A Comparative Study on the Effect of Reciprocal Agitation Thermal Processing, Acidified Thermal Processing and Acidified High Pressure Processing of Trout Fillets on the Product Quality Attributes

By

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ABSTRACT

The objective of this study was to investigate the effects of reciprocal agitation thermal processing (RATP) (commercial sterilization), acidified thermal processing (ATP), and acidified high-pressure processing (AHPP) (pasteurization treatments) on the texture and color properties of trout, a highly perishable and delicate food product.

RATP, which combines thermal treatment with continuous agitation, was found to enhance the heat transfer and thereby reduced the heating time resulting better preservation product firmness as compared to processing under static conditions. Additionally, the RATP showed significant color retention, protecting the characteristic pink hue of trout by limiting pigment degradation.

ATP, which integrated product acidification (to reduce the pH below 4.6) and mild heat effectively reduced the thermal severity and ensured microbial safety. Under the acidified conditions, a pasteurization treatment ensures the microbial safety by destroying the vegetative pathogens and spore forming bacteria will not grow under these acidic conditions there by ensuring commercial sterility. The acidic environment gave a softer texture in spite of milder heat treatment possibly caused by the hydrolysis of connective tissues. The heat exposure under acidified conditions also resulted in partial pigment loss and protein denaturation, which adversely affected not only the color but also texture.

AHPP, which used high pressure, instead of heat, for the pasteurization purpose showed better texture and color preservation. The product structural integrity was better preserved and heat-sensitive pigments were shielded from destruction under this processing situation. Furthermore, AHPP stopped browning reactions and reduced pigment deterioration, and maintained the trout's distinctive pink color.

These results demonstrated that AHPP is the best way to preserve the sensory qualities of trout, providing non-thermal processing technique to satisfy customer demand for a safe and high-quality product.

RÉSUMÉ

L’objectif de cette étude était d’investiguer les effets du traitement thermique par agitation réciproque (RATP) (stérilisation commerciale), du traitement thermique acidifié (ATP) et du traitement acidifié à haute pression (AHPP) (traitements de pasteurisation) sur les propriétés de texture et de couleur de la truite, un produit alimentaire hautement périssable et délicat.

Il a été constaté que le RATP, qui combine un traitement thermique avec une agitation continue, améliore le transfert de chaleur et réduit ainsi le temps de chauffage, ce qui se traduit par une meilleure conservation du produit par rapport au traitement dans des conditions statiques. De plus, la RATP a montré une rétention de couleur significative, protégeant la teinte rose caractéristique de la truite en limitant la dégradation des pigments.

L'ATP, qui intègre l'acidification du produit (pour réduire le pH en dessous de 4,6) et une chaleur douce, réduit efficacement la gravité thermique et garantit la sécurité microbienne. Dans des conditions acidifiées, un traitement de pasteurisation assure la sécurité microbienne en détruisant les agents pathogènes végétatifs et les bactéries sporulées ne se développeront pas dans ces conditions acides en garantissant la stérilité commerciale. L'environnement acide a donné une texture plus douce malgré un traitement thermique plus doux, probablement dû à l'hydrolyse des tissus conjonctifs. L’exposition à la chaleur dans des conditions acidifiées a également entraîné une perte partielle de pigment et une dénaturation des protéines, ce qui a affecté non seulement la couleur mais également la texture.

L'AHPP, qui utilisait une haute pression au lieu de la chaleur, à des fins de pasteurisation, a montré une meilleure préservation de la texture et de la couleur. L'intégrité structurelle du produit était mieux préservée et les pigments sensibles à la chaleur étaient protégés de la destruction dans cette situation de traitement. De plus, l'AHPP a stoppé les réactions de brunissement et réduit la détérioration des pigments, tout en conservant la couleur rose distinctive de la truite.

Ces résultats ont démontré que l'AHPP est le meilleur moyen de préserver les qualités sensorielles de la truite, en fournissant une technique de traitement non thermique pour satisfaire la demande des clients pour un produit sûr et de haute qualité.

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CONTRIBUTION OF AUTHORS

Mrs. Sathiyapraba Gurumoorthy is the M.Sc. candidate. She is enrolled in the Department of Food Science and Agricultural Chemistry, where she is doing her thesis under the supervision of Dr. Hosahalli S Ramaswamy. She did an elaborate literature review from the time she started the course, formulated the research protocol with the help of her supervisor, Research Associate and lab mates, conducted experiments, gathered and analyzed the data, and presented the results for seminars and poster and drafted the thesis for publication.

Dr. Hosahalli S. Ramaswamy is the supervisor under whose guidance the research was conducted. He guided throughout the research by providing funding for the research, providing the special processing equipment, supervising the experiments, reviewing the results, and final editing the thesis. He also supported in editing the poster and manuscripts prepared for conferences and publications.

Dr Ali R. Taherian is the research associate working at the laboratory. He supported the M.Sc. candidate by providing help with experimentation methods, reviewing results, and editing the thesis. He also guided the candidate in editing the poster and manuscripts prepared for conferences and publications.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this research has been presented as posters at conferences

1. Gurumoorthy, S., Taherian, A., and Ramaswamy H.S. 2024. Effect of Reciprocal Agitation Thermal Processing on Quality of Trout. Poster presentation in Northeast Agricultural and Biological Engineering Conference (NABEC), July 15-16, 2024, College Park, PA.
2. Gurumoorthy, S., Taherian, A., and Ramaswamy H.S. 2025. Effect of Conventional Vs Acidified Thermal Processing on Quality of Trout. Institute for Thermal Processing Specialists conference (IFTPS), Mar 10-12, 2025, Reno, Nevada.

Manuscripts from the thesis

Chapters 3-5 have also been prepared in the style of manuscripts so they can be revised and sent for publication once ready and formatted according to the journal style

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

In recent decades, there has been a significant growth in the demand for high quality fish products worldwide due to growing consumer awareness of the nutritional value of seafood and the need for sustainable protein sources (Anderson & Moore, 2019; Baker & Reynolds, 2020). Among a variety of fish species, trout is notably regarded for its balanced fatty acid profile, high protein content, and favorable sensory characteristics (Chen et al., 2021). But it's always difficult to preserve trout's qualitative characteristics throughout processing or without processing, particularly texture and color. Although conventional thermal processing is effective in microbial inactivation, it can also result in undesirable changes, including protein denaturation, moisture loss, and color degradation (D'Souza et al., 2020; EI-Sayed & Abou-Arab, 2018). Modern food processing research has shifted its focus to alternative methods that can preserve the inherent qualities of fish, while also assuring safety and extending the shelf life (Foster et al., 2019: Gupta & Verma, 2020).

Rising consumer knowledge of the nutritional value of seafood and the necessity of sustainable source of protein have driven a significant global demand for premium fish products to rise over the last years (Anderson & Moore, 2019; Baker & Reynolds, 2020). Among the several fish species, trout is especially prized for its balanced fatty acid profile (Chen et al., 2021), high protein level, and good sensory qualities. On the other hand, the persistent challenge is preserving the quality attributes of trout, particularly texture and color, in fresh state as well as after processing. Although conventional thermal processing is effective in microbial inactivation, it can also result in undesirable changes, including protein denaturation, moisture loss, and color degradation (D'Souza et al., 2020; EI-Sayed & Abou-Arab, 2018). In response, contemporary food processing research has shifted its attention to alternative methods that can preserve the inherent qualities of fish while also assuring safety and extending its shelf life (Foster et al., 2019; Gupta& Verma, 2020).

This thesis examines the impact of three processing method like reciprocal agitation thermal processing (RATP), acidified thermal processing (ATP) and nonthermal high processing (HPP) on the texture and color of trout. Unique mechanisms of action are provided by each method. RATP employs controlled, continuous agitation motion during heating, which may mitigate the adverse effect on protein structure and pigment stability by promoting uniform heat distribution and reducing localized overheating (Hernandez et al., 2017: Ivanov et al., 2019). Conversely, acidified thermal processing of low acid foods like trout and salmon, introduces acidulants into the product either before or during the heating process. Acidification has the potential to reduce the process severity and preserve the sensory and nutritional quality of fish by reducing the pH to levels that inhibit growth of spore forming bacteria and modulate enzymatic activity and use the milder processing conditions to kill vegetative bacteria (essentially involving a pasteurization process) (Rampurwala et al., 2025; Jain & Kumar, 2021; Kim & Lee, 2022).

High pressure processing (HPP) of acid or acidified low acid foods (a form of nonthermal processing) has been developed as a result of recent advancements in nonthermal processing. This process synergistically integrates the quality preserving characteristics of HP treatments with the antimicrobial benefits of acidification (Lopez et al., 2018; Martinez et al., 2020). In comparison to conventional thermal methods, HPP of acidified products preserves the structural integrity of muscle proteins and the natural pigments of fish, resulting in a product with superior texture and color (Kim & Lee, 2022; Nguyen et al., 2021). Many studies have demonstrated this (Kim & Lee, 2022; Nguyen et al., 2021). This discovery is noteworthy due to the fact that preservation of these quality attributed not only enhances the consumer acceptability but also preserves the nutritional value and shelf life of processed trout (Olsen et al., 2017; Patel & Singh, 2019).

The quality attributes of texture and pigment in fish are critical determinants of marketability. Protein denaturation, Collagen solubility, and hydration retention are among the factors that influence texture, which is associated with the product's structural integrity and mouth feel (Quinn et al., 2021; Rodriguez & Silva, 2020). Color, which is frequently linked to visual appeal and perceived freshness, is exceedingly susceptible to chemical reactions that reinduced by processing, such as Maillard browning and pigment oxidation (Smith et al., 2022; Thompson & Wu, 2021). The application of heat in thermal processing, for example, can result in irreversible changes to the structure of muscle proteins, which can contribute to discoloration and textural toughening (Taherian and Ramaswamy, 2009; Uddin et al., 2020; Venkatesh & Zhao, 2022)

Conversely, nonthermal processes, thereby maintaining the preferable qualities of the raw product (Williams et al., 2019; Xu & Park, 2021).

An innovative modification of conventional heating systems is the use of reciprocal agitation during thermal processing (RATP). This method improves the convective heat transfer to the fish by generating a continuous, back and forth motion, which may lead to a more uniform temperature distribution and diminished thermal gradients within the product (Abbatemarco and Ramaswamy, 1994; Sablani and Ramaswamy, 1996; Dwivedi and Ramaswamy, 2010; Singh et al, 2015a,b; Singh et al., 2016; Yoon et al., 2020; Zhao et al., 2023). The delicate structure of muscle fibres is preserved, and the likelihood of undesirable textural changes is reduced by this controlled agitation, which also reduces the risk of overcooking in localized regions and enhances the efficiency of heat penetration (Andrews et al., 2018; Bennett & Carter., 2022). Additionally, the intensity of natural color, which are otherwise susceptible to thermal degradation, can be significantly preserved through uniform heat distribution (Deng et al., 2022).

Acidification, either used in thermal or nonthermal procedures, has been widely acknowledged for its dual function in the preservation of quality and the control of microbial populations (EI-Morsy et al., 2019); Farooq et al., 2020). The addition of food-grade acids (such as citric or lactic acid) prior to heating in acidified thermal processing can reduce the pH, resulting in a reduction in enzymatic activity that could otherwise contribute to textural softening and pigment oxidation (Rampurwala et al., 2025; Gracia & Hernandez., 2021; Iqbal et al., 2019). Nevertheless, the concurrent application of heat still presents a risk for structural damage to proteins, despite the fact that acidification can enhance microbial safety and slow down decomposition reactions (Johnson & smith., 2021; Kaur et al., 2020). This constraint has incited additional investigation into nonthermal alternatives that can integrate the advantages od acidification with innovative processing technologies.

A promising technique for preserving the sensory and nutritional integrity of fish has emerged: acidified nonthermal processing, particularly acidified high-pressure processing (Kim & Lee, 2022). High-pressure processing (HPP) is a well-established method that successfully inactivates pathogens and spoilage microorganisms by applying pressures typically between 100 and 600 MPa, while largely preserving food’s original flavor, texture, and color (Lopez et al., 2018; Martinez et al., 2020). This method not only improves microbial safety but also establishes conditions that prevent enzymatic activities that are detrimental to muscle structure and pigment stability when combined with acidification (Nguyen et al., 2021; Patel & Singh, 2019). Recent comparative studies have indicated that acidified high-pressure processing is a more effective alternative for industrial applications than conventional thermal methods in preserving the desired textural firmness and vibrant color of trout (Kim & Lee, 2022; Roberts et al., 2023).

However, the literature is still lacking in a comprehensive comparative evaluation of reciprocal agitation thermal processing, acidified thermal processing, and acidified nonthermal (high-pressure) processing on trout fish, in spite of these advancements. Despite the fact that individual aspects of these processes have been investigated (Sanchez et al., 2018; Thompson & Wu, 2021), it is imperative to conduct a systematic investigation that explicitly compares their impacts on critical quality parameters. This assessment is essential for the optimization of processing protocols that balance sensory quality with microbial safety, thereby offering the industry practical insight into enhancing product shelf-life and consumer appeal (Uddin et al., 2020; Venkatesh & Zhao, 2022).

The general objectives this research are therefore:

1. To evaluate and compare the effect of conventional thermal processing and acidified thermal processing on quality of trout under RATP processing conditions
2. To evaluate and compare the conventional acidified thermal processing (ATP) and acidified nonthermal processing (HPP) for better retention of the quality of trout.

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CHAPTER 2

LITERATURE REVIEW

2.1. Thermal Processing

2.1.1. General Overview

Thermal processing is a widely used technique for microbial inactivation. It includes heating food for a predetermined amount of time inside a closed system. As it is done in hermetically sealed containers such as metal cans and containers, this procedure is also known as "canning". During storage, this closed system protects processed food from recontamination. "Commercially sterile" refers to food that has undergone thermal processing; this indicates that the product contains no active microbes that may multiply at room temperature. According to Owusu-Apenten and Viera (2022), thermal processing effectively eliminates just those pathogens that pose a risk of contaminating food under commercial storage conditions. It does not completely eliminate food and cause health problems when consumed. Mesophilic microbes are the main target when canned food is commercially kept on shelves at room temperature.

The cans/jars contain a particle-in-fluid phase, where solid pieces of food are suspended in a solution called the canning liquid. To allow the contents to expand and to aid in product agitation, a small portion of the container (headspace) is left empty under vacuum. This takes place as a result of air being eliminated to prevent oxidation reactions and the development of aerobic microbes. Therefore, anaerobic microorganism still has the potential to thrive. *Clostridium botulinum*, an anaerobic, spore forming, gram positive bacterium that causes botulism, a deadly disease, is the most hazardous anaerobic bacteria. It generates a neurotoxin (Lund and Peck, 2013), heat-processing containers provide an ideal environment for clostridium botulinum a mesophilic and anaerobic bacterium to reproduce.

2.1.2. History of Thermal Processing

Over the past years, terms such as canning, sterilization and preservation have been used to describe the process of microbial inactivation of food in sealed containers, although other containers like glass jars, retortable pouches, semi rigid plastic containers have all been included in the same context. As time passed, the use of a more appropriate and general term was imperative, and this process is now known as thermal processing.

The method was discovered in 1810 by French inventor Nicolas Appert. This was carried out in response to the French government's request for ideas on how to preserve food for the nation's military purposes. Appert used a glass jar with a tight-fitting lid to store food. The airtight jar was brought to a specific temperature, maintained there for a predetermined amount of time, and then sealed until needed. The simplicity of this method made it popular and led to its continued use. However, until French chemist and microbiologist, Louis Pasteur demonstrated in 1864 that heat inactivates germs, that contaminate food, but the explanation for this methods success remained unknown. Bigelow and Esty (1920) made the next significant find as they classified microbial spores based on the pH level at which they grow and the degree of heat they can tolerate. Bigelow and Ball created a method in 1920 determined the minimum level of sterilizing needed to process food. In 1923, Collin Ball improved upon the technique. During 1950's, Stumbo refined Ball's formula method and increase its accuracy and adaptability following a few further improvements. Over the past few years, this idea has been improved to become more effective.

Thermal processing has advanced significantly over the past three decades. A comprehensive study of the idea of agitated thermal processing has been conducted using a variety of agitation techniques, such as end-over-end, axial, and reciprocating motions (Singh et al., 2018). In addition, every agitation mode has been specifically designed to attain increased heat transfer rates and less quality loss. For instance, Dwivedi and Ramaswamy (2010) modified fixed axial agitation to free axial (bi-axial) agitation to provide a higher rate of heat transfer and prevent particle clumping on container walls. The effects of reciprocation agitation had also been studied for various can orientations (Singh and Ramaswamy, 2015).

2.1.3. Principles of Thermal Processing

For many different types of microorganism, the contents of can makes excellent growth media. Specifically, they will easily promote the growth of anaerobic over aerobic organisms, which sets them apart from other prepared food products for retail sale. Aerobe growth is reflected in the most common indicators of food spoiling, therefore contaminated can contents may become hazardous before they become distinctly spoiled. On the basis of temperature regime, thermal processing equipment or method, fish species, packaging technique, or the process's microbiological target, thermal processing can be further classified into a number of roughly overlapping groups.

For specific processing temperature, a specific amount of thermal processing time is needed to inactivate a microbe. These time-temperature combinations are measured as "lethality" and indicate the extent of heat treatment. It is equivalent to the entire process's heating time, measured in min at the reference temperature and is calculated by the following equation:

$$F_O = \int 10^{\frac{(T-T_O)}{10}} dt \qquad 2.1$$

where t is the time, T_0 is the reference temperature, and T is the sample temperature. 121.1℃ is the standard temperature for commercial sterilization. The thermal death time (D) is the amount of time needed to inactivate 90% of a microorganisms original count, or one log cycle of it. For each temperature, the D-value is hence distinct. The thermal sensitivity indicator, sometimes referred to as the z-value, is the temperature difference that represents one log cycle of a variation in the D-value.

Graphically, D-value and z-value are obtained as shown in Figure 2.1 and Figure 2.2, respectively. The z-value of microbial vegetative forms, microbial spores, and nutrients in food are approximately 5 to 8℃, 10 to 15℃, and 30 to 35℃, respectively (Holdsworth, 1985; Ramaswamy and Marcott, 2006; You, 2015). It is observed from equation 2.1 that a higher z value would indicate that a lower lethality was assigned in a specific amount of time. For the same thermal treatment, nutrients in food are therefore less destroyed than microbial spores and vegetative microorganisms. Thermal processing enables the rapid inactivation of food microorganisms while preserving much of the products nutrients because these bacterial are more sensitive to temperature changes. High temperature short time (HTST) and ultra high temperature (UHT) processing are the terms used to describe the concept that a higher processing temperature and a shorter processing time will preserve a greater percentage of the products quality.

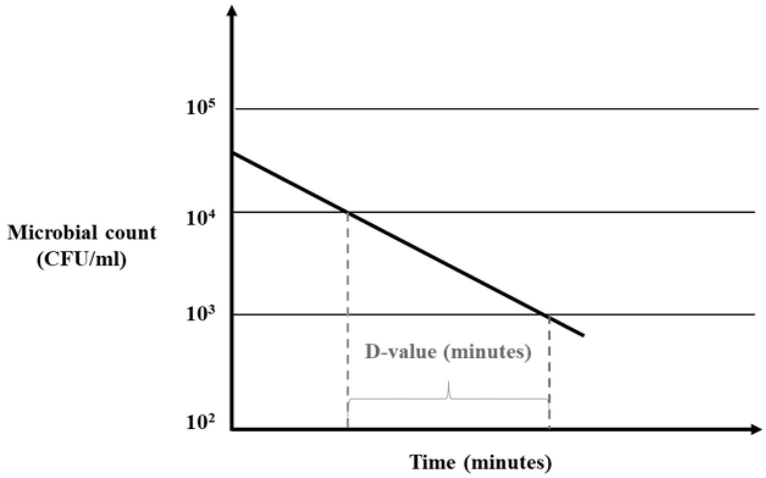

Figure 2.1. Microbial inactivation Survivor curve

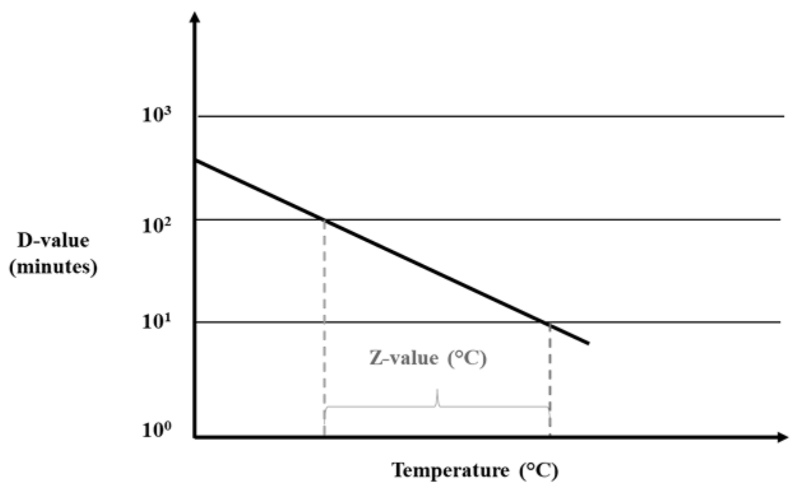

Figure 2.2. D-value - Temperature curve

Foods with high acidity require a milder procedure known as "pasteurization", as only vegetative pathogens an spoilage causing microbes must be rendered inactive. Bacterial spore activity is inhibited by the high acidity of the environment. According to Breidt et al. (2014) and Odlaug et al. (1977a,b), the desired lethality for pasteurization is F_{90} is 10 min. There could be a major risk if bacterial spores are not completely destroyed during acidified thermal processing. Even in highly acidic environments, *Bacillus licheniformis* spores could survive and proliferate, and its activities might raise the pH level (Rodriguez et al., 1993). The growth of *Clostridium botulinum* may be facilitated by this phenomenon. In order to prevent such risks, the target lethality for acidified thermal processing is set to inactivate *Bacillus licheniformis* spores (Tola and Ramaswamy, 2014a, b). To achieve a shorter shelf-life of two to six weeks, low-acid foods can also be pasteurized. It needs to be kept in a refrigerator, though. In these situations, the heat treatment renders vegetative microorganisms inactive, and maintaining the temperature below 4℃ inhibits the activity of bacterial spores.

