control. Samples treated for 30 min, had significantly higher firmness than all other treatments for all pressure levels studied across storage. This indicates that higher pressure induces higher firmness. Furthermore, the samples treated for 30 min at 350 MPa showed no significant difference between themselves

for 21 days, indicating better retention in their firmness property of the treated sample. An increase in firmness with the application of HPP has been demonstrated in several earlier studies. Chauhan et al. (2015) demonstrated an increase in firmness on Hilsa treated at 350 MPa, Tsai et al. (2022,) concluded an increase in firmness for yellowfish tuna treated at 300 MPa for 5 min, Cartagena et al. (2019) demonstrated increased firmness in albacore treated at 500 MPa for 2 min, Rode and Rotabakk (2021) observed increased firmness for cod treated at 400 MPa. This increase can be as a result of changes in proteins of the samples. As higher pressure induces greater denaturation of the proteins in them, a higher firmness value is obtained.

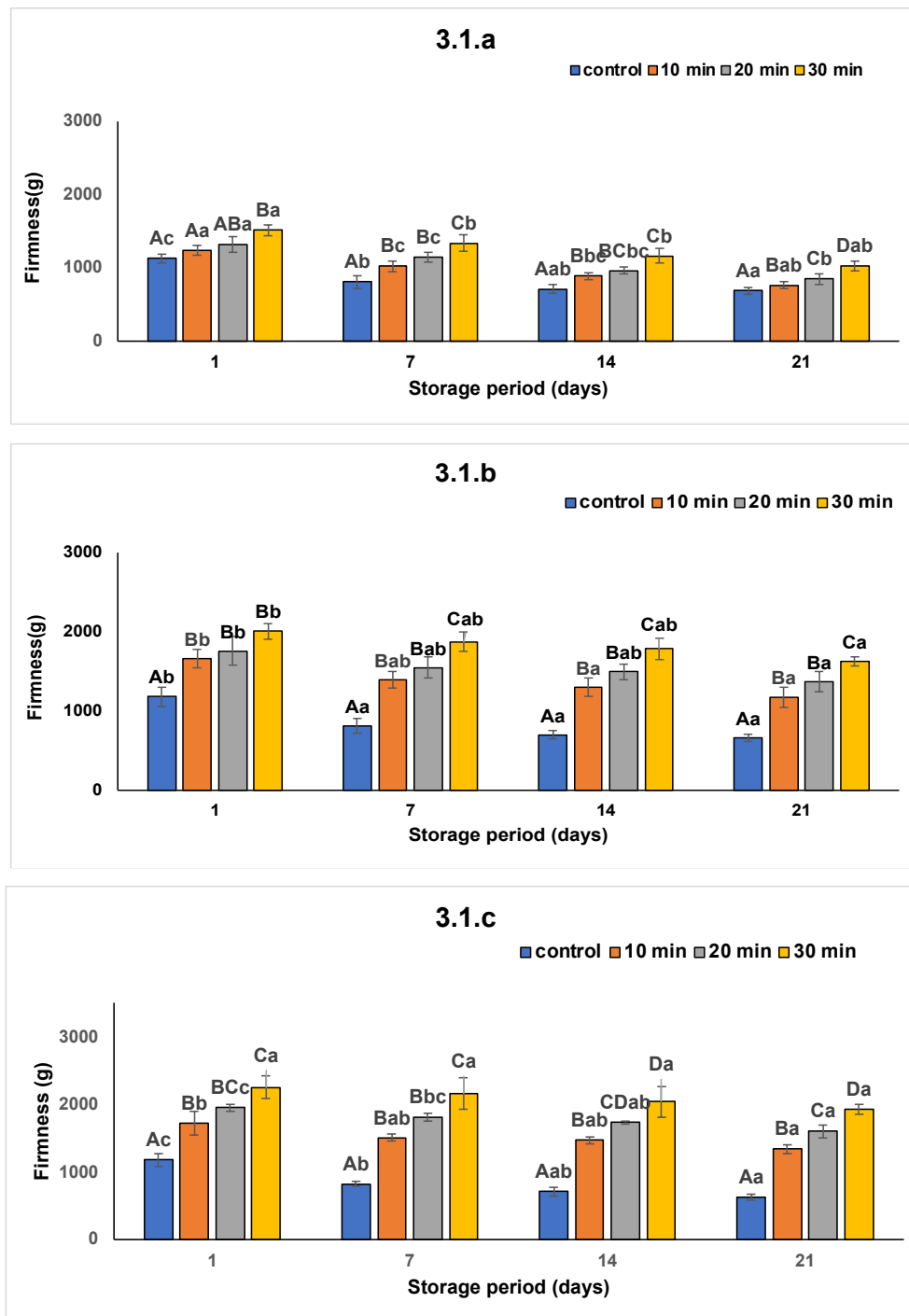


Fig. 3.1. Effect of HP treatment on firmness of treated fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa. Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

From this study it can be said that a treatment of at least 20 min is required for causing significant protein changes in salmon treated for 150 MPa, whereas for 250 and 350 MPa, 10 min treatments are capable of causing protein denaturation, that was reflected as increased firmness. The highest protein changes were observed for 30 min treatments across storage. Furthermore, 350 MPa treated samples for 30 min showed no significant difference during storage until day 14, indicating that the protein got altered the most for this treatment level and time and thereby underwent reduced changes, which is reflected as reduced changes across storage.

3.4.1.1.1. Quality retention percentage

In Table 3.3, the retention percentage for the firmness of all samples were calculated to understand the magnitude of texture loss undergone by each across storage. In the previous section, the variability associated with the control and sample were presented as observed in the form of bar graphs and their error bars. It is clear the error bars associated with each vary and therefore meaningful comparison may become difficult. In Table 3.3, these are presented based on texture values relative to what was observed immediately after the HP treatment on Day 1. The effect of pressure level, treatment time and changes during storage are more clearly depicted. For example, the pressure level and treatment time are highlighted in different rows and the storage effects in columns. It is clear the control sample had the least firmness as compared to all other test samples and the softening effect continued during the storage up to 21 days. Another clear observation is that the treatment time from 10 to 30 min at each pressure level contributed to texture firming of the samples by about 5%. Further, the pressure treatment caused a highly significant increase in texture firmness by about 25% at 150 MPa to 35% at 350 MPa. Overall, higher pressure and longer treatment times resulted in reduced loss in firmness.

Also, with respect to storage time, there were steady decreases in firmness as the storage time increased. For control and 150 MPa treated samples, this loss was about 25-27% which reduced to 14-17% after treatment at 250 MPa and was reduced to about 12% after treatment at 350 MPa. Overall, the pressure treated samples on an average had a maximum retention percentage of firmness from 28% after 150 MPa treatment, 52% after 250 MPa treatment and 60% after 350 MPa treatment for 30 min.

Table 3.3. Retention (%) for firmness of HP treated fresh salmon

Firmness Retention (%)	Day 7	Day 14	Day 21
Control	69.86± 8.26	60.29± 6.47	53.03± 3.52
150 MPa-10 min	82.48± 1.53	71.53±7.68	61.35±6.95
150 MPa-20 min	86.61±8.16	73.19± 8.03	64.33± 6.21
150 MPa-30 min	88.17±4.83	76.78± 9.7	68.02± 6.23
250 MPa-10 min	84.06± 6.68	78.22± 7.98	70.49± 9.55
250 MPa-20 min	88.10±9.56	84.96± 6.61	78.25± 8.44
250 MPa-30 min	93.33± 2.63	89.06±2.88	81.14± 2.24
350 MPa-10 min	87.77± 8.55	85.61±6.94	77.85± 6.95
350 MPa-20 min	92.7± 4.72	88.57± 1.48	81.97± 5.6
350 MPa-30 min	95.81± 3.43	90.58±4.94	85.44± 4.16

Values are the mean ± standard deviation (n=3) samples during 21 days of storage

3.4.1.1.2. Relative firmness

Figure 3.2 indicates yet another representation of the changes associated with the firmness by how they change relatively as compared to that for control sample with similar storage time. Since the changes associated with each parameter is different with respect to pressure level, treatment time and storage time, this type of representation provides a better

comparison of the performance of treated samples. This suggests the improvement achieved with each treatment conditions relative to control. The relative value will be 1 for control and deviation from 1 represents the degree of influence. Only Day 1 (Figure 3.2.a) and Day 21 (Figure 3.2.b) are shown with two way interactions between the pressure level and treatment time. For all pressure levels, a higher treatment time gave a higher retention. And this firmness retention increased with the pressure level. The systematic synergistic effect of pressure level and treatment time is clearly evident. The 30 min treatment time at 350 MPa, clearly showed a systematically achieved maximum relative firmness moving up from 150 MPa and 10 min treatment. The maximum relative values which was about 1.8 on Day 1 reached nearly 3.0 on Day 21 indicating the importance of the HP treatment. Obviously, without the treatment or with the minimal HP treatment, significant texture retention could not be achieved while the 30 min treatment at 350 MPa demonstrated a deep effect on texture preservation.

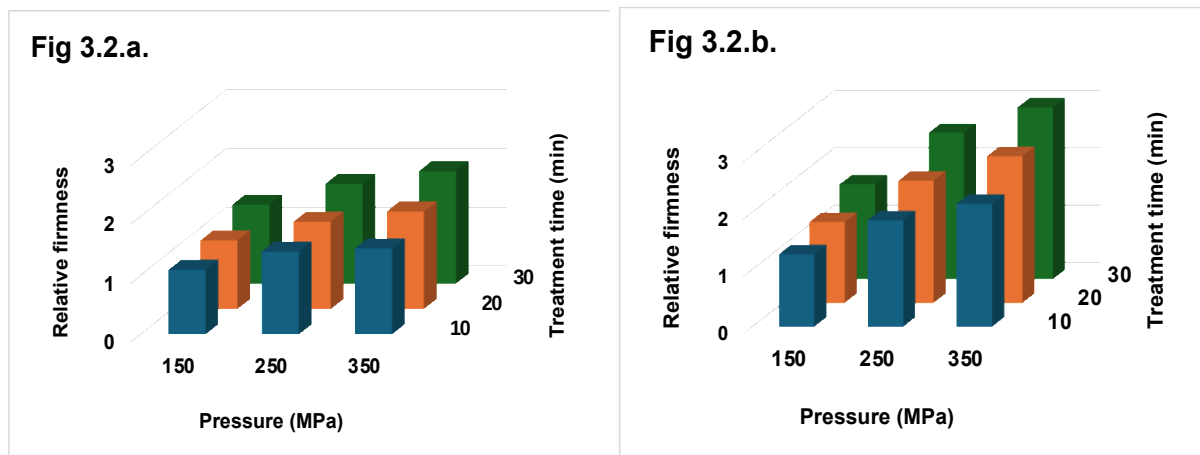


Fig. 3.2. Effect of HPP on relative firmness of 150, 250 350 MPa treated fresh salmon on day 1 (Fig 3.2.a) and day 21 (Fig 3.2.b). Values are the mean of 3 independent samples during 21 days of storage.

3.4.1.2. Effect on HPP on tenderness

Tenderness is a texture attribute for analysing how a sample yields to stress. It indicates values that reflect how soft a sample is when acted upon by force (Chen et al., 2022). For processed salmon this was important as it describes the pleasantness consumers are provided with during the chewing process. Samples having a higher softness provide a greater mouthfeel as they reflect, that they are chewier in nature (Mohd et al., 2023).

The effect of HP treatment and storage on the associated changes in tenderness are presented in Figure 3.3, Table 3.4, and Figure 3.4 similar to the way it was presented for firmness. In Figure 3.3, the effect on tenderness is presented as bar graphs in three subplots a, b and c representing treatment effects at 150, 250 and 350 MPa, with treatment time and storage time effects represented to cover 10-30 min HP treatment time and 21 day storage at 4 °C. Error bars are inscribed on each and the significance of difference indicated by letter codes.

As shown in Figure 3.3.a, after 150 MPa treatment of fresh salmon (Figure 3.3.a.), the tenderness increased significantly with treatment time for all samples but across the storage each sample and the control lost a significant amount of tenderness. As it can be seen from the significance (uppercase letters), the treatment effect was significant and the treated samples remained significantly different from each other after 7, 14 and 21 days of refrigerated storage ($p < 0.05$). Comparing the storage time effect, similar differences were also noticed (denoted by lowercase letter) and the tenderness softening effect continued during the storage.

Similar effects were observed for samples treated at 250 MPa (Figure 3.3.b) and 350 MPa (Figure 3.3.c) when treatment time effects were compared, but not all changes with respect to storage time were significant (some demonstrating same lower case letters, $p > 0.01$). For example, as indicated in Figure 3.3.b for 250 MPa treated samples, the tenderness increased

significantly on each day with the treatment time, and across storage there was a significant loss for all samples, except for 20 min treated samples that showed no significant difference between day 7 and 14. From Figure 3.3.c the tenderness for 350 MPa again increased significantly for all days, which was found to be similar to 150 and 250 MPa. And across storage, similar to 150 MPa, a significant loss was observed between all the samples across 21 days of study.

The tenderness retention percentage was calculated for 150, 250 and 350 MPa (Table 3.4). Higher pressure levels and treatment times resulted in greater tenderness significantly across refrigerated storage. However, the retention levels were exaggerated with the 14 and 21 day pattern with the 350 MPa-30 min treatment time demonstrating a significant 72-85% higher firmness retention as compared to the control sample.

In Figures 3.4 a & b, the effect of pressure treatment at 150 - 350 MPa for 10-30 treatment times are shown on day 1 and 21, respectively. Again, as observed with the firmness trends, higher treatment times and pressure levels resulted in a systematic increase relative tenderness values. The relative tenderness was calculated for Day 21 for all pressure level and treatment times. While the relative value after 30 min treatment at 350 MPa was about 1.8 times that of the control sample, the relative value after 21 days storage increased to almost 3.2, much better than observed on Day 1. These results show the importance of presenting data in these formats.

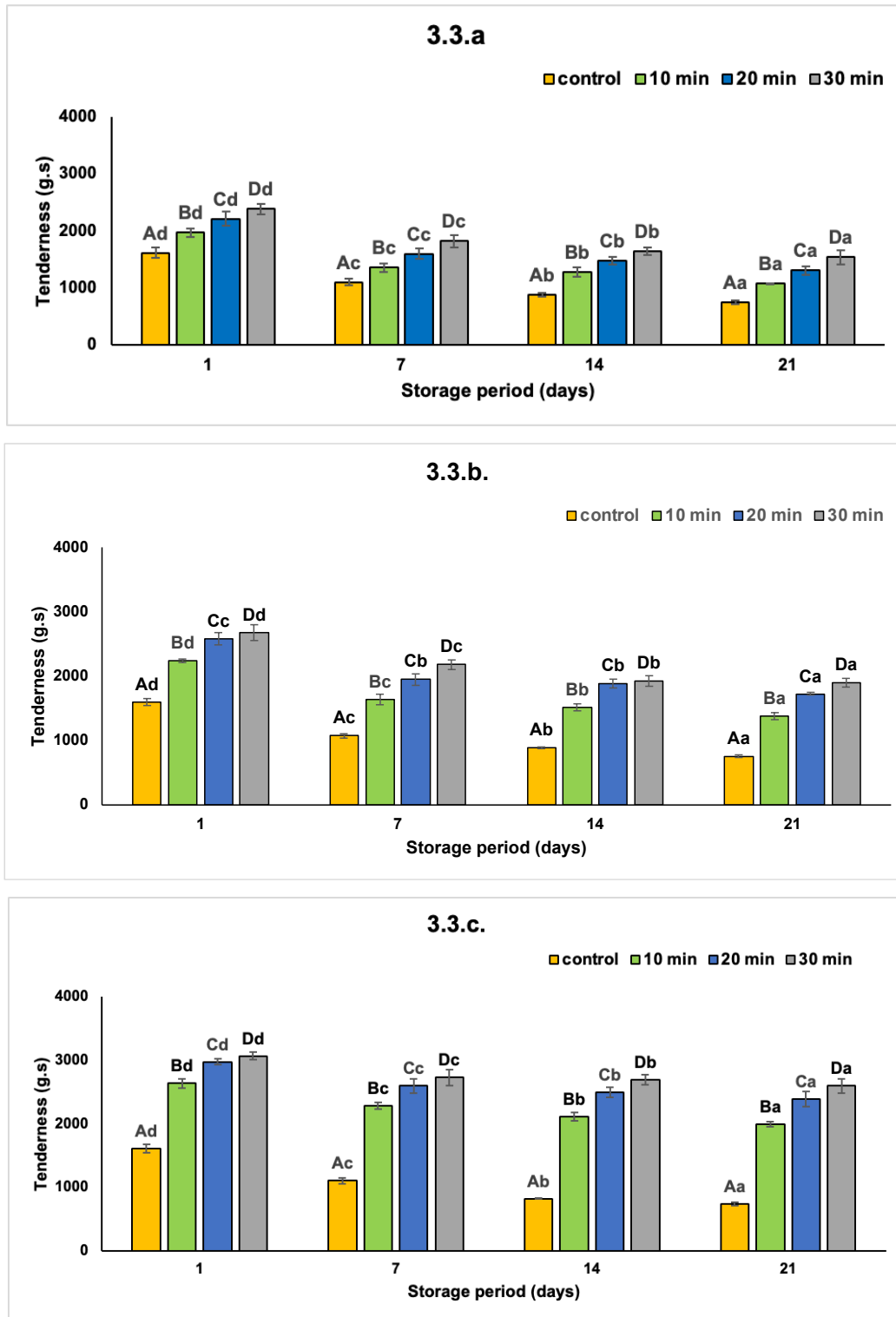


Fig. 3.3. Effect of HP treatment on tenderness of treated fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

Table 3.4. Retention (%) for tenderness of HP treated fresh salmon

Tenderness Retention (%)	Day 7	Day 14	Day 21
Control	68.7± 2.53	51.14± 0.3	45.97± 0.81
150 MPa-10 min	68.77±1.54	64.83±3.99	54.73±0.61
150 MPa-20 min	72.09±3.1	66.45±4.74	59.01±3.13
150 MPa-30 min	76.23±1.91	69.12±1.65	64.58±1.73
250 MPa-10 min	73.16±1.17	67.73±0.23	61.84± 2.17
250 MPa-20 min	75.6±1.13	73.07±1.97	66.86±1.46
250 MPa-30 min	81.56±1.08	71.85±1.18	70.73±1.72
350 MPa-10 min	86.79± 5.28	80.05±5.15	75.54±6.06
350 MPa-20 min	87.34± 2.07	83.97± 1.58	80.2±2.92
350 MPa-30 min	89.08±4.84	87.82±3.89	84.67±2.02

Values are the mean ± standard deviation (n=3) samples during 21 days of storage

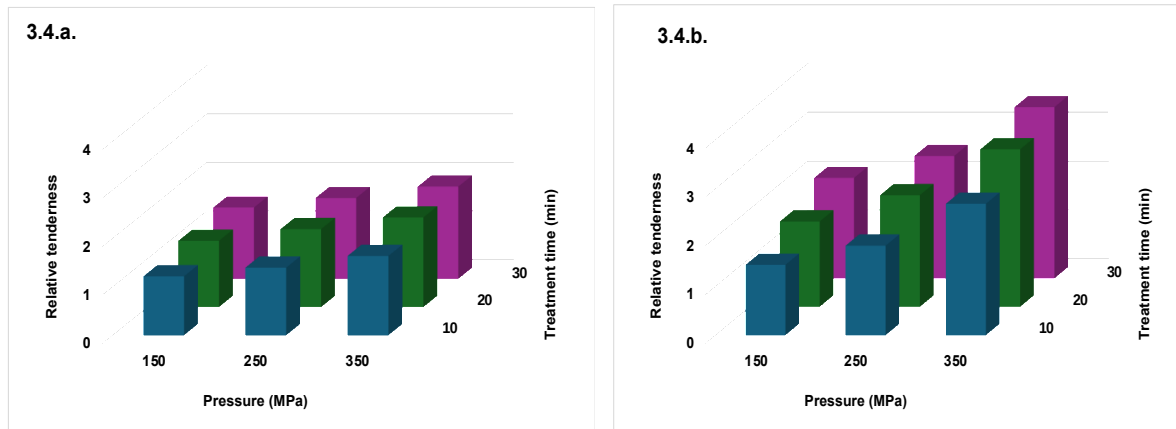


Fig. 3.4. Effect of HPP on relative tenderness of 150, 250, 350 MPa treated fresh salmon on Day 1 (Fig 3.4.a) and Day 21(Fig 3.4.b). Values are the mean of 3 independent samples during 21 days of storage.

