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Interactions Between High-Pressure Processing and Natural Antimicrobials in Ground White-Meat Chicken: An Analysis of Microbial, Textural Charactheristics and Physicochemical Changes

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INTERACTIONS BETWEEN HIGH-PRESSURE PROCESSING AND NATURAL ANTIMICROBIALS IN GROUND WHITE-MEAT CHICKEN: AN ANALYSIS OF MICROBIAL, TEXTURAL CHARACTHERISTICS AND PHYSICOCHEMICAL CHANGES.

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ABSTRACT

High Pressure processing is an innovative technique for preserving a wide variety of foods, including meat, and this method is increasingly used to satisfy consumer demand for quality food products. High-pressure treatment can impact, negatively or positively, various aspects of meat quality, including color, texture, protein denaturation, and microbial stability, particularly when food products are processed at ambient or low temperatures. By minimizing thermal exposure, appropriate high-pressure ranges help to prevent quality and nutrition degradation, reduce microbial contamination, and enhance tenderness.

The first objective of this research was to examine the influence of high-pressure processing and addition of natural antimicrobials on the safety and quality of ground chicken breasts, focusing on microbial aspects, lipid-oxidation, various texture attributes and color through different physicochemical analysis. Additionally, the second objective of this investigation was to explore quality attributes of cooked high-pressurized chicken patties incorporating natural antimicrobials through a sensory study involving untrained panelist, while compering results with the TPA analysis performed previously. To address this first objective, ground chicken breasts were processed with two different pressures treatments. Additionally, two natural antimicrobials were added: buffered concentrated vinegar with rosemary essential oil (V+REO) and a lemon juice-vinegar blend. Samples were inoculated with a non-pathogenic *E. coli* strain and individually packaged. The samples, with or without the antimicrobial additives, were refrigerated and then processed at either 300 MPa or 600 MPa. *E. coli* and *Pseudomonas spp*. counts were

performed, along with pH measurements, TBARS analysis, color, texture profile analysis (TPA) of raw samples, and TPA and yield of cooked samples. All samples treated at 600 MPa showed reductions in E. coli greater than 5 log cfu/g, while non-HPP samples saw more modest reductions, even in those treated with antimicrobials. Higher HPP treatments showed significant difference ($P \leq 0.05$) in color, primarily evidenced by an increase in whiteness.

To approach the second objective, a sensory study was conducted to investigate the acceptability and preference of nine chicken patties treatments, which involve combinations of three pressure conditions (0MPa, 300 MPa and 600 MPa) and two antimicrobials (Buffered concentrated vinegar with rosemary oil (V+REO) and a lemon-vinegar blend). A panel consisting of 63 untrained participants evaluated sensory attributes including juiciness, cohesiveness, tenderness, overall texture and color for each chicken patty cooked using sous vide and finished with surface searing. Panelists rated the chicken patties using a 9-point hedonic scale, with those processed at 600 MPa earning the lowest mean score. These results were significantly different ($P \leq 0.05$) compared to the samples processed at 300 MPa and those not processed using HPP. The samples processed at 300 MPa received significantly higher rankings ($P \leq 0.05$) compared to both the samples processed at 600 MPa and those processed without pressure. From this work, it is evident that high pressure processing can improve certain quality characteristics, however, pressures above 300 MPa can lead to undesirable changes. Further research is needed to explore various pressure levels and times to ensure adequate safety while maintaining quality characteristic.

DEDICATION

To my family and friends because they were always there at every step of this journey, even when the path seemed very difficult and impossible, they supported me and helped me find myself again. Truly, having them in my life is what helped me finish this stage of my life. Every day, I saw God through them, and for that, I thank Him for always helping me find the way and for helping me always to see something good in everything I experienced. Sometimes Clemson felt very lonely and other times I felt happy here, but thanks to life for every experience lived and for bringing me closer to a better version of myself.

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CHAPTER ONE

INTRODUCTION

To guarantee food safety, food is heated at elevated temperatures for fixed, often long times (Gómez, Janardhanan, Ibañez, & Beriain, 2020). Thermal processing can have several potential benefits- Preservation, development of taste and flavor and development of a desired material structure representing some of the more common potential benefits of food-processing operations such as canning, baking, pasteurization, drying, frying and others (Fryer & Robbins, 2005). Despite being a very effective, economical, and easily available technology, research has shown that there can be a loss of quality and nutritional characteristics when subjecting food to high temperatures (Téllez-Luis, Ramírez, Pérez-Lamela, Vázquez, & Simal-Gándara, 2001). It is for this reason that food technologists and food industries have developed alternative techniques that might have the possibility of reducing the microbial load while preserving nutritional and quality characteristics. These new processing methodologies align with the consumers' desires for food products that have an acceptable shelf life, are convenient to prepare and retain their nutritional value and flavor (Fryer & Robbins, 2005).

To ensure a food is "safe to eat" through thermal processing, first the food needs to reach a minimum temperature at every point in its geometry. The disadvantage with this procedure is that in the time required to reach that safe temperature in the center, the outside layers have been already heated for a considerable amount of time and may be overcooked, causing quality loss and dietary deficiency (Fryer & Robbins, 2005). That is

why in food industries is necessary to have complete knowledge of the complexity of food materials and their physical properties. Since high pressure is an isostatic process, the food is treated and preserved uniformly.

High pressure processing (HPP) is a technology defined as a non-thermal pasteurization that effectively inactivates foodborne pathogens in food, being independent of size and shape of food (Chuang, Sheen, Sommers, Zhou, & Sheen, 2020). In recent years, high pressure processing has been used commercially to extend storage life of a variety of products, such as milk, natural juices, meat, seafood and others (Liu, Betti, & Gänzle, 2012). High pressure has a long list of advantages, among which the most important is the way high pressure relates with food. High pressure does not break covalent bonds, maintaining the natural flavor of the products. Compared to traditional thermal processing, HPP operates at room temperature, which lowers energy consumption required for heating and subsequent cooling. Moreover, since the food is processed in its packaging, it avoids direct contact with processing equipment, preventing post-pasteurization contamination. The pressure transfer medium used in this method can also be recycled after processing. Due to its lower energy requirements, HPP technology is considered an environmentally friendly processing method (Srinivas, Madhu, Srinivas, & Jain, 2018).