As described in Figure 2.3, foods are at least divided into 2 groups based on their pH level. Acidic and high acid foods have a pH ≤ 4.6, while low-acid food have a pH > 4.6. Vegetative pathogens need lower heat treatment to inactivate and bacterial spores, which are more heat-resistant but do not turn active in high-acid environment, are the target microorganisms in food. In order to inactivate bacterial spores and vegetative pathogens, we need to use a higher temperature while working with low-acid foods. This procedure necessitates a lethality of $F_{121.1C}$ = 5 min, which is known as "commercial sterilization". In order to statistically lower the survivor probability to 1 in 10^{12}, this log reduction guarantees that the starting count of *Clostridium botulinum* decreases by 12 log reductions. The term "*Bot cook*" refers to inactivating *Clostridium botulinum* by 12 log reductions.

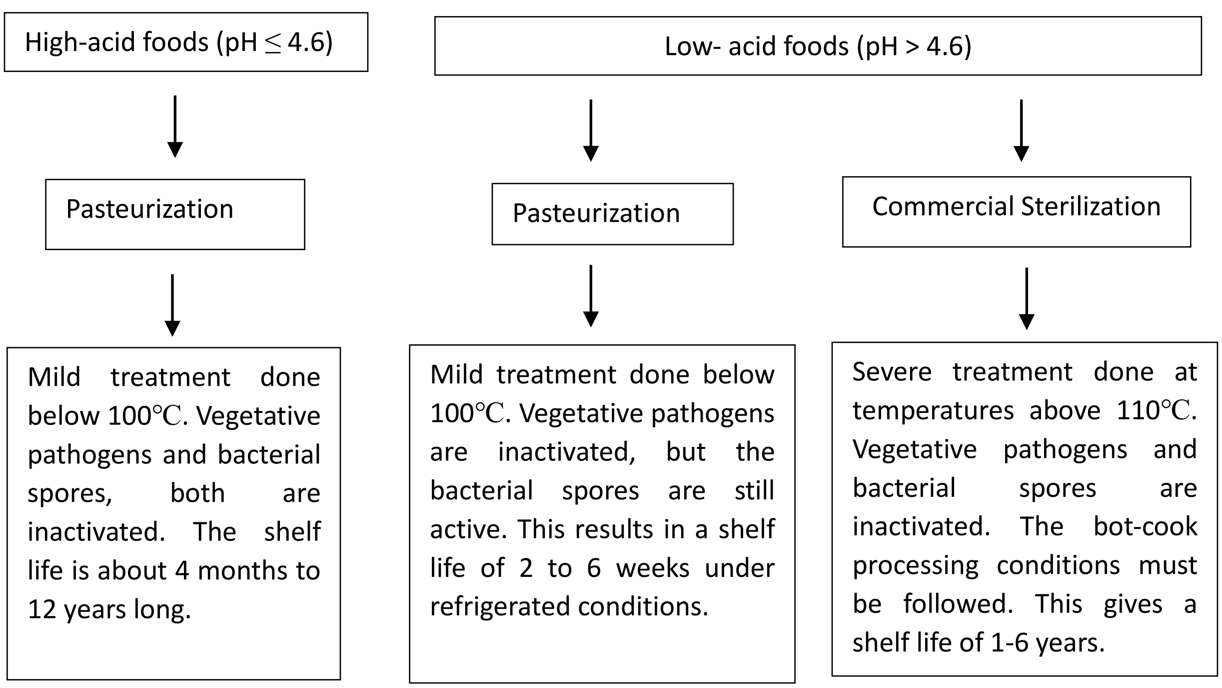

Figure 2.3. Schematic diagram of the thermal processing

2.1.4 Rate of heat transfer during thermal processing

The heating lag factor (j_{ch}) and heating rate index (f_h) are calculated to determine the rate of heat transfer of a specific process. Although it is a well-known indication of heat transfer rate, the heating rate index does not actually measure heat transfer rate. It is defined as the amount of time needed to complete one log cycle for the logarithmic temperature differential between the sample and the retort as a function of time. Therefore, a lower f_h number indicates a higher rate of heat transmission. As a result, the desired lethality would be reached more quickly, resulting in a shorter processing time and higher product quality. Figure 2.4 shows a typical heating curve diagram, where the slope of line 1 gives the heating rate index. The heating lag factor, which measures the time it takes to reach constant phase heating, is another crucial indicator of the heat penetration properties. It takes less time to reach steady phase heating when the heating lag factor is lower. Similar to the heating rate index, a lower heating lag factor value indicates a higher rate of heat transmission. From Figure 2.4, we obtain the values of Log (T_R-T_{ph}) and Log (T_R-T_{ih}). The ratio of (T_R-T_{pih}) to (T_R-T_{ih}) gives the heating lag factor.

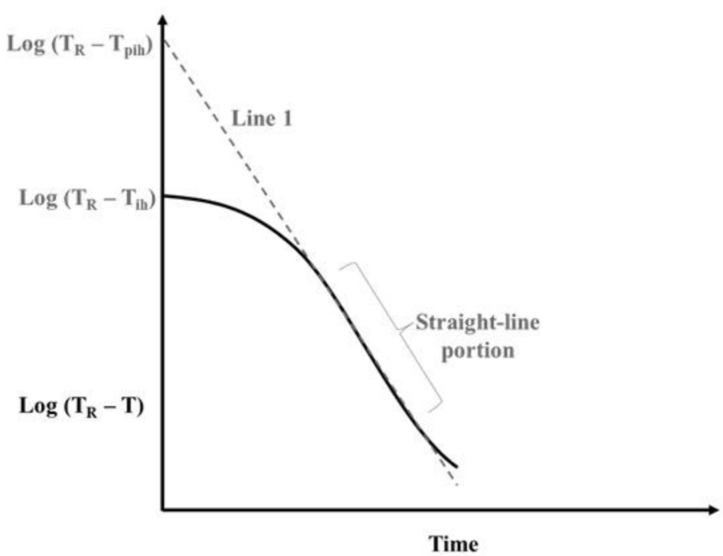

Figure 2.4. Thermal processing heating curve diagram

The rate at which heat penetrates the food item must be accelerated in order to shorten the processing time. Several studies have proposed methods for introducing agitation into the system during thermal processing in order to speed up the rate of heat transfer. By increasing forced convection and creating turbulence in the containers, this speeds up the canning liquid's intake of heat. As a result, food particles heat up more quickly. According to Abbatemarco and Ramaswamy (1994), processing times for green beans, potatoes, and carrots are shortened when the rate of agitation is increased during end-over-end rotating thermal processing. Similar outcomes were demonstrated by Dwivedi (2008) for both fixed axial and free-axial agitation modes. Pratap Singh et al. (2017) also showed that reciprocating agitation increases the rate of heat transfer for tomato puree as the frequency of agitation was stepped up. Another study on the effect of reciprocating agitation on processing shrimps showed similar results (Dixon el al., 2020).

2.2. Types of Thermal Processing

2.2.1. Based on Method of Agitation

2.2.1.1. End-Over-End Agitation

The containers are rotated so that one end is switched with the other, flipping the container lengthwise. Figure 2.5 provides clear explanation of this motion. Containers are often put in cages and the cage is turned end over end. Because the central container would rotate around its central horizontal axis while the containers positioned on the end of the cage would span the widest circumference relative to the others, this compels the containers to be handled in slightly different ways.

2.2.1.2. Fixed-Axial Agitation

The vertical axis of the container is rotated. The orientation of the containers is the only difference between end-over-end agitation with this. Free axial agitation is subject to some variability, just like end-over-end agitation. Particle would clump together on the container wall as a result of the increased centrifugal force experienced by the particles in the particle-in-fluid phase. As a result of the clumps reduced mixing and thermal resistance, the fluid's total rate of heat transmission is decreased.

2.2.1.3. Free-Axial / Biaxial Agitation

The containers rotate and are aligned similarly to those in free axial agitation. The sole distinction is that the rotation is not a full 360^0 revolution, as seen in Figure 2.5. Rather, the containers are slowed to roll over their lateral surface throughout the rest of the rotation after the cage has been allowed to rotate at a certain angle. The containers are allowed to roll over their surface during the bottom portion of rotation because of the presence of gravitational pull. By switching to the opposite direction of rotation, the container can prevent clumps from forming on the walls. Because the containers rotate in two different directions throughout each full revolution, this is also known as biaxial rotation.

2.2.1.4. Reciprocating Agitation

All of the containers are treated similarly because they are agitated back and forth. Figure 2.5 describes this kind of agitation. There are several techniques to perform reciprocating agitation thermal processing, depending on how the containers are oriented. The long axis of the containers could be inclined, parallel, or perpendicular to the direction of agitation. The containers might also be oriented in two different ways when positioned perpendicularly: either parallel to the direction of gravity or perpendicular to it. The impact of container orientation on the rate of heat transfer during RATP was demonstrated by Singh and Ramaswamy (2015). Additionally, Singh et al. (2018) found that increasing the rate of agitation enhanced the overall rate of heat transfer for reciprocating agitation by the largest percentage. This suggests that the most effective way to speed up heat transmission is by this type of agitation.

2.3. Methods of Processing

2.3.1 Conventional Thermal Processing

Low-acid foods are processed to achieve a lethality of F_{121C} = 5 min. This assures a 12-log reduction in the initial *Clostridium botulinum* count. This is a severe treatment as the processing temperature is higher than 110°C. The treated foods have a shelf life of one to six years and are regarded as commercially sterile. Most of the vegetables falls under the category of low-acid foods.

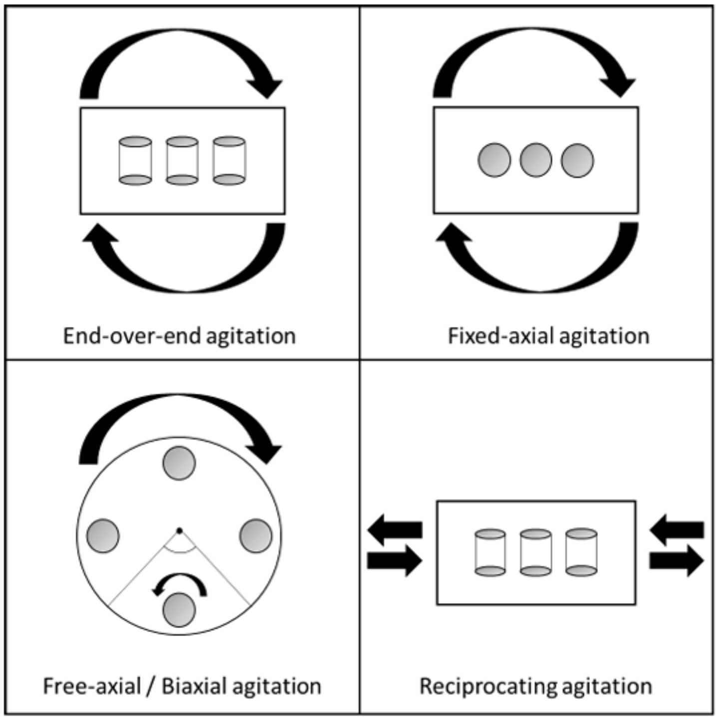

Figure 2.5. Agitation methods for thermal processing

2.3.2 Acidified Thermal Processing

Food-grade acids such as citric, acetic, tartaric, and others are used to acidify low-acid foods to high-acid foods. Glucano-d-lactone is a milder acid that doesn't give food an intense sour taste (Tola and Ramaswamy, 2018). Acidified thermal processing (ATP), which involves acidifying low-acid foods to a pH≤ 4.6, has the advantage of pasteurizing food instead of commercially sterilizing it because the high-acid environment already inhibits bacterial spore activity. Pasteurization is a milder process than the conventional method since it uses process temperatures lower than 100℃. A lethality of F_{90} = 10 min must be attained. The bacteria that are targeted include fungal spores, *Bacillus licheniformis, Listeria monocytogenes,* and vegetative pathogens such as *Escherichia coli.* It is anticipated that Acidified Thermal Processing will keep greater product quality than the traditional approach because of the lower processing temperatures.

2.4. Factors Influencing the Rate of Heat Penetration

The factors influencing the rate of heat penetration into food particles during thermal processing in a particle-in-fluid phase have been assessed by a number of researchers. Understanding these characteristic's ability to speed up heat transfer and, in turn, reduce processing time was the primary goal of the study. More quality would be preserved with a shorter processing time. As a result, these factors affect the product's quality. The majority of the research showed that the size, shape, and density of the food particles, the container's headspace, the fluid viscosity, and the agitation speed and mode all had the most effects on how quickly heat penetrated the food particles (Lenz and Lund, 1978; Deniston et al., 1987; Fernandez et al., 1988; Sablani, 1996; Sablani et al., 1997; Sablani and Ramaswamy 1995, 1996, 1997, 1998; Ramaswamy and Sablani 1997, 1999). These components are typically examined based on how they affect the overall heat transfer coefficient (U). Some of these investigations used particles of other materials, such as nylon, aluminium, lead, and so forth, to avoid fluctuations brought on by the food's biological system. Others, however, utilized actual food. The following section has covered the variables that affect the rate of heat penetration.

2.4.1. Agitation Method

Different agitation techniques affect the rate of heat transfer in different ways. According to Singh et al., (2018), reciprocal agitation had the highest total heat transfer coefficient. End-over-end agitation, fixed-axial agitation, and biaxial agitation follow, with the latter having the lowest. Compared to any agitation processing method, the still mode has a substantially lower overall heat transfer coefficient. According to Quast and Shiozawa (1974), fixed axial rotation increased heat transfer rates by two to four times compared to static processing. Additionally, it was said that end-over-end agitation had a heat transfer coefficient that was two to three times greater than static axial rotation (Naveh and Kopelman, 1980). The impact of various agitation modes on the rate of heat transfer is further explained by Dwivedi and Ramaswamy (2010) and Rattan and Ramaswamy (2014). According to Dwivedi (2008), these variations are particularly noticeable in the early phases of processing when there is a significant temperature differential between the sample and the heating source.

2.4.2. Rate of Agitation

According to numerous studies, the rate of agitation and the rate of heat transmission are directly proportional (Van Loey et al., 1994; Sablani and Ramaswamy 1996; Dwivedi and Ramaswamy, 2010; Singh et al., 2015). Abbatemarco and Ramaswamy (1994) demonstrated that when the end-over-end rotational speed was increased from 0 to 20 RPM, the heating rate index decreased. For potatoes and radishes, You et al. (2016) found that when the reciprocation frequency rose from 0 to 3 Hz, the heating rate index decreased. The impact of the agitation rate on the heating rate index during axial agitation thermal processing was described by Stoforos (1988). Additionally, the fluid to particle heat transfer coefficient for potato pieces in water during end-over-end agitation was determined by Lekwauwa and Hayakawa (1986).

2.4.3. Viscosity of Canning Liquid

It has been noted that the rate of heat transmission was reduced when the canning liquid's viscosity increased (Hassan, 1984). Being a more viscous fluid, water contributed to a higher rate of heat transfer, according to a study that evaluated the rates of heat transfer between water and a 60% sucrose solution (Lenz and Lund, 1978). Similarly, Sablani (1996) found that a water canning liquid had a higher total heat transfer coefficient than an oil canning liquid. Nylon spheres were used as food particles in this investigation. According to these studies, lesser viscosity canning liquids have a higher rate of heat transmission because the particles move more quickly relative to one another. This raises the total rate of heat transmission by causing more turbulence.

2.4.4. Food-Particle Concentration

The rate of heat transfer between the particles and the canning liquid is significantly influenced by the particle concentration in the particle-in-fluid phase. The canning liquid's flow pattern is influenced by the quantity of particles withing the jar. Furthermore, these particles disperse the heat uniformly and produce additional agitation. Research has shown that the rate of heat transmission increases in accordance with the particle concentration (Hassan, 1984). The rate of heat transfer eventually starts to drastically decrease as the particle concentration rises further. Consequently, for a process to be effective, the particle concentration must be optimized. According to Deniston et al. (1987), the rate of heat transmission decreased at high food particle concentrations because the particles were closely packed together and had no space to move. This lowers the rate of heat transfer by reducing the secondary agitation that particles produce.

2.4.5. Food-Particle Size

Finding a relationship between food particle size and heat transfer rate has been the focus of several investigations, but the results have been inconsistent. The rate of heat transfer was shown to decrease as the potato sphere's diameter increased from 2.22 cm to 3.49 cm. Additionally, Sablani and Ramaswamy (1997) demonstrated that when nylon particle size increased from 19.05 mm to 25 mm, the rate of heat transmission reduced. However, Lenz and Lund (1978) found that when the particle size expanded, so did the total rate of heat transfer. As particle size increases, the surface area to volume ratio of these particles falls, which lowers the rate of heat transfer. Particles that are too tiny, on the other hand, clump together when agitated, and lowers the rate of heat transfer.

2.4.6. Food-Particle Shape

Sablani and Ramaswamy (1997) investigated the impact of particle shape on heat transfer rate using nylon particles in the shapes of cylinders, cubes, and spheres. They showed that cubical particles transferred heat at a slower pace than spherical and cylindrical particles. Different forms allowed for different levels of mixing inside the can by creating different empty spaces between the particles. According to Astom and Bark (1994), spherical particles had the maximum rate of heat transfer, while cubical particles interlock with one another is influenced by their shape, which in turn impacts how quickly heat penetrates.

2.4.7. Food-Particle Density

The particle density may have an effect on the heat transfer coefficient, altering the fluid motion pattern of the particles within the container. According to Sablan (1996), particle density had a major impact on the rate of heat transfer because denser particles settled in the container more quickly, causing the fluid to move more and enhancing convection. According to Meng and Ramaswamy (2007), as particle density rose, so did the rate of heat transfer.

2.4.8. Container Headspace

It was found that the headspace of the container increased the rate of heat transmission up to a certain extent (Mohamed, 2007). This is due to the fact that rapidly heating the water's surface results in higher heating rates (Singh and Ramaswamy, 2016). In contrast, a very big can headspace starts to function as insulation, which lowers the rate of heating (Singh et al., 2018).

2.5. Quality Loss During Thermal Processing

Although thermal processing has many benefits for food safety and preservation, the quality of the processed product is compromised. Food's texture, color, and nutritional value are all deteriorated by heat treatment. Additionally, it changes the taste and smell of food, which is usually not a good thing. Optimizing processing parameters and preserving product quality require an understanding of the mechanisms underlying these changes.

2.5.1. Texture

A major issue that impacts the end products marketability and sensory quality is texture loss during the thermal processing of fish. The structural integrity of muscle proteins and connective tissues is the primary determinant of fish texture, which is defined by firmness, tenderness, springiness, and chewiness. These proteins may become denature and degrade as a result of thermal processing, even though it is necessary to maintain microbiological safety and increase shelf life. The normal structure of myofibrillar protein is disrupted by high temperatures and extended exposure period of time, which causes collagen and filament lattice to shrink. As a result, the fish becomes solid or hard. The breakdown of cell membranes and sarcoplasmic protein aggregation are another effect (Alipour et al., 2010). Since texture is directly related to protein denaturation, measurements of texture should correlate with water content. However, this relationship is not obvious and may sometimes be technically challenging (Skipnes, 2014). During processing, collagen, an essential component of connective tissues, changes thermally. Collagen turns into gelatin at moderate temperatures, which adds to the texture's tenderness. But too much heat can cause collagen to breakdown to a level where the fish's structural integrity is no longer supported. Furthermore, high temperatures can cause the lipids in fish tissue to oxidation, changing the texture and flavor. The degree of texture loss depends heavily on variables including species, beginning quality, and the particular heat processing settings used.

The "texturometer", which measures all parameters using two compression-decompression cycles on a force vs. distance graph, is the tool used to measure texture parameters. Two bites are simulated by these two cycles. The probe is the component of the apparatus that comes into direct contact with the product. In order to analyze the texture of various product types, the following variables must be changed: the type of probe, the probe's pre-test, and post-test speeds, and the degree of compression, which is expressed as a fraction (in percentage) of the product's height. The term Texture Profile Analysis (TPA) refers to the complete test.