A secondary process that occurs during HP treatment is the alteration in texture of the sample treated with pressure. Main factors that cause these textural changes include myofibrillar protein denaturation and enzymatic changes. This can also lead to the fish samples having a gel-

like structure (Yagiz, et al., 2007). Disruption of microbial cell structure and breakdown of fish enzymes can also affect these textural changes. The observed trend of increase in salmon firmness with pressure condition could be as a result of denaturation of the fish protein that causes shrinkage of fish muscle (Tsai et al., 2022). In this study it was observed that even a 10 min treatment could induce changes in the samples to give a higher tenderness. In comparison to the firmness, all the treatment times for each pressure level, resulted in an increased tenderness. This indicates that pressure treated samples have a greater chewiness and provided better palatability to consumers. As observed from Table 3.4, higher pressure level associated with higher treatment times, resulted in causing myofibrillar changes in the salmon, that gave an increased tenderness. In terms of quality of fish muscle, softening of fish tissue was considered as an indicator towards degradation of fish quality. It represents the loss in firmness of the fish muscle (Tavares et al., 2021). Hence, as 350 MPa has the lowest tenderness loss towards the end of the study, it undergoes the least quality deterioration, followed by 250 MPa and then finally 150 MPa that is observed from the relative tenderness graph (Figure 3.4). A previous study by Yagiz et al. (2009) on Atlantic salmon treated for 350 MPa, indicating having an increased chewability as a result of protein denaturation and myofibrillar shrinkage (Gomez et al., 2020).

3.4.2. Color of pressure treated samples

Color is an indicator used by consumers to visually determine the quality of the fish that they purchase. When it comes to fish such as salmon, color is an important parameter that is used as an indicator for determining fish quality (Zhu et al., 2024). This makes it a determining factor for consumers while making the purchasing decision of the fish products. Though HP treatment aims in reducing microbial count, inducing enzymatic changes, and extending the storage, a secondary factor that occurs during this process is the change in fish color (Tavares et al., 2021). This is

also a reason as to why higher pressure levels cannot be opted for during the processing of the sample as the color factor is to be considered while increasing the pressure levels. In addition, the color values also provide information on the carotenoids and heme pigments which are associated with proteins (Bharatbhai and Shyni, 2024). In terms of fish color analysis, the L^* value is an indicator of fish lightness or brightness, a^* represents the redness of the fish, chroma represents the intensity of a color, and hue helps in identifying the color of the sample in the color wheel.

3.4.2.1. Effect of HPP on lightness (L^*)

As with the effect of pressure on texture discussed in the previous sections, this section deals with the pressure effects on color parameters starting with the lightness L^* value. Again, the data are presented in three formats - the general variation in the L^* values of control and treated samples as influenced by pressure treatment time and storage time grouped according to the treated pressure level (150-350 MPa) (Figure 3.5), the retention percentage (Table 3.5) and relative retention values (Figure 3.6).

From Figure 3.5, in general, it can be observed that the pressure treatment caused the lightness of treated samples to increase significantly following the treatment. The lightness (L^*) of 150 MPa treated fresh salmon is represented in Figure 3.5.a. Irrespective of the length of storage time at 4 °C, the treated samples had a significantly higher L^* value than the control. Within the storage period, the changes were small, but still an increasing trend was observed. On days 7 and 21, there was no significant increase between 20 and 30 min treatments. Across storage, all samples had a significant increase in L^* . The samples were becoming somewhat paler with HP treatment and refrigerated storage.

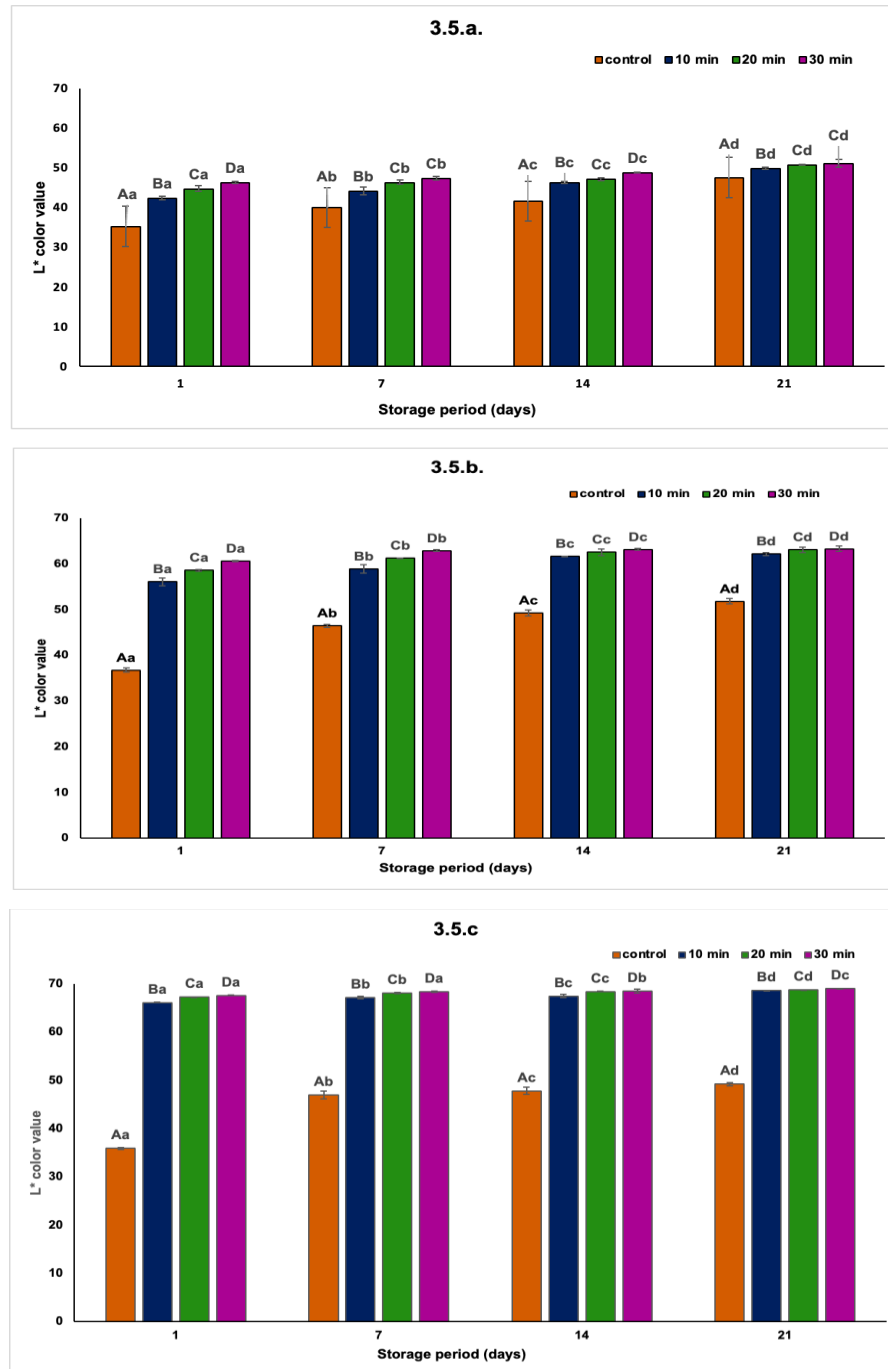


Fig. 3.5. Effect of HP treatment on the L^* (lightness) of treated fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

At 250 and 350 MPa treatments (Figure 3.5.b &c), it was observed that the L* value increased significantly for all treatments and across storage, just as it did with 150 MPa. The 30 min treatments had the highest increase in lightness for all days. However, across storage it was observed that 30 min treatment did not undergo significant changes between Day 1 and Day 7. This indicates that this higher pressure levels and treatment times helps to reduce changes in L* values creating better stability. During storage all samples significantly had increased L* values and from day 14 there was significant difference for 30 min treatment time.

The retention percentage of L* (Table 3.5) was calculated to observe the increase in lightness of fresh salmon across refrigerated storage as compared to the values found immediately after the HP treatment. Similar to the texture characteristics, an increase in L* retention was observed for samples treated with higher pressure and longer treatment time. The magnitude of the increase was higher. For example, on Day 7, the increase in L* over the control was about 33% while on Day 21, the difference increased to 66% demonstrating a long lasting protective effect. With storage time however, the lightness value retentions decreased although the differences between the control and treated samples got enlarged. This was because during the storage the changes associated with the control samples were more rapid than in treated samples.

The relative retention values are shown as two parameter bar graphs in Figure 3.6 as observed on the first and last day of storage. Again, a steady pattern was noticed with the effect of pressure treatment level and treatment time with progressive systematic increase in L* with an increase in pressure level from 150 to 350 MPa as well as treatment time 10 to 30 min. In other words, the L* value increased with treatment time for each treatment pressure level, and as the pressure levels increased, an increase in L* value was observed as well. The overall increase in

relative L^* was about 1.8 over the control. It was observed that after the storage study, there was a further increase in relative L^* from day 1. With increased pressure levels and pressure holding times, a relative value of about 2.2 was reached on Day 21.

Table 3.5. Retention (%) for L^* of HP treated fresh salmon

L^* Retention (%)	Day 7	Day 14	Day 21
Control	73.58 $\pm$ 0.29	65.96 $\pm$ 0.17	58.9 $\pm$ 0.63
150 MPa-10 min	95.83 $\pm$ 0.32	90.81 $\pm$ 1.41	82.42 $\pm$ 1.13
150 MPa-20 min	96.34 $\pm$ 0.16	94.25 $\pm$ 0.09	86.3 $\pm$ 0.74
150 MPa-30 min	97.72 $\pm$ 1.28	94.62 $\pm$ 0.06	89.66 $\pm$ 0.53
250 MPa-10 min	94.92 $\pm$ 0.92	90.08 $\pm$ 0.5	89.13 $\pm$ 0.1
250 MPa-20 min	95.39 $\pm$ 0.81	93.27 $\pm$ 0.24	92.36 $\pm$ 0.26
250 MPa-30 min	96.17 $\pm$ 0.16	95.72 $\pm$ 0.16	95.48 $\pm$ 0.15
350 MPa-10 min	98.4 $\pm$ 0.74	97.93 $\pm$ 0.37	96.24 $\pm$ 0.41
350 MPa-20 min	98.75 $\pm$ 0.7	98.28 $\pm$ 0.18	97.76 $\pm$ 0.77
350 MPa-30 min	98.81 $\pm$ 0.43	98.58 $\pm$ 0.1	97.8 $\pm$ 0.72

Values are the mean $\pm$ standard deviation (n=3) samples during 21 days of storage.

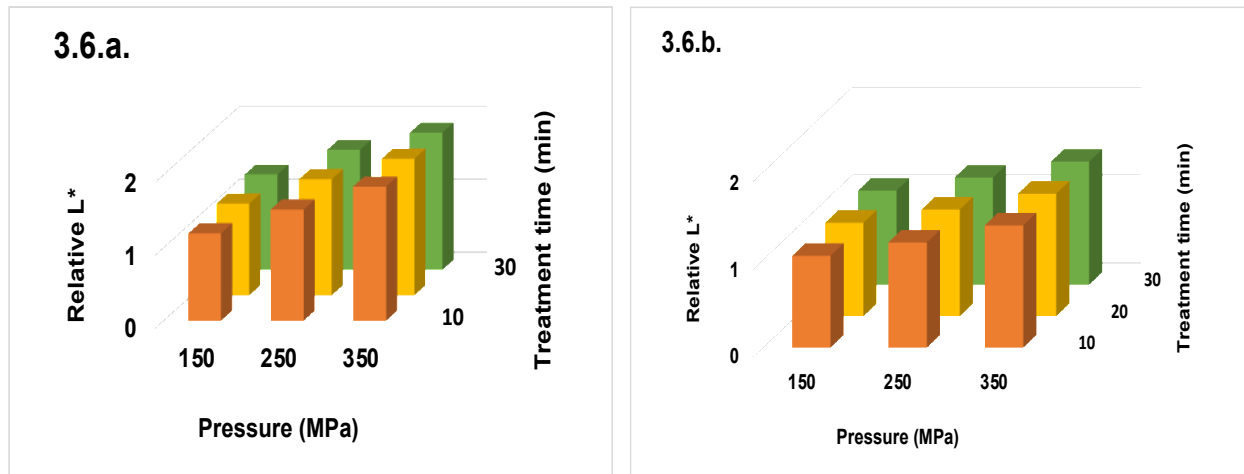


Fig. 3.6. Effect of HPP on relative L^* of 150, 250 350 MPa treated fresh salmon on day 1 (Fig 3.6.a) and day 21 (Fig. 36.b). Values are the mean of 3 independent samples during 21 days of storage.

An increase in L^* at higher pressure level treatments is as a result of protein changes such as denaturation and unfolding (Bharatbhai and Shyni, 2024). It was observed from the texture results that the firmness and tenderness increased significantly on Day 1 for all treatment times and pressure levels. This indicates the possible reason as to why the L^* increased on Day 1. Across storage, the lightness further increases as all the treated samples undergo loss in firmness and tenderness significantly. 350 MPa treated fresh salmon had the lowest increase in L^* (Table 3.5.) and during storage the relative firmness and tenderness were highest for the same. A study by de Alba et al. (2019) on mackerel samples treated at 300 MPa for 5 min saw an increase in L^* as a result of changes in myofibrillar and sarcoplasmic proteins along with an increase in textural properties. The same observation of an increase in L^* with the texture was observed by Tsai et al. (2022) in yellowfish tuna treated at 400 MPa for 5 min.

3.4.2.2. Effect of HPP on redness (a^*)

The redness (a^*) value results as influenced by pressure are presented in a similar format in Figure 3.7, Table 3.6 and Figure 3.8 as described for L^* values. As compared to L^* values, the pressure effect of a^* was reversed. The treated samples had a lower a^* value as compared to the control and as the storage time increased, the a^* decreased further for all samples.

The effect of HP treatment at 150 MPa on fresh salmon is shown in Figure 3.7.a. 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. The observations are similar at 250 MPa (Figure 3.7.b.) and at 350 MPa (Figure 3.7.c). During storage, the control, 10 and 20 min treatment times had further significant decrease in a^* , however for 30 min treatments, no significant difference was observed between Day 7 and Day 14. This indicates the ability of 30 min treatment time to retain the a^* during refrigerated storage.

From the retention percentage in a^* (Table 3.6) demonstrated similar qualitative trends. The higher treatment levels and pressure holding times resulted in values with reduced losses across storage. However, the retention of a^* pattern was very different from that observed from L^* value. The change in a^* in control from Day 7 to Day 21 was very small 96.5% to 93%. Similar margin of changes were observed even for treated samples. At 150 MPa the change improved slightly from 97% to 94%, 98% to 97% at 250 MPa and 99% to 98% in 350 MPa treated samples. Overall, the retention percentage of a^* values was excellent with HP treatment.

The relative a^* values calculated for 150, 250 and 350 MPa for Day 1 and Day 21 showed nearly similar results (Figure 3.8). It was observed that for all pressure levels, 10 min treatments had the highest relative a^* , and at all treatment times, the a^* retention value was highest at 150 MPa, the lowest pressure level used.

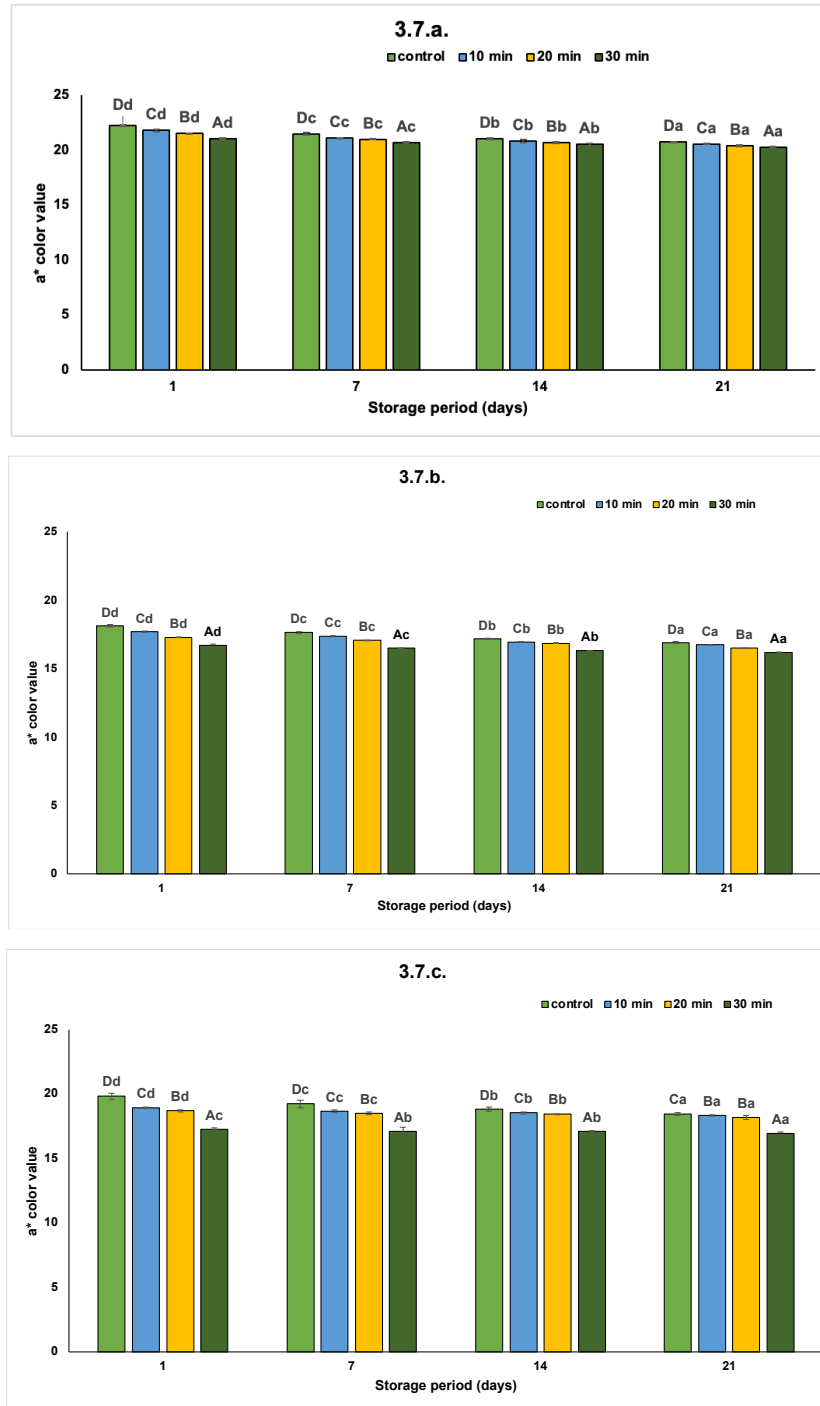


Fig. 3.7. Effect of HP treatment on the a^* (redness) of treated fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

Table 3.6. Retention (%) of a^* of HP treated fresh salmon

a^* Retention (%)	Day 7	Day 14	Day 21
Control	96.49 $\pm$ 0.15	94.52 $\pm$ 0.12	93.07 $\pm$ 0.14
150 MPa-10 min	96.91 $\pm$ 0.31	95.54 $\pm$ 0.17	94.41 $\pm$ 0.12
150 MPa-20 min	97.61 $\pm$ 0.02	96.1 $\pm$ 0.14	94.78 $\pm$ 0.15
150 MPa-30 min	98.44 $\pm$ 0.1	97.53 $\pm$ 0.05	96.35 $\pm$ 0.2
250 MPa-10 min	98.1 $\pm$ 0.07	95.66 $\pm$ 0.2	94.58 $\pm$ 0.28
250 MPa-20 min	98.76 $\pm$ 0.21	97.47 $\pm$ 0.36	95.47 $\pm$ 0.31
250 MPa-30 min	98.84 $\pm$ 0.12	97.57 $\pm$ 0.35	96.9 $\pm$ 0.33
350 MPa-10 min	98.65 $\pm$ 0.09	97.92 $\pm$ 0.14	96.79 $\pm$ 0.25
350 MPa-20 min	98.88 $\pm$ 0.15	98.49 $\pm$ 0.21	97.23 $\pm$ 0.24
350 MPa-30 min	99.02 $\pm$ 0.21	98.98 $\pm$ 0.11	98.02 $\pm$ 0.91

Values are the mean $\pm$ standard deviation ($n=3$) samples during 21 days of storage.