Foodborne pathogens cause illnesses and deaths, making this a public health and safety problem. For this reason, food technologists have been continually interested in techniques which could reduce microbial loads while preserving quality characteristics. The relationship between microbiota and high-pressure treatment is very complex and

depends on many factors, including type of bacteria, food matrix, presence of spores, cell wall thickness, etc. Radovčić et al., (2019), Thames & Theradiyil Sukumaran (2020) and Mor-Mur & Yuste (2010) have shown that bacterial resistance to high pressure is particularly variable even among strains of the same species. In the case of bacteria, the inhibition or inactivation depends on multiple factors correlating to the gram type, physiological and morphology state, strain particularities, presence of absence of spores, etc. This microbial inactivation is caused by denaturation of proteins, enzyme inactivation and membrane damage (Radovčić et al., 2019). High-pressure processing (HPP) has emerged as the most commercially viable non-thermal processing technique to address those needs and has been shown to inactivate vegetative pathogenic and spoilage microorganisms in countless studies on a wide variety of products.

However, high-pressure has limitations that are important to mention. Food enzymes and spores are very resistant, so it requires an extremely high pressure to kill or inhibit those spores, exposing the color and texture of the food to degradation. One of the most critical effects of high pressure is the enzymatic and oxidative deterioration of certain food components. High pressure in the food industry is usually used in ranges of 200-800 MPa, whether it is for improving functional and rheological properties (typically in the lower end of the range) or to cause inactivation of microorganisms (most commonly toward the higher end). It is fundamental to study different pressure ranges and processing times to determine the one that offers the best results for safety and quality of the food product.

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CHAPTER TWO

LITERATURE REVIEW

FOOD PROCESSING TREATMENTS.

Food processing treatments are necessary to maintain food safety and. These processes are known as food preservation techniques, which their major emphasis is to preserve or extend shelf life by controlling chemical, biological and physical activities (Abera, 2019). After Amit, Uddin Rahman, Islam, & Khan (2017), food processing may be classified according to the mechanisms used to achieve them.

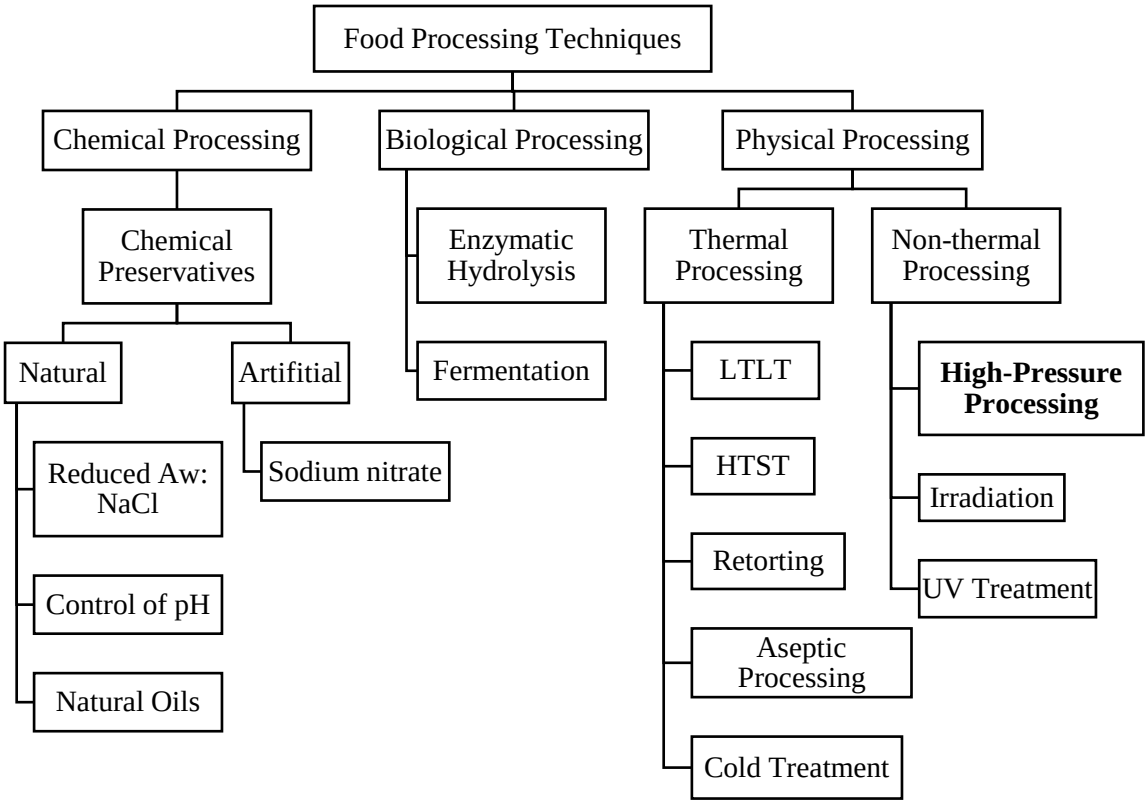

Figure 2.1. Classification of food preservation methods (Amit, Uddin, Rahman, Islam, & Khan, 2017).