Hardness, fracturability, cohesion, springiness, gumminess, and resilience are the textural criteria that are imparted by heat treatment. Hardness is determined by measuring the first compression's maximum force. For the majority of products, this is the case, but it need not be at the first notable peak indicates fracturability. When a product is fracturable, its hardness is determined by measuring the first peak rather than the first significant peak. The ratio of work completed during the second compression cycle to that of the first compression cycle is used to compute cohesiveness. It shows how resilient a product is to return to the second compression in comparison to the first one. A product's springiness indicates its capacity to return to its initial height following compression. It is expressed as a percentage of the initial height and is calculated as the ratio of heights recorded by the probe at the second compression to the first. Gumminess which only applies to semi-solid items, is a measure of how gummy a product is. It is computed by multiplying cohesiveness by hardness. We measure chewiness rather than gumminess if the product is solid. As a result, these two criteria cannot coexist. Hardness, cohesiveness, and springiness are multiplied to determine chewiness. It is equivalent to the amount of energy needed to chew the product. Finally, resilience refers to a product's capacity to withstand the probe and regain its initial height. It is determined by dividing the initial compression's upstroke energy by its downstroke energy.

In addition, there is another textural parameter called adhesiveness. However, since there are more ways to calculate this parameter, it can be perceived as secondary parameter. The negative work performed between the two compression is how it is measured. The product must adhere to the probe following the initial compression and be raised with the probe in order for adhesiveness to be measured. It is not required that the product stay in place while the probe moves. Many investigations have been conducted to learn more about how heat treatment softens food. Cheng et al. (1979) investigated how the texture of minced fish gel was affected by heat processing. According to Mohan et al. (2006), pouch-processed goods were palatable overall and had a harder texture than canned goods.

2.5.2. Color

One important fish quality that has a big impact on how consumers view and accept fish is its color. The distinctive colors of fish species are determined by a variety of pigments, such as myoglobin, hemoglobin, and carotenoids. However, heat processing frequently results in unnecessary hue changes even though it is necessary to ensure microbiological safety and extend shelf life. The denaturation and oxidation of pigments as well as the breakdown of other chemical components in the fish tissue are the main causes for these alterations.

Food color characteristics are measured using an instrument known as a colorimeter, with 100 representing the lightest or brightest. The scale goes from +a to -a, with +a denoting redder. On the other hand, blueness ranges from +b to -b with -b denoting more blueness. The following formula is used to calculate the total color difference, or ΔE:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2.2)$$

A major cause of color loss is the change in myoglobin and hemoglobin, which give many fish species their red and pink colors. These pigments undergo structural alterations during heat processing, changing into oxidized forms such as metmyoglobin. This change gives the product a dark, brownish hue that makes it less consumer-friendly. A loss of vivid orange or pink color is also a result of the deterioration of carotenoids, such as astaxanthin, which is present in salmon and trout. Prolonged processing times and high temperatures can accelerate pigment degradation, giving them a pale or faded appearance. According to Kong et al. (2007), the color of the salmon's muscles whitened during the first ten min of treatment and then turned brown as the heat increased.

2.6. High-Pressure Processing vs Thermal Processing

High-Pressure processing (HPP) is a revolutionary method of food preservation that has distinct benefits over conventional thermal processing, especially when it comes to preserving food quality while guaranteeing safety and increasing shelf life. HPP employs incredibly high hydrostatic pressure (up to 600 MPa) to inactivate microbes and enzymes, in contrast to thermal approaches that depend on high temperatures. This novel method preserves the nutritional value and sensory qualities of food products, especially fish, while achieving microbiological safety without the negative impacts of heat. The capacity of HPP to preserve food's original sensory properties is among its most important benefits. High temperatures during thermal processing denature proteins, break down pigments, and change the texture of food, this frequently results in cooked flavor, fading of color, and softening or drying of the texture. Thermal processing, for example, can discolor fish items and eliminate their delicate, flaky texture, which consumers associate with freshness. On the other hand, HPP keeps fish's original flavor, texture, and appearance. The product maintains a fresh-like quality that closely matches its raw state because uniform pressure keeps the cellular structure mostly intact.

Additionally, HPP is excellent at maintaining nutritional value, which is frequently compromised by heat-intensive thermal processing. Nutrients that are particularly vulnerable to deterioration during heat treatments include antioxidants, omega-3 fatty acids, and vitamins B and C. These nutrients are abundant in fish, and preserving it is essential to providing customers with health advantages. HPP keeps the nutritional profile of the product close to that of fresh fish by using cold or ambient temperatures, which reduces nutrient loss. Because of this feature, HPP is a desirable choice for health-conscious shoppers looking for premium, nutrient-dense foods. From a safety view, HPP does not require heat to efficiently inactivate a variety of harmful pathogens, such as *Salmonella*, *Vibrio* species, and *Listeria monocytogenes*. By focusing on spoiling organisms, this technique also prolongs the shelf life of food items. HPP reduces the possibility of overprocessing, which can lower quality, in contrast to thermal techniques. Furthermore, because pressure is applied isotopically, HPP is very effective at delivering homogenous treatment, guaranteeing constant microbial inactivation throughout the product. Another benefit of HPP versus thermal processing is its sustainability. Since HPP systems don't need frequent cycles of heating or cooling, they use less energy than heat-based techniques. In brief, HPP combines strong safety and sustainability qualities with outstanding food to provide a strong advantage over thermal processing. HPP satisfies contemporary customer needs for fresh, minimally processed, and nutrient-rich foods by maintaining the inherent sensory qualities and nutritional value of fish.

2.7. High Pressure Processing

HPP is an inventive non thermal technique, opens the door for companies to abandon the usage of dangerous chemical preservatives and traditional heat treatment techniques. According to Galanakis (2021), it introduces the concept of minimally processed fresh meals. The pressure treatment of seafood helps to induce changes in cell membrane and genetic mechanisms, altered biochemical reactions, longer shelf life, and the inactivation of pathogenic and spoilage microorganisms. All of these effects occur while maintaining the availability of nutritious food that is not significantly impacted in terms of color, texture, quality, or other sensory parameters (Levy et al., 2021).

2.8. Evolution of HPP

Despite the fact that pressure has been used for food applications since the nineteenth century, a review of the literature shows that study on high pressure processing has steadily increased over the past 20 years (Singh and Sit, 2021). The pioneers Ohshima et al. (Ohshima et al., 1993) and Lanier (Lanier, 1998) conducted the first successful investigations involving the application of HP treatment for seafood. The impact of HPP on fish color, texture, and other quality criteria was investigated later in the early 2000s (Matser et al., 2000). By 2005, HPP was well-known for its capacity to reduce harmful microbiological germs in fish, improving the stability and quality of food. According to recent reviews, HPP can be utilized to increase the digestion of seafoods and reduce its allergens. According to Zhang et al. (2019), this was accomplished by altering the immunoglobulin's binding location or epitopes to increase binding capability.

Utilizing HPP (600 MPa for 10 min) to prevent the oxidation of cholesterol in minced mackerel is another method that has been used over the years. HPP (300 MPa at 20℃ for 15 min) is used for biofilms to extend the shelf life of smoked sardines (Gunlu et al., 2014); HP treatments (450 and 600 MPa for 15 min) have the potential to replace the steam precooking, particularly in the canned tuna industry (Jiranuntakul et al., 2018). In addition to increasing firmness, low pressure HPP treatment of hilsa fillets at 200 MPa for 10 min at 30℃ reduced the microbiological count by 2 log units (Chouhan et al., 2015). Additionally, research has been done on the use of plant extracts or phytochemicals to reduce oxidation in this food group (Roobab et al., 2022). Food companies operating in Japan were the first to introduce food products subject to the HPP to the market in 1993. The HPP technology has currently been used to commercialize a number of goods on the market, including commodities related to beverages, meat products, vegetables, and seafood. Along with the pathogenic and quality aspects, research has been done on the potential of HPP to change food ingredients and create foods with new functions (Huang et al., 2020).

Many studies were carried out on HPP including, the use of pressure for studying endogenous enzymes of pressurized fish resulted in improved 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 in comparison to conventional thawing (Li, 2024), reduced drip loss was observed in HP thawed salmon (Zhu et al., 2024), HPP in Atlantic salmon also resulted in improved structure stability of myofibrillar protein (Li, 2024), and pressure-shift freezing in carp resulted in reduced TBA content and reduced drip loss (Sequeira-Munoz et al., 2005).

2.9. How HPP Works?

Water is the medium used in the HPP application to transfer pressure to the seafood product. The product is sealed or vacuum packaged, and any flexible packaging material can be utilized. The sample undergoes pressure treatment after being put into a chamber (Levy et al., 2021). Generally speaking, there are two ways to treat HP: the batch technique and the semi-continuous method. The batch method, which may be used for both liquid and moist solid food products, is typically used by companies. The equipment includes conveyors for loading and unloading baskets that hold the products, a vessel for processing the products under high pressure, plugs and wedges to close the vessel, a yoke to withstand the pressure generated, intensifier pumps for creating pressure, and a system for monitoring and managing temperature and pressure. The pressure chamber and intensifier pumps, which aid in producing pressure, makeup a significant portion of the apparatus (Nabi et al., 2021).

After placing the seafood into the vessel, also known as loading of products, the vessel aligns with the yoke. The food sample must be packed before loading since pasteurization causes a volume loss of roughly 10% to 20% and once the pressure is released, the volume returns to almost its initial level. After that, low pressure water is injected into the vessel, and the wedges and plugs close it off. More water is then forced into the vessel by the high-pressure intensifiers. A few pressure-transmitting substances besides water are sodium benzoate, ethanol, glycol, and castor oil. A piston in each of these intensifiers is propelled by hydraulic oil. Given that the piston’s are is significantly larger than the plunger, the fluid pressure inside the vessel is increased as a result of this sectional difference (Huang et al., 2017). After then, the pressure is maintained for a short while, when microorganisms are destroyed, enzymes are inactivated, and some food quality is altered, largely maintaining food freshness without the use of heat. After the pressure is finally released, depressurization takes place, and the vessel is then opened. The product is unloaded, allowing it to be delivered to customers without being heated. Cycle time and loading factor determine the equipment's performance (Ramaswamy and Shao, 2010). The total amount of time needed for pasteurization, pressure holding, and subsequent de-pasteurization is known as the cycle time. The percentage of the vessel volume used to store the sample and the package shape is known as the loading factor. As the process progresses, adiabatic heating from the fluid compression causes a notable increase in temperature as the pressure rises (Zhu et al., 2004). Avure Technologies from USA, NC Hyperbaric from Spain UHDE from Germany, Kobelco from Japan, Stansted from UK, Bao Tao Kefa from China, and others are well-known providers of HP processing equipment. There are currently both horizontal and vertical vessel configurations on the market. While research equipment can reach pressures of up to 1400 MPa, the majority of commercial HPP equipment has a processing pressure limit of 700 MPa (Nabi, 2021).

2.10. Governing Principles of HPP

The HP treatment is applied to food samples using two scientific principles. The first is based on Le Chatelier's principle, which is also known as the thermodynamic equilibrium law. It claims that when a system in equilibrium is upset, it responds by trying to minimize the disruption by moving in a direction that lessens the change as it settles back into equilibrium. This aids in the research of how temperature and pressure may affect the equilibrium position in industries. According to Evrendilek (2023), this indicated that in HP, pressure enhances phenomena associated to a decrease in reaction volume while inhibiting those connected to an increase in volume. According to the principle, a system's equilibrium shifts in proportion to changes in temperature and pressure, as evidenced by the volume reduction. Pressure is therefore advantageous for reactions involving the volume decrease. Because pressure transmission in this case is independent of mass and time, treatment times are shortened, and the technology can be further scaled up for commercial use (Barba et al., 2020).

The second theory relies on the isostatic rule, which states that pressure is quickly and uniformly distributed throughout a sample while it is under pressure. This is in light of the sample being either hermetically sealed in a flexible packaging material or in direct contact with the pressure transmitting medium (Abera, 2019). This due to the fact that a fluid known as hydrostatic can transfer pressure without creating friction when a specific amount of pressure is applied. In this case, applying pressure changes the space between the food molecules, which causes the material's volume to decrease without changing its shape. Covalent bonds are less impacted by this pressure than electrostatic, hydrogen bonding, or hydrophobic interactions because their working distance is unaffected. And that's the main reason why actions for food functional groups can't be prevented effectively. Additionally, it shown that pressure does not have a macroscopical effect on nonporous foods. All things considered; it is seen that the pressure transfer is isostatic and almost immediate. Unlike thermal processing, HPP is not influenced by the size of the equipment or the shape of the sample (Naveena and Nagaraju, 2020).

2.11. Current Status of HPP for Thermal Processing

Numerous diverse studies have been conducted in the field of HPP to improve food's safety and nutritional value as well as its shelf life. The use of HPP has been studied for a broad range of food products, including dairy, meat, seafood, drinks, and vegetables. Other food research topics include packaging design, new and improved goods, and most popularly increased food safety. Increased milk shelf life through high-pressure, high-temperature spore destruction of *Clostridium botulinum* in milk (Shao et al., 2022) and pressure destruction of L. monocytogenes (Mussa and Ramaswamy, 1999), HP caused Clostridium sporogenes in milk to be destroyed at quasi-elevated temperatures (Ramaswamy et al., 2010); HP caused ascorbic acid to be impregnated into apple cubes for nutritional fortification and decreased browning (Vatankhah and Ramaswamy, 2019); HP reduced retrogradation of HP treated lentil slurry (Ahmed et al., 2009); HP treated Basmati rice samples had lower gelatinization (Ahmed et al., 2009); HPP treated soybeans showed limited protein structural changes following HP treatment (Alvarez et al., 2008); HPP treated pork improved texture (Singh and Ramaswamy, 2012); high pressure caused destruction of effective inactivation of avidin in eggs (Singh et al., 2015); and improved rheological characteristics of egg components, HPP was utilized to determine the kinetics of E. coli (O157:H7) and L. monocytogenes destruction in mackerel fish slurry (Ramaswamy H. S., et al., 2008), and HP treated tuna showed decreased oxidation and texture degradation (Zare and Ramaswamy, 2004); enhanced mango juice storage via HP destruction kinetics (Hiremath and Ramaswamy, 2005); enhanced orange juice microbiological stability (Basak and Ramaswamy, 2001); HP induced pectin inactivation in apple juice (Riahi and Ramaswamy, 2003); and enhanced aquafaba functional properties (Alsalman and Ramaswamy, 2020) are some of the numerous studies employing HPP in food Products.

In addition to food processing, HPP has advantages in a number of other fields. Using HPP, the thermal characteristics of polylactides for the creation of food packaging materials were investigated (Ahmed et al). Among the non-food processing, uses of HPP are the following: high-pressure steaming of cellulose fabrics, which showed improved shrinkage and fabric hand (Ohshima, 2003); high-pressure and high-temperature graphene inlaying of fabric for anti-ststic and anti-ultraviolet properties (Zhang, 2019); biotechnological application for improved homogenization of high-pressure homogenizer (Shirgaonkar, 1998); and wood dyeing, where better intensity and uniform dyeing was achieved through HPP in comparison to conventional hot dip method (Yu Y et al., 2019).

2.12. Effect of HPP on Color

Visual aspects of color are a key sensory factor that aid in assessing the quality of the seafood. It serves as a sensory indicator for customers to assess the freshness of seafood products while thwy are being purchased. Products made from seafood that have been treated with HPP take on a whiteness or opaque appearance that is similar to that of cooked seafood. This is caused by myoglobin denaturation in fish muscles as well as further protein denaturation of sarcoplasmic and myofibrillar proteins at pressures higher than 150-300 MPa. Along with these protein alterations, additional elements that may also play a role in the color shift of shellfish muscles include pigments, lipid oxidation, and muscle moisture levels. Additionally, a crucial factor in this color change is the impact of time. Tilapia fillets were used in the investigation, and they were pressure treated with 200 MPa for 1 and 3 min in order to notice this. At longer treatment durations, a noticeable change in fish color was seen (Suemitsu & Cristianini, 2019). Furthermore, the type of fish also affects color changes. For example, hake did not change color when subjected to a 200 MPa pressure treatment, whereas turbot showed signs of cooking after the same pressure processing treatment (Puertolas & Lavilla, 2020).

For Scientific or commercial uses, the CIELAB system is used to examine color changes in seafood. Using three color values, CIELAB is a 3-D color space that aids in precisely quantifying color. Here, color change is determined by numerical values, which are obtained using the equation L*a*b*. Here, L stands for the lightness parameter on a scale of 0 to 100; green is denoted by an a* negative, red by an a* positive, and blue by b*negative and yellow by b* positive. When seafood products undergo HP treatment, they show an increase in L* value, which translates into a lighter fish color. For some fish species, such ah mackerel, tuna, tilapia, hake, salmon, and cod, the L* value rises after HPP treatment. When analyzing this seafood quality metric, L* is thought to be more significant than a* and b*. Certain species, such tuna, fresh cod, and mackerel, have a lower redness or a* value, while species like tilapia, tuna, cod, and salmon have higher b* values (Puertolas & Lavilla, 2020).

For Oysters, an HP treatment of 300 MPa at 20℃ for 10 min resulted in an increased L* or opaque look, while the redness value dropped at around 100 MPa. The color of the pressured prawns started to whiten around 100 MPa; the species diversity also affected the results. Sulfites are added to prawns to prevent blackening caused by polyphenol oxidase (PPO), which is seen to be a bad organoleptic quality and results from the oxidation of phenols to quinones. It has been demonstrated that HP treatment between 300 and 400 MPa, conducted for 10 min at a temperature below 10℃, reduces prawn PPO activity by around 80% (Duranton et al., 2014).

2.13. Effect of HPP on Texture

The denaturation effects of high-pressure processing on proteins are primarily responsible for the textural alterations in seafood. Hardness is induced by pressure, and the more pressure applied, the harder the product will be. Thus, pressure application causes a number of processes that impact the product’s texture, such as oligomer disassociation, precipitation, denaturation, unfolding, and gelatinization. Because seafood products are so delicate, they must be cohesive or hard in order to be consumed. The water loss brought on by protein denaturation is another element that influences textural alterations. Textural alterations in the fish muscle can also be brought on by myofibril structure fragmentation and sarcomere length reduction. Studying characteristics like chewiness, springiness, or hardness can effectively characterize these textural changes. These parameters were found to be unaffected by mild pressure of 100 MPa for 2 to 5 min on mackerel fillets, but to be significantly altered by high pressure of roughly 500 MPa for the same amount of time (de Alba et al., 2019). According to de Oliveira et al. (2017), the enhanced hardness and hydrogen bonding network development may be the cause of the fish muscle’s increased springiness. An investigation into adhesiveness was conducted on albacore tuna, where pressure treatment at 50-150 MPa did not alter the treated and control samples; treatment at 200-250 MPa increased adhesiveness; and treatment at 300-500 MPa caused the gradually increased adhesiveness to disappear. Myosin fiber loss may be the cause of increased adhesiveness at mid-pressures, while actin and sarcoplasmic protein unfolding and the hydrogen-bonded networks may be the cause of decreased adhesiveness at high pressures (Cartagena et al., 2019).

2.14. Effect of HPP on Storage Life

The initial suggestion for using HPP on food samples is preferably based on their capacity to increase product shelf life through microbial inactivation and protein denaturation. Additionally, using this method raises the prospects of significant sensory modification in minimally processed food. Gram negative bacteria that are sensitive to pressure are primarily linked to seafood, including fish and shellfish. As a results, using HPP as a processing method decontaminates these bacteria, as evidenced by the presence of gram-negative bacteria (LAB) following pressure treatment. Additionally, LAB aids in the inhibition of additional harmful organisms. HPP can inhibit enzymes that cause food spoiling, extending the shelf life of marine goods (Lee at al., 2021). HPP has been shown to reduce E. coli growth in apple juice (Ramaswamy et al., 2003), increase milk destruction rates (Mussa and Ramaswamy, 1999), improve textural properties of fruits and vegetables (Basak and Ramaswamy, 1998), and increase storage of mango pulp with improved consistency (Ahmed et al., 2005), resulting in longer storage life for pressurized products.

HP treatments of 250 to 600 MPa in seafood reduce microbiological contamination and alter the color of the product, giving it the appearance of being cooked due to increased opaqueness. As a result, HPP is used as an alternative for fish species like cod or hake, where color change is less noticeable. HPP treatment of seashells not only extracts flesh from crustaceans and shucks mollusks, but also extends shelf-life through pressure (Puertolas &Lavilla, 2020).

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Bridging Text to Chapter 3

Chapter 2 reviews the theoretical foundations of thermal and non-thermal processing, detailing how factors such as heat transfer, agitation, acidification, temperature, and pressure affect microbial inactivation and quality loss (e.g., protein denaturation, moisture loss, and pigment degradation) in fish. Building on this literature, Chapter 3 presents the experimental evaluation of conventional thermal processing versus acidified thermal processing on trout quality. This chapter applies the principles from Chapter 2 to measure the effects of processing conditions on texture and color, providing practical insights and validation for the concepts discussed in the literature review.

CHAPTER 3

EFFECT OF CONVENTIONAL THERMAL PROCESSING AND ACIDIFIED THERMAL PROCESSING ON QUALITY OF TROUT

3.1. Abstract

Trout is a highly perishable fish species that necessitates efficient processing methods to guarantee food safety and preserve quality: Conventional Thermal Processing (CTP) and Acidified Thermal Processing (ATP) are both frequently employed methods; however, their effects on texture, pigment, and microbial control are distinct. CTP is a process that involves the use of high temperature to eradicate pathogens, which ensures safety. However, this process results in protein denaturation and moisture loss, which results in firmer, drier fillets. Pigments are also degraded by prolonged heat exposure, which diminishes their visual appeal.