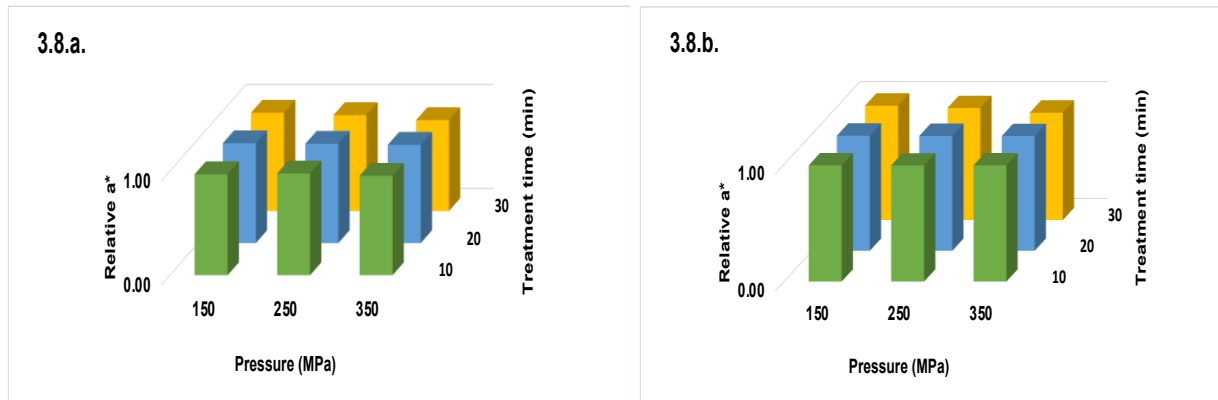


Fig. 3.8. Effect of HPP on relative a^* of 150, 250 350 MPa treated fresh salmon on day 1 (Fig 3.8.a) and for day 21 (Fig 3.8.b). Values are the mean of 3 independent samples during 21 days of storage.

The observed a^* plots (Figure 3.7) in general indicated significant decrease in a^* with the application of HP when presented as mean values. However, when the data were treated in relative terms, the changes were less dramatic (Figure 3.8). Furthermore, across the storage, all

samples underwent a decrease in a^* , except for 30 min treatment of 350 MPa that showed no significant difference during storage on Days 7 and 14. The reduction in a^* with treatment level and time, correlates with the observed increase in L^* and decrease in texture qualities across storage. Similar to the L^* value of 30 min treatment of 350 MPa, which observed no significant difference between Day 1 and Day 7, a^* values indicated no significant difference for Day 7 and Day 14. Therefore, this study for a^* indicates that higher pressure and holding time results in decreased loss of color parameter across refrigerated storage (Table 3.6.).

Singh et al. (2022) determined that the changes in Myoglobin (Mb), which are color pigments bounded to the proteins, undergoes changes with the application of pressure in fish. As the firmness and tenderness increased with the application of pressure in this study, it can be said that the samples undergo changes in protein. This change could account for the reduced a^* value. Chen et al. (2020) studied that in *Trichiurus Haumela*, the a^* decreased and the texture increased with the application of 450 MPa to the fish, as a result of disassociation of proteins. Arnaud et al. (2018) observed increased L^* and reduced a^* for 450 MPa treated cod and Giannoglou et al. (2021) observed the same changes in lightness and redness in sea Bram fillet treated at 300 MPa for 5 min. Enzymatic changes and lipid oxidation that occur during storage in fish with time, could result in this decreased loss of a^* (Singh et al., 2022).

3.4.2.3. Effect of HPP on Chroma and Hue

In color analysis, it is important to determine the chroma and hue to understand the position of a sample's color in the color space chart. Chroma and hue are fundamental parameters of color analysis that help in determining its intensity and categorising a color. Chroma values help in analysing the colorfulness of a sample, relative to the brightness of its surrounding, and in chroma, it defines how much the sample differs from the bright centre of the

color chart (Flachot and Gagenfurtner, 2021). With a sample having a higher pigment concentration, the chroma increases from the bright centre denoted as zero to the extremes, where the values increase (Cairone et al., 2020). Hue angle are values that help in distinguishing the samples amongst the color charts such as red, green, yellow etc. It is generally denoted as degrees around the color chart ranging from 0° to 360° (Suriano, 2021).

The effect of high-pressure processing on the chroma of the fresh salmon is summarized in Table 3.7. Upon applying HPP, on all the days of study, the HP treated samples had a significantly lower C* value. For 150 MPa treated samples, for all 21 days of study, the higher treatment times resulted in a lower C* value. And across storage, all the holding times had a significant decrease in C*. For 250 MPa, on Day 7, the 20 and 30 min treated samples showed no significant difference. For all other days the C* significantly decreased, except for 30 min treatment that showed no significant difference across storage from Day 7. This indicates that the changes in chroma were well controlled for the 30 min treatment. For 350 MPa, the observations were similar to 150 MPa where the C* decreased with treatment time, and but remained steady during storage.

These observation of C* were similar to a* across study where the redness decreased with pressure level and holding time and further decreased across storage. This conveys that a reduction in intensity of the color occurs during HPP (Gómez et al., 2020). This denotes that pressure treated samples have a more cooked appearance which is in accordance with the increased L* values across the study. Across the 21 days of storage, it was noted that C* decreases, however the treated samples had a lower loss in comparison to the control. A decrease in C* has been observed in other studies such as in white shrimp processed at 900 psi for 5 min

by Kustyawati et al. (2021), minced albacore treated at 310 MPa for 6 min by Ramirez-Suarez and Morrissey (2006) and sliced cod treated at 450 MPa for 5 min (Arnaud et al., 2018).

Table 3.7. Effect of HPP on chroma (C*) of fresh salmon

Chroma	Day 1	Day 7	Day 14	Day 21
Control	27.92 ± 0.17 ^{Dd}	27.18±1.26 ^{Dc}	26.28±1.35 ^{Db}	25.24±1.63 ^{Da}
150 MPa-10 min	26.67±0.66 ^{Cd}	26.17±1.56 ^{Cc}	25.77±1.03 ^{Cb}	25.15±1.27 ^{Ca}
150 MPa-20 min	25.69±0.94 ^{Bd}	25.35±1.69 ^{Bc}	24.87±1.41 ^{Bb}	24.46±1.47 ^{Ba}
150 MPa-30 min	24.53±0.87 ^{Ad}	24.20±0.84 ^{Ac}	24.08±2.36 ^{Ab}	23.89±1.29 ^{Aa}
250 MPa-10 min	25.92±1.03 ^{Cd}	25.64±1.15 ^{ABc}	25.31±1.42 ^{Cb}	24.86±1.22 ^{Ca}
250 MPa-20 min	24.36±1.62 ^{Bd}	24.17±1.47 ^{Ac}	23.97±1.98 ^{Bb}	23.79±1.12 ^{Ba}
250 MPa-30 min	22.42±0.99 ^{Aab}	22.16±0.98 ^{Ab}	21.75±0.60 ^{Ab}	21.48±1.26 ^{Ab}
350 MPa-10 min	25.69±1.30 ^{Cd}	25.37±0.35 ^{Cc}	25.12±0.97 ^{Cb}	24.97±0.63 ^{Ca}
350 MPa-20 min	23.98±1.17 ^{Bd}	23.77±0.99 ^{Bc}	23.58±1.54 ^{Bb}	23.38±1.31 ^{Ba}
350 MPa-30 min	21.54±1.63 ^{Ad}	21.41±0.78 ^{Ac}	21.25±0.47 ^{Ab}	21.05±1.23 ^{Aa}

Values are the mean ± standard deviation (n=3) 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 ($p < 0.05$).

The hue angle results (Table 3.8) indicated that the 20 min treatment could cause the same effect of 30 min sample, only for Day 1. Furthermore, 10 and 20 min treatments did not have significant difference on Day 21. Across storage there was a significant increase in hue angle for 30 min; however, 10 and 20 min treatments showed no significant difference between Days 14 and 21. For 250 MPa on Day 1, there was a significant increase between all treatments. However, on Day 7, 20 and 30 min treatments showed no significant difference, and by Day 14 all the three treatments had no significant differences between the holding times. On Day 21, the 10 and 20 min treatments had no significant difference, which was observed in 150 MPa also.

Across storage the hue angle increases for all samples significantly. For 350 MPa on Day 1, all the treatments showed a significant increase in hue angle. Similar to Day 7 of 250 MPa, the 350 MPa treated samples for 20 and 30 min showed no significant difference. For Day 21, no significant difference was observed between 20 and 30 min treatments.

Further for the hue values it was noticed that the treated samples had a higher value than the control. This denotes that the control was more towards the desired color of salmon such a pinkish red and the treated more towards yellowish green. During storage we notice that all the samples have an increased hue angle but the rate of increase slower for pressurised samples. A study on albacore muscles indicated similar results where the pressure treated samples observed a light yellowish-grayish hue for the samples (Ramirez-Suarez, 2006).

Table 3.8. Effect of HPP on hue of fresh salmon

Hue	Day 1	Day 7	Day 14	Day 21
Control	37.93± 0.68 ^{Aa}	39.09± 1.07 ^{Ab}	39.43± 1.00 ^{Ac}	39.60± 1.01 ^{Ad}
150 MPa-10 min	38.25± 1.01 ^{Ba}	39.32± 1.10 ^{Bb}	39.79± 0.51 ^{Bc}	39.83± 1.3 ^{Bc}
150 MPa-20 min	38.65± 1.91 ^{Ca}	39.42±1.23 ^{Cb}	39.85± 1.06 ^{Cc}	39.88± 0.34 ^{Bc}
150 MPa-30 min	38.74± 1.16 ^{Ca}	39.50±1.73 ^{Db}	39.90± 1.05 ^{Dc}	39.97± 1.42 ^{Cd}
250 MPa-10 min	42.84± 0.65 ^{Ba}	43.23± 0.74 ^{Bb}	43.51± 0.58 ^{Bc}	43.78± 1.67 ^{Bd}
250 MPa-20 min	42.98± 1.21 ^{Ca}	43.33±0.15 ^{Cb}	43.54± 0.97 ^{Bc}	43.80± 1.48 ^{Bd}
250 MPa-30 min	43.09± 0.77 ^{Da}	43.4± 0.81 ^{Cb}	43.57±0.94 ^{Bc}	43.9± 1.4 ^{Cd}
350 MPa-10 min	43.45± 1.69 ^{Ba}	43.63± 0.5 ^{Bb}	43.78± 1.39 ^{Bc}	43.89± 0.77 ^{Bd}
350 MPa-20 min	43.59± 1.5 ^{Ca}	43.71± 1.01 ^{Cb}	43.89± 0.98 ^{Cc}	43.94± 1.67 ^{Cd}
350 MPa-30 min	43.78± 1.23 ^{Da}	43.83± 0.39 ^{Ca}	43.9± 1.92 ^{Db}	44.01± 0.31 ^{Cb}

Values are the mean ± standard deviation (n=3) 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 ($p < 0.05$).

3.4.3. Protease activity in pressure treated samples

Proteolytic activity of the high pressure treated sample was compared against untreated control during 21 days of refrigerated storage condition of 4 °C (Figure 3.9). The proteolytic activity (PA) was directly proportional to the absorbance value (280 nm per 30 min). For 150 MPa, the protease activity was calculated (Figure 3.9). It was observed that a HP treatment of 150 MPa, could induce significant decrease in PA for Day 1. However, the three treatment times had no significant differences between them. Across storage, except for Day 14, there is significant difference between the control and the treated samples on all other days. On Day 7, 10 and 20 min treatments showed no significant difference between treatment times. Furthermore, on Day 21, the 20 and 30 min treatment times had no significant difference. Across storage, it was observed that 10 min treatment had no significant difference between Day 1 and Day 21. For all other treatments and control, there was significant difference across the refrigerated storage.

In Figure 3.9.b, the changes in PA across storage for 250 MPa is displayed. It was observed that for all days, there was a significant difference after HP treatment. However, on day 7, 20 and 30 min treatment times had no significant difference. In comparison to 150 MPa that yielded no significant difference for Day 21 between 20 and 30 min treatment, for 250 MPa, all three treatment times showed no significant difference. During storage, for all samples, a significant difference was observed. A much higher stability in the PA was observed for 250 MPa in comparison to 150 MPa. At 350 MPa, on all days, the treated samples showed a significant difference from the control.

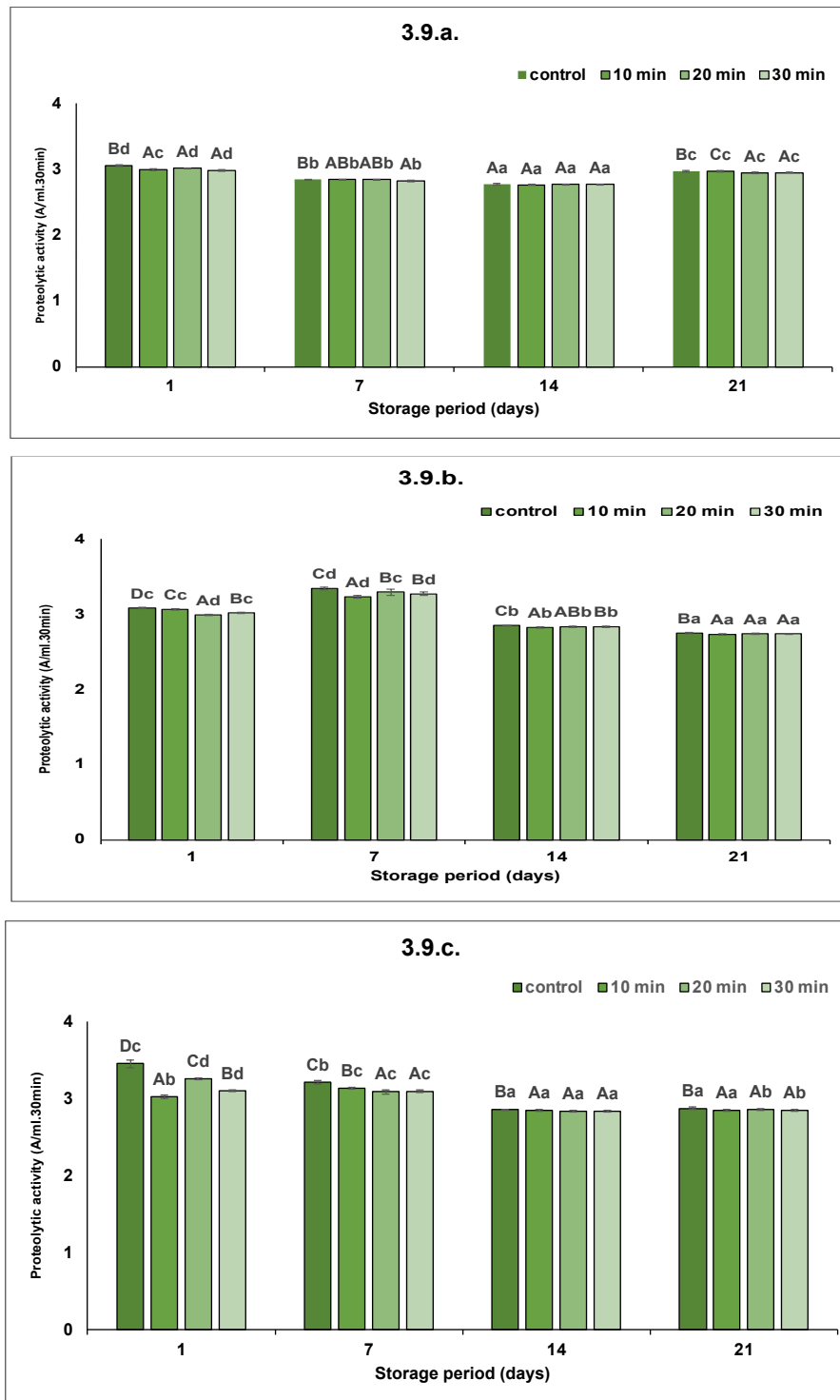


Fig. 3.9. Effect of HP treatment on the protease activity fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

For Day 1, the PA was reduced on application of HP treatment. Similar to 250 MPa, on day 7, 20 and 30 min treatments showed no significant difference. For Day 14, no significant difference was observed between the different treatment times of HP. And on Day 21, again similar to 250 MPa, there were no significant difference observed between the treatment times. Across storage, 10 min treatment showed no significant difference between Day 14 and Day 21.