Chemical Processing

All components of food are chemicals- proteins, carbohydrates, fats, minerals, and water are constituted by chemical elements bonded in various ways that create and define their function in food products. However, because of the natural variation, it is often necessary to adjust the composition to provide a product of consistent quality. In addition to those chemicals that are part of food products, other chemicals can be incorporated during the growing, storage, or processing (National Research Council, 1973). The effectiveness of chemical processing on enhancing food safety depends on the concentration and selectivity of chemical reagents, the type of spoilage mechanisms, the microorganism(s) of concern, and physical and chemical characteristics of the food. Although chemical compounds can preserve or improve food quality, these need to be monitored and regulated by different rules and government authorities (Amit, Uddin, Rahman, Islam, & Khan, 2017).

Chemical Preservatives

Preservatives are substances capable of inhibiting, retarding, or arresting the growth of microorganisms. Food preservatives extend the shelf life of various food products by retarding degradation caused by microorganisms, thereby maintaining color, texture, and flavor. Food preservatives can be classified into natural and artificial categories. Animals, plants and microorganisms contain various chemicals that have the potential to preserve foods, these natural preservatives also function as antioxidants, flavorings, and antibacterial agents. Artificial preservatives, on the other hand, are

produced industrially and can be classified as antimicrobial antioxidant and anti-enzymatic.

- **Reduced Aw: NaCl**

Sodium chloride is the most common salting agent used in the food industry. NaCl is essential for achieving favorable texture, characteristic flavors, and extended shelf life in traditional salted products. It binds to muscle myofibrils, enhancing water binding and retention, which promotes tenderness and juiciness, resulting in an ideal texture. NaCl influence on biochemical reactions such as proteolysis, lipolysis and lipid oxidation provide a highlight of flavors. Furthermore, NaCl effectively hinders the proliferation of pathogenic bacteria by reducing water activity (Aw) and suppressing biogenic amine formation, extending shelf life (Jia et al., 2024).

Most foods have a water activity (Aw) above 0,95, which provides sufficient moisture and available water to support the growth of bacteria, yeasts, and molds. Each microorganism has a specific Aw threshold below which it cannot grow, form spores, or produce toxins. Understanding the minimum Aw values that permit microbial growth is crucial for ensuring microbial stability in food. For instance, common spoilage bacteria are inhibited at an Aw around 0.97, clostridial pathogens at 0.94, and most Bacillus species at 0.93. Staphylococcus aureus is notably Aw-tolerant, growing aerobically at an Aw of 0.86 and anaerobically at 0.91 (Barnard, 2023). Reducing the amount of available moisture can inhibit the growth of these microorganisms. Bacterial cells transfer nutrients and waste materials out through the cell wall, requiring materials to be in soluble form to permeate the cell wall. In food, some water is strongly bound to specific sites, such as the hydroxyl

groups of polysaccharides and the carbonyl and amino groups of proteins and does not act as a solvent. Fully dehydrated foods have an Aw of about om3 to control microbial growth and other reactions that affect food quality. The solute used to control Aw also affects the minimum Aw for growth, with certain solutes like glycerol having lower inhibitory effects due to their ability to permeate cell membranes, other solutes like NaCl and sucrose primarily lower Aw, while substances like ethanol exert antibacterial effects through other mechanisms (FDA, 2014).

- **Control of pH: Use of citrus juices.**

Several recent studies have reported different alternatives using citrus juices as antimicrobials solutions instead of synthetic ingredients like calcium sulfate, ammonium phosphate, sodium nitrate, red #3, propyl gallate and others (FDA, 2024). The use of natural additives can have a great impact on flavor, odor profile and color, as well can be used as preservatives. The antimicrobial properties of citrus juices have been acknowledged for centuries. With the rising demand driven by changes in legislations, consumer preferences, and the increasing prevalence of antibiotic-resistant pathogens, there is a need to find alternatives to chemical-based bactericides. Citrus oils, which are generally recognized as safe, are suitable for use in food and have demonstrated inhibitory effects on Gram-positive and Gram-negative bacteria (Fisher & Phillips, 2008). Consumers are leaning towards more natural foods that can be considered as health-promoting food. Health-promoting food protects consumers against malnutrition in all its forms, from cardiovascular diseases to other conditions. Citrus juices reduced pH value, changing the auspicious environment of microorganism. They also increase tenderness in

meat products and improved microbiological quality and sensory properties (Augustyńska-Prejsnar, Kačániová, Ormian, Topczewska, & Sokołowicz, 2023).

- **Natural oils**

Essential oils are volatile organic compounds produced by plants in response to stress, ecological factors, pathogen attacks and as a form to attract pollinators. They are obtained from natural vegetable material through steam distillation, mechanical processes, or dry distillation. These oils are characterized by complex compositions of low-molecular-mass molecules, including monoterpenes, sesquiterpenes, alcohols, aldehydes, esters, ethers, ketones, and various volatile organic compounds. Several essential oils and their individual components exhibit antimicrobial activity against foodborne pathogens in vitro and, to a lesser extent, in actual food products. Phenolic components are particularly effective due to their ability to permeabilize microbial membranes (Khosravi, 2013).

Essential oils and plant extracts are considered GRAS (Generally Recognized as Safe), allowing their use in food products without consumer risk. They have various biological activities such as antioxidants and antimicrobials. The antioxidant activity is mainly due to phenolic compounds, alcohols, aldehydes, phenylpropanoids, terpenes, and ketones, which protect against pro-oxidants in meat. The antimicrobial activity is primarily attributed to aromatic oxygen compounds. These natural compounds act by increasing microbial cell membrane permeability, inhibiting substrate absorption essential for microbial growth and interfering with cellular metabolism (Campolina, Cardoso, Caetano, Nelson, & Ramos, 2023).

- **Sodium Nitrite**

Artificial preservatives are substances or mixture of substances incorporated, directly or indirectly, during the growing, storage, or processing of foods. Many additives are used to accentuate flavor and color, like in the case of sodium nitrate used to preserve meat products (National Research Council, 1973). Additives perform many functions in the food product and may be classified into four general categories: preservatives, nutritional additives, sensory agents, and processing agents. These ingredients can improve the nutritional value, taste, texture, consistency, and color, making food more appealing, especially, to address changes that might occur during the transportation of food from the production facility or farms to the markets. Additives can decelerate the growth of some pathogens microorganisms since affect their environment and combat enzymatic deterioration (less rancidity) (Vaclavik & Christian, 2014).