ATP facilitates microbial lethality at lower temperatures by combining moderate heat with pH reduction (≤4.6). This reduces thermal degradation, but it can soften the texture as a result of acid-induced hydrolysis of connective tissues. It also partially influences pigment retention, which results in a change in color. This investigation emphasizes the necessity of optimizing processing conditions to ensure food safety and quality retention, thereby accentuating the trade-offs between CTP and ATP. The results contribute to the development of enhanced processing strategies that ensure an extended shelf-life while preserving sensory and structural integrity.

Keywords: Thermal Processing, Conventional, Acidified, Food safety, pH, Shelf life.

3.2. Introduction

Fish, particularly trout (Oncorhynchus mykiss), are highly perishable food commodities due to their high moisture and protein content. Preservation techniques that are effective, are indispensable for the preservation of the quality and safety of trout products. CTP is frequently implemented to prolong the shelf life of fish by eliminating decomposition and pathogenic microorganisms (Smith et al., 2018). Nevertheless, thermal interventions can have a detrimental impact on the sensory attributes, nutritional profile, and texture of fish (Jones & Brown, 2020). As a result, there has been a growing interest in alternative preservation strategies, such as ATP, which involves acidification before heating to improve microbial inactivation at lower temperatures, potentially preserving quality attributes (Garcia et al., 2019).

The impact on product quality, including texture, color, nutritional retention, and sensory characteristics, varies depending on the procedure used, despite the fact that thermal processing is a critical intervention in fish preservation. Acidified thermal processing relies on the application of acidulants, such as acetic, citric, or lactic acid, to reduce pH levels, thereby increasing microbial lethality at reduced thermal intensities (Kim et al., 2021). Conventional thermal processing is wholly dependent on heat application. The purpose of this study is to conduct a critical assessment of the impact of CTP and ATP on the quality of trout with a particular focus on consumer acceptability like texture and color.

CTP typically entails the heating of fish to temperatures ranging above 100℃ for varying durations, contingents upon the intended microbial safety levels and shelf life extension (Ahmed et al., 2017). One of the primary benefits of CTP is its capacity to effectively inactivate spoilage bacteria and pathogens (Lee & Park, 2020). However, the fish's texture can undergo substantial modifications as a result of the application of heat alone, which can result in protein denaturation and muscle hardening (Rahman et al., 2016).

Heat treatment also affects lipid oxidation, resulting in the formation of oxidation products that can have a detrimental impact on the nutritional quality and flavor of fish (Hernandez et al., 2022). Consumer acceptability is compromised by the development of rancidity, which is the consequence of the oxidation of polyunsaturated fatty acids (PUFAs) (Morris et al., 2019). Furthermore, the degradation of pigments, including astaxanthin, results in color changes that contribute to the distinctive hue of trout flesh (Zhang et al., 2018). These challenges require the investigation of alternative or complementary preservation techniques that reduce the effects of thermal degradation.

ATP has emerged as a promising method for preserving the quality attributes of fish while improving its microbial safety. ATP mitigates protein denaturation and lipid oxidation by reducing the microbial load at reduced heat intensities by lowering the pH of fish muscle prior to thermal treatment (Guan et al., 2021). Acetic acid, citric acid, and lactic acid are among the most frequently employed acidulants in ATP. These acids also contribute to the enhancement of flavor profiles and the extension of the shelf life (Fernandez & Martinez, 2018).

Protein conformation and water-holding capacity can be influenced by acidification prior to heating, which can lead to enhanced textural properties in comparison to conventional heating (Torres et al., 2020). Furthermore, the controlled acidification process has been demonstrated to restrict lipid oxidation by functioning as an antioxidant, thereby safeguarding the nutritional integrity and sensory quality of trout (Yildiz et al., 2022). Furthermore, the nutritional value of processed fish products may be further enhanced by ATP's capacity to modify protein interactions, thereby increasing the bioavailability of specific nutrients (Singh et al., 2017).

The results of numerous studies that have contrasted the effects of CTP and ATP on fish quality have been inconsistent, as they are contingent upon the fish species, pH adjustment level, and processing conditions (Gonzalez et a., 2021). Although both methods effectively mitigate microbial contamination, ATP provides substantial benefits in terms of preserving desirable nutritional and textural characteristics (Rodriguez at al., 2019). The synergistic effect of acidification and heat results in a reduction in processing temperatures while maintaining equivalent microbial lethality, thereby reducing thermal degradation (Silva et al., 2020).

Nevertheless, ATP also poses certain obstacles, such as the potential for change in flavor and increased acidity, which could potentially impact consumer preferences (Kumar & Patel, 2019). It is imperative to optimize acid concentrations and processing parameters in order to achieve a balance between safety and sensory acceptability in acidified thermal procedures (Liu et al., 2016). In order to guarantee food safety and optimize quality retention for species fish species, future research should concentrate on the refinement of ATP protocols (Navarro et al., 2021).

3.3. Materials and Methods

3.3.1. Sample Preparation

Fresh rainbow trout (*Oncorhynchus mykiss*) of uniform size were obtained from a local supermarket (Smith, 2022). The fish were promptly transported to the laboratory on ice, where they were eviscerated, filleted, and cleaned with chilled potable water (Garcia et al., 2023). The fillets were uniformly divided into portions (100±5 g) and stored at 4℃ before being subjected to thermal processing. A subset of the fillets was acidified for 5 hours with 1% (v/v) citric acid solution, and then drained and blotted dry prior to thermal treatment (Jones et al 2021). The objective of this pretreatment was to preserve the sensory qualities of the fish while simultaneously improving microbial inactivation. The control group consisted of the remaining fillets that were subjected to conventional thermal processing without acidification. It was essential to guarantee a consistent sample size and initial freshness in order to acquire reproducible results (Kim et al., 2022).

3.3.2. Retort Equipment

Thermal processing was conducted using a pilot-scale, vertical, static retort (Loveless Manufacturing Co., Tulsa, OK). The retort was converted into a reciprocating agitation retort by incorporating a slider crank assembly, magnetic motor, and basket arrangement at a height of one-third of the retort from the top. The motor's input voltage was adjusted to regulate the frequency of reciprocation. A combination of steam and air was employed as the heating medium, while frigid water was employed for cooling. Prior to each experiment test, the equipment was calibrated to guarantee uniform heat distribution.

3.3.3. Reciprocal Agitation Thermal Processing

The trout fillets are cut into the circular shape of 7 cm diameter and inserted into the glass jar. The glass jar is filled with an brine solution leaving headspace. These containers were subsequently heated in the retort at 115, 120, and 125℃ at static, 1 Hz, and 1.5 Hz (Brown at al., 2021). The goal was to improve microbial lethality and obtain uniform heat penetration (Zhao et al., 2023). Clark et al. (2020) observed that agitation during thermal processing was effective in preventing overheating at the sample's surface and assuring uniform cooking. The samples were over-processed in the first run to provide time-temperature data, through which the processing time was observed. The F-values were established by recording the internal temperature of each sample at regular intervals, which verified the adequacy of thermal lethality (Choi et al., 2023), using the following equation:

$$F_0 = \int L\, dt = \sum 10^{\frac{T_S - T_O}{z}} \frac{5}{60} \tag{3.1}$$

where T_s represents the sample temperature and T_O stands for the reference temperature. The cumulative lethality was also calculated. The point at which the target lethality was achieved was noted as the processing time for those processing conditions. The target lethality for the conventional method of thermal processing is F_{121}=5 min. The fillets were swiftly cooled in an ice-water bath during post-processing to prevent further heat-induced degradation (Patel at al., 2022).

3.3.4. Acidified Thermal Processing

In a similar manner, acidified thermal processing was implemented, with the inclusion of a pre-treatment phase that involved immersing fillets in a 1% (v/v) citric acid solution for 5 hours prior to treatment (XU et al., 2021). These fillets were subsequently subjected to thermal processing at a lower temperature of 90°C for the same agitation speed (Wu et al., 2023). The target lethality for acidified thermal processing is F_{90} = 10 min. The microbial survival threshold was decreased by the acidification phase, which eliminated the necessity for increased thermal intensities (Kimura et al., 2022). In order to evaluate the impact of acidification on texture and color, changes in protein denaturation and lipid oxidation were closely monitored (Hassan et al., 2023). Following the processing, the fillets were promptly chilled in an ice bath to prevent any residual thermal effects (Lee et al., 2021).

3.3.5. Texture Analysis

Texture profile analysis (TPA) was performed with TA. XT plus texture analyser (Texture Technologies Corp, New York, USA). 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 mmm 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 analysis was conducted at room temperature (Gonzalez et al., 2023). The texture parameters that were evaluated are Firmness and tenderness (Singh et al., 2022). These measurements were essential for comprehending the impact of processing on the mechanical properties of the fish muscle (Das et al., 2021). A minimum of three replicates per sample were analysed to guarantee the accuracy of data (Ahmed et al., 2023). These values for firmness and tenderness obtained directly from the Exponent software (Texture Technologies, New York, USA). The findings contributed to a better understanding of the impact of various processing methods on the structural integrity of trout fillets (Park et al., 2020). Different texture parameters were evaluated from the force deformation curves as follows:

$$Firmness = \frac{maximum\ force}{maximum\ deformation} = \frac{MF}{MD} \tag{3.2}$$

$$Tenderness = \frac{Force/crosssectional\ area}{deformation/initial\ length} = \frac{F/A_0}{\Delta L/L} \tag{3.3}$$

3.3.6. Color Measurement

Color measurement was conducted by Minolta Tristimulus Colorimeter (Minolta Crop, Ramsey, NJ, USA) against a standard white and black reference plate prior to each set of measurements (Rahman et al., 2021). In order to account for potential color variations, the L* (lightness), a* (red-green), and b* (yellow to blue) values were recorded at three distinct locations on each fillet (Silva et al., 2023). To quantify the effects of thermal processing, the browning index (BI) and total color difference (ΔE) were determined (Morris et al., 2022). It was particularly important to investigate the influence of acidification and heat on pigment degradation in trout flesh, as it naturally contains carotenoids that contribute to its unique coloration (Chen et al., 2021). The analysis was reliably and random errors were minimized through the use of triplicate measurements (Hernandez et al., 2023). Measurement was taken 10 times for each sample after which the average value was considered. L* is measured on a scale of lightness to darkness, 100 being the lightest and 0 being the darkest. a* is measured on a scale of redness to greenness, with +a being redder and -a being greener. b* is measured on a scale of yellowness to blueness, with +b denotes yellowness and -b denotes blueness. The lightness (L*), redness (a*), and blueness (b*) were measured and displayed by the software (SpectraMagic, Minolta Corp., Ramsey, NJ, USA).

The parameters were derived as shown below:

$$BI = \frac{100}{0.172} \times \left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} - 0.31\right) \quad (3.4)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3.5)$$

3.3.7. Statistical Analysis

The data were analyzed using Microsoft excel for two-way analysis of variance (ANOVA) (Gomez et al., 2020). Significant differences ($p<0.05$) between treatment groups were determined using the Tukey’s post-hoc test (Singh et al., 2022). The mean ± standard deviation was used to present the result of each experiment, which was conducted in triplicate (Das et al., 2023). Statistical analysis enabled the identification of the substantial impacts of acidification and various thermal interventions on the quality parameters of trout fillets (Ahmed et al., 2023). Correlations between thermal exposure and quality deterioration were investigated, and trends in texture and color variations were assessed (Park et al., 2020).

3.4. Results and Discussion

3.4.1. Processing Time

Thermal processing is a critical process in the food industry, particularly for low-acid and acidified products, as it is designed to preserve desirable quality attributes, including flavour, texture, and nutritional value, while also guaranteeing microbiological safety (Rao et al., 2020). The attached time-temperature graph (Figure 3.1) for trout processing demonstrates the variety of methods that can be employed to achieve these objectives, including Conventional Thermal Processing (CTP) at 115, 120, and 125℃ and acidified thermal processing at 90℃ (Figure 3.1). The data also account for the impact of reciprocal agitation at three rates (0Hz, 1Hz, and 1.5 Hz) in addition to the variation of the processing temperature.

Figure 3.1 demonstrates shorter come-up times and steeper temperature rise profiles at higher retort temperature under CTP conditions. This is due to the fact that microbial inactivation kinetics typically exhibit a logarithmic relationship with temperature, resulting in the faster destruction of microorganisms at elevated heat levels (Fellows, 2017). As a result, treatments that are conducted at 125℃ can achieve the desired internal temperature in a shorter amount of time than those that are treated at 115℃. In contrast, the curves at 115℃ exhibit more gradual slopes, which suggests that the heat penetrates the product at a slower rate. In order to accomplish the same level of microbial inactivation, a longer holding period is required due to the slower come-up time.

Li et al. (2019) have observed that the temperature differentials and associated lethality rates in the graph are consistent with established heat transfer theories, which claim that both conduction and convection heat transfer mechanisms intensify at elevated temperatures, thereby accelerating the heating rate. Nevertheless, the retention of heat-resistant vitamins and the structural integrity of fish proteins can be adversely affected by the prolonged use of excessively high temperatures (Juneja et al., 2020).

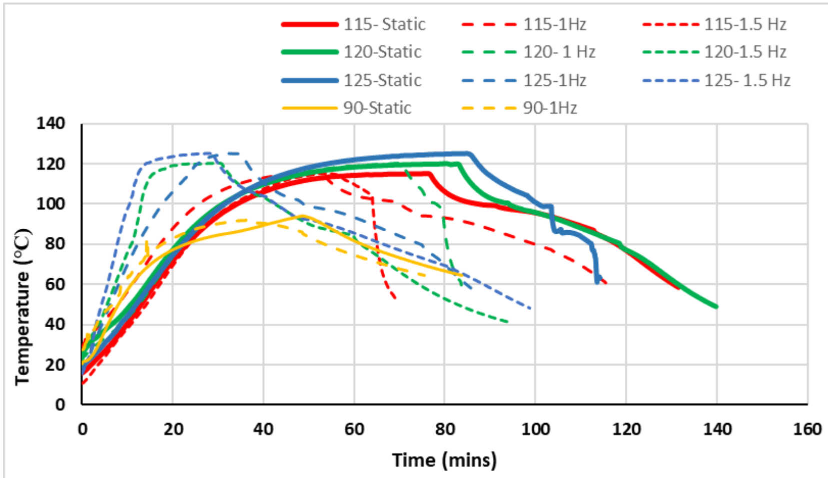

Figure 3.1. Time-Temperature graph of trout processed under conventional and acidified thermal processing

In parallel, acidification alters the microbial inactivation curve by decreasing the pH of the product, which increases the sensitivity of bacteria to heat (Silva & Gibbs, 2021). This synergy between mild heat and low pH is leveraged by ATP at 90℃ to inactivate pathogens that would necessitate more intense heat interventions under non-acidified conditions (Dimitrijevic et al., 2020). As a result, the curves at 90℃ reach a lower final temperature than the CTP curves. However, the reduced pH environment mitigates this temperature disparity by decreasing microbial heating resistance. Additionally, the preservation of certain heat-resistant nutrients, including omega-3 fatty acids and vitamins, is frequently facilitated by milder temperatures, as they are susceptible to degradation at elevated retort temperatures (Peleg, 2019).

Thermal processing is also significantly influenced by time. The come-up phase, the holding or plateau phase, and the chilling phase are the three general phases of each curve in the graph (Ramaswamy & Marcotte, 2006). The internal product temperature increases during the come-up phase until it reaches the target processing temperature. The rate at which this occurs is contingent upon factors such as agitation, retort temperature, and product characteristics, including density and heat conductivity (Martinez-Hernandez et al., 2018). The holding phase commences when the product’s core reaches the intended lethal temperature. This stage is crucial for achieving the necessary microbial inactivation, ensuring that the target temperature is maintained at or above the coldest point in the container for an adequate period of time to satisfy the required F-value. The plateau in the graph may be more apparent at higher temperatures, as processors typically maintain the product at these temperatures for a brief but precise period to achieve commercial sterility (Fellows, 2017). Cooling is initiated following the holding phase to expedite the temperature reduction and prevent overprocessing. The retort system's cooling water flow, agitation speed, and packaging geometry can all influence the cooling slope (Li et al., 2019).

Another significant factor that is evident in the graph is the pace of reciprocal agitation. Heat transmission within the product is primarily reliant on natural convection and conduction at 0Hz (static), As an outcome, the temperature profiles at static conditions are less steep, suggesting a slowed rate of heat penetration. The movement within the retort or container disrupts boundary layers surrounding the product and facilitates a more uniform heat distribution when agitated is applied, particularly at higher frequencies such as 1.5 Hz (wang et al., 2021). As a result, the temperature curves at 1 Hz and 1.5 Hz typically have a reduced total process time and a faster come-up phase than their static counterparts. This enhanced heat transmission is also advantageous during the cooling phase, as it may mitigate quality degradation and reduced thermal gradients within the container (Martinez- Hernandez et al., 2018). Optimizing agitation speed can result in increased throughput and energy savings from an industrial standpoint, as shortened processing times are generally more efficient (Rao et al., 2020).

3.4.2. Texture Profile Analysis

3.4.2.1. Hardness

The structural and functional properties of fish muscle are unavoidably altered by thermal processing, which is a critical step in extending the shelf life of fish products. The primary components of fish muscle are myofibrillar proteins (e.g., myosin, actin), connective tissue (collagen), and sarcoplasmic proteins (Fletcher, 2019). These proteins denature when heated, and they can either form a denser protein matrix (which increases firmness) or expel water (which could result in a drier, tougher texture). The force necessary to deform the fish sample is indicted by the hardness parameter, which is influenced by protein denaturation, water-binding capacity, and collagen solubilization (Tornberg, 2005).

In the bar graph (Figure 3.2), the hardness values of trout are compared across various thermal processing conditions. Focusing on the differences between CTP (115-125℃) and ATP (90℃), it is evident that the lower temperature acidified samples (90℃) exhibit relatively high hardness than CTP. This result indicates that protein functionality and water-binding capacity can be modified by acidification, which enables pasteurization rather than complete sterilization. Acidification effectively eliminates the necessity for temperatures exceeding 100℃ by decreasing the thermal resistance of spore-forming bacteria. Nevertheless, the isoelectric points of fish proteins can be altered by acid, resulting in a denser aggregation and higher hardness. Parallel to this, the CTP samples at 115-125℃ experience significant heat-induced protein denaturation and collagen solubilization, which may result in a decrease in hardness in comparison to the control sample. However, there are significant differences in CTP conditions that are associated with agitation speed.

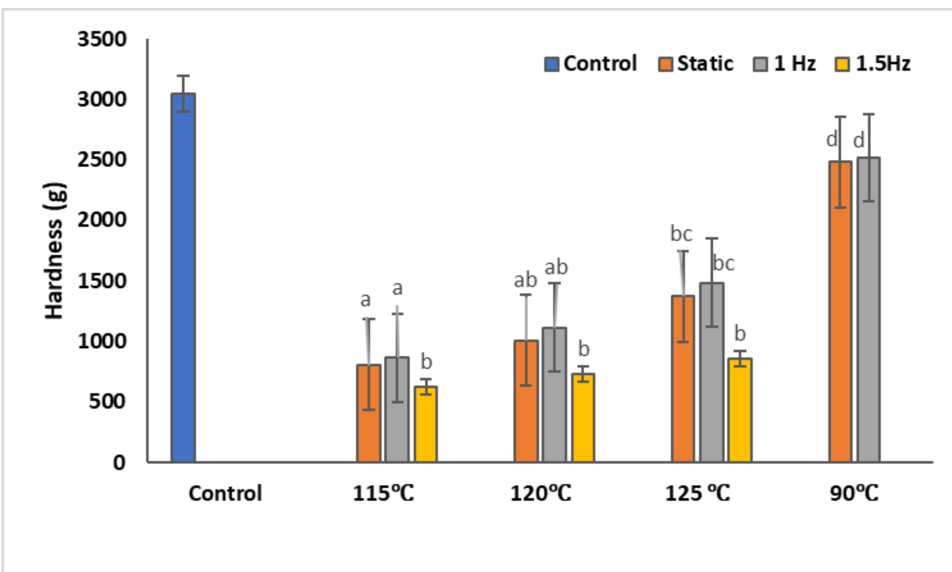

Figure 3.2. Effect of CTP and ATP on hardness of trout.

Different letters indicate significant differences in reciprocal frequencies(p<0.05).

The effect of thermal and acidification on the protein and connective tissue of fish is observed in the result of hardness. High temperature in CTP denatures myofibrillar proteins (actin and myosin), resulting in contraction and water loss. However, they also promote collagen solubilization, which can contribute to a more tender product (Tornberg, 2005). On the other hand, pasteurization is facilitated by acidified conditions at 90°C, as the pH is reduced and the heat resistance of spore-forming bacteria decreases, thereby eradicating the necessity for the higher temperatures that are characteristics of CTP (Silva & Gibbs, 2010). Protein-protein interactions can be either facilitated or prevented by acidification, based on the proximity of the muscle protein's isoelectric points to the final pH (Wang et al., 2018). On occasion, the texture of the fish muscle may be softened by partial collagen hydrolysis, while in other instances, it may become firmer as the proteins aggregate more densely under acidic conditions. In addition, the formation of protein networks and heat transfer are influenced by agitation during thermal treatment. A moderate level of agitation (1 Hz) results in higher hardness value due to lower treatment time of trout, on the other hand, higher agitation (1.5 Hz) in CTP shows lower hardness than static conditions, where localized overcooking can occur (Holdsworth & Simpson, 2016). In general, the ability of acidification to inactivate spores at lower temperatures provides ATP with a competitive advantage over CTP in terms of maintaining a desirable texture, provided that the acid levels are carefully controlled to prevent excessive protein aggregation.