Proteases are enzymes in the fish muscle that occur as a sign of biodegradation. It acts as a bio sensory indicator to indicate the deterioration in quality of fish muscle during post-mortem storage (Chen et al., 2022). Although this enzyme degradation helps with the softening of the muscle it was not as much desired for fish as this indicates a certain level of degradation (Olsen et al., 2023). In this study, it was observed that the pressure levels applied did not induce a much higher control of PA than the untreated control. However, in the untreated control of fresh salmon, from Day 7 there was an increase in proteolysis thus indicating the degradation and tissue softening of the salmon. A higher loss in firmness, tenderness, L^* and a^* could be the result of this significant increase in enzyme across storage. For higher pressure levels of 250 and 350 MPa, on Day 21, the different treatments times showed no significant difference between them. This indicates that the higher pressure used for treating the samples could provide more stable conditions in controlling enzymes across storage. Though the treatment times are not significant amongst themselves on Day 1 for 150 MPa treated samples, it was observed that by Day 21, 20 and 30 min treated samples had a significant difference, further showing the stability of higher treatment times. For several studies that aimed for a significant reduction in PA, pressure levels above 350 MPa are employed such as by Fidalgo et al. (2015), who used 450 MPa for causing significant reduction in PA for Atlantic horse mackerel. However, some studies

also indicate the possible activation of protease with the application of pressure, such as by Montiel et al. (2013) who observed an increase in protease in salmon.

This increase in proteolytic activity causes disruption in proteins of the fish thereby inducing changes in texture and color of the sample (Cropotova et al., 2020). It was observed from the graphs that the use of HP helps in achieving a certain level of reduced proteolysis amongst the pressure treated fresh salmon samples, therefore improving the storage quality.

3.4.4. Microbiology of pressure treated samples

The microbial effect of the untreated fresh salmon and the pressure treated samples are illustrated in the Figure 3.10 for 150, 250 and 350 MPa treated samples. The figures are represented as logarithmic growth (CFU/g) of all samples across 21 days of 4°C refrigerated storage.

For 150 MPa treated fresh salmon (Figure 3.10.a.), the treatment results in significantly reduced growth than the untreated control all across 21 days of study. Except for Day 7, where there was no significant difference between 20 and 30 min treatments, all other days had significantly reduced growth with the higher treatment time. Across storage, there was no significant increase in microbial growth for 30 min treatment from Day 1 to Day 7, indicating better control of microbial growth with increased holding time.

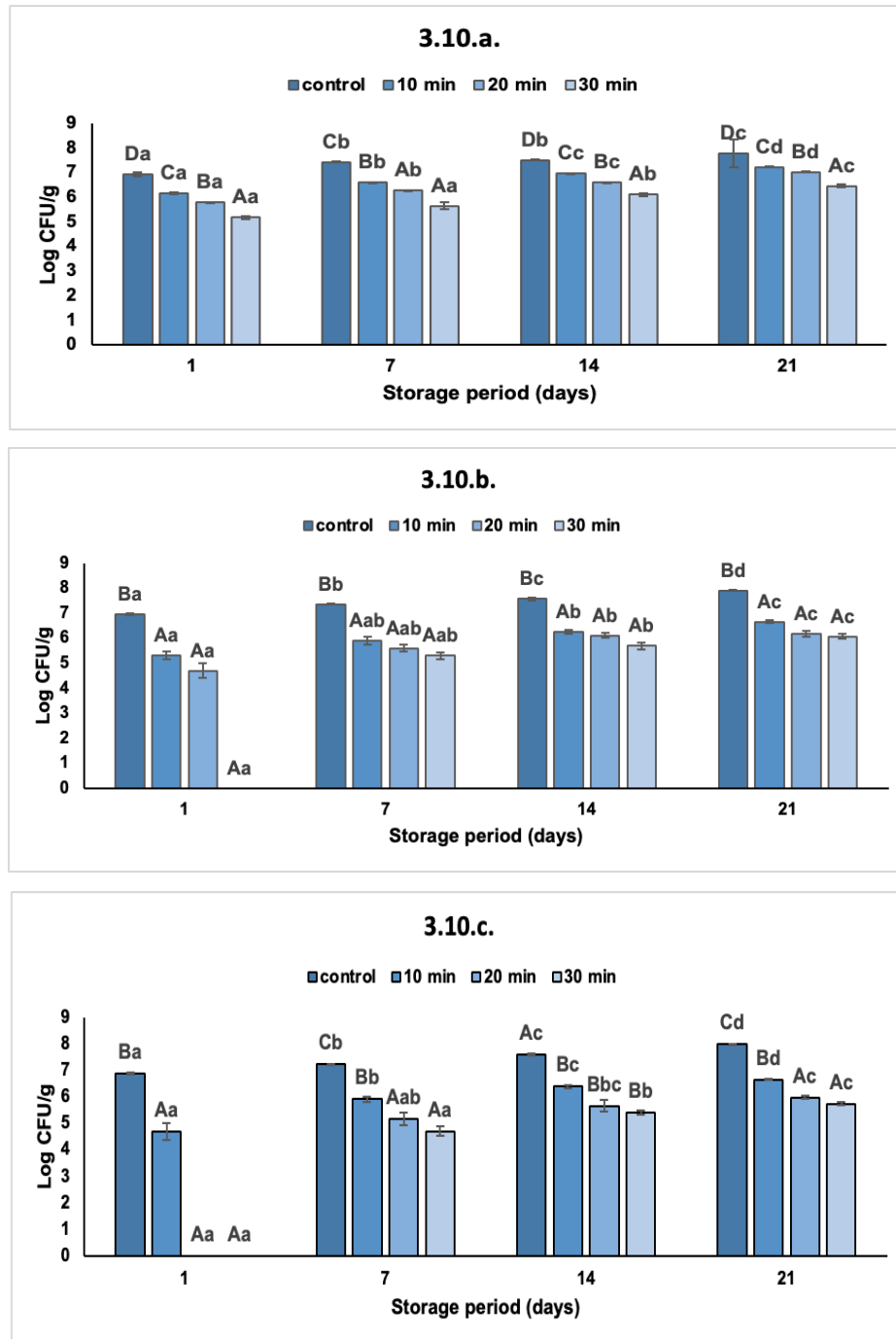


Fig. 3.10. Effect of HPP on microbial activity of fresh salmon as a function of treatment time (min) and 4 °C storage time (days) at (a) 150 MPa, (b) 250 MPa and (c) 350 MPa.. Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

From Figure 3.10.b, a 250 MPa treatment resulted in significantly lower growth than the control for all days of storage. For all 21 days, the three different treatment times had no

significant reduced growth between them for the holding time. Across storage, the microbial growth increased significantly for all samples with significantly reduced growth for higher treatments. HP treatment of 350 MPa on fresh salmon (Figure 3.10.c) resulted in significantly reduced microbial growth for all days of refrigerated storage. On Days 1 and 14, there was no significant difference between all three treatment times. For Days 7 and 21, the holding times of 20 and 30 min showed no significant difference. Across storage, all samples undergo a significant increase in microbial growth, however no significant increase was observed between Day 1 and Day 7 for 30 min treatment. This indicates that longer holding time resulted in greater microbial control across storage.

Aerobic plate count (APC) is an important parameter for indicating the quality of seafood products stored at low temperature. By the application of HP treatment to the fresh salmon, an inactivation of the microbial cells occurs. This phenomenon is as a result of the compression and decompression of the sample on pressure application which causes the bursting and leaching of the cells. Study by Podolak et al. (2020) indicated that simultaneous bursting of cell organelles causes the leaching of microbial cells and their inactivation. Other changes in protein and enzymatic activities also take place during the pressure release process (Yang et al., 2021). Overall, it can be observed from Figure 3.10 that the application of HP resulted in significantly reduced microbial growth than the untreated fresh salmon. For 150 MPa treated salmon, 20 min treatment produced the same effect as 30 min treatment. However, after 21 days, the 30 min treatment indicated having a significantly lower growth. For 250 MPa, there was no significant difference between all three treatments from Day 1 to Day 21. This indicated that the 10 min treatment at this pressure level could result in the same reduced microbial growth as 30 min treatments. In case of 350 MPa treatments, all three holding times showed no significantly

reduced growth between them. However, on Day 21, the 10 min treatment time showed a significantly reduced growth. This indicated that 20 min treatment at 350 MPa could result in same effect of microbial control as 30 min treatment.

In this study, HP treatment of fresh salmon at 150, 250 and 350 MPa for 30 min, resulted in having growths of 6.46, 6.06 and 5.74 log CFU/g after 21 days. These values were similar to previous studies on HPP of grass carp by Chen et al. (2024) where 300 MPa treated fish had a growth of 5×10^4 CFU/g. In European sea bass fillets, Tsevdou et al. (2023) showed that there was a 3 log cycle reduction from 5.05 CFU/g for treated fish. These studies confirm the outcome as a higher pressure level helps in achieving a greater inactivation of the microbial load across the refrigerated shelf-life study.

3.5. Overall Interaction Effects

ANOVA two-way and three-way plots were generated using the IBM SPSS software. The model demonstrated that the high pressure treatment process overall induced significant changes for all output parameters which are supported by the two-way and three-way interaction effects (Table 3.9). The table indicates that there were significant changes from HPP treatment from the individual pressure level, treatment time and storage days. Furthermore, from the two-way and three-way interactions also, the significant changes are noticed. Thus, this conveys the significant changes resulted from HP treatment to the fresh salmon samples. The interaction effect plots were generated for all output parameters with respected to the dependent variables of pressure, treatment time and storage time in Figure 3.11. Much of this has been described earlier. These plots help to demonstrate the specific effect plots to give an overall picture.

Table 3.9. ANOVA 2-way and 3-way interactions

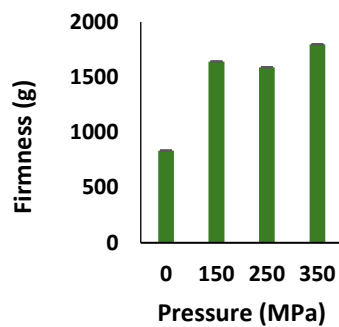
Origin	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	L	10945,756 ^a	39	280,660	10676,911	<,001
	a	343,088 ^b	39	8,797	10816,132	<,001
	Chroma	317,068 ^c	39	8,130	102,588	<,001
	Hue	552,592 ^d	39	14,169	18421,284	<,001
	Firmness	16618678,104 ^e	39	426119,951	31,335	<,001
	Tenderness	40519323,880 ^f	39	1038957,023	1335,754	<,001
	Protease	3,843 ^g	39	,099	725,524	<,001
	AM (microbial activity)	11872671,700 ^h	39	304427,479	1163,343	<,001
Intersection	L	333671,715	1	333671,715	12693572,724	<,001
	a	37337,701	1	37337,701	45907009,333	<,001
	Chroma	64885,582	1	64885,582	818762,731	<,001
	Hue	184272,157	1	184272,157	239573768,521	<,001
	Firmness	240758326,785	1	240758326,785	17704,112	<,001
	Tenderness	363928917,663	1	363928917,663	467891,676	<,001
	Protease	947,202	1	947,202	6973264,447	<,001
	AM	4088012,926	1	4088012,926	15621,984	<,001
Pressure	L	8135,249	2	4067,624	154740,974	<,001
	a	306,286	2	153,143	188290,268	<,001
	Chroma	50,400	2	25,200	317,987	<,001
	Hue	420,885	2	210,443	273598,368	<,001
	Firmness	839503,178	2	419751,589	30,866	<,001
	Tenderness	15213721,187	2	7606860,594	9779,895	<,001
	Protease	,177	2	,089	652,808	<,001
	AM	119834,130	2	59917,065	228,968	<,001
Day	L	522,338	3	174,113	6623,611	<,001
	a	11,768	3	3,923	4822,923	<,001
	Chroma	24,464	3	8,155	102,901	<,001
	Hue	16,421	3	5,474	7116,167	<,001
	Firmness	962339,521	3	320779,840	23,588	<,001
	Tenderness	9756498,545	3	3252166,182	4181,205	<,001
	Protease	2,119	3	,706	5200,980	<,001
	AM	3032083,562	3	1010694,521	3862,281	<,001
Time	L	91,506	2	45,753	1740,542	<,001
	a	15,473	2	7,737	9512,214	<,001
	Chroma	151,591	2	75,795	956,429	<,001

	Hue	,743	2	,371	482,691	<,001
	Firmness	4834395,516	2	2417197,758	177,748	<,001
	Tenderness	4069032,427	2	2034516,213	2615,712	<,001
	Protease	,002	2	,001	8,432	<,001
	AM	83284,685	2	41642,343	159,133	<,001
Pressure * Day	L	63,748	6	10,625	404,186	<,001
	a	,926	6	,154	189,683	<,001
	Chroma	2,733	6	,455	5,748	<,001
	Hue	3,333	6	,556	722,281	<,001
	Firmness	1900979,981	6	316829,997	23,298	<,001
	Tenderness	275427,360	6	45904,560	59,018	<,001
	Protease	1,057	6	,176	1297,444	<,001
	AM	76290,093	6	12715,015	48,589	<,001
Pressure Time	* L	12,345	4	3,086	117,407	<,001
	a	5,141	4	1,285	1580,339	<,001
	Chroma	15,667	4	3,917	49,424	<,001
	Hue	,030	4	,007	9,650	<,001
	Firmness	66353,808	4	16588,452	1,220	,309
	Tenderness	41107,880	4	10276,970	13,213	<,001
	Protease	,010	4	,003	18,432	<,001
	AM	44268,926	4	11067,231	42,292	<,001
Day * Time	L	12,526	6	2,088	79,417	<,001
	a	,409	6	,068	83,879	<,001
	Chroma	1,987	6	,331	4,179	,001
	Hue	,172	6	,029	37,171	<,001
	Firmness	112331,893	6	18721,982	1,377	,234
	Tenderness	43263,953	6	7210,659	9,271	<,001
	Protease	,013	6	,002	16,449	<,001
	AM	46273,315	6	7712,219	29,472	<,001
Pressure Day * Time	* L	6,071	12	,506	19,247	<,001
	a	,076	12	,006	7,833	<,001
	Chroma	5,581	12	,465	5,868	<,001
	Hue	,115	12	,010	12,494	<,001
	Firmness	48684,971	12	4057,081	,298	,988
	Tenderness	28326,842	12	2360,570	3,035	,001
	Protease	,082	12	,007	50,061	<,001
	AM	25636,630	12	2136,386	8,164	<,001
Error	L	2,103	80	,026		
	a	,065	80	,001		
	Chroma	6,340	80	,079		
	Hue	,062	80	,001		
	Firmness	1087920,481	80	13599,006		

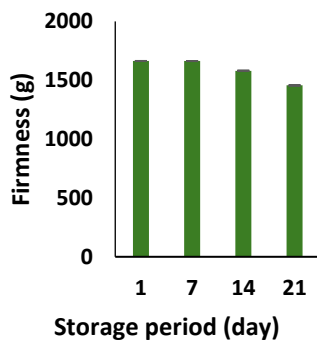
Total	Tenderness	62224,474	80	777,806		
	Protease	,011	80	,000		
	AM	20934,667	80	261,683		
	L	406002,274	120			
	a	42087,821	120			
	Chroma	71770,024	120			
	Hue	211173,901	120			
	Firmness	321467509,948	120			
	Tenderness	496979638,625	120			
Corrected total	Protease	1056,312	120			
	AM	13570628,000	120			
	L	10947,859	119			
	a	343,153	119			
	Chroma	323,408	119			
	Hue	552,654	119			
	Firmness	17706598,585	119			
	Tenderness	40581548,354	119			
	Protease	3,854	119			
	AM	11893606,367	119			

- a. R squared = 1,000 (adjusted R squared = 1,000)
- b. R squared = 1,000 (adjusted R squared = 1,000)
- c. R squared = ,980 (adjusted R squared = ,971)
- d. R squared = 1,000 (adjusted R squared = 1,000)
- e. R squared = ,939 (adjusted R squared = ,909)
- f. R squared = ,998 (adjusted R squared = ,998)
- g. R squared = ,997 (adjusted R squared = ,996)
- h. R squared = ,998 (adjusted R squared = ,997)

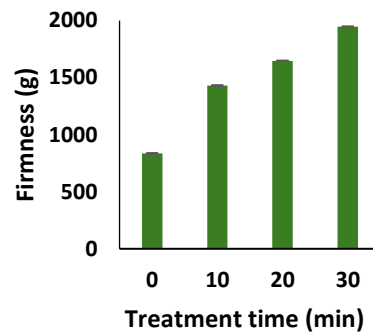
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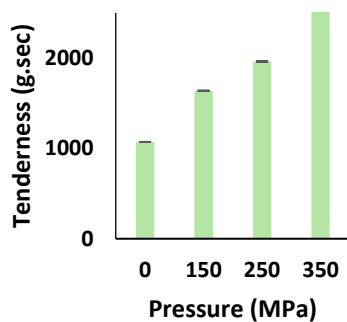
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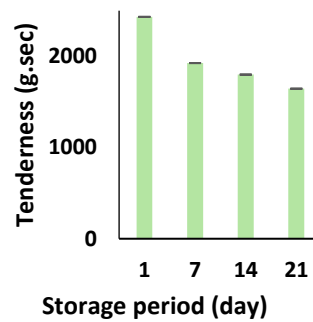
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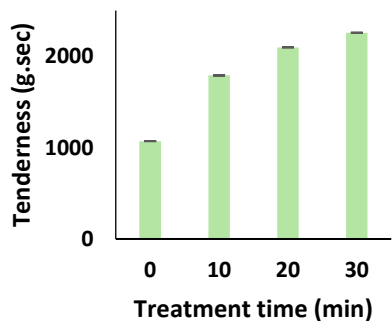
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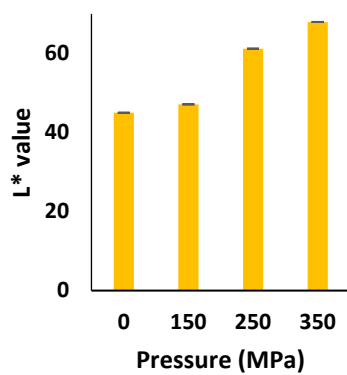
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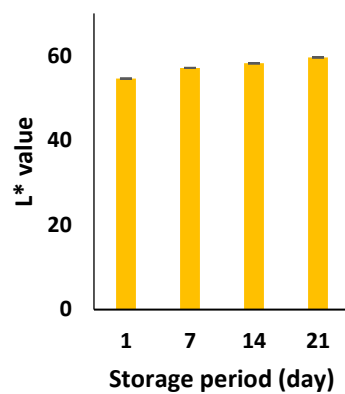
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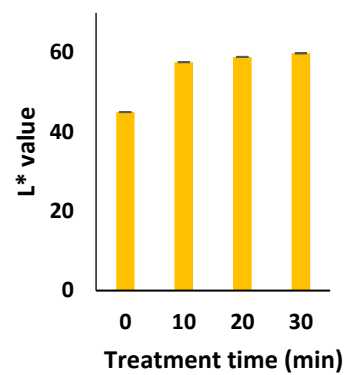
g.



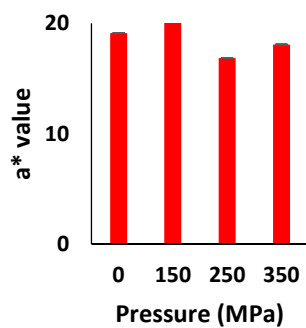
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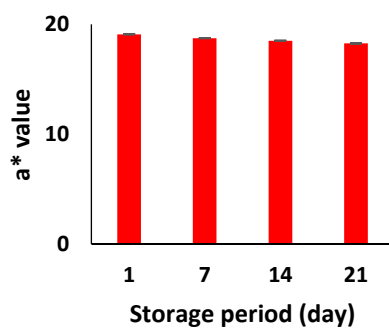
i.