The current use of nitrate and nitrite salts in cured meat products has its roots in ancient salting practices for meat preservation. Scientific advancements later identified nitrate and nitrite as the key ingredients responsible for meat preservation. Sodium and potassium salts of nitrite and nitrate are now common food preservatives in meat processing. These substances inhibit microbial growth, delay rancidity, enhance cured meat flavor and aroma, and stabilize the meat's red color. Sodium nitrite is well known to produce a pinkish-red color via interaction with the myoglobin in muscle. Also, the NO component can deplete oxygen by self-oxidation, binding to the iron ion present in hemoglobin, this prevents oxidation and eliminate the radical chain reactions of lipid oxidation (Zhang et al., 2023)

Biological Processing.

Biological processing uses enzymatic hydrolysis and fermentation. Compared to chemical and physical modification, biological processing can be advantageous because it is often more environmentally friendly and energy-saving. Moreover, as chemical processing has increasingly created a negative impact due to the use of undeclared additives, consumers have increased their level of distrust in those methods (Wu et al., 2020). Recent studies in biological preservation intend to reduce health risks without changing the sensory quality of the food product (Holzapfel, Geisen, & Schillinger, 1995).

- **Enzymatic hydrolysis**

Enzymatic hydrolysis uses commercial enzymes derived from plants, animals, or microorganisms, offering several advantages for the food industry. These advantages include mild temperature and pH conditions, selective enzyme action, no secondary products like those produced in microbial fermentation, and an absence of chemical compounds, making it environmentally friendly. The process is straightforward, easy to inactivate, and can yield high-quality bioactive peptides, which are used as supplements and to forficate and improve nutritional and sensorial characteristics (Hajfathalian, Ghelichi, García-Moreno, Moltke Sørensen, & Jacobsen, 2017). Small peptides are particularly valuable as their size helps them resist hydrolysis by gastrointestinal enzymes. Current trends involve using combined commercial enzymes and studying peptide bio accessibility. Enzymatic hydrolysis is typically carried out in reactors that enhance efficiency, increase efficiency and yield and ensure stable batch-to-batch

production. Using membranes or immobilized enzymes reactors can significantly save enzymes and lower processing costs (Mora & Toldrá, 2023)

- **Fermentation**

Initially developed by ancient humans to preserve perishable foods, fermentation has since evolved into a method for improving the organoleptic, nutritional, and functional qualities of food products (Netsanet & Terefe, 2016). Fermentation is a food preservations processing technique that relies on microorganisms to stabilize and transform food materials. The metabolites produced during fermentation foster the growth of beneficial organisms while inhibiting harmful ones, extending shelf life and suppressing spoilage. For example, lactic acid, acetic acid, carbon dioxide, ethanol, some peptides, antifungal compounds and others collectively create an environment hostile to spoilage and pathogenic organisms. Beyond preserving food, fermentation enhances aroma, flavor, and texture, depending on the substrate, microbial strains and environmental conditions like temperature. Now, fermentation serves to create desirable organoleptic properties and improve food palatability. Th unique flavor and textures resulting from microbial metabolism during fermentation are difficult to replicate through physicochemical processes (Di Cagno, Coda, De Angelis, & Gobbetti, 2012).

Physical Processing.

Physical processing of food are those methods that use physical treatments to inhibit, kill or remove undesirable and threatening microorganisms. Foods are physically complex substances, made up of fluids, solids and semisolids, and many times the structures provide localized areas that have suitable characteristic to induce microbial

growth. Additionally, the rheological features of many foods are often time and process dependent, which make addressing the physical. Inside this category, food processors can find different methods that can inhibit microbial growth; from dehydration processes (drying and freeze-drying) to treatment such as heating, to bacteriostatic temperature lowering methods like refrigeration and freezing (Doyle & Beuchat, 2007).

- **Thermal Processing**

Thermal processing relies on the application of heat, and is mainly used for preservation, development of taste and flavor, and development of material structure. Currently, food preservation by elevation of temperature for a short time is the most common form of food preservation. The majority of thermal processes were discovered by empirical investigation related with the effect of temperature and time of exposure on microbial survival (Fryer & Robbins, 2005). However, current knowledge is not sufficient to define a universal physiological model of how the amount and duration of applied heat relates to microbial inactivation and/or inhibition kinetics. That said, models that relate certain physicochemical parameters and information about physiological effects of heat on microorganisms can provide important guides to improve the development of preservation systems that incorporate controlled variables of temperature elevation (Earnshaw, Appleyard, & Hurst, 1995).

- **Non-thermal Processing**

Nowadays, the food industry is facing a new reality where consumers want minimally processed products that retain nutritional characteristics, have an acceptable shelf life, and are convenient to prepare (Fryer & Robbins, 2005). Many traditional

processes, particularly thermal methods, can cause a significant loss of nutrients and flavors, affecting the quality of the final product. Therefore, the food industry and processors are looking for preservation alternatives and non-thermal technologies that ensure food safety and avoid extreme changes in sensory, nutritional, physicochemical, and antioxidant characteristics (Calderón-Santoyo, López-Quintana, Ramírez-de-León, Jiménez-Sánchez, & Ragazzo-Sánchez, 2019) Emerging technologies for extending shelf life and improving food safety have revolutionized the food-processing sector. Different studies have shown that techniques like supercritical carbon dioxide, high hydrostatic pressure, cold plasma, and ozone technology can ensure the freshness of the food and keep nutritionally-heat-sensitive compounds inherent to their nature (Allai, Azad, Mir, & Gul, 2023). These processing methods have the potential to be considered excellent alternatives, principally for consumer and trade demands as well as economic and regional changes.