Statistically significant differences ($p< 0.05$ or an analogous threshold) among treatments are indicated by the letters above the bars (a, b, c, d). Bars that share the same letter are not significantly different from one another, whereas those with different letters exhibit a significant difference in hardness. It is clear from the chart that the control group is statistically distinct, as its hardness values exceed those of all other treatments. Certain bars within the CTP range (115°C-125°C) possess same letters, indicating that while numerical differences may exist, they do not reach the level of statistical significance in certain comparisons, In contrast, the ATP samples (90°C) form distinct groups from the CTP treatments, which serves as an illustration of the distinctive function that acidification can have in controlling texture. This statistical classification emphasizes the combination of acidification, thermal load, and agitation to produce comparable textural outcomes, despite the nominal differences in process parameters.

3.4.2.2 Tenderness

The graph (Figure 3.3) illustrates the tenderness of trout under various thermal processing conditions, comprising a control sample, three levels of CTP at 115, 120, and 125°C, as well as ATP at 90°C. In each temperature group, the trout samples were either maintained in a static state

(0 Hz, indicated by the orange bars) or subjected to reciprocal agitation at 1 Hz (grey bars) and 1.5 Hz (yellow bars). The y-axis represents tenderness, with increased values signifying a more tender product. The control bar exhibits the highest tenderness value, exceeding 3500 g.s, indicating its potential as a reference standard that has preserved significant moisture and structural integrity. Among the processed samples, ATP treatments at 90°C exhibit notably high tenderness values, especially the 1 Hz sample, which approaches 3000 g.s. In contrast, numerous CTP samples demonstrate reduces tenderness, although variation persists within their respective categories.

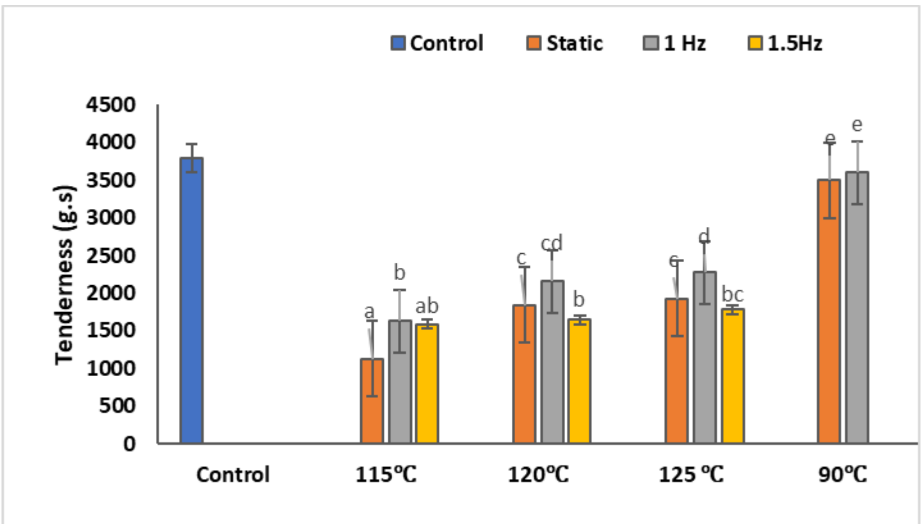

Figure 3.3. Effect of CTP and ATP on tenderness of trout.

Different letters indicate significant differences in reciprocal frequencies($p<0.05$).

Analysis of the temperature sets reveals a distinct trend, as the processing temperature increases from 115°C to 125°C, tenderness values typically improve as it reduces processing time which helps trout to retain higher tenderness property. At 115°C, the majority of bars range from 1600 to 1800g.s At 120°C, certain bars exceed 2000g.s, and at 125°C, several bars achieve or exceed 2000-2400g.s. On the other hand, the ATP treatment at 90°C further improve the tenderness measurements, by avoiding sterilization treatment. This indicates that processing at a lower temperature, when paired with acidification, can effectively preserve or improve tenderness. This underscores the advanced properties of ATP, demonstrating its ability to ensure microbial safety through the inactivation of spore-formers in acidic environments, while simultaneously maintaining the delicate texture of fish.

The graph also illustrates the impact of agitation speed on tenderness. Each temperature category is represented by three bars, static(orange), 1 Hz (grey), and 1.5 Hz (yellow). At 115°C, static processing yields a marginally lower tenderness value than the 1 Hz condition as the trout is processed for a long time, while 1.5 Hz ends up with intermediate tenderness level as a result of broken sample due to higher speed of agitation. At 120°C, a comparable trend s observed, with the 1 Hz bar exhibiting a significant increase in tenderness relative to static and 1.5 Hz conditions, At 125°C, the 1 Hz sample exhibits a relatively high level of tenderness. However, the static sample does not show a significant decrease across treatments. In the 90°C (ATP) group, a frequency of 1 Hz results in highest tenderness values, whereas static yield moderately lower results. The findings indicate that moderate agitation at 1 Hz enhances uniform heat transfer and mitigates localized overprocessing, thereby improving tenderness. Excessive agitation at 1.5 Hz or the absence of agitation may result in suboptimal distribution of heat and acid, consequently leading to reduced tenderness.

Multiple factors contribute to the differences in tenderness observed across the treatments. Temperature significantly affects the denaturation of myofibrillar proteins and the solubilization of connective tissue (Tornberg, 2005). At elevated temperatures (115-125°C), fish muscle proteins, including actin and myosin, experience notable structural alterations that may result in either product toughening due to excessive protein aggregation or tenderization through collagen breakdown. The overall outcome frequently results from the interaction of these two processes, with collagen solubilization becoming increasingly significant at elevated temperatures or extended holding period (Yin & Pan, 2002). Conversely, acidification at 90°C (ATP) reduces the thermal resistance of spore-forming bacteria, enabling sterilization safety at pasteurization temperatures (Silva & Gibbs, 2010). The acidified environment can alter the isoelectric point of proteins, bringing it nearer to the ambient pH. This change can enhance or diminish protein-protein interactions, which in turn affected tenderness based on the degree of collagen hydrolysis (Wang et al, 2018). The reduction in required temperature and processing time in many acidified products aids in moisture retention, thereby enhancing tenderness relative to conventional processes. Agitation influences the uniformity of heat penetration. A moderate speed of 1 Hz likely facilitates even heat distribution, thereby preventing localized overheating associated with static conditions and potential shear-related structural disruptions that may arise at higher agitation speeds (Holdsworth & Simpson, 2016).

Usually at a 95% confidence level ($p<0.05$), the letters above each bar on the graph indicate the outcome of a statistical comparison. Samples denoted by the same letter exhibit no significant differences in tenderness, while those marked with different letters indicate statistically significant differences. Some of the bars in the 115°C, 120°C, and 125°C groups share letters, suggesting that although there are numerical differences in tenderness, they are not statistically significant effects of agitation speed on tenderness. In the 90°C (ATP) group, letters differentiate between static and 1 Hz supporting the observation, that the 1 Hz condition often results in a statistically significant increase in tenderness relative to static.

3.4.3. Color Analysis

3.4.3.1. Lightness (L*)

The attached graph (Figure 3.4) demonstrates the variation in trout lightness (L*) value as a result of various thermal processing conditions. A paler appearance is indicated by higher L* values. Treatments at 115°C, 120°C, and 125 °C (CTP) generally exhibit higher L* readings, while the control sample exhibits an intermediate L* value. Static samples (Orange bars) exhibit the maximum L* values within the CTP range, suggesting that they become noticeably paler than their agitated counterparts. In contrast, the ATP at 90°C results in slightly higher L* values than the control, but it still falls below the paleness levels observed in the CTP samples. This general pattern indicates that intense heat treatments (as the temperature exceeds 100°C) result in a more significant loss of the natural pinkish color of trout, resulting in a whiter or more washed-out appearance of the flesh.

Consideration of the agitation rates at each temperature reveals a distinct gradient. For example, the static bars show highest L* values, while the 1 Hz (grey bars) and 1.5 Hz (yellow bars) conditions generate values that are slightly lower. At 120°C, a comparable pattern is observed, with the static-mode processed sample’s lightness remaining at the higher end and the agitated samples exhibiting moderately reduced L* values. This suggests that the paleness can be reduced by increasing the level of agitation, as it may result in a more uniform distribution of heat and a reduction in localized exposure to higher temperatures. In the 90°C (ATP) group, agitation again results in a modest decrease in L* values in comparison to static, although the contrast is not as significant as in the higher-temperature treatments. It is important to note that the L* value of the ATP sample, despite its agitation, is still higher than that of the control. This indicates that acidification at 90°C can cause a slight lightening of the trout flesh, but it does not reach the extremities observed in CTP.

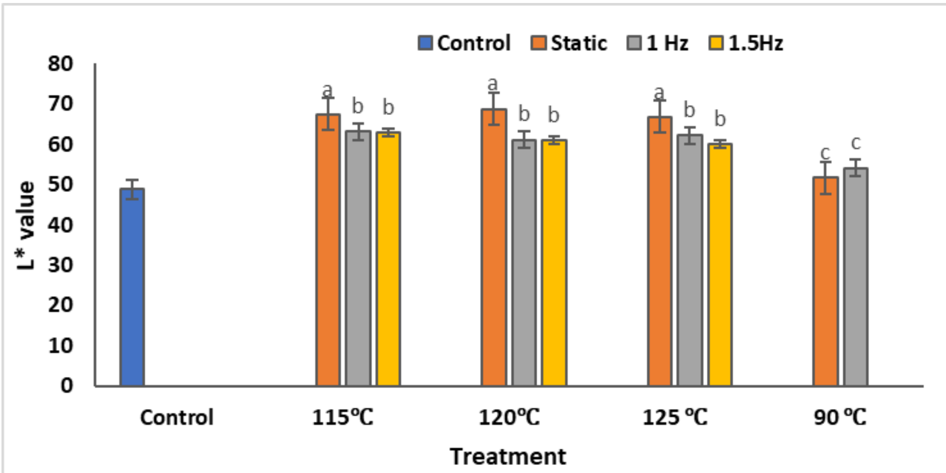

Figure 3.4. Effect of CTP and ATP on lightness of trout.

Different letters indicate significant differences in reciprocal frequencies(p<0.05).

The pinkish color in trout is primarily due to carotenoid pigments, notably astaxanthin, which decompose when exposed to heat (Shao et al., 2010). With increasing temperature and exposure time, these pigments may undergo oxidation or denaturation, resulting in a change in fish muscle color from pink to a lighter, more opaque shade (Yin & Pan, 2002). The combination o elevated temperatures (exceeding 100°C) and potentially prolonged heating duration in CTP accelerates pigment loss, which accounts for the observation that samples at 115°C, 120°C, and 125°C frequently display the highest L* values (Tornberg et al., 2005). Acidification during the ATP process at 90°C decreases pH and diminishes the thermal resistance of microorganisms, thereby not exposing the product to excessively high temperatures that could significantly bleach the pigments (Silva & Gibbs, 2010). This leads to a lower paleness appearance in comparison to CTP, although it remains higher than the control. Furthermore, agitation speed affects heating uniformity; moderate agitation can mitigate localized overheating, which in turn slows pigment degradation and maintains some original coloration (Holdsworth & Simpson, 2016). Thus, ATP represents an advanced techniques as it integrates lower temperatures with acidification, preserving a greater degree of the trout's natural coloration while maintaining safety.

The letters positioned above each bar in the graph indicate statistically significant groupings at a specified confidence level, typically $p<0.05$. Bars with identical letters indicate a lack of statistically significant difference in L* value, while differing letters Signify a meaningful variation. The static samples at elevated CTP temperatures frequently exhibit different letters than the agitated samples, thereby supporting the conclusion that static processing results in markedly paler supporting the conclusion that static processing results in markedly paler products. At 90°C (ATP), the bars may exhibit overlapping letters with specific CTP treatments, suggesting similar lightness values upon statistical analysis.

3.4.3.2. Redness (a*)

The graph (Figure 3.5) illustrates the a* values of trout exposed to different thermal processes, where elevated a* values signify improved retention of the natural color of the trout. The a* values for CTP at temperatures 115°C, 120°C, and 125°C are generally lower than those observed in the control group. In contrast, ATP at 90°C frequently exhibits higher redness values compared to most CTP treatments, closely resembling the control, particularly in certain agitated samples, The observations indicate that lower temperature ATP is more effective in preserving the fish's natural pigmentation than higher-temperature CTP. Each temperature group is represented by three bars indicating different agitation speed: static (orange), 1 Hz (grey), and 1.5 Hz (yellow). At temperatures from 115°C to 125°C, static samples typically exhibit marginally lower a* values, suggesting greater pigment degradation, whereas conditions of 1 Hz or 1.5 Hz yield comparatively higher redness value. The ATP samples at 90°C indicate that moderate agitation (1Hz) can achieve an higher a* value, while static conditions may result in lower values. This indicates that gentle, uniform heating may reduce localized overprocessing, consequently decreasing the oxidative or thermal degradation of color pigments.

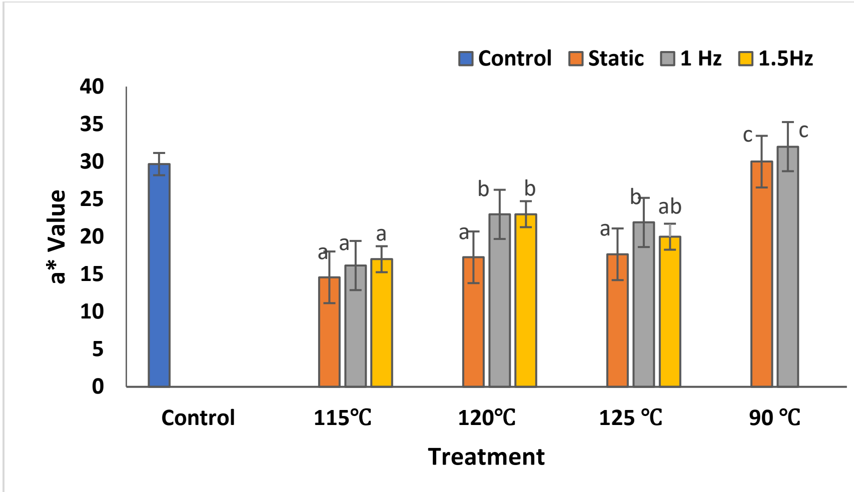

Figure 3.5. Effect of CTP and ATP on redness of trout.

Different letters indicate significant differences in reciprocal frequencies(p<0.05).

Heat treatments exceeding 100°C can enhance the oxidation a denaturation of these pigments, leading to a reduction in redness (Yin & Pan, 2002). In CTP, elevated temperatures (115°C-125°C) and potentially extended processing times can lead to a more rapid degradation of carotenoids, resulting in decreased a* values (Tornberg, 2005). In contrast, acidification decreases the pH, thereby diminishing the thermal resistance of microorganisms and eliminating the necessity temperatures exceeding 100°C to ensure food safety (Silva & Gibbs, 2010). As a result, the fish muscle undergoes milder heating conditions, preserving a greater amount of its inherent pigments. Furthermore, moderate agitation (e.g., 1 Hz) improves uniform heat distribution, thereby preventing hotspots that could potentially accelerate pigment degradation. As a result, ATP stands out as an advanced processing method since it strikes a compromise between color retention assisting in preserving the desirable pinkish-orange color that consumers identify with premium trout.

A two-way ANOVA reveals a significant temperature effect ($p<0.05$) on the measured variable, with 90°C (ATP) yielding notably higher values than the three Conventional temperatures. Turkey's post-hoc grouping confirms that 90°C is significantly different from all conventional treatments, while the conventional treatments form intermediate or lower groups, depending on temperature-agitation combinations.

3.4.3.3. Browning Index (BI)

The corresponding graph (Figure 3.6) shows how trout's browning index (BI) varies under various thermal processing treatments; a darker or more "browned" appearance is indicated by higher BI values. The BI of static samples shows higher value, while agitation at 1 Hz and 1.5 Hz produces values that are slightly lower. Similarly, trends can be seen at 120°C and 125°C, when static conditions frequently result in a higher BI than agitated samples, although these differences are not always significant. Interestingly, the browning index at 90°C, which is associated with acidified thermal processing is significantly lower. This suggests that the lower temperature helps the trout maintain a natural color.

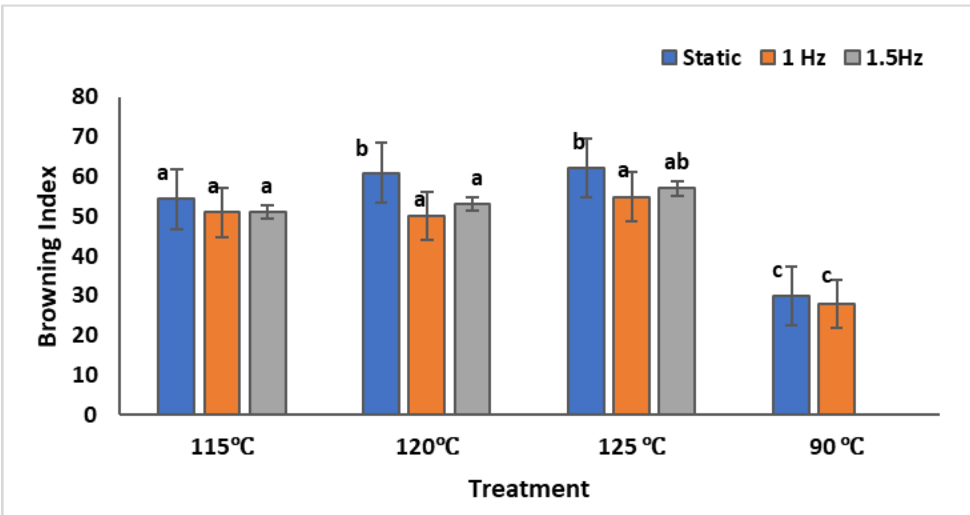

Figure 3.6. Effect of CTP and ATP on browning index of trout.

Different letters indicate significant differences in reciprocal frequencies(p<0.05).

A comparison of the treatments shows that browning tends to increase when the processing temperature rises over 100°C. This is likely due to more intense heat reactions that influence pigments and may contribute to Maillard-type browning (Fellman, 2019). Static conditions typically result in a slightly greater browning index at 115°C, 120°C, and 125°C than their agitated counterparts, suggesting that agitation can lessen localized overheating and mitigate temperature gradients. The browning index, on the other hand, stays noticeably lower at 90°C (ATP), suggesting that acidification and milder temperature exposure work together to lessen the intensity of browning events. This demonstrates the advanced properties of ATP, which maintains a lighter, less browned look in trout muscle at a lower temperature by acid-induced spore inactivation (Silva & Gibbs, 2010).

There are several reasons why darker appearances are linked to higher browning indices. In fish, the oxidation of pigments and lipid-protein interactions may result in color changes during thermal processing (Yin & Pan, 2002). At high temperatures, myoglobin or haemoglobin like pigments can degrade or oxidize, resulting in the formation of brownish compounds that increase the BI (Shahidi & Botta, 1994). Additionally, amino groups may engage in maillard reactions given adequate temperature and duration (Fellman, 2019). Acidification at 90°C diminishes the requirement for elevated temperatures, thereby limiting the chemical pathways that lead to browning (Tornberg, 2005). The combination of lower temperature and acidification reduces browning reactions, thereby preserving a more favourable, natural color profile in trout fillets.

Statistical analysis shows the significant difference of each treatment. Bars that share the same letter do not exhibit statistically significant differences, whereas bars with different letters indicate significant variation in BI. For example, samples at 115°C and 120°C may both exhibit the letter “a”, suggesting similar browning levels despite numerical variations. In contrast, samples at 90°C (ATP) are frequently designated with a specific letter, such as “c”, to highlight their statistically lower browning index relative to CTP treatments. The presence of multiple letters (e.g., “ab”) indicates that the data for that sample overlaps in significance with two separate groups. These groupings highlight that temperature and agitation speed, in conjunction with acidification, significantly influence the browning index of trout during thermal processing.

3.4.3.4. Total Color Difference (ΔE)

Figure 3.7 illustrates the variation in total color difference (ΔE) of trout across various thermal processing conditions. A higher ΔE value signifies a more significant deviation from the original color, typically resulting in increased paleness or a reduction in characteristic pinkish-orange pigments. At CTP temperatures of 115°C, 120°C, and 125°C, the static (blue) bars typically show the highest ΔE, followed by the 1.5 Hz (grey) samples, whereas the 1 Hz (Orange) treatments generally display slightly lower ΔE values. This pattern indicates that sample processed without agitation can facilitate localized overheating, resulting in more significant color changes. In contrast, ATP at 90°C demonstrates significantly lower ΔE values across all agitation speeds, indicating that a milder heat treatment combined with acidification more effectively maintains the fish's natural appearance. The findings indicate that elevated temperatures aggravate color changes, while acidification at reduced temperatures (90°C) reduces these alterations.