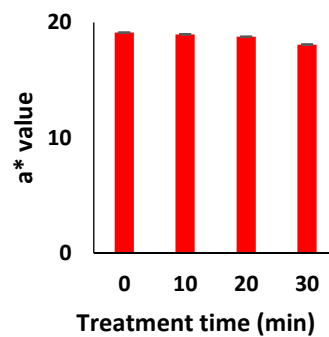
j.



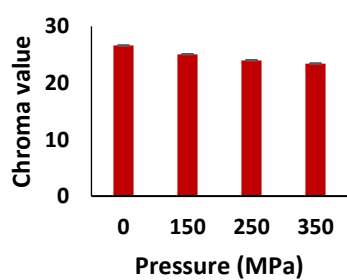
k.



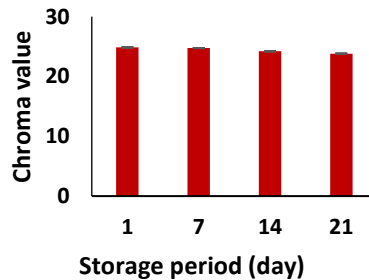
l.



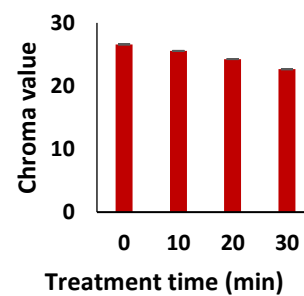
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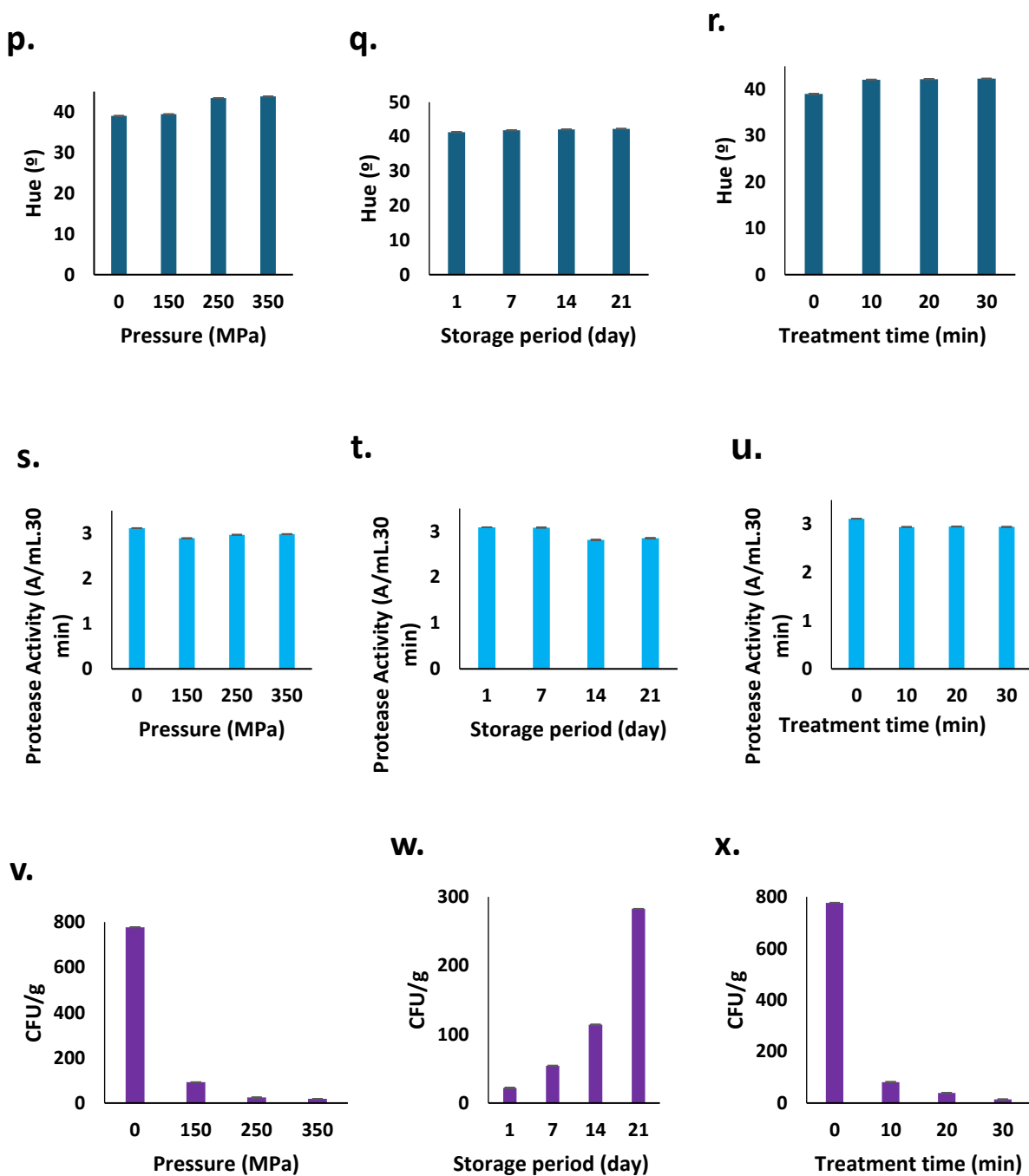


Fig 3.11. (a-x; Left to right) Effect response plots for each quality parameter analysed for pressure, storage period and treatment time. Rows 1-8 are for firmness, tenderness, lightness, redness, chroma value, hue angle, proteolytic activity, and microbial growth, respectively.

3.6. Conclusions

This comparative study was conducted to evaluate the refrigerated storage stability of fresh salmon treated with high pressure. For 150 MPa treatment, at least a 20 min treatment was required to significantly improve textural properties. The 30 min treatment showed better stability across storage for color, protease, and microbial growth. In 250 MPa treated samples, the 20 and 30 min treatment showed no significant differences between the holding times for certain attributes such as firmness, protease, and microbial growth by the end of 21 days. However, for color quality parameters, significantly improved retention was obtained for 30 min treatment. Salmon samples treated with 350 MPa had significant improvement in texture with higher holding times across refrigerated storage. Furthermore, 30 min treated samples had significantly improved stability across storage for color, protease, and microbial control. It also noted from the ANOVA table that the interaction effects support the significant changes within the samples. Overall, it was revealed that the quality of pressure treated samples retain superior characteristics in texture and color, as well as greater protease and microbial inactivation over 21 days of refrigeration. This study could be helpful in practical application for extending the refrigeration life of fresh salmon fillet.

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Connecting Text to Chapter 4

The previous chapter focused on the use of HPP on fresh market salmon to extend the refrigerated shelf-life of the fish. The use of three pressure levels and three holding times were performed for the previous study. The highest pressure level of 350 MPa used for the fresh salmon was found to have the highest retention of quality characteristics across storage. The focus of this chapter was to use this highest pressure level from the previous chapter on industry provided smoked salmon samples. Treatment of samples at 350 MPa was performed, and quality analysis was done across 21 days of refrigerated storage. Texture (firmness and tenderness), color (lightness, redness, chroma and hue angle) and microbial analysis were the quality analysis tests performed across storage.

CHAPTER 4

Evaluation of Quality and Storage Stability of Smoked Salmon Treated with High Pressure Processing

4.1. Abstract

Smoking is a traditional method that has been performed for several years in the seafood industry to enhance the flavour of the products. It is a method of exposing fish to smoke from burning the green or wood chips. The smoking process not only helps in inducing unique flavours to the fish, but it also removes the moisture and reduces microbial growth therefore improving the preservation of the highly perishable fish commodity. High Pressure Processing (HPP), on the other hand, is a non-thermal technique that helps in the microbial control and also retains the nutritional benefits of the food without inducing much changes to it. In this work, the effect of HPP on storage stability and changes in quality attributes of smoked salmon fillet were investigated. A pressure level of 350 MPa was applied to the smoked salmon for three different holding times of 10, 20 and 30 min. The untreated and HP treated samples were assessed for textural properties (firmness and tenderness), color (lightness, redness, chroma and hue angle), as well as microbial analysis on day 1, 7, 14 and 21 at refrigerated storage of 4 °C. It was revealed that the use of HPP could help to retain superior texture and lower the degree of contamination while color parameters slightly changed within 21 day refrigerated storage.

Keywords: Smoked salmon, high pressure processing, texture, color, microbial.

4.2. Industrial relevance

Through the study it was demonstrated that the use of HPP, could be beneficial for obtaining superior quality and extension of the refrigerated life of smoked salmon. The microbial

growth was controlled, the texture in terms of firmness and tenderness improved, and the color qualities were better retained during refrigerated storage. Most importantly as the salmon was smoked, the change in color parameters were minor. This control in color change after HP processing is extremely important in terms of a consumers' perception and merchandizing the fish. As the color change after applying pressure did not increase much, it was an achievement in combining the techniques of smoking and HPP which allows for products from combination treatment to be available in the market with improved quality characteristics.

4.3. Introduction

The seafood industry offers a valuable market because of the different fish species that play an important role in providing high nutritional value to the diet and the accompanied health benefits obtained from consuming it (Ekonomou et al., 2020). Amongst several fish species, salmon is an extremely popular type of fish due to several health benefits it provides. The vitamins B & D, minerals, high quality protein and omega-3 fatty acids obtained from its consumption are popularly known for providing protection against cardiovascular diseases (Landry et al., 2023). Salmon is also popularly known as a superfood due to the nutritional profile it carries and the delicious taste it provides (Nhamo and Talabi, 2024). However, the high perishability of seafood with very short shelf-life even under refrigerated conditions makes it difficult for both the producers in meeting the supply chain demands and for consumers in being provided with products that have an extended shelf-life (Ray et al., 2022). Furthermore, the short shelf life of fish makes it a problem where the quality deterioration begins with the oxidative changes that occur from the time the fish is caught (Muñoz et al., 2020). Therefore, appropriate preservation techniques are required in this sector that focus on increasing the extension of the fish without inducing much changes to it. Thermal processing is employed for a variety of fish to

provide safe and shelf-stable product but the quality is generally considered poor due to thermal degradation of quality. Drying suffers from similar concerns due to changes during drying and lack of appropriate quality up on rehydration. Freezing is perhaps the best technique - most gentle and can keep the product for extended time under frozen conditions - but requires the more expensive frozen chain and can still have associated oxidative changes continuing. Refrigerated/ice storage is the best practiced technique but only with very limited shelf-life of less than a week. Minimal processing with few weeks of extended refrigerated shelf-life is an ideal situation for the need of the day, for providing high quality products that can reach the consumer within a few weeks.

Smoking is an age old traditional preservative technique that helps in intensifying the flavour of the fish, adds aroma, in addition to extending its preservation. Salmon is one amongst the most popular fish that is used for smoking where unique flavours are induced, and its perishability is reduced (Bienkiewicz et al., 2022). During the smoking process, the salmon is partially cooked and smoked at the same time, as a result of which the taste is improved due to changes in protein and lipids. Smoking also helps with removing the moisture and therefore provides a protective function with the reduction in microbial growth.

Smoking of salmon is popularly done either as a cold or hot smoking technique, each providing its own unique flavour and texture to the fish (Andhikawati and Pratiwi, 2021). In case of hot smoked salmon, higher air/smoke temperatures of 75-121°C is used which cooks the fish in addition to inducing a smoky flavour to it. For cold smoking, lower temperatures of 20 to 30 °C is used, where the fish is exposed to lower temperatures (Hagos, 2021). Due to this, the salmon absorbs the smoke flavors and salts across a period of time and generally takes longer time than hot smoking. Hot smoked salmon tends to have an increase in firmness as compared to

cold smoked salmon, where the fish is not fully cooked (Parker and Pontin, 2021). Hot-smoked salmon are also often available as ready-to-eat products popularly in North American, Asian, European, and Australian markets where the products have a pinkish-opaque color (Lerfall and Hoel, 2021).

High pressure (HP) processing is another preservation technique that has gained wide popularity in the food industry for being a nonthermal processing method that can replace part of the low end of the thermal processing like pasteurization. The use of pressure to achieve the processing of food makes it a unique technique that induces minimal changes to the food and protects the nutritional properties of food. As a result of this, this method has become popular amongst consumers for providing food products as naturally as possible to the consumers (Terefe et al., 2023). The HP treatment technique employs water for transmitting pressure to the packaged food and this gives it a unique advantage of achieving isostatic processing where the food is uniformly processed with the use of water (Nath et al., 2023). In case of industries this is extremely beneficial where economic advantages are achieved from the uniform processing of the food.

HPP can also induce changes in textural properties of processed fish. Several studies indicate that the firmness and chewiness of HP treated fish are affected after processing (Zhu et al., 2022). Furthermore, HPP can provide greater retention of quality properties during refrigerated storage (Kårlund et al., 2023). The microbial control is another popular advantage of HPP where the induced pressure helps with the bursting and leaching of the vegetative bacterial cell's membrane, therefore improving its storage (Mulla et al., 2022).

Smoked salmon can be considered as a ready-to-eat product and the incorporation of HPP to smoking can further enhance its quality and decrease the microbial load for longer periods of

time (Valø et al., 2020). Several work has been carried out in the area of incorporating HPP to smoked salmon. HPP induced a reduced growth of *Listeria innocua* in cold smoked salmon when a combination of subzero temperature with HPP was performed for controlling *Listeria monocytogenes* resulting in reduced bacterial growth, an increase in water holding capacity. Several studies demonstrate that in cold-smoked salmon, the combination of lactoperoxidase system with HPP helps in the inactivation of *Listeria monocytogenes*, reduce myosin effects, and inactivate calpain enzyme (Gudbjornsdottir et al., 2010; Ritz et al., 2008; Lakshmanan et al., 2007; Montiel et al., 2012; Lakshmanan et al., 2005). However, the effect of HP treatment on smoked sample varies with the HPP conditions used, pressure levels studied, and the type of smoking performed on the fish. Therefore, the aim of this work was to investigate the effect of HPP on hot-smoked industry supplied salmon. In addition, the quality attributes and refrigeration stability of smoked salmon were compared to those of fresh salmon presented in the previous chapter.

4.4. Material and Methodology

4.4.1. Sample preparation

Smoked salmon fillets were received from an industry and placed in the lab under frozen storage conditions. Before pressure treatment, the samples were thawed overnight under refrigerated conditions. On the day of the treatment, the smoked salmon fillets were deskinning using a sterile knife and cut into uniform shaped cubes of size 20 mm x 20 mm x 15 mm. The smoked salmon pieces were then packed into HDPE pouches and sealed with a vacuum sealer to remove air. This air removal ensures that the pouches do not float inside the chamber of the HPP equipment that contains water for pressure transmission. Each pouch contained samples of about 45 g and separate pouches were prepared for all samples for 21 days of storage study after the

treatment. All the pouches except for the control were then given a HP treatment. The control, which was the smoked salmon fillets without HP treatment was transferred directly for refrigerated storage. The HP treatment was given using a cold isostatic press (AE 400 MPa - Isostatic Press, Autoclave engineering, Columbus, Ohio) at a pressure level of 350 MPa for three different holding times, 10, 20 and 30 min. Immediately after pressure treatment all samples were analysed on the first day for texture (firmness and tenderness), color (lightness, redness, chroma and hue angle) and microbial growth. The samples were then stored under refrigerated conditions at 4 °C for 21 days during which analysis was performed on Day 7, 14 and 21.

4.4.2. Experimental setup

The high pressure processing equipment is the same as the one discussed in Chapter 3.

4.4.3. High pressure treatment

For applying pressure treatment on the smoked salmon samples, a pressure level of 350 MPa was selected. This was because 350 MPa was the highest pressure level used for the processing of fresh salmon samples as detailed in the previous chapter. For the preliminary tests conducted at 350 MPa, the color of the HPP treated smoked salmon was not much affected in comparison to the control, which was only smoked salmon not subjected to HP treatment, and hence this pressure was used for treating smoked salmon. The prior smoking performed on the salmon had induced some stability in color even in the control sample, and after pressure application, the color change was not much different from the control. Hence, the smoked salmon was opted for treatment at 350 MPa for 10, 20 and 30 min. However, the storage stability with other quality parameters and microbial growth could be influenced by the HP treatment.

4.4.4. Texture measurement

For texture analysis, a TA.XT plus texture analyser (Texture Technologies Corp, New York, USA) was used. The probe used for conducting the analysis was a multi-wired probe similar to chapter 3 (Figure 4.1).

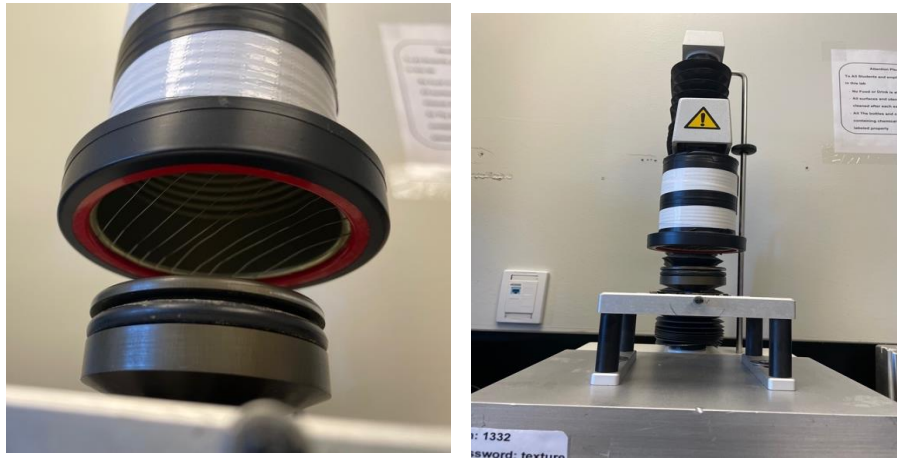


Fig. 4.1. Probe used for performing texture analysis to the smoked salmon samples treated at 350 MPa.

4.4.5. Color measurement

The color measurement on the smoked salmon 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 chapter 3.

4.4.6. Microbial analysis

For estimation of aerobic plate count (APC), enumeration of the aerobic bacteria was done as similar to chapter 3.

4.4.7. Statistical Analysis

The analysis was done in triplicates for all tests. The data were subjected to a one-way analysis of variance ($\alpha=0.05$) using the IBM SPSS Statistics Version 21 software (IBM

Corporation, USA). For the significant differences observed, mean treatments were compared using Tukey's test. Two-way and three-way ANOVA were also performed, and the interaction plots were generated using IBM SPSS.

4.5. Results and discussion

4.5.1. Texture of smoked salmon

In food analysis, texture measurement helps in understanding consumer acceptance and market preference (Guimarães, et al. 2020). In addition, it further helps in quality control by ensuring the consistency and variations in processing or storage conditions (Nawaz et al., 2020). Texture-modified diets are also tailored by health professionals for patients with swallowing difficulties (Wu et al., 2020). In this study, the firmness and tenderness of smoked salmon treated with HPP was analysed to study its changes across 21 days of refrigerated storage at 4 °C.