NON- THERMAL PROCESSING: HIGH PRESSURE PROCESSING.

High-pressure processing is a cold preservation system that uses high hydrostatic pressures to deactivate pathogens in food, maintaining food safety, extending shelf life, and reducing or eliminating the use of additives. High-pressure does not depend on heat, chemicals, reduced water activity, refrigeration or freezing temperatures to control pathogens or spoilage microbes (Gómez, Janardhanan, Ibañez, & Beriain, 2020). Temperature, pressure, and time are the three critical parameters to control in the design of any high-pressure treatment. Regarding time, it is not only important to consider the

duration of the treatment but also the time required to reach that pressure and the post-treatment decompression time to return to atmospheric pressure (Bertó Navarro, 2018)

The effectiveness of high-pressure processing in deactivating vegetative pathogens and spoilage microorganisms depends on various factors, including pressure, temperature, and processing time, as well as product-related factors such as pH, water activity, salt content, presence of other antimicrobials, physiological state, matrix and form of food. Various studies have shown that HPP can achieve a reduction of greater than 4 log units in common vegetative pathogenic and spoilage microorganisms when pressures of 400-600 MPa are applied for short periods of time (3-7 min at room temperature) (Gómez, Janardhanan, Ibañez, & Beriain, 2020).

HPP technology can operate at low temperatures, room temperature, or processing temperatures above 60 °C. During application of high pressure, adiabatic heating occurs, which is equivalent to 3 ◦C per 100 MPa in water and up to 8.7 °C for fats and oils at 25 °C (Tadapaneni, Edirisinghe, & Burton-Freeman, 2015). This is considered an advantage because preserves the nutritional value and final product quality since low molecular weight compounds responsible for odor, taste, pigments, and certain vitamins remain unaltered. Furthermore, the pressure has minimal effects on the covalent bonding of these compounds, maintaining the product's texture and preventing antioxidant degradation. Additionally, HPP could offer food products with reduced use of additives, allowing the incorporation of prebiotic cultures and the production of fermented products. This responds to the consumer demand for processed foods that are healthier and more innovative. Another advantage is that this technology can be applied as a final

preservation measure in packaged foods, serving as a cold pasteurization step directly within the product container. This provides a level of microbiological safety that ensures the safety of ready-to-eat products (Figueroa-Sepúlveda, Castillo-Robles, & Martínez-Girón, 2021).

HPP has been used potentially for the processing of fruit juices such as passion fruit, cucumber, mango, and apple (Calderón-Santoyo, López-Quintana, Ramírez-de-León, Jiménez-Sánchez, & Ragazzo-Sánchez, 2019). On the other hand, HPP for raw meat has not seen widespread adoption as a commercial application. This is mostly because HPP has an undesirable effect on the color, texture, and lipid oxidation of raw meat (Sert & Coşkun, 2022). These changes in fruit juices are not seen as a disadvantage by consumers and do not affect their preference for them. However, HPP is considered an emergent technology that has favorable expectations for white meat processing (Bak, Bolumar, Karlsson, Lindahl, & Orlien, 2019).

High-Pressure processing background.

In 1883, Certes became the first in history to relate the effect of high pressure on organisms. Nevertheless, it was not until 1899 that the effects of high hydrostatic pressures on food were initially discovered by Bert Hite and his colleagues at the agricultural experiment station at West Virginia University. Hite employed high hydrostatic pressures of up to 600 MPa to processed milk and subsequently applied this technique to vegetables and fruits in 1914 (Elamin, Endan, Yosuf, Shamsudin, & Ahmedov, 2015).

Since then, a considerable number of research papers have been published, indicating the effect of high pressure on microbes, starches, and various proteins. However, in that moment of history, food processors did not consider high pressure as a practical method to commercially preserve food because more robust pressure vessels, pumps, and instrumentation needed development. Consumer preferences were leaning toward frozen foods because they were synonyms of freshness, quality, and convenience. The availability of frozen foods was increasing, and their preservation technologies were easily acquired to the point of becoming a marketing strategy for convenient meals through the 1950s. As years passed, Macfarlane (1973) and Bouton et al. (1977), published research work on the effect of high pressure (140 MPa) on cuts of red meat pre and post rigor mortis. Their commercial trials indicated that these pressures could potentially help to tenderize cuts of meat, following the mechanism of pressure's action on the activation of natural proteolytic enzymes in the beef muscle. In 1974, Wilson reported that high pressure could also be employed to preserve fruits like apricots when packaged in flexible hermetically sealed pouches (Farkas, 2016).

Sale et al. (1970) provided a comprehensive description of the impact of high pressure on the survival of spores by pressure causing the initiation of germination followed by inactivation of the germinated forms. In 1986, Kyoto University in Japan began to work with high-pressure processing on food products. Later, the Japanese Society for High Pressure was formed, and with this, the launching of the first commercial products was a reality in 1990. Subsequently, many academic and industrial high-pressure activities have been carried out (Knorr, 1995).

Table 2.1 provides an overview of some existing industrial applications of high-pressure treated foods. While there may be ongoing developments of other products, the lack of publicly available data is commonly attributed to the confidential nature of these projects. In the seafood industry, high pressures are used for fresh, processed, or cooked fish products, and easy opening of bivalve mollusks and crustaceans, both for the product and for the extraction of their meat without cooking. In the meat industry, this technology allows the production of preservative-free products, known as clean-label products. This post-packaging lethality treatment enables the elimination of artificial ingredients. In the dairy and eggs industries, high pressures are opening doors to new products due to induced modifications in the functional properties of whey proteins as well as in other milk components. High pressure improves the microbiological quality of milk without substantially changing its native enzymes. In fruits and vegetables, high pressures extend shelf life while preserving the intrinsic qualities of the food. Finally, high-pressure has been shown to increase cheese yield when is processed at 300-400MPa (Srinivas & King, 2018). After Bello, Martínez, Ceberio, Rodrigo, & López (2014), present a general overview of alternative use of HPP in different commercial food products.