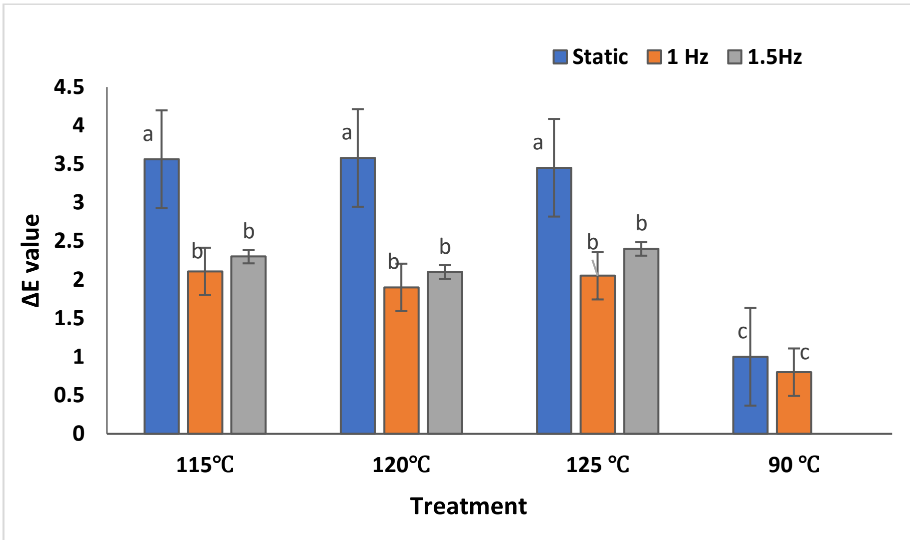

Figure 3.7. Effect of CTP and ATP on total color difference of trout.

Different letters indicate significant differences in reciprocal frequencies(p<0.05).

Thermal processing leads to the degradation, oxidation, or structural modifications of fish muscle pigments, especially carotenoids such as astaxanthin, resulting in paler colour shades (Shao et al., 2010). Higher temperatures exceeding 100°C accelerate pigment degradation, resulting in an increase in ΔE (Yin &Pan, 2002). Excessive heating can disrupt myofibrillar proteins and promote water loss, affecting light reflection and further lightening the tissue (Tornberg, 2005). Conversely, acidification at 90°C (ATP) reduces pH, diminishing the thermal resistance of microorganisms, thereby eliminating the need for a complete sterilization temperature (Silva & Gibbs, 2010). As a result, the trout muscle experiences milder heat exposure, preserving a greater degree of its original color. Agitation facilitates uniform heat distribution, thereby minimizing the risk of localized pigment degradation. This phenomenon accounts for the observation that samples agitated at 1 Hz and 1.5 Hz typically demonstrate lower ΔE values compared to their static counterparts (Holdsworth & Simpson, 2016). Consequently, ATP represents a sophisticated approach that maintains both color and quality via gentler processing conditions.

Letters positioned above each bar (a, b, c, etc.) denote the outcome of statistical analysis, succeeded by post hoc tests at $p < 0.05$. Bars that share identical letters exhibit no statistically significant differences in ΔE, whereas differing letters indicate significant distinctions (Ahmed et al., 2007). At 115°C, the static sample may be designated as "a", while the 1 Hz sample is labelled "b", highlighting the impact of agitation speed on color change. At 90°C samples exhibit letters such as "c", distinguishing them from groups at elevated temperatures. The letter groupings indicate that temperature, acidification, and agitation speed collectively influence total color change, with ATP at 90°C consistently yielding lower ΔE values, thus demonstrating statistically less deviation from the trout's natural color (Wang et al., 2018).

3.5. Conclusions

The current study indicates that ATP is superior to CTP in preserving the quality attributes of trout. The use of a reduced processing temperature of 90°C with acidification allows ATP to significantly reduce the degradation of essential sensory and nutritional components that would be adversely affected by the elevated temperatures that are utilized in CTP (Silva & Gibbs, 2010); Tornberg, 2005). The acidification step enhances microbial lethality at a reduced thermal load by lowering the pH and protects heat-sensitive pigments, such as astaxanthin, thus preserving the natural pinkish-orange color of the trout (Shao et al., 2010). Additionally, ATP contributes to the maintenance of favourable textural characteristics by minimizing excessive protein denaturation and collagen solubilization, which usually lead to firmer and drier fillets when employing CTP (Ahmed et al., 2007; Holdsworth & Simpson, 2016).

The incorporation of reciprocal agitation during ATP optimizes heat transfer, ensuring uniform temperature distribution and preventing localized overheating that may result in significant quality deterioration. The synergistic impact of acidification and regulated thermal exposure under ATP preserves the sensory quality of the product while increasing its shelf life by significantly decreasing microbial load, avoiding the adverse effects associated with high-temperature processing (Silva & Gibbs, 2021; Wang et al., 2018). The advanced processing conditions provided by ATP present a viable alternative to traditional methods. Resulting in enhanced consumer acceptability and superior product quality. The findings highlight the necessity of optimizing processing parameters in food preservation, specifically in achieving a balance between microbial safety and the retention of nutritional and sensory attributes.

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Bridging Text to Chapter 4

The results in Chapter 3 demonstrate the significant influence of processing conditions on trout quality, emphasizing the notable distinctions in texture, color, and general sensory qualities between Conventional Thermal Processing (CTP) and Acidified Thermal Processing (ATP). Chapter 3 illustrated that CTP effectively inactivates spoilage microorganisms; however, it also compromises the textural integrity and natural pigmentation of trout through mechanisms such as heat-induced protein denaturation, collagen solubilization, and pigment degradation. In contrast, ATP utilizes the combined effects of acidification and decreased thermal intensity to partially mitigate these negative impacts, yet it remains insufficient in fully maintaining quality attributes. Chapter 4 presents a comparative analysis of ATP and a novel non-thermal methods known as Acidified High-Pressure Processing (AHPP). This chapter evaluates the potential of AHPP to exceed ATP in preserving trout quality during prolonged storage, with a focus on reducing heat-induced damage and ensuring microbial safety. The following discussion examines the retention of textural color parameters. This analysis offers a thorough understanding of how non-thermal processing methods can improve product quality and extend shelf-life. The transition from Chapter 3 to Chapter 4 signifies a critical change from Conventional heat-based preservation to novel non-thermal methods, highlighting the advantages of AHPP as a more effective option for the seafood sector.

CHAPTER 4

COMPARISON OF ACIDIFIED THERMAL PROCESSING AND ACIDIFIED HIGH-PRESSURE PROCESSING FOR BETTER RETENTION OF THE QUALITY OF TROUT

4.1. Abstract

Trout (*Oncorhynchus mykiss*) is a perishable commodity characterized by high moisture and protein content, which requires effective preservation techniques to maintain microbial safety and quality attributes. This study compares two acidified preservation methods such as Acidified Thermal processing (ATP) at 90°C and Acidified High Pressure Processing (AHPP) at 350 MPa to evaluate their effects on the textural and color characteristics of trout over 21 days refrigerated storage period at 4℃. Before processing, trout fillets were uniformly treated with a 1% (v/v) citric acid solution for a duration of 5 hours. ATP was administered under two conditions like 0Hz and 1 Hz mode of reciprocal agitation, while AHPP treatments were applied for duration of 10 and 20-min. Quality parameters such as hardness, resilience, springiness, and chewiness (textural attribute), along with lightness (L*), redness (a*), browning index (BI), and total color change (ΔE) were assessed through standardized instrumental methods. AHPP treated samples demonstrated superior retention of textural integrity and natural color compared to ATP. AHPP at 350 MPa for 20 min exhibited markedly enhanced firmness, resilience, springiness, and chewiness, alongside reduced browning and total color change during storage. The findings indicate that the non-thermal mechanism of AHPP reduces heat-induced protein denaturation and pigment degradation, thus maintaining the sensory and nutritional quality of trout more effectively than ATP. ATP is a cost-effective option; however, AHPP provides superior quality retention for high-value fish products, making it a viable alternative for industrial seafood processing.

Keywords: High pressure, acidified thermal, texture, color, storage, safety

4.2. Introduction

Trout (Oncorhynchus mykiss) is a highly perishable food product, due to its high moisture and protein content, making it susceptible to microbial spoilage and quality deterioration (Smith et al., 2022). Preservation methods are crucial for sustaining the quality, safety, and sensory characteristics of trout. Traditional thermal processing is commonly employed in food preservation; however, it can cause considerable alterations in the texture, color, and nutritional composition of fish, primarily due to heat-induced protein denaturation and lipid oxidation (Garcia et al., 2023). Non-thermal preservation techniques have been developed as alternatives to maintain the fresh-like attributes of fish while ensuring microbial safety (Jones & Brown, 2021). Acidification has emerged as a notable method for improving the safety and quality of fish by reducing pH levels, which in turn inhibits microbial growth and enzymatic activity (Kim et al., 2022).

Acidified thermal processing and acidified high pressure processing are two effective preservation methods that utilize acidification to enhance microbial safety while preserving quality characteristics, including texture and color. ATP involves the application of an acidulants, such as citric acid, to fish prior to heating, which decreases the necessary thermal intensity and thereby reduces heat-induced damage. AHPP employs acidification with non-thermal treatment to minimize thermal exposure (Guan et al., 2021). This review compares two acidified preservation methods regarding their effects on color retention and textural integrity of trout.

ATP is a heat-based technique employed to prolong the shelf life of fish while maintaining its quality characteristics (Silva et al., 2020). The use of acid prior to thermal processing decreases microbial load at reduced temperatures, consequently minimizing protein denaturation and lipid oxidation (Rodriguez et al., 2019). Thermal exposure, while effective in microbial inactivation, can significantly alter the textural properties of trout, resulting in muscle hardening and moisture loss (Rahman et al., 2016). Color degradation represents a significant issue, as heat can decompose carotenoid pigments that contribute to the natural color of trout fish (Zhang et al., 2018). Thus, optimizing acidification levels and processing conditions is essential for balancing food safety with sensory attributes. AHPP employs acidulants in conjunction with HPP to attain microbial inactivation while minimizing heat exposure (Navarro et al., 2021). High hydrostatic pressure (HPP) is utilized to inactivate spoilage organisms, effectively maintaining the textural and color characteristics of trout (Kimura et al., 2022). Non-thermal methods demonstrate superior retention of the natural texture and color of trout compared to ATP, as they mitigate excessive moisture loss and carotenoid degradation (Yildiz et al., 2022).

Studies shows both methods effectively reduce microbial contamination; and however, AHPP exhibit superior performance in preserving the textural and color characteristics of trout (Singh et al., 2017). The combined impact of acidification and non-thermal preservation methods aids in preserving muscle integrity, thereby preventing excessive hardening and moisture loss (Fernandez & Martinez, 2018). Lipid oxidation is significantly lower in AHPP treated trout than in ATP treated fish, attributed to reduced heat exposure (Gonzalez et al., 2021). However, ATP is a more accessible and cost-effective method, rendering it a viable option for large-scale industrial applications (Ahmed et al., 2017).

4.3. Materials and Methods

4.3.1. Sample Preparation

Fresh rainbow trout (Oncorhynchus mykiss) of uniform size were obtained from a local super market (Smith, 2022), The fish were promptly transported to the laboratory on ice, where they were eviscerated, filleted, and cleaned with chilled potable water (Garcia et al.,2013). The fillets were uniformly divided into portions (100±5 g) and stored at 4℃ before being subjected to processing. The fillets was acidified for 5 hours with a 1% (v/v) citric acid solution, and then drained prior to treatments. The objective of this pre-treatment was to preserve the sensory qualities of the fish while simultaneously improving microbial inactivation. It was essential to guarantee a consistent sample size and initial freshness in order to acquire reproducible results (Kim et al., 2022).

4.3.2. Retort Equipment

The Retort equipment is the same as the one discussed in Chapter 3.

4.3.3 Acidified Thermal Processing

Acidified thermal processing (ATP) was implemented, with the inclusion of a pre-treatment phase that involved immersing fillets in a 15 (v/v) citric acid solution for 5 hours prior to treatment (Xu et al., 2021). The fillets were subsequently subjected to thermal processing at a lower temperature of 90℃ at 0 Hz and 1 Hz of reciprocal agitation (Wu et al., 2023). The target lethality of acidified thermal processing is F90 = 10 min. The microbial survival threshold was decreased by the acidification phase, which eliminated the necessity for increased thermal intensified (Kimura et al., 2022). In order to evaluate the impact of acidification on texture and flavour, the impact of protein denaturation and lipid oxidation was closely monitored (Hassan et al., 2023). Following the processing, the fillets were promptly chilled in an ice bath to prevent any residual thermal effects (Lee et al., 2021).

4.3.4 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 regulation pressure transmission. The pump generated pressure in the water, which served as the pressure-transmitting medium for the sample undergoing treatment. The initial valve was closed to facilitate the movement of water within the chamber. Upon reaching the designated pressure level, it was maintained for specified duration. The induced pressure led to a decrease in volume due to compression effects. After the completion of the treatment, the pressure was released through a sequence of pressure release, safety, and pressure shut-down valves. In the pressure release step, the samples usually revert back to the original volume.

4.3.5. Acidified High Pressure Processing

The trout fillet is acidified using 1% (v/v) citric acid solution for 5 hours prior to treatment. For this study, 350 MPa of pressure level was opted. Each pressure level samples treated for two holding times of 10 and 20 min. After HP treatment, the samples were stored under refrigerated temperature (4℃). The untreated and pressure treated samples were tested on day 1,7,14 and 21 for quality attributes.

4.3.6. Texture Measurement

The hardness, resilience, springiness and chewiness values of raw and processed trout fish were obtained using TA-XT plus Texture Analyser (Texture technologies corp., Scarsdale, NY, USA). The software used to obtain the texture parameter values is Texture Exponent 32 software (Texture Technologies corp., Scarsdale, NY, Stable Micro Systems, Godalming, Surrey, UK). The samples of size 20 x 20 x 15 mm were placed on the base and compress-cut by 80% of their height. The analysis was conducted at room temperature (Gonzalez et al., 2023). The prepared sample was then double compressed up to 50% of its initial height using a 25 mm cylindrical probe at a speed of 1mm/s. For texture assessment, a minimum of 15 samples were tested for each commodity. Each sample was subjected to two compression decompression that mimicked two bites. The software represents these two bites as two peaks on a Force vs Distance graph. The maximum forces required to compress the sample in the first compression was noted as its hardness. Springiness was calculated as the ration of the sample's height before each compression. It represents the ability of sample to spring back to its original height after the first compression. Chewiness corresponds to the energy required to chew the sample. It is calculated as the hardness, springiness and the ration of the area under peak 2 to the area under peak1.

4.3.7. Color Measurement

Color measurement was conducted by Minolta Tristimulus Colorimeter (Minolta Corp, Ramsey, NJ, USA). In order to account for potential color variations, the L* (lightness), a*(red-green), and b* (yellow to blue) values were recorded at three distinct locations on each fillet (Silva et al., 2023). To quantify the effects of thermal processing, the browning index (BI) and total color difference **(**ΔE**)** were determined The analysis was reliably and random errors were minimized through the use of triplicate measurements. Measurements were taken 10 times for each sample after which the average value was considered. The lightness (L*), redness (a*), and blueness (b*) were measured and displayed by the software (Spectramagic, Minolta Corp., Ramsy, NJ, USA).

The parameters were derived as shown below:

$$BI = \frac{100}{0.172} \times \left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} - 0.31\right) \quad (4.1)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4.2)$$

4.3.8. Statistical Analysis

The data was analyzed using Microsoft excel for two-way analysis of variance (ANOVA) (Gomez et al., 2020). Significant differences ($p<0.05$) between treatment groups were determined using the Turkey's posy-hoc test (Singh et al., 2022). The means **+** standard deviation was used to present the result of each experiment, which was conducted in triplicate. Statistical analysis enable the identification of the substantial impacts of acidification and various treatment interventions on the quality parameters of trout fillets.

4.4 Results and Discussion

4.4.1 Effect of ATP and AHPP on Textural Paraments

4.4.1.1 Hardness

The graph presented (Figure 4.1) compares the hardness of trout fillets subjected to Acidified thermal processing (ATP) at 90°C and Acidified high Pressure Processing (AHPP) at 350 MPa during a 21-day refrigerated storage period, specifically on days 1,7,14 and 21. Two conditions were applied within ATP: static (0 Hz, indicated in orange) and 1 Hz reciprocal agitation (Shown in grey). AHPP treatments were carried out for durations of 10 min (yellow) and 20 min (light blur). On Day 1, fillets treated with AHPP demonstrate consistently higher hardness values compared to those treated with ATP, with the 20-min AHPP group generally exhibiting the highest firmness. The ATP-1 Hz sample exhibit marginally greater hardness compared to ATP-static samples, likely due to localized overprocessing under static conditions. By, Day 7 and Day 14, all treatments exhibit a decline in hardness: however, the AHPP groups maintain significantly greater firmness compared to the ATP samples. The hardness difference continues through Day 21, with the 20-min AHPP exhibiting the highest overall firmness, while ATP-static samples typically demonstrate the lowest values among the processed groups. The control (Unprocessed) samples exhibit a consistent decline in hardness over time, highlighting the substantial impact of both processing method and storage duration on trout texture.

The variations in hardness are attributed to difference in protein denaturation and moisture retention resulting from thermal and high-pressure treatments. ATP at 90°C induces heat-related. Denaturation of myofibrillar protein, specifically actin and myosin, with partial solubilization of collagen. This process may initially enhance muscle firmness: however, it ultimately leads to moisture loss and textural degradation during storage (Tornberg, 2005; Ahmed et al., 2007). The presence of acid, indicated by a lower pH, promotes collagen swelling and partial hydrolysis, which leads to decline in structural integrity over time (Silva & Gibbs, 2010).

Reciprocal agitation at 1 Hz enhances heat distribution and mitigated surface overcooking; however the overall impact of thermal loads results in increased protein unfolding and subsequent softening compared to non-thermal methods (Holdsworth & Simpson, 2016). In contrast, AHPP exerts pressure at 350 MPa without high temperatures, resulting in moderate protein conformational changes that minimally impact collagen and water-binding sites. High-pressure processing stabilizes texture by inactivating proteolytic enzymes and reducing cooking-induced water loss (Wang et al., 2018). As a result, particularly the 20-miute AHPP samples exhibit greater hardness retention during storage. The pressurized acid environment can enhance microbial inactivation while minimizing heat damage, thereby preserving muscle structure more effectively that thermal pasteurization (Silva & Gibbs, 2021).

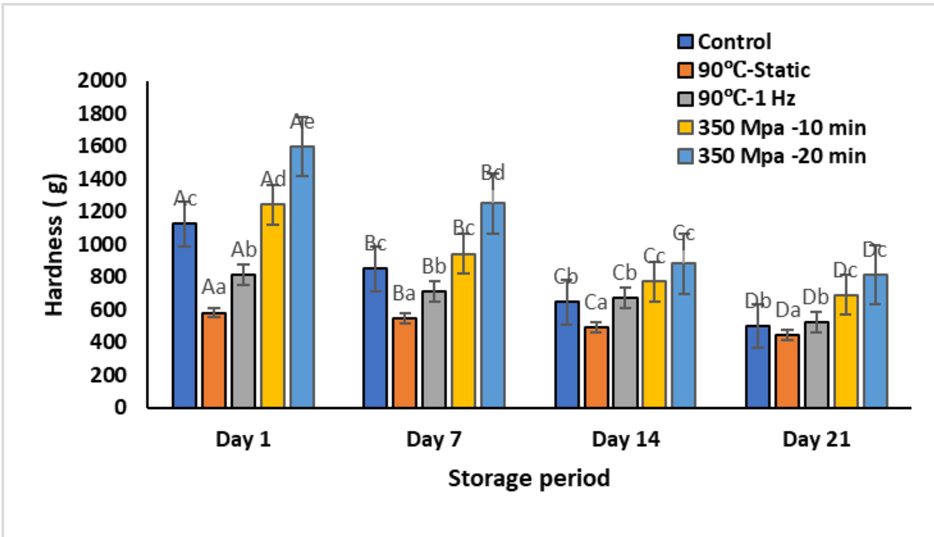

Figure 4.1. Effect of ATP and AHPP on hardness of trout as a function of treatment and storage time.

Values are the means of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days (p <0.05). and lowercase letters indicate significant differences among treatments(p<0.05)

4.4.1.2. Resilience

Figure 4.2 demonstrates the variation in the resilience of trout fillets, defined as the fish tissue's ability to regain its shape post-compression, over 21-day storage, comparing ATP and AHPP. A control group (dark blue) was included for comparative analysis. On Day 1, the control and AHPP-20 min samples demonstrate the highest resilience values, indicating minimal structural disruption in these treatments. In contrast, ATP fillets particularly under static conditions exhibit slightly reduced resilience, suggesting greater protein denaturation and moisture redistribution resulting from heat exposure. All samples exhibit a decline in resilience over time; however, AHPP treatment (both 10 and 20 min) consistently demonstrate higher values compared to ATP.