4.5.1.1. Firmness of smoked salmon

Firmness refers to how much deformation the sample can resist when applied with force acting from the TA (Rahman et al., 2021). All these outcome parameters were analyzed in the same format as done in Chapter 3 with fresh salmon treated by HP. The firmness of smoked salmon subjected to various HP treatments and stored for different lengths of time is shown in Figure 4.2. This format of figure clearly describes the variation in sample texture both with respect to HP treatment time (min) and each also influenced by the storage time (days) at 4 °C. It can be observed that on each day of storage, the HP treated samples had a significantly higher firmness than the control demonstrating the influence of HP treatment influence on salmon texture. On day 1, it was observed that the 10 and 20 min treatments did not have significant difference, However, after 21 days, each treatment displayed a significantly higher firmness than the other pressure holding time. This clearly indicated that higher holding times helped in having

significantly higher firmness across the refrigerated storage. It was interesting to note that from day 7, the control sample did not show a significant change in firmness. However, all the treated samples demonstrated a slight but steady and significant decrease in their value, but still maintaining a far higher firmness values as compared to the control.

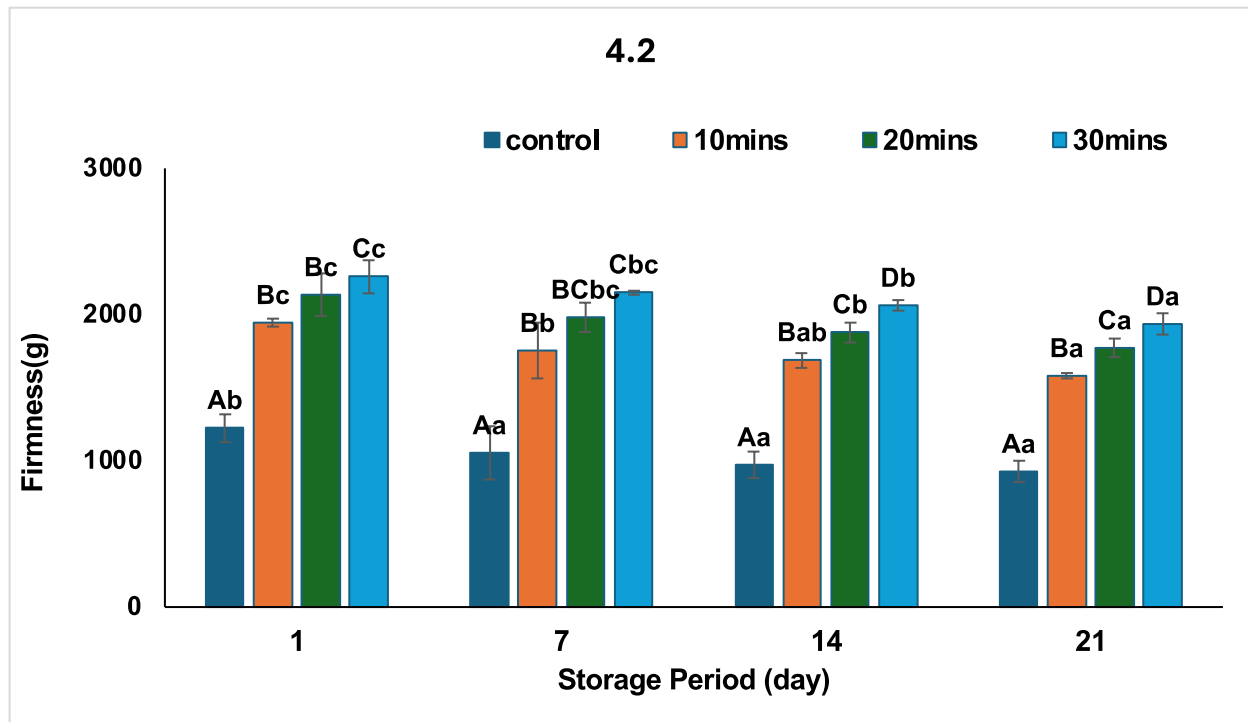


Fig. 4.2. Effect of HP treatment on the firmness of 350 MPa treated smoked salmon as influence by treatment and storage times. Values are the mean of 3 independent samples during 21 days of storage at 4 °C. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

From Table 4.1. the firmness retention (%) was studied for each treatment across storage. It was observed that the smoked control itself had a good retention of 75.8% after 21 days, whereas the fresh salmon control, as detailed in the previous chapter only had a retention of 53.0%. Smoke curing itself, therefore, helps in improving the firmness and its retention during storage as compared to fresh salmon; however, the incorporation of both smoking and HP

treatment yields much better firmness properties across refrigerated storage. Across all storage days, the treated samples had a significantly higher retention which undergoes a significant decrease across refrigerated storage. The retention was 10% higher in HP treated samples at 350 MPa for 30 min after refrigerated storage for 21 days.

Table 4.1. Retention (%) for firmness of HP treated smoked salmon

Firmness Retention (%)	Day 7	Day 14	Day 21
Control	86.1 ± 4.83	79.5 ± 6.86	75.8 ± 5.38
350 MPa-10 min	90.1 ± 7.01	86.7 ± 1.58	81.3 ± 2.10
350 MPa-20 min	92.9 ± 2.96	87.9 ± 3.27	83.0 ± 1.26
350 MPa-30 min	95.1 ± 4.41	91.4 ± 4.43	85.7 ± 3.60

Values are the mean ± standard deviation (n=3) samples during 21 days of storage

In Figure 4.3, the relative firmness of each treatment time was compared as related to the changes with respect to the control. With higher treatment time, a higher relative firmness was obtained. Across storage, while all the treatment times underwent a significant reduction in firmness as shown earlier in Figure 4.2. In relation to that happening with the control, the relative value steadily increased demonstrating the positive influence of the HP treatment. The relative firmness almost doubled when HP treated sample was compared after a 21 day storage period.

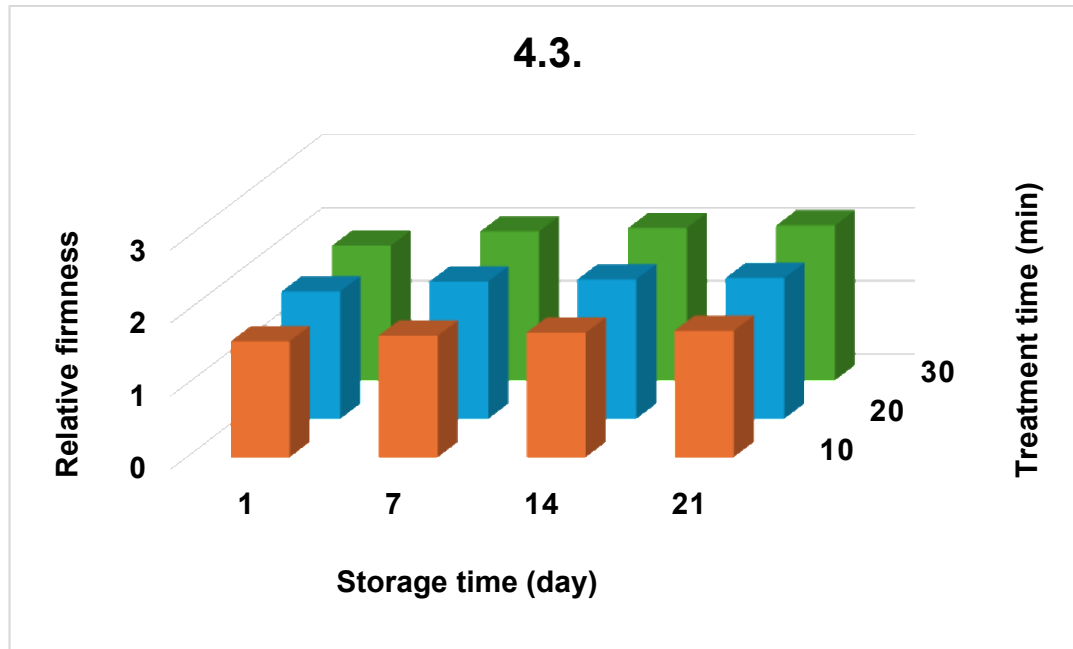


Fig. 4.3. Effect of HPP on relative firmness of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C.

Overall, from observing the firmness results, it can be said HP treatment definitely helps to improve the firmness of smoked salmon. Though on day 1, treatment times of 10 and 20 min showed no significant difference, by day 21 it was observed that a higher firmness was obtained with higher treatment times. This increase in firmness with HP application, could be as a result of denaturation of proteins in the fish muscle and with the formation of a new hydrogen-bonded network during HPP. In this study, even 10 min treatment induced HP related protein denaturation. Other studies on the application of HPP on fish also reported an increase of firmness. Renaud et al. (2022) observed increased firmness in salmon with a pressure application of 400 MPa for 5 min, Tsironi et al. (2019) observed the same increase in European sea bass fillets processed at 600 MPa for 5 min, Yagiz et al. (2007) in Atlantic salmon processed for 300 MPa for 15 min, HP treatment at 310 MPa for 4 min in albacore tuna by Ramirez-Suarez and Morrissey (2006) and Chouhan et al. (2015) in hilsa treated at 350 MPa for 10 min. By the end

of the refrigerated storage study, the pressure treated samples indicated having a clearly higher retention of firmness.

4.5.1.2. Tenderness of smoked salmon

Tenderness refers to the texture attribute of how easily a sample can break apart from the force applied from the texture analyser (Ricardo-Rodrigues et al., 2024). This is an important parameter as it helps indicate how easily the food can be chewed. In Figure 4.4, it was observed that even an application of 10 min treatment induced a significantly improved tenderness in smoked salmon. On each day, the HP treated samples had a significantly higher tenderness than the control. Furthermore, on all days, the treatments were significantly different from each other. Across storage, however, there was a significant gradual loss in tenderness in all samples, the loss being even higher with control samples.

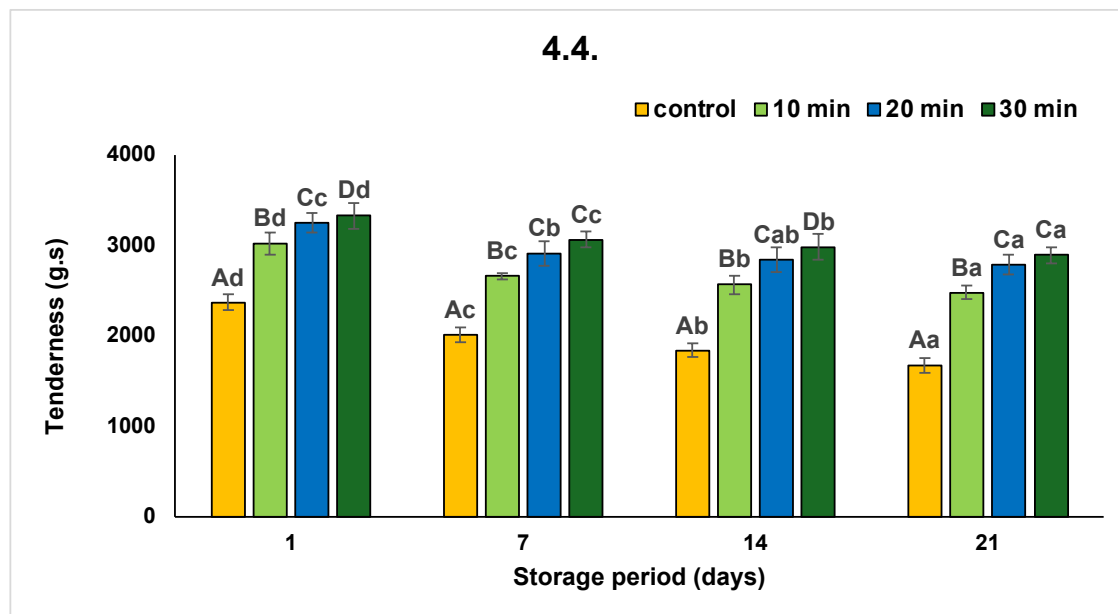


Fig. 4.4. Effect of HPP on tenderness of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

From the percentage retention table for tenderness (Table 4.2), the control sample lost about 15% of its firmness during the storage. While it was only 3-6% for 10-30 min treated samples. Treatment for 10-30 min improved the texture by about 6% over the control on Day 7, while over 16% after storage for 21 days. Hence the HP treated samples had significantly higher retention than the untreated smoked salmon across refrigerated storage, and with increased pressure holding time, an increased tenderness retention was observed.

Table 4.2. Retention (%) for tenderness of HP treated smoked salmon

Tenderness Retention (%)	Day 7	Day 14	Day 21
Control	85.06±1.96	77.71±1.44	70.74±0.53
350 MPa-10 min	88.01±1.7	84.96±2.68	82.11± 2.88
350 MPa-20 min	89.72± 4.82	87.57±1.6	85.9±3.85
350 MPa-30 min	91.91±4.48	89.53±3.5	86.8±2.33

Values are the mean ± standard deviation (n=3) samples during 21 days of storage

The relative tenderness graph was plotted for 350 MPa treated smoked salmon is shown in Figure 4.5. HP treatment for 10-20 min demonstrated a relatively minor change in firmness as compared to those existed with the control on the same day, while the 30 min treated samples showed a gradual better performance going up by about 50% firmness as compared to control with the same storage time. All the treatments showed a higher value with increased pressure holding time. Across storage the values decreased significantly for all treatment times. However, higher treatment times had a greater relative tenderness.

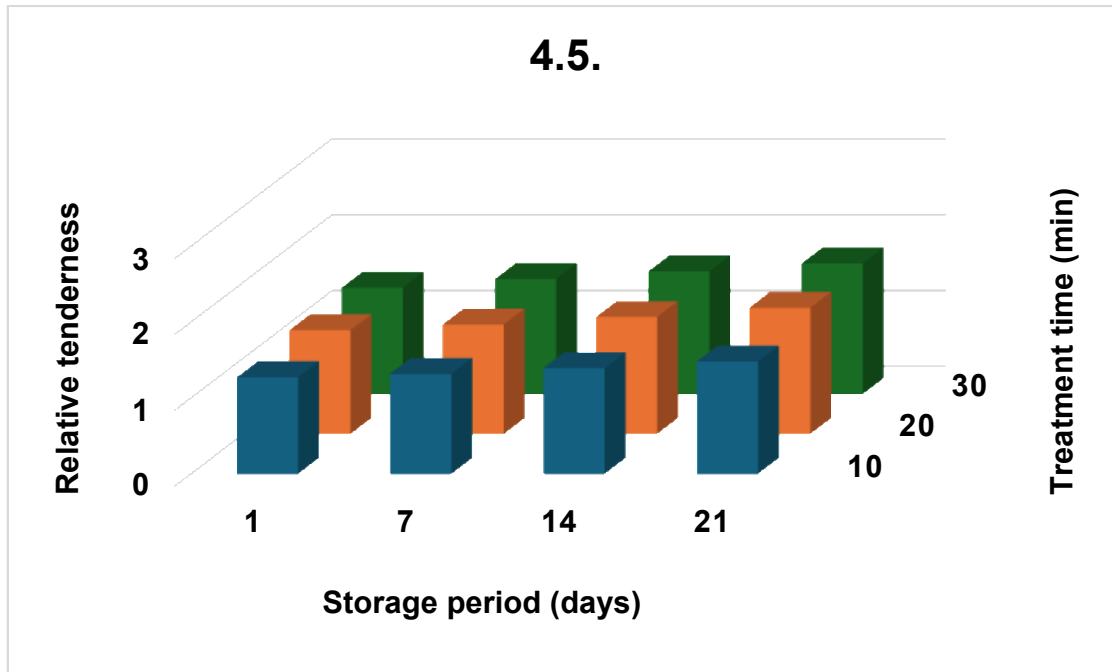


Fig. 4.5. Effect of HPP on relative tenderness of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C.

From the observed results for tenderness of smoked salmon treated at 350 MPa, it can be said that HP treatment significantly helps in improving the tenderness in addition to having reduced loss across storage. For the tenderness, unlike firmness of day 1, every increased treatment time resulted in generating a significant increase in tenderness. This pattern was observed until the last day of study, which was similar to firmness, where higher treatment times gave higher tenderness values. On comparing untreated smoked salmon control with the tenderness of fresh salmon from the previous chapter, it was noted that the smoked salmon control had a higher retention of 70.7% as compared to fresh salmon having 46% tenderness retention after 21 days of refrigeration. Better controlled enzymatic activity can be why the rate of loss of tenderness is slower in HP treated smoked salmon (Olsen et al., 2023). Hence it was

observed that smoked salmon samples treated with HP had a higher tenderness on pressure application and greater retention during storage.

4.5.2. Color of smoked salmon treated with HP

Color is an important sensory attribute that affects consumers' purchasing choices. For this reason, for the smoked salmon, color was analysed using Minolta Tristimulus Colorimeter. The lightness/brightness (L^*) and redness (a^*) that were selected to represent the color changes in the samples across storage because they are the ones more closely associated with the visual color. Chroma (C^*) and hue angle were also calculated to study the intensity and angle of color of the sample (Nawaz and Talabi, 2020).

4.5.2.1. Lightness (L^*)

Again, the discussion was based on the presentation of result in three formats as has been used earlier. Figure 4.6 shows the effect of HP treated smoked salmon on the L^* values as affected by HP treatment time (min) and subsequent storage at 4 °C (days). HP treatment application resulted in an immediate increase in L^* and this increase remained significantly high for all days of storage, except for Day 7, where no significant increase was observed between 20 and 30 min treatments. Across storage a significant increase in L^* was observed for all samples. However, for 30 min treated samples, no significant change occurred during storage, indicating it to be a better treatment to control of the color of treated salmon across refrigeration.

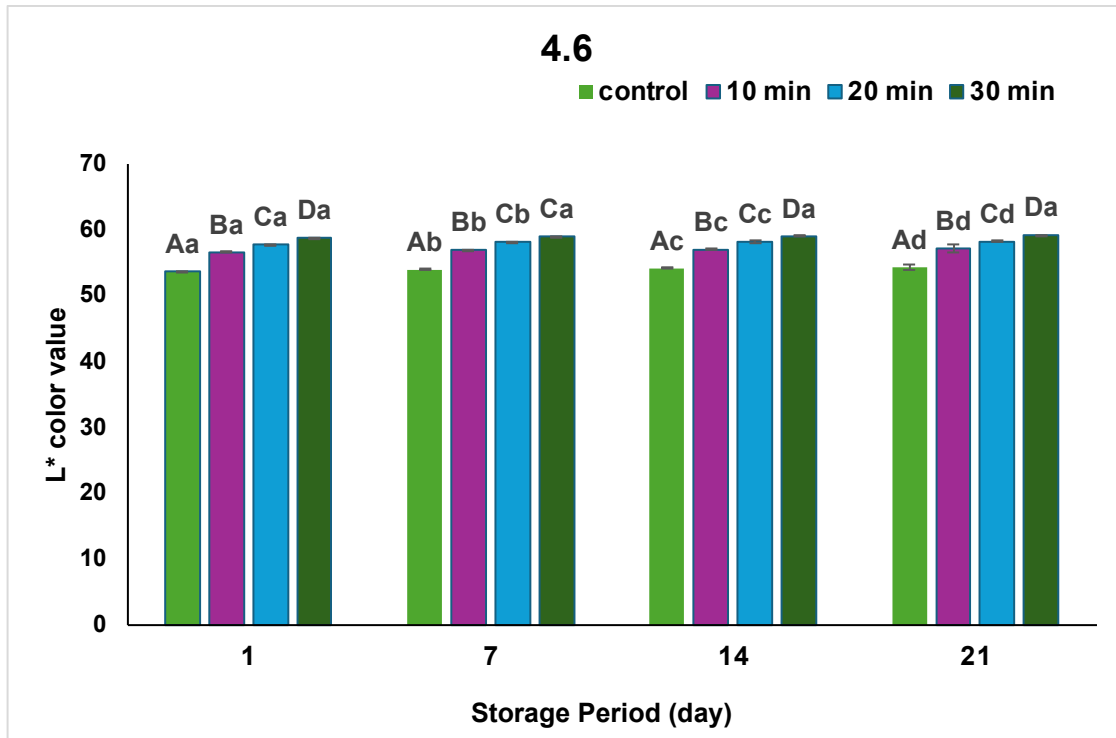


Fig. 4.6. Effect of HPP on L^* of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

The percentage retention of L^* is summarized in Table 4.3 and demonstrates how the parameter changed during the storage relative to what was observed on Day 1. As observed, the changes in L^* for both HP treated and untreated samples were very minimal. Even the smoked salmon control sample which was not subjected to HP treatment demonstrated a very high retention value of 98.6% changing by less than 1% over the period. This was in contrast to what was observed for fresh salmon control samples as shown in Table 3.5 in the previous chapter which showed a retention value of only 58.9%. This is obviously not resulting from the HP treatment but due to the process of smoke curing of the samples. The smoking process which resulted in some moisture loss and incorporation of smoke components into the product has

stabilized the color of the product likely by interfering with the protein denaturation process or suppressing chemical changes that results in the color parameter. In samples of smoked salmon that were subjected to pressure treatment, the L* values retention was almost complete demonstrating less than 0.5% change over the storage period. The time effect also was minimal.