Table 2.1. Overview of some existing industrial applications of high-pressure treated foods (Bello, Martínez, Ceberio, Rodrigo, & López, 2014).

Product	Pressure (MPa)	Holding time (min)	Temperature (°C)
Jams, fruit dressings, fruit sauce, yogurt, fruit jelly.	400	10-30	20
Grapefruit and orange juice,	120-400	2	20
Cooked chicken	300-600	2-3	20
Beef	100-150	30-40	20
Cheese	50-800	20	10-30
Apple-broccoli juice	50–400	5–20	21
Pineapple juice	550–600	3–15	20–80
Tomato sauce	100–800	10	25-60

High-Pressure principles and considerations

There are two main principles on which the application of high pressure is based, Le Chatelier's principle and Pascal's principle. According to Le Chatelier's principle, if a reaction at equilibrium is disturbed from the outside, the system evolves to counteract the effects of that disturbance. During this event, there is an inverse relationship between volume and pressure, characterized by a decrease in volume and an increase in pressure. The application of high pressure accelerates reactions that involve a reduction in volume. Pascal's principle explains that the applied pressure is uniformly and almost instantaneously transmitted to all parts of the food, regardless of its composition, size, or geometric shape. This property prevents the deformation of the product, even when the pressure is too high, the product remains the same (Bertó Navarro, 2018). Following Pascal's principle, the temperature of homogenous food will increase uniformly due to compression. Compression will increase the temperature of foods by approximately 3°C per 100 MPa. Investigations have shown that a combination between initial temperatures

of 90°C-100°C and applied pressures of 500-700 MPa can inactivate spore-forming bacteria such *as Clostridium botulinum* (Kadam, Jadhav, Salve, Machewad, 2012). High pressure does not modify foods shape because the product is getting pressure all the way around and all the way through it (Abera, 2019).

High-pressure processing uses two principal types of industrial equipment and after ACCUA HPP solutions, four principal stages conform HPP system. The first equipment needed is a batch system for processing packed foods (Kadam, Jadhav, Salve, Machewad, 2012). A standard high-pressure system is formed by a pressure vessel and a pressure-generating device. Packaged cold products are placed in their respective containers, is important to notice that flexible and airtight packaging is required, such as plastic bottles, vacuum bags, and trays. The containers are moved into the high-pressure vessel and sealed to initiate the process. The vessel is filled with potable water, which is the pressure medium most often used in HPP. Electric high-pressure pumps increase the pressure in the vessel until the desired pressure can be maintained without further need for energy, effectively eliminating germs and microorganisms. After the pressure decreases, the water is drained. The high-pressure vessel is opened, and the containers with the treated products are unloaded. The process is isostatic, so the pressure is transmitted rapidly and uniformly throughout both the pressure medium and the food with little or no heating (ACCUA HPP solutions, 2023).

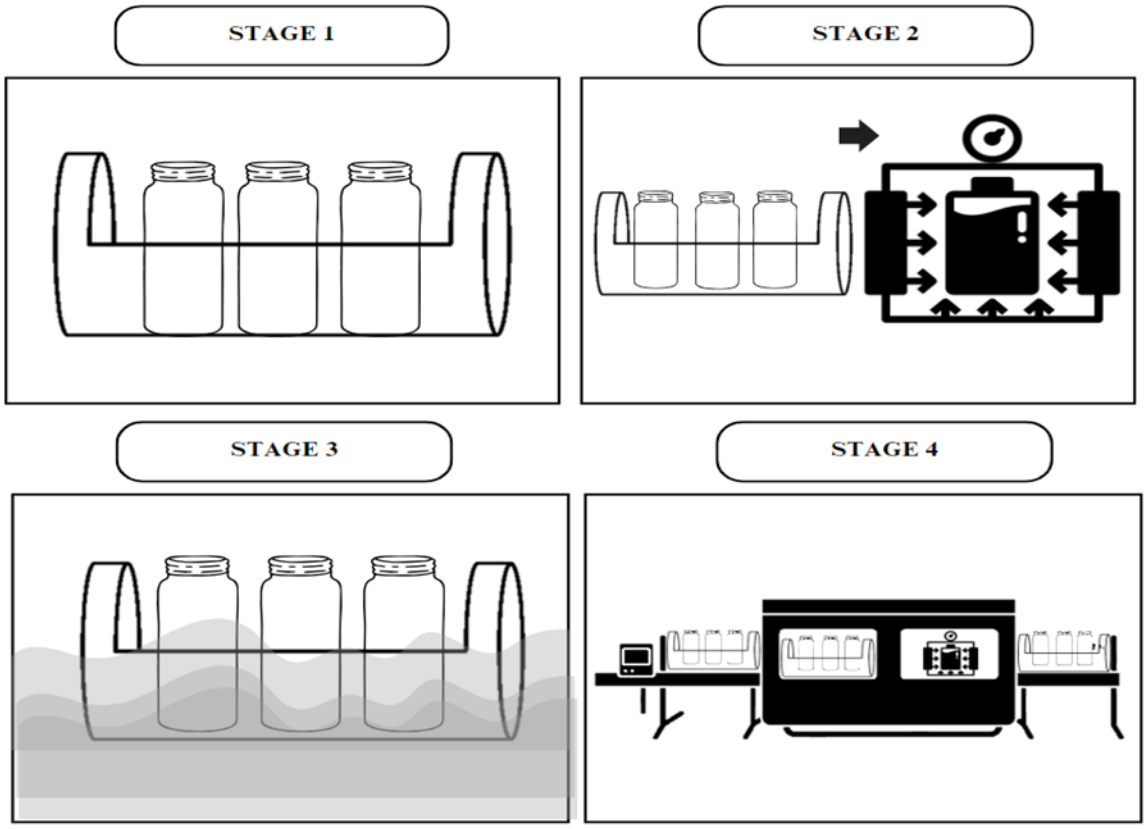

Figure 2.2. High-Pressure processing stages (ACCUA HPP solutions, 2023)