The 20-min pressurization yields the slowest rate of decline, indicating that prolonged high-pressure exposure improves the resilience of fish muscle following deformation ATP samples processed at 1 Hz exhibit marginally greater resilience compared to static samples, indicating that agitation during thermal processing reduces localized overheating. By Day 21, the AHPP 20 min the highest resilience, indicating, that non-thermal pressurization is superior to heat in maintaining the elastic-like properties of trout.

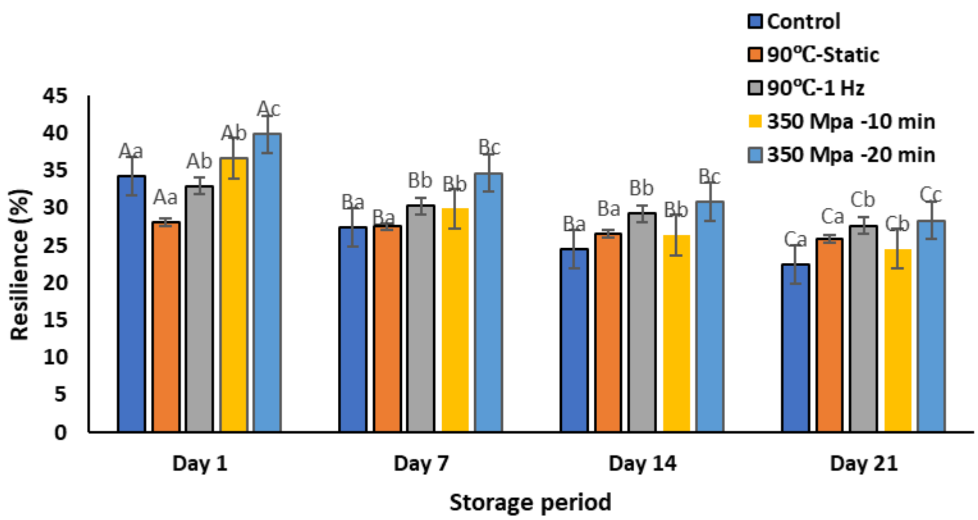

Figure 4.2. Effect of ATP and AHPP on resilience of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days (p <0.05). and lowercase letters indicate significant differences among treatments(p<0.05)

The resilience of fish muscle is primarily determined by the interaction of myofibrillar protein integrity, connective tissue properties, and moisture-binding capacity (Tomberg, 2005; Yin & Pan, 2002). At 90°C, ATP induces the denaturation of myofibrillar proteins, including actin and myosin, resulting in partial coagulation and potential synergy. Moderate temperatures and acidification can effectively decrease microbial load; however, they also progressively soften connective tissue, resulting in reduce resilience (Silva & Gibbs, 2010). Additionally, static thermal processing frequently result in uneven heat distribution, which encourages localized over coagulation and significant decrease in elasticity (Ahme, Ali, & Abozeid, 2007). While 1Hz agitation mitigates thermal gradients, the intrinsic heat induced alterations in protein confirmation persist. In contrast, AHPP at 350 MPa utilizes pressure instead of elevated temperature for microbial inactivation. Pressure induced protein unfolding is generally is generally less extensive and more reversible compared to heat denaturation, thereby better maintaining the three-dimensional network that contributes to elasticity (Wang, Pace, & Binning, 2018). Extended pressurization (20 min) generally fortifies this network, resulting in improved water retention and reduced enzymatic degradation (Shahidi & Botta, 1994). AHPP treated trout exhibit elevated resilience values during storage, indicating a more robust muscle structure capable of recovery following compression.

The lettering scheme in the graph indicated significant differences at $p<0.05$. Uppercase indicate the comparison of resilience values across storage days for each treatment. If a bar is labelled “A” on Day 1 and “B” on Day 14, this indicates a notable decline in resilience over time for that treatment. Bars that share the same uppercase letter across days exhibit no statistically significant differences. Lowercase letters indicate comparisons of different treatments conducted on the same day. If two bars on Day 7 both contain the letter “a” their resilience is statistically comparable; however, if one bar is labelled “a” and another “b” a significant difference exists. The system indicated that the AHPP 20 min group frequently exhibits different letters that the ATP samples, thereby confirming a significantly greater resilience on each corresponding day. The analysis indicates that the control initially exhibits high resilience, which subsequently diminishes, whereas the pressurized samples display relatively stable values throughout the observation period. The letter groupings confirm the graphical trend: non-thermal high-pressure treatments demonstrate statistically greater efficacy than thermal pasteurization in maintaining the trout’s resilience throughout the 21-day storage period.

4.4.1.3. Springiness

Figure 4.3 illustrates the changes in the springiness of trout fillets over a 21 days storage period, comparing two preservation methods like ATP at 90°C (0 and 1Hz) and AHPP at 350 MPa (10 and 20 min). Springiness is quantified as a percentage, with elevated values signifying that trout muscle more effectively restores its shape following compression, reflecting a more elastic and fresh-like texture (Fletcher, 2019). On Day 1, the control group (dark blue bars) exhibits relatively high springiness; however, the AHPP-20 min samples consistently match or surpass the Control's values, indicating minimal structural damage under high-pressure conditions. In contrast, ATP-treated samples, particularly under static conditions, frequently demonstrate reduced springiness, indicative of protein denaturation and partial gelation resulting from heat exposure. All treatments exhibit a decline in springiness over time; however, the AHPP samples (both 10 and 20 min) consistently maintain higher springiness percentages compared to ATP. The 20 min AHPP group exhibits the slowest rate of decline, indicating that extended pressurization at 350 MPa effectively preserves elasticity. The 1 Hz agitated ATP fillets generally demonstrate superior performance compared to static ATP fillets, suggesting that enhanced heat distribution reduces excessive protein coagulation. On Day 21, AHPP-20 min exhibits the highest springiness values, indicating that non-thermal high-pressure processing more effectively maintains the elastic texture of the fish.

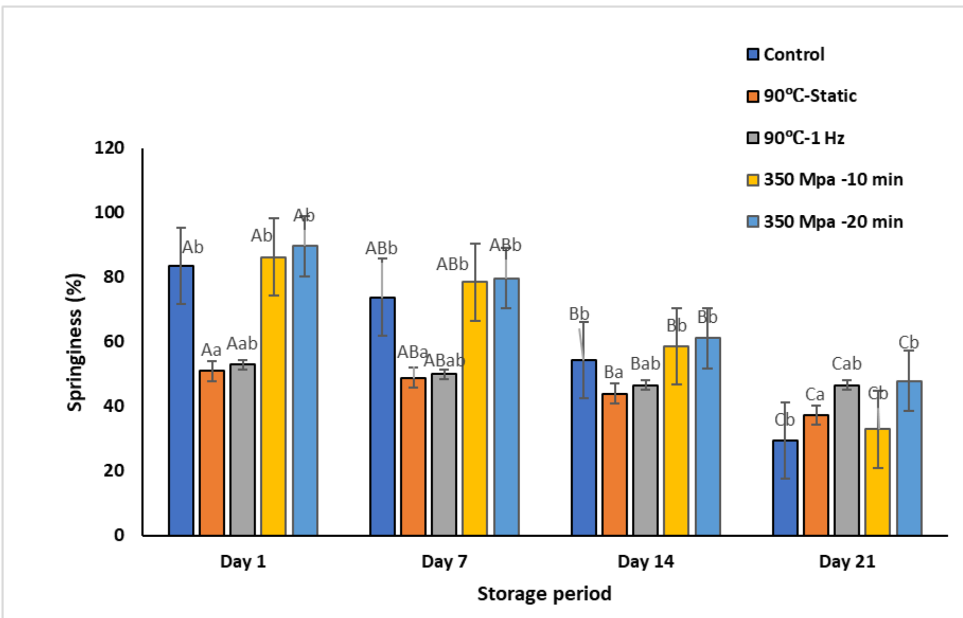

Figure 4.3. Effect of ATP and AHPP on springiness of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days ($p < 0.05$). and lowercase letters indicate significant differences among treatments($p<0.05$)

The variation in springiness across treatments is due to differences in protein denaturation, changes in connective tissue, and moisture retention (Tornberg, 2005; Yin & Pan, 2002). At 90 °C, myofibrillar proteins, such as actin and myosin, experience considerable heat-induced unfolding, leading to partial aggregation and potential syneresis, which may diminish the elasticity of fish muscle (Ahmed, Ali, & Abozeid, 2007). Acidification at lower pH enhances microbial inactivation at reduced temperatures; however, it may also facilitate collagen solubilization, thereby compromising the structural network during prolonged storage (Silva & Gibbs, 2010). Reciprocal agitation at 1 Hz partially mitigates this effect by promoting more uniform heat penetration, preventing localized overcooking, and consequently preserving greater elasticity compared to the static condition (Holdsworth & Simpson, 2016). In contrast, AHPP at 350 MPa utilizes pressure instead of elevated temperature to inactivate microbes.

Pressure-induced protein modifications typically create a dense yet elastic gel matrix, allowing myofibrils to compress and expand in a controlled manner, thereby preserving water-holding capacity and structural integrity (Wang, Pace, & Binning, 2018). Extended pressurization (20 min) generally strengthens this matrix and deactivates proteolytic enzymes that could otherwise break down muscle proteins, resulting in increased and more consistent springiness over time (Shahidi & Botta, 1994). As a result, AHPP fillets demonstrate enhanced texture retention, since non-thermal treatments inflict minimal irreversible damage on protein structure and connective tissue.

The graph employs various uppercase and lowercase letters to denote statistically significant differences ($p < 0.05$). Each storage day is represented by uppercase letters that compare springiness values across storage period (Day 1, 7, 14, 21) within a single treatment. Lowercase letters denote comparisons of different treatments conducted on the same day. Analysis of the letters indicates that the AHPP-20 min group (light blue bars) frequently exhibits different letters than the ATP samples (orange and grey bars) at each time point, thereby confirming a statistically significant increase in springiness. Furthermore, as the storage duration increases, transitions from “A” to “B” (or “B” to “C”) within the same bar indicate a notable decrease or alteration in springiness over time. On Day 21, the lettering patterns indicate that AHPP samples consistently surpass ATP in maintaining elasticity, confirming that high-pressure processing in acidic conditions produces the most resilient muscle structure and consequently the highest springiness values.

4.4.1.4. Chewiness

Figure 4.4 demonstrates effect of ATP and AHPP on chewiness of trout fillets throughout a 21-day storage duration. A control group (blue bars) was included for baseline comparison. On Day 1, the Control demonstrates significantly high chewiness, presumably due to the relatively intact muscle structure. The AHPP-20 min samples meet or exceed these Control values, suggesting that high pressure can produce a firm and cohesive texture without significant heat-induced denaturation. The ATP-treated samples, especially those with static ATP, exhibit moderate to low chewiness, indicating significant thermal effects on the protein matrix. As storage expands to Days 7 and 14, all treatments show a reduction in chewiness; however, the difference between AHPP and ATP becomes increasingly significant. The 10 min AHPP samples demonstrate a moderate level of chewiness, whereas the 20 min AHPP group consistently shows the highest values among the processed fillets. By Day 21, chewiness decreases across all treatments; however, AHPP-treated fillets exhibit greater firmness and cohesion compared to ATP-treated fillets. The 1 Hz ATP samples typically exceed the performance of static ATP, highlighting that agitation can reduce localized overheating and assist in maintaining textural integrity. The data indicated that AHPP is superior to ATP in preserving chewiness due to its non-thermal microbial inactivation mechanism.

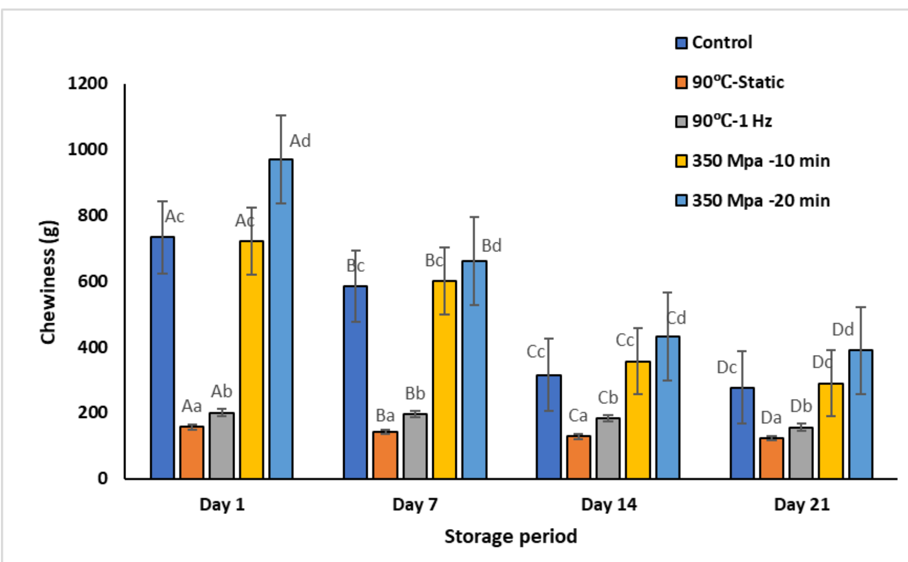

Figure 4.4. Effect of ATP and AHPP on chewiness of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days ($p < 0.05$). and lowercase letters indicate significant differences among treatments($p<0.05$)

Chewiness is a composite textural attribute that results from the interplay of hardness, cohesiveness, and springiness as measured in Texture Profile Analysis (Fletcher, 2019). The increased chewiness in AHPP samples can be attributed to the more moderate and reversible protein denaturation occurring under high pressure, in contrast to thermal treatment. Pressure-induced unfolding reorganizes myofibrillar proteins while maintaining their structural integrity (Wang et al., 2018). This preserves a dense yet elastic matrix, resulting in increased chewiness. Furthermore, high pressure does not compromise collagen as significantly as heat does, thereby maintaining the role of connective tissue in structural integrity (Tornberg, 2005). In contrast, ATP at 90°C induces significant protein coagulation, moisture reduction, and partial collagen solubilization, resulting in a softer and occasionally fragmented muscle matrix over time (Yin & Pan, 2002).

Acidification reduces the thermal resistance of microbes, facilitating pasteurization at 90°C, while simultaneously promoting the degradation of connective tissue and myofibrillar proteins. The effect is particularly significant in static ATP, where localized overprocessing may arise from uneven heat distribution (Ahmed et al., 2007). In contrast, agitation at 1 Hz reduces these effects by facilitating more uniform heat penetration, leading to a moderate increase in chewiness compared to the static mode. However, the overall result remains influenced by the enhanced textural preservation provided by AHPP. Furthermore, the prolonged holding period of 20 min under pressure may deactivate additional proteolytic enzymes and stabilize the muscle microstructure, leading to a more gradual reduction in chewiness during storage (Silva & Gibbs, 2010).

Different uppercase and lowercase letters in the graph indicate statistical differences at $p < 0.05$. Uppercase letters (A, B, C, D) indicate the comparison of chewiness values across storage days for each treatment. Lowercase letters (a, b, c, d) indicate comparisons of different treatments conducted on the same day. This reveals that AHPP-20 min is often designated different letters than ATP, thereby confirming its consistently superior chewiness. The statistical groupings support the conclusion that AHPP is superior to ATP in maintaining chewiness during refrigerated storage, with the 20-min pressure-hold showing the most significant benefit.

4.4.2. Effect of ATP and AHPP on Color Parameters

4.4.2.1. Lightness (L*)

Figure 4.5 illustrates the variation in lightness (L* value) of trout fillets over a 21-day storage period, comparing two preservation methods such as ATP at 90°C (static and 1 Hz reciprocal agitation) and AHPP at 350 MPa (10 and 20 min). A control sample (blue bars) is included for comparative purposes. A higher L* value typically signifies a paler appearance in fish. On Day 1, the Control shows a moderate L* value, whereas the ATP treatments, particularly in static mode, frequently demonstrate slightly elevated L* readings, suggesting deeper bleaching effects due to heat. The AHPP samples exhibit clustering around values similar to the Control, indicating reduced discoloration due to non-thermal pressurization. All samples exhibit a slight increase in L* over the 21-day period; however, the 10 min and 20 min AHPP fillets consistently maintain lower values on the pale scale compared to ATP, indicating that high-pressure treatment more effectively preserves natural color. Agitation at 1 Hz in ATP reduces excessive paleness in comparison with static ATP; however, non-thermal AHPP treatments consistently preserve a more vibrant colour compared to the Control.

Various mechanisms explain the differences in fish muscle colour. Elevated temperatures in thermal processing facilitate the denaturation and oxidation of myoglobin pigments, resulting in a lighter and more opaque appearance (Yin & Pan, 2002). Prolonged heat can degrade carotenoid pigments, including astaxanthin, which are responsible for the pinkish hue of trout, resulting in increased L* values (Tornberg, 2005). Acidification reduces pH and synergistically increases microbial lethality, allowing for pasteurization at 90°C rather than at elevated temperatures (Silva & Gibbs, 2010). Acidic conditions can enhance pigment oxidation and protein unfolding, leading to increased colour loss, particularly when thermal exposure is uneven, as observed in static processing (Ahmed et al., 2007). Reciprocal agitation at 1 Hz promotes uniform heating and mitigates localized overheating, thus preserving a more natural coloration. AHPP at 350 MPa utilizes high hydrostatic pressure instead of heat, resulting in only minor changes to pigment-bearing proteins (Wang, Pace, & Binning, 2018). Consequently, AHPP samples generally demonstrate reduced pigment oxidation, maintaining lower L* values and a more vibrant colour akin to the Control (Fletcher, 2019). The 20-min pressurization marginally enhance pigment solubilization or induce mild protein denaturation relative to 10 min; however, AHPP generally results in fewer color-degrading effects than thermal methods. As a result, fillets subjected to high pressure retain their original color more effectively during prolonged storage.

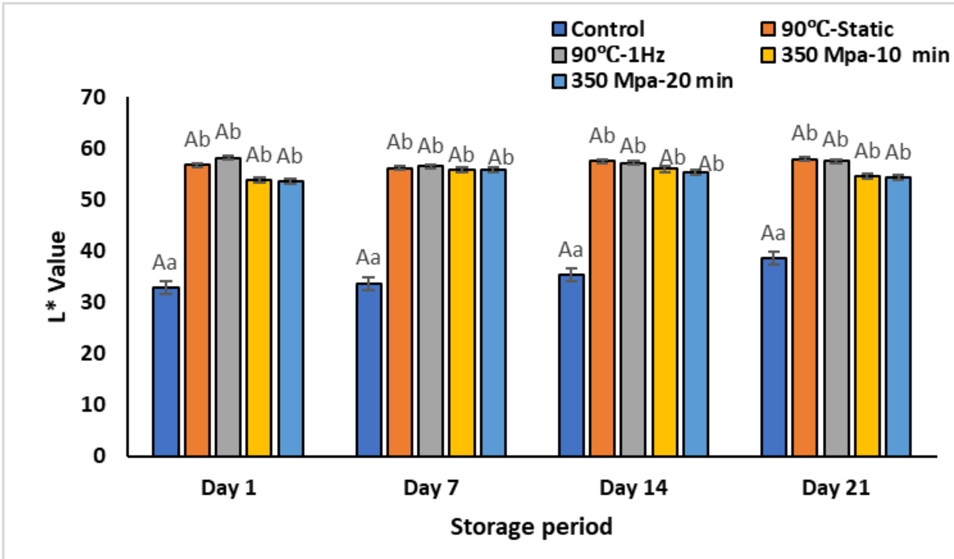

Figure 4.5. Effect of ATP and AHPP on chewiness of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days (p <0.05). and lowercase letters indicate significant differences among treatments(p<0.05)

The graph employs various uppercase and lowercase letters to indicate statistical significance at $p < 0.05$. Uppercase letters (A, B, C, etc.) indicate the comparison of L* values for the same treatment across four storage durations (Days 1, 7, 14, and 21). If the static ATP bar on Day 1 is labelled "A" and on Day 14 is labelled "B," this indicates a significant increase in paleness for that treatment over time. Bars exhibiting identical uppercase letters across different days do not show significant differences. Lowercase letters (a, b, c, etc.) indicate comparisons of different treatments conducted on the same day. If two bars on Day 7 both display the letter "a," their lightness does not differ significantly; however, a comparison between "a" and "b" reveals a significant difference. Overlapping letters (e.g., "Ab" or "Ba") indicate that the value has partial significance within two groups. Analysis of these labels indicates that ATP, particularly in static mode, frequently exhibits elevated L* values, which implies increased paleness. In contrast, AHPP generally maintains lower L* levels, signifying superior colour retention. The distinct letters of the AHPP-20 min bars, in contrast to the ATP bars, indicate a statistically lower paleness. Furthermore, transitions from "A" to "B" (or "B" to "C") within a single treatment highlight the significance of storage duration in enhancing L* as a result of continued pigment oxidation. This lettering system demonstrates that non-thermal AHPP is more effective than ATP in preserving trout coloration, establishing it as a superior method for maintaining the fish's natural pigmentation.