Table 4.3. Retention (%) for L* of HP treated smoked salmon

L* retention (%)	Day 7	Day 14	Day 21
Control	99.3±0.01	90.0±0.01	98.6±0.02
350 MPa-10 min	99.4±0.01	99.2±0.01	99.0±0.04
350 MPa-20 min	99.5± 0.03	99.4±0.02	99.3±0.07
350 MPa-30 min	99.8±0.16	99.6±0.03	99.5±0.02

Values are the mean ± standard deviation (n=3) samples during 21 days of storage.

The relative L* as compared to the control on each day is demonstrated in Figure 4.7 showing differences that could be better visualized. Higher pressure treatment times influenced the relative L* values demonstrating a small but steady increase over the control. Across storage, however, all treatment times had a slight decrease in L* relative value as compared to control, more so with samples treated for longer times. The net change was less than 5%.

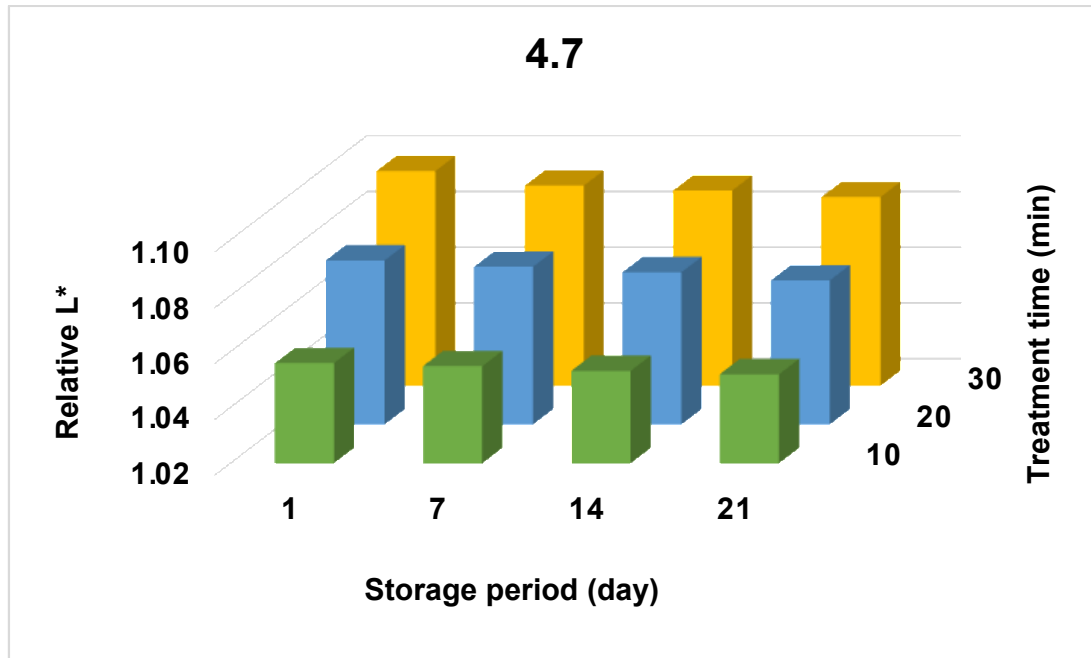


Fig. 4.7. Effect of HPP on relative L^* of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage.

Studies on smoking has reported an increase in L^* such as in smoked salmon by Chan et al. (2020), or smoked hagfish having an increased L^* by Zeng et al. (2022). As in this study the samples were smoked prior to HP treatment, and the associated changes were reported to be very minimal. This proves a beneficial for industries to adopt HPP for smoked salmon permitting treatment at higher pressures for longer times without the concerns of inducing the protein denaturation associated color changes.

4.5.2.2. Redness (a^*) of smoked salmon

Figure 4.8 shows the effect HP treatment on smoked salmon treated at 350 MPa as influenced by treatment and storage times. With the application of HP treatment, a significant decrease in a^* was observed in all test samples, for all days when compared with the control. Clearly the HP treatment had an effect on a^* values, however small it was in the magnitude

(about 2-3 color units). For Day 14, no significant difference was observed between 10 and 20 min treatments. Across storage, both treated and untreated control samples underwent a significant decrease in a^* .

In observing the retention (%) for a^* (Table 4.4.), the retention was better in treated samples with the percentage slightly improving with treatment time. However, there was a gradual decrease in the retention value for all treated samples during the 21 days of refrigerated storage, still the highest retention was observed for samples treated for longer times. Overall, there was 2-3% better retention in treated samples over the control.

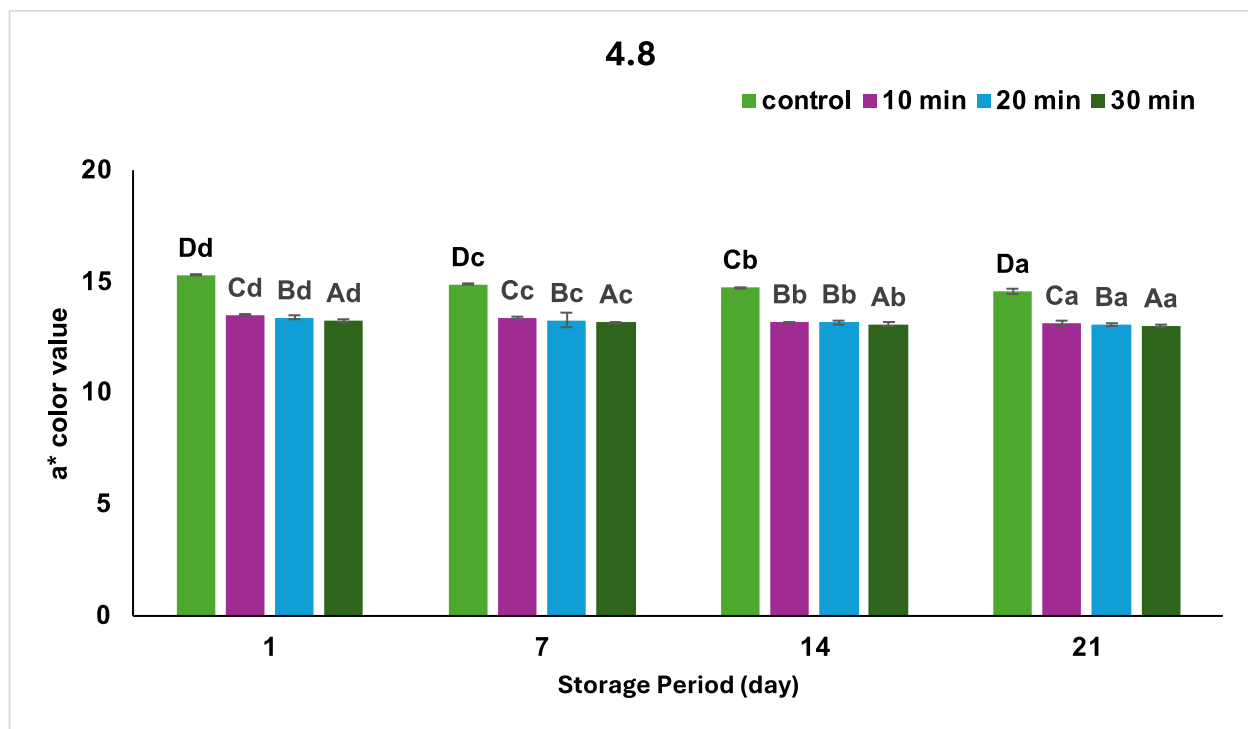


Fig. 4.8. Effect of HPP on a^* of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C. For each evaluation day, different uppercase letters indicate significant differences among treatment times ($p < 0.05$) and lowercase letters indicate significant differences of treatments across evaluation days ($p < 0.05$).

Table 4.4. Retention (%) for a^* of HP treated smoked salmon

a^* retention (%)	Day 7	Day 14	Day 21
Control	97.3 $\pm$ 0.17	96.1 $\pm$ 0.15	95.3 $\pm$ 0.21
350 MPa-10 min	98.9 $\pm$ 0.09	97.5 $\pm$ 0.06	97.1 $\pm$ 0.07
350 MPa-20 min	99.1 $\pm$ 0.15	98.3 $\pm$ 0.14	97.6 $\pm$ 0.02
350 MPa-30 min	99.4 $\pm$ 0.14	98.8 $\pm$ 0.08	98.3 $\pm$ 0.02

Values are the mean $\pm$ standard deviation (n=3) samples during 21 days of storage.

The relative a^* retention data are shown in Figure 4.9 for the three different pressure holding times. All relative values were lower than 1.0 demonstrating a decreasing trend for the values. It was observed that the higher treatment times induced a significantly lower a^* values. With respect to the treatment time there was a steady decline in the relative values while across the storage the a^* gradually recovered. The greatest retention was observed for longer pressure treatment times.

Overall, it was evident that the application of HPP for smoked salmon caused a decrease in a^* (Figure 4.8). Myoglobin (Mb), a pigment responsible for redness in fish is associated with the fish proteins. As it was observed from the firmness and tenderness results, which increased with HPP, the Mb pigments could have possibly been affected, resulting in reduced Mb concentration and therefore a lower a^* value (Singh et al., 2022). In this study, as texture results increased significantly even for 10 min treatment, the treatment can be considered to be associated with L^* increased and a^* decreased because of changes in the myofibrillar proteins of the smoked salmon. A reduced a^* value was observed by Ramirez-Suarez and Morrissey (2006)

in 310 MPa treated albacore tuna for 6 min and in 300 MPa treated mackerel by Cropotova et al. (2020). A study by Zeng et al. (2022) conveyed that probable enzymatic changes could have also been the reason for color changes across refrigerated storage.

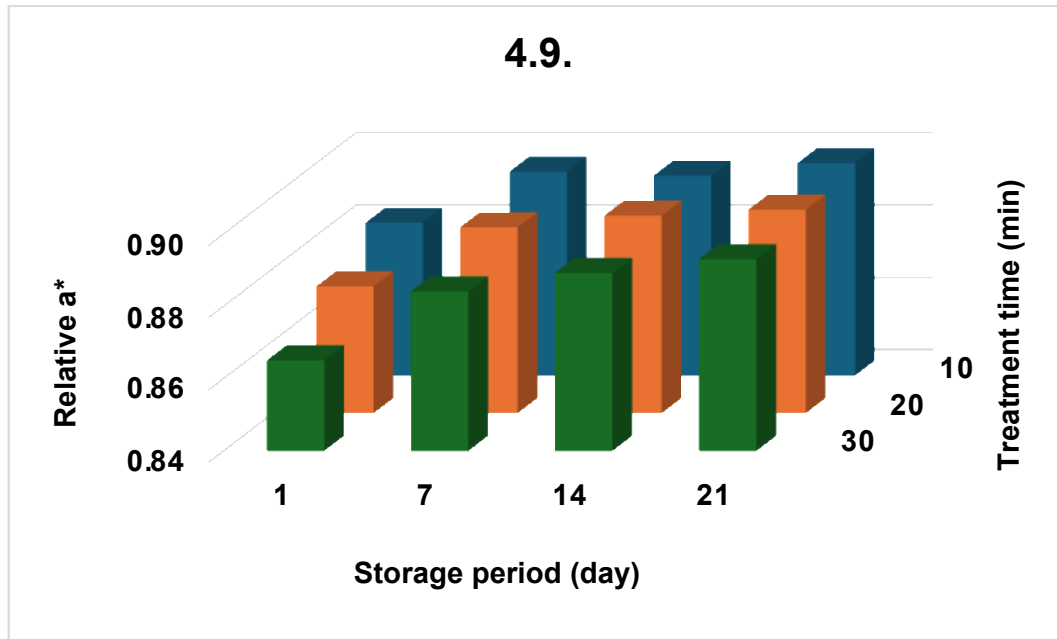


Fig. 4.9. Effect of HPP on relative a^* of 350 MPa treated smoked salmon. Values are the mean of 3 independent samples during 21 days of storage at 4 °C.

4.5.2.3. Chroma (C^*) of smoked salmon

From Table 4.5, the C^* values of smoked salmon treated with 350 MPa indicates that the application of pressure resulted in significantly lowering C^* value with an increase in pressure treatment time for all storage days, except for Day 14, where 10 and 20 min treatments showed no significant difference. Across storage, it was observed that the C^* continued to significantly decrease for all samples, except for 20 min treatment that showed no significant difference between Day 1 and 7.

Table 4.5. indicates that the control has a higher intensity of color in comparison with the HP treated samples. Furthermore, the C* decreased for all samples which is supported by the increase in L* and decrease of a* of pressure treated smoked salmon samples. However, it was also observed that the treated samples went through a slower reduction of C*. Tsironi et al. (2019) observed in 400 MPa treated sea bass fillets that the C* reduced as a result of HPP, Arnaud et al. (2018) observed the same reduction in sliced cod treated at 450 MPa. In comparing the C* of smoked salmon with HP treated fresh salmon from chapter 3, it was observed that greater C* values are observed in smoked salmon samples, thus indicating better intensity of color.

Table 4.5. Effect of HPP on chroma (C*) of smoked salmon

Chroma	Day 1	Day 7	Day 14	Day 21
Control	35.0 ± 1.63 ^{Dd}	34.7 ± 0.54 ^{Dc}	34.5 ± 0.76 ^{Cb}	34.1 ± 0.36 ^{Da}
350 MPa-10 min	29.8 ± 0.04 ^{Cd}	29.7 ± 0.12 ^{Cc}	29.5 ± 0.81 ^{Bb}	29.1 ± 1.1 ^{Ca}
350 MPa-20 min	28.8 ± 0.43 ^{Bab}	28.5 ± 0.43 ^{Bab}	28.3 ± 0.26 ^{Bb}	28.2 ± 0.52 ^{Ba}
350 MPa-30 min	27.2 ± 1.38 ^{Ad}	27.1 ± 0.77 ^{Ac}	26.9 ± 0.76 ^{Ab}	26.9 ± 1.42 ^{Aa}

Values are the mean ± standard deviation (n=3) 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 ($p < 0.05$).

4.5.2.4. Hue angle of smoked salmon

The hue angle is an angular value that denotes the position of the color in the color chart, where 0° indicates the color red and 90° denotes the color yellow. Hence, the hue value provides information of fish samples color position between the colors red and yellow (Yeşilayer, 2020). The hue angle values of 350 MPa treated smoked salmon samples are presented in Table 4.6 and

varied between 52.4 and 55.2 somewhat a narrow range indicating only a small change in the red color. It was observed that the hue angle increased significantly upon HP treatment application on all days and in addition, significantly increases further during storage.

The obtained hue angle values of smoked salmon indicated that as the products are treated with pressure, the samples are inclined to move a bit towards the color yellow. In previous studies conducted on smoked salmon, it has been stated that smoking induces yellowness in samples because of the interaction of several organic acids, phenolic compounds, carbonyls, and other reactions from the smoking process (Lingbeck et al., 2014). The hue angle values obtained from this study indicate a similar observation of increased yellowish color. Skredge and Storebakken (1986) observed the same increased yellowness and reduced a^* across refrigerated storage. In another study conducted on smoke-flavoured Atlantic Bonito, it was observed that the samples resembled a yellowish color upon storage (Selçuk and Ayvaz, 2022).

Table 4.6. Hue of smoked salmon as influenced by the HP treatment and storage times

Hue	Day 1	Day 7	Day 14	Day 21
Control	52.4 ± 0.7 ^{Aa}	52.7 ± 2.27 ^{Ab}	53.0 ± 1.51 ^{Ac}	53.0 ± 1.08 ^{Ad}
350 MPa-10 min	54.0 ± 0.06 ^{Ba}	54.3 ± 1.42 ^{Bb}	54.6 ± 0.75 ^{Bc}	54.7 ± 1.03 ^{Bd}
350 MPa-20 min	54.3 ± 0.2 ^{Ca}	54.9 ± 0.99 ^{Cb}	54.9 ± 2.31 ^{Cc}	55.0 ± 0.25 ^{Cd}
350 MPa-30 min	54.6 ± 1.83 ^{Da}	55.0 ± 0.56 ^{Db}	55.0 ± 0.32 ^{Dc}	55.2 ± 1.4 ^{Dd}

Values are the mean ± standard deviation (n=3) samples during 21 days of storage at 4 °C. 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 ($p < 0.05$).

4.5.3. Microbial analysis of smoked salmon treated with HPP

The microbial results of smoked salmon samples treated with HPP is presented in Table 4.7 as logarithmic CFU/g influenced by HP treatment time over storage up to 21 days. It was observed from the table that by the application of HPP on smoked salmon, there was no growth detected on day 1 and 7 for all samples, and on Day 14 for 20 and 30 min treatments. On day 14, the smoked salmon and 10 min treatment did indicate growth, but the 10 min treatment had significantly lower growth. By Day 21, all samples showed growth, however 10 and 20 min treatment showed no significant difference. 30 min treatment was found to have the least growth detected by Day 21.