One important consideration when applying HPP to solid and liquid foods in flexible vacuum packaging is that it cannot be applied to foods packaged in rigid containers (glass or cans) or to solid foods that contain excessive amounts of air. One significant drawback of this technology is that it does not allow the subsequent preservation of foods at room temperature without affecting food safety. Therefore, high-pressurized food must undergo further preservation stages, either by storing them at temperatures that prevent bacterial spores' germination or by lowering the pH below 4.5. More recently some investigators have explored the synergistic combination between pressure and elevated temperatures which can inactivate bacterial spores. This

combination process results in food products that do not require refrigerated conditions or the addition of acidifying agents same (Bertó Navarro, 2018).

Effect of Pressure on Foodborne Pathogens.

The inactivation of microorganisms through HPP results from a combination of different factors, including alterations in cell membranes/ walls, structural changes in proteins, and disruption of enzyme-mediated cellular processes. The primary sites of damage induced by pressure are cell membranes, which subsequently lead to a malfunction in cell permeability, transport systems, loss of osmotic responsiveness, disruption of organelles, and an inability to maintain intracellular pH. Furthermore, high pressure causes biochemical and genetic modifications by inactivating enzymes involved in DNA replication and transcription. Studies involving proteins and lipid membranes have observed that even pressures of 100 MPa or lower caused a decrease in the fluidity of the lipid bilayer and reversible conformational changes in transmembrane proteins, resulting in a dysfunction of membrane-bound enzymes (Woldemariam & Emire, 2019).

Microorganisms can survive by being sub-lethally injured and developing sensitivity to unfavorable physical and chemical environments to which normal cells are resistant. The recovery of damaged cells will depend on the conditions after treatment, and this has implications for microbiological counts and food safety. Following this, high-pressure processing needs an alternative processing method to maintain safety limits. The degree of inactivation depends on various factors: the microorganisms and their strain, the level of pressure, treatment temperature and time, and the composition of the dispersion medium. In addition, it is known that the phase of the cell cycle, the shape

and characteristics of the membrane of the bacteria under study, can influence the effectiveness of high-pressure processing (Torres Mejía, 2011).

The pressurization phase usually takes less than 4 minutes, followed by a dwell time of 4-12 minutes, while the depressurization process typically occurs almost instantly. High pressure has the effect of coagulating proteins, including those that are vital for microbial cell functions. The rapid depressurization generally affects gram-negative bacteria, such as nontyphoidal *Salmonella* and *Escherichia coli* more acutely than *Gram*-positive bacteria, like *Listeria* and *Clostridium*, which are more resistant to high pressure. Because of this, process validation plays a crucial role in ensuring the proper functioning of food safety systems, and it is a fundamental aspect of Hazard Analysis Critical Control Point (HACCP) systems. Since it is not practical or reasonable to introduce pathogenic bacteria into food processing operations, non-pathogenic bacteria, called surrogates, can be used to represent specific pathogenic bacteria (Woerner et al., 2018).

It can be stated that vegetative cells are sensitive to pressure, with inactivation occurring at pressures between 300-600 MPa, while bacterial spores are more resistant and are only inactivated at pressures greater than 1200 MPa. In food preservation, one of the most challenging activities is the inactivation of these bacterial spores. Under high pressure, spores can germinate into vegetative cells and then can be inactivated. This inactivation is strongly influenced by temperature, pH, water activity, and ionic strength (Torres Mejía, 2011).

Effect of pressure on color and texture of muscle food products.

High-pressure (HP) treatment frequently leads to changes in beef, lamb, pork, turkey and poultry color. The magnitude of these color alterations depends on the physical and chemical properties of the meat, particularly myoglobin (oxymyoglobin, metmyoglobin), as well as the surrounding atmospheric conditions during and after the pressurization process. Culturally, beef is commonly referred to as "red meat" due to its distinct red hue, followed by turkey, pork, and poultry, which is often termed "white meat". This is primarily because beef has a greater concentration of myoglobin in its muscle structure compared to those called “white meat”. Meat color is one of the most critical quality attributes relied on by consumers when making purchase decisions. For instance, a consistently pinkish or white-yellowish color is typically preferred chicken, but these colors are less acceptable in ground beef or lamb (Bak, Bolumar, Karlsson, Lindahl, & Orlien, 2019).

Meat color is influenced by both the myoglobin content within the muscle and the optical characteristics of the meat’s surface. The increase in high-pressure can lead to significant visual changes in the color parameters of fresh meat, which typically manifests as an increase of lightness (L*) and reduction of redness (a*). The decrease in a* in fresh meat is due to myoglobin oxidation and heme displacement and release, while the alteration in L* can be attributed to changes in sarcoplasmic and myofibrillar proteins, subsequently affecting meat surface properties. One method of moderating these changes is the use of vacuum packaging with an oxygen scavenger, which can partially preserve meat color. However, the loss of meat color induced by HPP can hinder the

commercialization of fresh meat since a departure from the expected color can affect consumer acceptance (Bolumar et al., 2021). Nevertheless, the adverse impact of HPP on fresh meat color can be less significant if the meat is further processed into other meat products or other consumption alternatives (Tao, Sun, Hogan, & Kelly, 2014).