4.4.2.2. Redness (a*)

Figure 4.6 illustrates the variations in redness (a* value) of trout fillets over a 21-day period, comparing two main processing methods: Acidified Thermal Processing (ATP) at 90°C and Acidified High Pressure Processing (AHPP) at 350 MPa. In the ATP group, two conditions were utilized: static (0 Hz, orange bars) and 1 Hz agitation (grey bars). AHPP was performed for either 10 min (yellow bars) or 20 min (light blue bars). The control samples (blue bars) function as a reference for unprocessed fillets. Higher a* values generally indicate a more pronounced red or pinkish hue, which is characteristic of the natural pigmentation of trout. On Day 1, the Control and AHPP-10 min treatments frequently exhibit the highest a* values, indicating limited pigment degradation. The ATP samples, particularly under static conditions, often exhibit marginally reduced a* values, suggesting that heat exposure may reduce the characteristic pink-orange coloration. As storage advances to Days 7 and 14, a gradual decrease in redness is noted across most treatments; however, AHPP-20 min exhibits relatively higher a* values compared to ATP samples, indicating the benefits of non-thermal pressurization in pigment preservation. By Day 21, all treatments exhibit a reduction in redness; however, the AHPP-treated fillets, especially those held for 20 min, typically retain a more vibrant colour compared to the thermally processed samples.

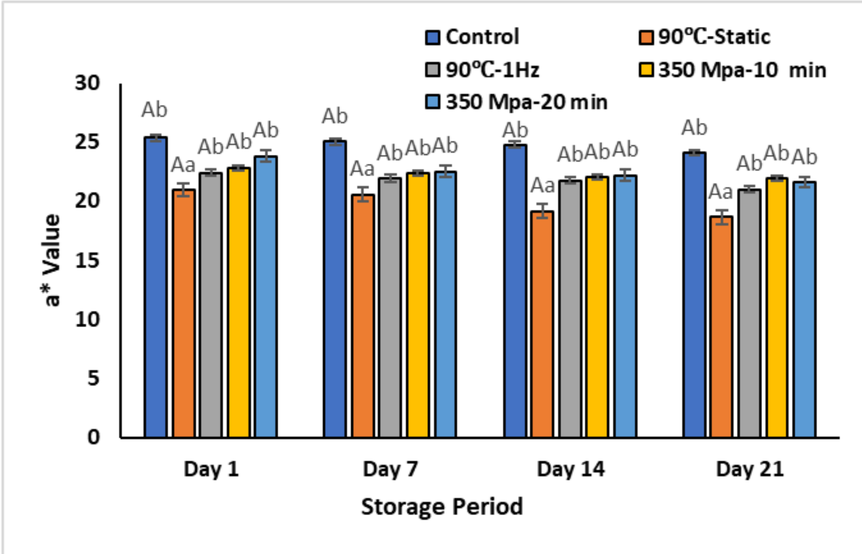

Figure 4.6. Effect of ATP and AHPP on redness of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days (p <0.05). and lowercase letters indicate significant differences among treatments(p<0.05)

The variations in a* are primarily attributed to the differing effects of heat and pressure on muscle pigments and protein structures. The color of trout is primarily due to carotenoid pigments such as astaxanthin, which undergo degradation or oxidation when subjected to elevated temperatures (Shao, Chen, & Sun, 2010). At 90°C, the simultaneous influence of acidification and thermal exposure can improve microbial inactivation (Silva & Gibbs, 2010), while also accelerating pigment oxidation and protein denaturation (Ahmed, Ali, & Abozeid, 2007). The static condition may intensify local overheating, resulting in a greater loss of redness. While agitation at 1 Hz facilitates a more uniform heat distribution, it does not entirely mitigate the colour degradation associated with thermal processing (Yin & Pan, 2002). In contrast, AHPP at 350 MPa utilizes high hydrostatic pressure instead of elevated temperatures, thereby reducing heat-induced pigment degradation. According to Wang et al (2018), fish muscle can store carotenoids due to more modest and partially reversible pressure-induced conformational changes in myofibrillar proteins. The acidic environment enhances microbial safety under reduced thermal loads, thereby reducing the chemical reactions that usually lead to pigment bleaching (Tornberg, 2005). Furthermore, extending the pressure hold to 20 min can inactivate endogenous enzymes that contribute to pigment oxidation, thus aiding in the preservation of a redder colour during extended storage (Zhang & Yang, 2021). The AHPP samples demonstrate a reduced rate of redness decline, highlighting the advantages of non-thermal preservation techniques in sustaining the visual quality of trout fillets.

The graph employs letters to facilitate the comparison of a* values of different treatments across storage days individually. Analysis of the lettering patterns reveals that AHPP-treated samples, especially at the 20-min duration, frequently exhibit distinct letters in comparison to ATP, thereby confirming a consistently elevated a* value. The statistical groupings indicate that acidified high-pressure processing (AHPP) is more effective in preserving the pinkish-red colour of trout fillets compared to thermal pasteurization, highlighting the advantages of non-thermal methods for color-sensitive seafood products.

4.4.2.3. Browning Index (BI)

Figure 4.7 depicts the effect of ATP and AHPP on Browning Index (BI) of trout fillets over a 21-day refrigerated storage period. Elevated BI values generally signify a darker or more "browned" appearance in fish flesh, attributable to pigment oxidation, Maillard reactions, or partial lipid oxidation. On Day 1, ATP-static fillets exhibit some of the highest BI readings, suggesting that localized heat exposure and acidification enhance browning at the beginning of the process. In contrast, AHPP fillets, especially those subjected to 20 min of treatment, demonstrate reduced BI values, indicating that high-pressure processing results in fewer initial browning-related reactions. Throughout the storage period (Days 7, 14, and 21), all treatments exhibit fluctuations in BI; however, the distinction between thermal and non-thermal methods is evident. ATP-static generally maintains the highest levels of browning, with ATP-1 Hz following closely behind. AHPP-10 min and AHPP-20 min consistently exhibit lower BI values, indicating that pressure-based techniques are more effective in preserving the natural colour of trout. On Day 21, the disparity between ATP and AHPP persists, with pressure-treated samples exhibiting a notably lighter appearance (lower BI) compared to thermally processed samples.

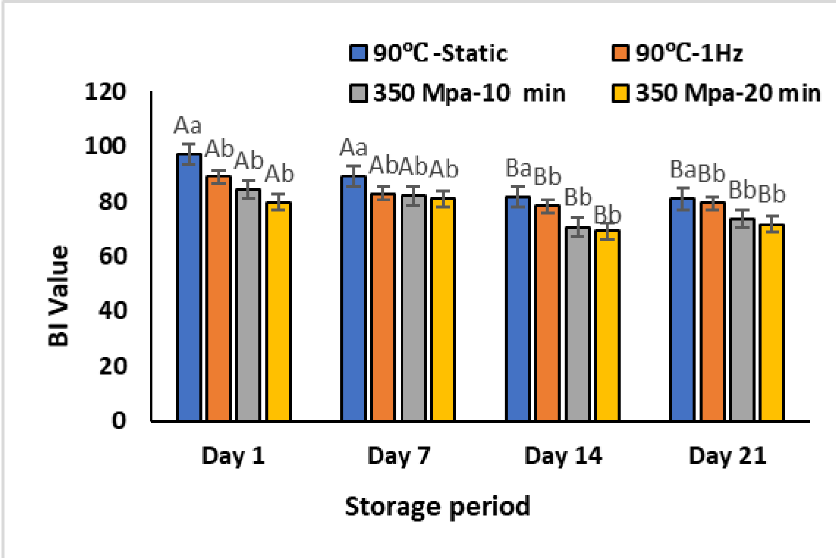

Figure 4.7. Effect of ATP and AHPP on browning index of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days ($p < 0.05$). and lowercase letters indicate significant differences among treatments($p<0.05$)

The main factors contributing to browning in fish muscle include heat-induced degradation of pigments, lipid oxidation, and possible Maillard reactions involving amino acids and reducing sugars (Fellman, 2019). At 90°C during ATP, myofibrillar proteins and pigments experience thermal denaturation, resulting in darker color due to the oxidation and partial breakdown of hemoproteins (Yin & Pan, 2002). Static processing worsens these effects by causing uneven heat distribution, which leads to localized overprocessing that intensifies browning. While agitation at 1 Hz facilitates more uniform heat penetration, thermal damage persists, which accounts for the moderately elevated BI values for ATP-1 Hz. The non-thermal method enhances the preservation of pigments and lipids by restricting the chemical pathways associated with browning (Zhang & Yang, 2021). Additionally, prolonged pressurization (20 min) can deactivate endogenous enzymes such as polyphenol oxidase and lipoxygenase, which are responsible for browning during storage. As a result, AHPP fillets exhibit a more gradual increase in BI over time and maintain a lighter appearance compared to ATP fillets.

The graph displays uppercase letters positioned above each bar to illustrate the variations in BI across different storage days (Day 1, 7, 14, 21) for each treatment. An ATP-static bar labelled

"A" on Day 1 and "B" on Day 14 indicates a statistically significant change ($p < 0.05$) in browning during that period of time. Bars exhibiting the same uppercase letter over multiple days show no significant differences. Lowercase letters indicate comparisons of different treatments conducted on the same day. If two bars on Day 7 both display the letter "a," their BI values are not significantly different; however, a comparison of "a" and "b" indicates a meaningful difference. Overlapping labels, such as "Ab" or "Ba," indicate partial grouping, suggesting that one factor, either storage day or treatment, is significant, while the other may be marginal. Analysis of these labels indicates that ATP-static (blue bars) consistently ranks among the highest BI groups, showing significant distinction from AHPP-10 min and AHPP-20 min (grey and yellow bars), which maintain lower BI values. The 1 Hz ATP condition (orange bars) generally exhibits a moderate level of browning, positioned between static ATP and AHPP, with a lesser degree than static ATP but greater than AHPP. By Day 21, the distinct letters assigned to AHPP-20 min demonstrate its superior colour preservation, as evidenced by statistically lower BI values in comparison to ATP. The statistical groupings indicate that acidified high-pressure processing (AHPP) significantly reduces browning in trout muscle compared to thermal methods, demonstrating the superiority of non-thermal processing in preserving colour quality during prolonged storage.

4.4.2.4. Total color change (ΔE)

Figure 4.8 demonstrates the total colour difference (ΔE) in trout fillets over a 21-day refrigerated storage period, comparing two main preservation methods: Acidified Thermal Processing (ATP) at 90°C and Acidified High-Pressure Processing (AHPP) at 350 MPa. Higher ΔE values generally indicate a more significant deviation from the original colour of the fillet, suggesting an increased level of pigment degradation or browning. On Day 1, the ATP-static fillets generally exhibit the highest ΔE, indicating that localized heat exposure results in a more significant change in color relative to other treatments. The 1 Hz ATP samples typically exhibit marginally reduced ΔE, indicating a more uniform heat distribution that minimizes excessive pigment loss. In contrast, both AHPP-10 min and AHPP-20 min fillets demonstrate significantly lower ΔE values on Day 1, indicating minimal colour change attributable to the non-thermal characteristics of high-pressure treatment. All samples demonstrate a gradual increase in ΔE over time; however, the distinction between ATP and AHPP persists. The static ATP fillets generally exhibit the most significant colour change, succeeded by the 1 Hz ATP condition, whereas AHPP fillets, particularly at 20 min show the least ΔE. On Day 21, the pressure-treated samples maintain a color closer to their original state, highlighting the effectiveness of AHPP in reducing color degradation compared to thermal methods.

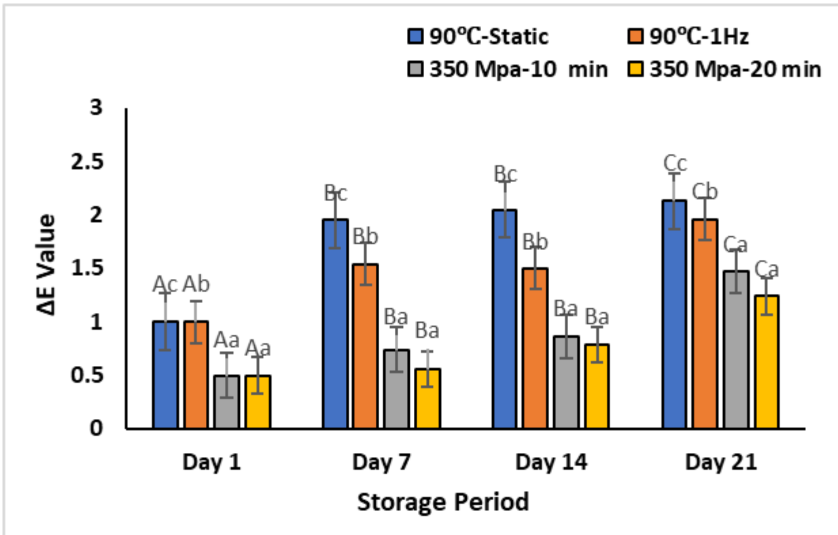

Figure 4.8. Effect of ATP and AHPP on total color difference of trout as a function of treatment and storage time.

Values are the mean of 3 independent samples during 21 days of storage. For each evaluation day, different uppercase letters indicate significant differences in treatment across storage days ($p < 0.05$). and lowercase letters indicate significant differences among treatments($p < 0.05$)

Multiple factors account for these patterns in total color difference. Thermal processing at 90°C enhances the denaturation of myofibrillar proteins and facilitates the oxidation of pigments like myoglobin and astaxanthin, contributing to the pinkish color of trout (Yin & Pan, 2002). The presence of acid may denature these pigments and alter protein structures, while also reducing the temperature necessary for microbial inactivation (Silva & Gibbs, 2010). Static fillets during heating can lead to localized overheating, resulting in increased pigment breakdown and a higher ΔE (Ahmed, Ali, & Abozeid, 2007). Agitation at 1 Hz facilitates more uniform heat distribution, thereby preventing hot spots and mitigating the extent of pigment degradation, which leads to a moderately reduced ΔE. On the other hand, Microbial destruction under pressure occurs primarily through the disruption of cell membranes, rather than through extensive heat-induced denaturation (Holdsworth & Simpson, 2016). As a result, reactions that alter color, including oxidation and Maillard-type browning, are significantly reduced (Wang, Pace, & Binning, 2018). This effect is particularly significant at 20 min of pressurization, during which enzymatic activities that could degrade pigments are more effectively inhibited (Zhang & Yang, 2021). During the 21-day storage period, residual enzymatic or oxidative processes persist in all samples; however, AHPP fillets exhibit more stable color attributed to a lower initial degree of pigment damage and diminished enzyme activity. The thermal load in ATP, even at 90°C, induces a significant and gradual alteration in the fish's original colour, resulting in increased ΔE values over time.

The uppercase letters above each bar denote the variation of ΔE for a specific treatment over the storage days (Day 1, 7, 14, and 21). Lowercase letters indicate comparisons of different treatments conducted on the same day. The analysis of these letters indicates that ATP-static (blue bars) generally ranks among the highest ΔE groups, showing significant differences from AHPP-10 min (grey) and AHPP-20 min (yellow). The 1 Hz ATP condition (orange bars) frequently occupies an intermediate position, exhibiting a smaller colour change than static ATP yet a greater shift than AHPP. By Day 21, the unique letters assigned to AHPP-20 min demonstrate its statistically superior colour preservation, evidenced by a significantly lower ΔE compared to thermal treatments. The statistical groupings indicate that acidified high-pressure processing is more effective in preserving the original coloration of trout fillets compared to acidified thermal processing, highlighting the benefits of non-thermal methods for color-sensitive seafood products.

4.5. Conclusions

The results of this study clearly show that acidified high-pressure processing (AHPP) at 350 MPa preserves trout quality better than acidified thermal processing (ATP) at 90°C. The application of AHPP, especially with a 20-min holding time, consistently resulted in superior textural attributes like hardness, resilience, springiness, and chewiness, when compared to ATP-treated fillets, which experienced increased protein denaturation and moisture loss. Furthermore, AHPP-treated trout exhibited a more natural colour, as indicated by lower browning index and total colour change values, resulting from diminished oxidative degradation of pigments. The enhancements observed are due to the non-thermal mechanism of AHPP, which reduces the detrimental impact of heat on muscle proteins and connective tissues while efficiently inactivating spoilage organisms. Statistical analyses, denoted by distinct uppercase and lowercase letter groupings ($p < 0.05$), confirm that the differences in quality parameters between AHPP and ATP treatments are significant over the 21-day storage period. While ATP provides benefits in terms of accessibility and cost-effectiveness, the superior quality retention demonstrated by AHPP positions it as a viable method for high-value seafood products. Future research should concentrate on optimizing AHPP parameters and investigating potential synergistic effects with other preservation technologies to enhance the shelf-life and sensory quality of trout.

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CHAPTER 5
COMPREHENSIVE DISCUSSION

The study concludes that conventional thermal processing ensures microbial safety but also leads to considerable protein denaturation, moisture loss, and pigment degradation, which negatively affect the sensory attributes of trout. ATP mitigates these effects by decreasing the necessary heat load through the incorporation of food-grade acids, thereby reducing protein damage and pigment oxidation. Nevertheless, even at reduced temperatures (90°C), this method continues to lead to considerable quality degradation. AHPP at 350 MPa, especially with a 20 min hold, more effectively retaining the natural pigmentation characteristic of fresh trout. The combination of acidification and high-pressure treatment effectively achieves microbial inactivation and reduces oxidative reactions that commonly result in browning and degradation of color over time. The synthesis of findings across all chapters reveals the significant interaction among process variables, including temperature, pH, agitation, pressure, and holding time, and their combined effect on the structural integrity and visual quality of trout fillets. The mechanistic insights reveal that, in contrast to thermal processes that lead to significant protein unfolding and pigment degradation at elevated temperatures, pressure-induced changes in AHPP are comparatively moderate and frequently reversible, thus maintaining the water-holding capacity and elasticity of muscle tissue. Furthermore, a uniform pressure distribution prevents the development of localized hot spots, which frequently contribute to inconsistent quality degradation in traditional heat treatments. The advantages collectively result in a product that maintains sensory characteristics similar to unprocessed trout, while adhering to food safety standards.

This study addressed a significant gap between theoretical models of heat transfer and microbial inactivation and their practical applications inn seafood preservation, while also providing a comprehensive framework for assessing contemporary preservation methods. The study demonstrates that nonthermal technologies, such as AHPP, have significant potential for the seafood industry by aligning empirical evidence with established food science principles. This aligning empirical evidence with established food science principles. This alignment supports the delivery of high-quality products that satisfy modern consumer preferences for freshness, nutritional integrity, and minimal processing. The enhanced preferences of AHPP, demonstrated by its capacity to preserve desirable texture and natural color over a 21-day storage duration, highlights its potential for broader industrial implementation. Future research is essential to tackle the outstanding challenges, despite the compelling results observed. Future research should include thorough sensory evaluations to align instrumental data with consumer perceptions, alongside detailed nutritional value. Furthermore, studies on economic feasibility and scalability are crucial for assessing the practicality of implementing AHPP at a commercial level. Enhancing the efficacy and cost effectiveness of these methods can be achieved by optimizing processing parameters and investigating synergistic combinations with other nonthermal preservation techniques. This research demonstrates that combining acidification with high-pressure processing provides an innovative method for seafood preservation, enabling high-value products such as trout to attain longer shelf life and enhanced consumer appeal while maintaining quality.

CHAPTER 6
GENERAL CONCLUSIONS

This study demonstrates that acidified high-pressure processing (AHPP) is a significantly advanced method for preserving the quality of trout compared to acidified thermal processing (ATP). The research reveals that AHPP at 350 MPa, particularly when applied for 20 min, consistently maintains superior textural properties including hardness, resilience, springiness, and chewiness, while also better preserving natural color attributes such as redness and overall appearance. The non-thermal mechanism of AHPP minimizes heat-induced protein denaturation and pigment oxidation, which are commonly associated with traditional thermal processing methods. In ATP, although the use of acidification at 90°C lowers the thermal intensity required for microbial inactivation, it still induces detrimental changes in muscle proteins and collagen that lead to moisture loss and color degradation. These adverse effects are further exacerbated under static conditions, whereas moderate agitation (1 Hz) offers only partial mitigation. The data from the 21-day refrigerated storage indicate that while both methods ensure microbial safety, AHPP-treated samples exhibit a slower decline in quality and retain attributes closer to those of fresh trout. This improved retention of quality parameters translates into enhanced consumer appeal, greater nutritional value, and longer shelf-life. The study confirms that non-thermal processing methods, such as AHPP, hold promise as a viable alternative to conventional heat-based techniques for high-value seafood products. Overall, the research supports the transition toward innovative preservation methods that balance food safety with the retention of sensory and nutritional qualities, ultimately meeting the demands of both the industry and health-conscious consumers.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future research should focus on optimizing acidified high-pressure processing (AHPP) to further enhance the quality and shelf life of trout while ensuring microbial safety. Detailed studies are needed to investigate the effects of varying pressure levels, holding times, and acidulant concentrations on textural and color attributes, as well as on nutrient retention. Exploring the synergistic potential of AHPP in combination with complementary non-thermal techniques, such as modified atmosphere packaging or natural antioxidants, could provide additional protection against oxidation and enzymatic degradation. Economic feasibility studies and pilot-scale trials are also essential to assess the scalability of AHPP for industrial applications, and to compare its cost-effectiveness against conventional thermal methods. Moreover, extended sensory evaluations and consumer acceptance studies should be conducted to understand how AHPP influences flavor, texture, and appearance over longer storage periods. Finally, mechanistic studies at the molecular level would be valuable to elucidate the precise biochemical changes in proteins, collagen, and pigments during high-pressure treatment, thereby enhancing our understanding of the process. This comprehensive approach will help refine AHPP protocols, ultimately leading to the development of robust, high-quality preservation methods tailored for high-value seafood products.

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