Table 4.7. Microbial Log growth of smoked salmon

Log CFU/g	Day 1	Day 7	Day 14	Day 21
Control	*ND ^a	*ND ^a	5.39±0.07 ^{Cb}	5.77±0.03 ^{Cc}
350 MPa-10 min	*ND ^a	*ND ^a	4.68±0.17 ^{Bb}	5.3±0.07 ^{Bc}
350 MPa-20 min	*ND ^a	*ND ^a	*ND ^{Aa}	5.17±0.2 ^{Bb}
350 MPa-30 min	*ND ^a	*ND ^a	*ND ^{Aa}	4.69±0.17 ^{Ab}

*Values are the mean ± standard deviation (n=3) samples during 21 days of refrigerated storage at 4 °C. 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 ($p < 0.05$). * ND refers to growth not determined.*

Aerobic plate count helps in enumerating the total viable plate counts of the HP treated samples stored under refrigeration conditions (4 °C). Smoking is a preservation technique that helps with inducing flavor and aroma to the smoked product. And during the smoking process, the removal of moisture also occurs, thereby helping with the preservation of the food (Lerfall and Hoel, 2021). In this study, the control being the smoked salmon, itself has a good

refrigeration stability for 2 weeks, where no growth was detected. And by 21 days of storage, all the samples had microbial growth control, with the pressure treated samples having growth across storage ($p < 0.05$). The smoking treatment performed on the samples helped in maintaining good microbial control. A possible reason could be the removal of moisture, smoking ingredients, temperature, or humidity that helps in inhibiting the microbial growth (Gómez-Estaca et al., 2007). Therefore, as seen from Table 4.7., the smoked control has no growth detected until 14 days of refrigeration. By 21 days of storage, it only had a growth of 5.77 CFU/g. Furthermore, the control did not indicate any off odour after 21 days of storage. On application of HPP to the smoked salmon, the compression and decompression of microbial cells occur during processing. This helps with further bursting of vegetative bacterial spores thereby providing an increased microbial control (Chen et al., 2024). Therefore, in the HP applied smoked salmon samples, a lower bacterial growth is observed across storage.

4.6. Interaction effects

The interaction effects of all the variables were analysed using the IBM SPSS software. The effect response curves were also generated. It was observed from the response curves that an overall interaction of pressure, treatment time (min) and storage time (day) resulted in an improved texture, color and reduced microbial growth after treatment. The main interaction effects of pressure, treatment time and storage days, resulted in significant changes in all the parameters analysed except for redness under the main effect of treatment time. The two-way effect showed significant difference only for aerobic microbial analysis. However, the individual interaction effect of all attributes measured showed having a significant difference, indicating improved quality of HP treated smoked salmon samples.

Table 4.8. ANOVA two-way interaction table for HP treated smoked salmon

Origin	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	L	6477,926 ^a	15	431,862	5,103	<,001
	a	49,182 ^b	15	3,279	1,028	,429
	Chroma	312,148 ^c	15	20,810	12,758	<,001
	Hue	251,554 ^d	15	16,770	4,191	<,001
	Firmness	21382485,649 ^e	15	1425499,043	10,012	<,001
	Tenderness	40869704,176 ^f	15	2724646,945	11,366	<,001
	AM	24977824,588 ^g	15	1665188,306	14,493	<,001
Intersection	L	525652,764	1	525652,764	6210,787	<,001
	a	68275,087	1	68275,087	21407,128	<,001
	Chroma	122111,205	1	122111,205	74860,972	<,001
	Hue	319828,789	1	319828,789	79928,088	<,001
	Firmness	327897342,579	1	327897342,579	2303,060	<,001
	Tenderness	505920632,587	1	505920632,587	2110,421	<,001
	AM	21861853,509	1	21861853,509	190,277	<,001
Day	L	1760,535	3	586,845	6,934	<,001
	a	28,209	3	9,403	2,948	,034
	Chroma	70,770	3	23,590	14,462	<,001
	Hue	41,165	3	13,722	3,429	,018
	Firmness	2756403,365	3	918801,122	6,453	<,001
	Tenderness	18638188,262	3	6212729,421	25,916	<,001
	AM	11312203,512	3	3770734,504	32,819	<,001
Time	L	4442,688	3	1480,896	17,497	<,001
	a	18,616	3	6,205	1,946	,124
	Chroma	233,122	3	77,707	47,639	<,001
	Hue	208,241	3	69,414	17,347	<,001
	Firmness	17967885,176	3	5989295,059	42,067	<,001
	Tenderness	22129054,420	3	7376351,473	30,770	<,001
	AM	9109502,316	3	3036500,772	26,429	<,001
Day * Time	L	274,702	9	30,522	,361	,952
	a	2,357	9	,262	,082	1,000
	Chroma	8,257	9	,917	,562	,826
	Hue	2,149	9	,239	,060	1,000
	Firmness	658197,108	9	73133,012	,514	,863
	Tenderness	102461,494	9	11384,610	,047	1,000
	AM	4556118,759	9	506235,418	4,406	<,001
Error	L	14895,839	176	84,635		

	a	561,328	176	3,189		
	Chroma	287,086	176	1,631		
	Hue	704,256	176	4,001		
	Firmness	25057938,260	176	142374,649		
	Tenderness	42191599,555	176	239724,997		
	AM	20221491,879	176	114894,840		
Total	L	547026,528	192			
	a	68885,597	192			
	Chroma	122710,439	192			
	Hue	320784,600	192			
	Firmness	374337766,488	192			
	Tenderness	588981936,317	192			
	AM	67061169,976	192			
Corrected total	L	21373,764	191			
	a	610,510	191			
	Chroma	599,234	191			
	Hue	955,811	191			
	Firmness	46440423,909	191			
	Tenderness	83061303,730	191			
	AM	45199316,467	191			

a. R squared = ,303 (adjusted R squared = ,244)

b. R squared = ,081 (adjusted R squared = ,002)

c. R squared = ,521 (adjusted R squared = ,480)

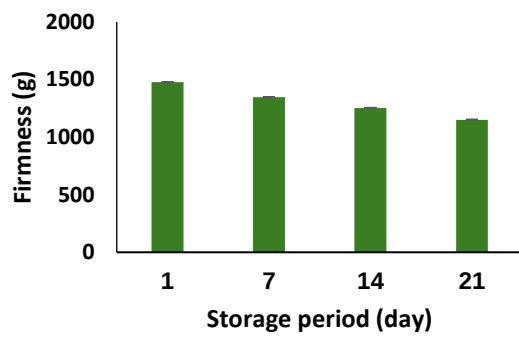
d. R squared = ,263 (adjusted R squared = ,200)

e. R squared = ,460 (adjusted R squared = ,414)

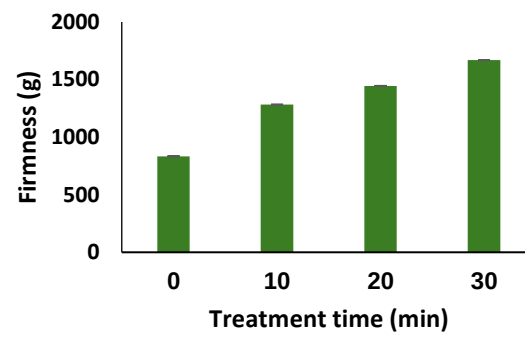
f. R squared = ,492 (adjusted R squared = ,449)

g. R squared = ,553 (adjusted R squared = ,514)

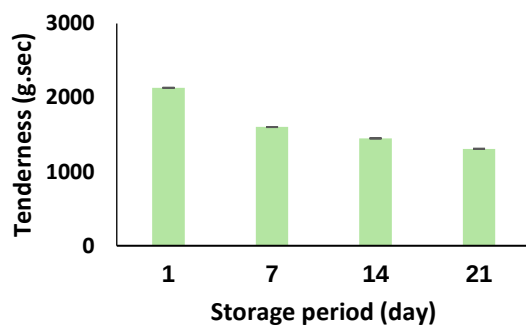
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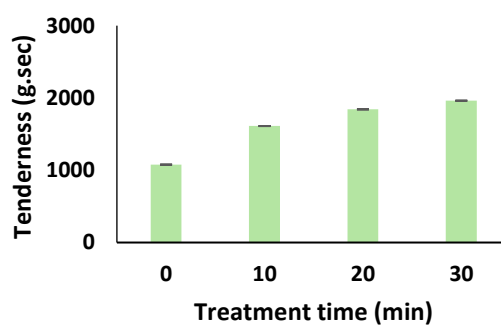
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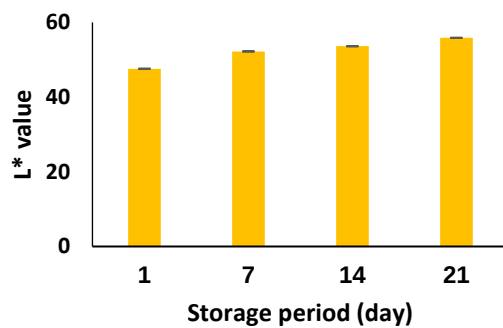
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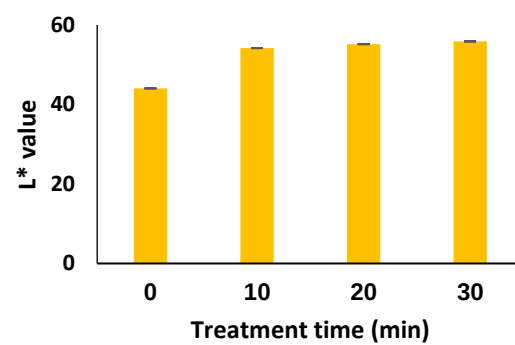
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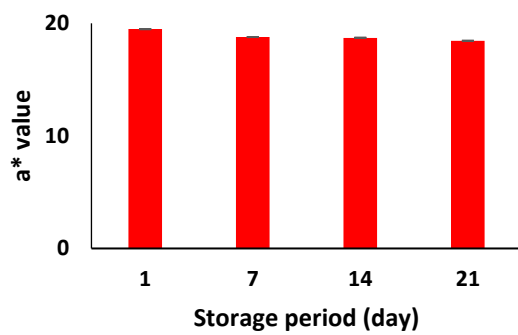
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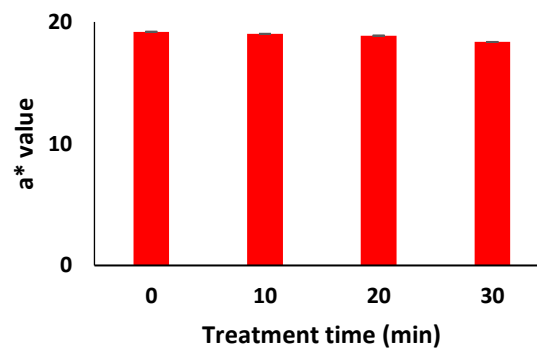
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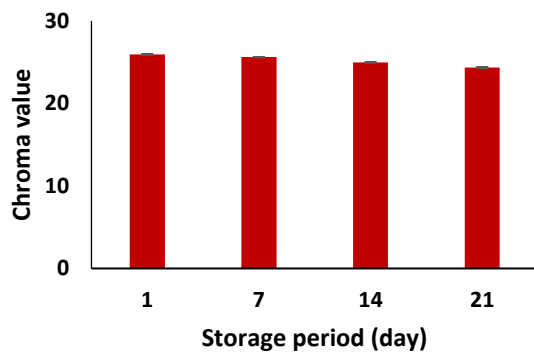
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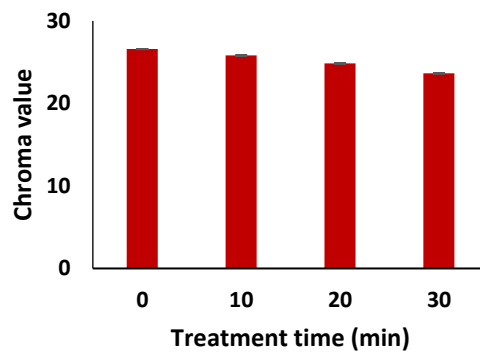
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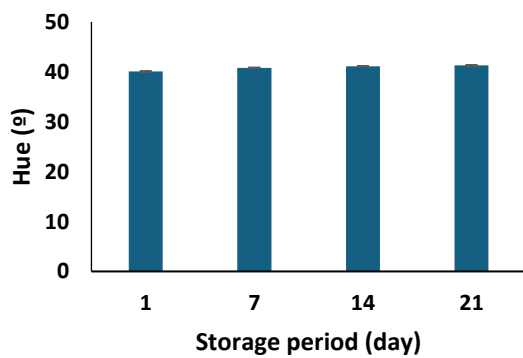
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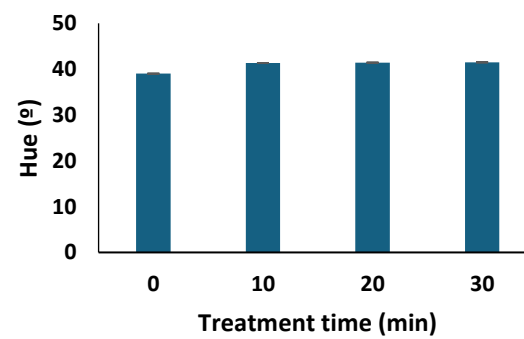
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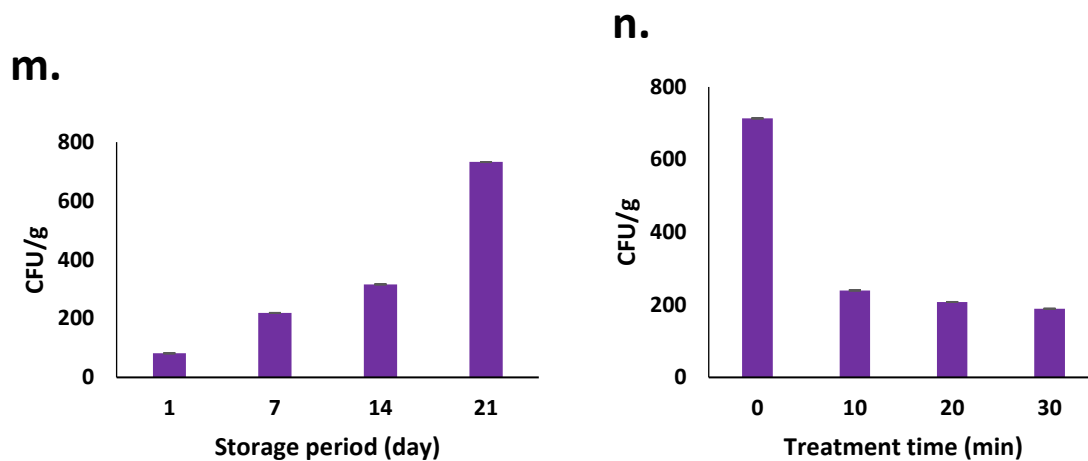


Fig. 4.10. (a-n; Left to right) Effect response graphs of pressure on storage period and treatment time for firmness (a and b); tenderness (c and d); L^* (e and f); a^* (g and h); Chroma (i and j); Hue (k and l); and microbial growth (m and n).

4.7. CONCLUSIONS

Several food processing techniques have been developed over the years to provide consumers with food with enhanced nutrition and an extended shelf-life. Combining different processing methods help in maintaining the nutritional profile of food with enhanced sensory attributes. In this study, the combination of HPP with smoking treatment of salmon samples proved to have effects on the salmon's firmness, tenderness, lightness, redness, chroma, hue and microbial growth. A pressure of 350 MPa applied on smoked salmon for 10, 20 and 30 min showed to have better physical characteristics of the product and reduced microbial growth. Textural properties such as firmness and tenderness were found to be higher for HP treated samples in comparison to the smoked control. Furthermore, the loss of these textural properties was slower across 21 days of refrigerated storage as compared to the smoked control. In the pressure treated samples, the lightness increased, redness decreased, chroma decreased and hue increased. Overall, the HP treated samples became lighter and their hue values were closer to a

yellowish color. This could also be as an effect of smoking where samples after the treatment have a golden-yellowish color. Microbial analysis of the samples demonstrated that combining HP treatment of smoked salmon helped in achieving reduced microbial growth during refrigerated storage. Furthermore, the samples did not produce any off odor. Overall, it can be concluded that the combination of smoking and HPP is a highly advantageous processing technique as the physical attributes are enhanced and microbial growth is reduced. A main advantage could also be the not so much visible color change after HP treatment as in the previous chapter, due to the smoking itself imparting increased L^* from the treatment.

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CHAPTER 5

General Conclusions

The overall objective of this study was to apply high pressure processing to salmon to achieve extended shelf-life with adequate quality retention under refrigerated storage at 4 °C for 3 weeks. Salmon is a very popular seafood preferred by many and its nutritional profile makes it an even more widely consumed food. However, the rapid deterioration of salmon even under refrigerated storage conditions raises a quality problem across supply chain and for consumption standards. Hence efforts to prolong the refrigerated shelf-life of salmon, both fresh and hot smoked, were the two general objectives of this study.

Fresh salmon was used initially for HP treatments and pressure levels of 150, 250 and 350 MPa were used with treatment times of 10, 20 and 30 min. It was found, in general, that with higher intensities of pressure and time, the textural and color changes could be minimized, proteolytic stability could be stabilized, and retarded microbial growth could be achieved. Overall, the interaction effects of pressure, treatment time and storage time were significant and their evaluations helped in creating conditions that enhanced the achievement of better retention of quality and microbial stability for an extended shelf-life of fresh salmon.

Hot smoked salmon obtained from industry partners treated was selected for the second objective of this study. As the color change of salmon was a concern in the first objective of this study, a parameter highly sensitive to pressure, these were evaluated under different HP treatment conditions to establish optimal HP processing conditions. In smoked salmon, color stability is generally better achieved through the use of smoke salts and it less of a concern in HPP. Therefore, only the most intense and successful pressure treatment level of 350 MPa was

selected for the HPP of smoked salmon samples, and the focus was on HPP effects on other quality parameters and microbial stability. As observed in the fresh salmon samples, the smoked salmon samples treated with HP also demonstrated improved textural and color retention across refrigerated storage, accompanied with reduced microbial growth providing a significant advantage over the control.

The comparative evaluation of HP treated fresh and smoked salmon, permitted opportunities for improved quality retention in texture, color, enzyme and microbial growth during the refrigerated storage for up to 3 weeks.

Overall, HPP helped achieving extended refrigerated shelf-life for salmon (both fresh and smoked) and for maintaining better quality in comparison with the untreated control both after HP treatment and after periodic refrigerated storage for up to 3 weeks. HP treatment for smoked salmon provided an additive quantitative synergy between two treatments each aimed at better sensory quality/stability for the refrigerated storage of salmon, providing a potential opportunity that could help seafood industry for better marketing of their seafood samples.

Recommendations for Future Research

There has been a continuously increasing consumer demand for more naturally or minimally processed procedures for fresh / smoked salmon. The application of HPP for salmon helps in achieving better quality retention and storage stability providing opportunities for high quality preservation and efficient marketing. As HPP helps in achieving an extension in refrigerated shelf-life of salmon, this technique can be adopted by industries in reducing supply chain losses that occur during transportation. However, the specific technique(s) or their combinations require a careful study in order to assess their usefulness and advantages. This research has demonstrated such opportunities.

Further research can be carried out in applying this technique in combination with other nonthermal processing techniques such as pulsed light, plasma, pulsed electric field, magnetic fields etc., for even better quality retention across refrigeration. When several processing techniques already are involved with inducing color stability in fish, and the application of HPP would result in better quality changes even at higher pressure levels which is one of the main constraints for HPP of seafoods.

Increased stability across refrigeration helps in improved cost economics within companies and therefore enable a positive impact on consumer satisfaction with the supply of minimally processed salmon. HPP equipment requires a high initial investment of about \$0.5-2.5 million that could vary depending on the processing capacity and operation. Though the production cost of HPP could be higher than thermal processing methods, an improved product with the absence of chemical preservatives and improved nutritional quality is made available through HPP. In the long run, processing benefits could be achieved though reduced losses in

products from HPP. Use of cryoprotectants could permit the use of further lower storage temperatures without the dangers of product freezing.

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