Texture is a fundamental property of food that not only influences its quality but also impacts consumer preferences and nutritional aspects. Textural properties are defined as the physical characteristics resulting from the structural components of food. These characteristics are perceived through the sense of touch and are related to how food deforms, disintegrates, and flows when a force performs. These textural properties are essential for ensuring food quality. The way food feels in the mouth is a crucial sensory attribute, moreover, the mechanical attributes of food texture serve as indicators of a product's freshness (Gokul Nath, Pandiselvam, & Sunil, 2023). Through a physical mechanism, high-pressure processing (HPP) can start changes in the characteristics of meat products, including tenderization and softening. HPP induces alterations in functional and physical aspects like water holding capacity, color, and the structure and function of myofibrillar proteins. Additionally, has effect on myofibrillar proteins, leading to their unfolding, agglomeration, aggregation, and the formation of protein networks

Meat texture is essentially defined by myosin and actin, and these properties are closely linked to the meat’s structure, with significant changes occurring at pressures exceeding 200 MPa. The primary functional protein in meat is myosin, either alone or in its complex form with actin, known as actomyosin. Myosin possesses a balanced

hydrophobic nature and a long fibrous structure, enabling it to form elastic gel matrices and a cohesive membrane around fat globules in minced and emulsified meats. The concentration of proteins, including myosin and actomyosin, in the aqueous protein extract drawn to the surface of meat particles during processing depends on several factors. This includes extraction time, salt concentration (ionic strength), and pH levels. Additionally, whether the meat is processed pre-rigor or post-rigor affects the type oof protein exudate obtained. Pre-rigor muscle, being less contracted, tends to yield more free myosin, while post-rigor muscle, more contracted, yields more actomyosin (Xiong & Kenney, 2001).

High-pressure processing (HPP) has demonstrated its ability to enhance textural qualities in a variety of food items while causing minimal deterioration. Raw chicken presents a notable rise in the firmness, toughness, cohesiveness, gumminess, and chewiness subjected to HPP at pressures exceeding 200 MPa (Vervisch, D'hondt, & De Potter, 2021). In the intricate composition and structure of processed muscle foods, proteins are pivotal components responsible for crucial functions such as water-binding, gelation, emulsification, meat particle binding, and overall consistency in meat products.

Effect of pressure on oxidation.

Oxidation is one of the key factors contributing to the non-microbial degradation of meat. High-pressure processing (HPP) induces the transformation of myoglobin and oxymyoblogin from their reduced stated to the ferric form, which in turn, promotes fat oxidation through the catalytic effect of iron, however this initiation factor has been disputed in various arguments. Scientists argued that the lipid oxidation resulting from

HPP application is not primarily due the catalytic influence of metmyoglobin, which is linked to the release of iron ions because of pressure, but rather stems from membrane damage (Sert & Coşkun, 2022). The formation of radicals and reactive oxygen species, that participate in tissue injuries and diseases, during high-pressure processing is another potential factor contributing to lipid oxidation. It is influenced by various treatment parameters such as pressure, temperature, and holding time, as well as the type of meat, including the species and muscle involved. Metmyoglobin appears to have little relevance to lipid oxidation, indicating that membrane damage caused by HPP is primarily responsible for initiating lipid oxidation. This observation is supported by evidence of mutual oxidative effects between lipids and hemeproteins. Studies on the effects of HPP have focused on phospholipid membranes composed of phosphatidylcholine bilayers and both saturated and unsaturated fatty acids as a model system. These biological components are commonly targeted in the pressure-based inactivation of microorganisms (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014).

Wiggers, Kroger-Ohlsen, & Skibsted (2004) concluded that high-pressure treatment at 400 and 600 MPa results in an elevated level of oxidation, with no impact at 200 MPa. This demonstrates that the pro-oxidative influence of high pressure becomes evident between 200 and 400 MPa when dealing with chicken meat subsequently stored under refrigeration. They also noticed that pressure applications of 500 MPa or lower for 10 minutes on chicken breast meat do not result in rancidity, and 500 MPa is considered a critical threshold in this regard. When comparisons are made between raw-pressurized chicken with heat-treated pressurized chicken, it is evident that the latter exhibits

increased susceptibility to oxidation, particularly in samples treated above 500MPa. High pressure also leads to the formation of secondary lipid oxidation products and the development of distinct off-flavors. The presence of Warmed-Over-Flavor (WOF) in poultry is related to the meat's fatty acid profile, the presence of natural antioxidants (especially α-tocopherol), cooking temperature, and storage conditions, like atmosphere and storage temperature. The practical application of high-pressure treatment for poultry meat will depend on establishing conditions that ensure minimal microbial risk and safety while maintaining the acceptability of the cooked product.

Some literature has established that vacuum packaging is commonly used before high-pressure processing (HPP) to mitigate the impact of HPP on meat oxidation. It has been indicated that during cold storage, the formation of Thiobarbituric Acid Reactive Substances (TBARS), which are secondary products of lipid oxidation in pressurized meat, can be reduced by utilizing antioxidant-active packaging. Regardless of the meat type (beef, chicken, or pork) and the packaging method used (vacuum, with rosemary extract), lipid oxidation is more pronounced at the meat's surface than its interior. In connection with the addition of antioxidants such as polyphenols, metal-chelating agents, or proteins control the lipid oxidation process (Bolumar et al., 2021).

POULTRY

Poultry industry continues to be a major supplier of protein in the United States and the world. The primary market distinction lies between the domestic and end export markets. In the United States, there is a preference for white meat domestically, while most of the dark meat is designated for exportation. The export market is subject to

various external factors, including oil prices, conflicts, environmental calamities, currency fluctuations, political concerns, and other global issues. The U.S. market is further categorized into two segments: chicken for household consumption and chicken intended for use in restaurants, educational institutions, and other similar settings (Hood, Myles, Peebles, & Thornton, 2012).

As the economy has grown rapidly and people's incomes have improved, there have been notable shifts in people's consumption and preferences related to food decisions. Chicken products, known for their nutritional benefits like low-fat content and high protein, have garnered increasing attention, leading to a surge in global demand. In 2016, the United States was the largest chicken producer worldwide, accounting for 20.42% of global chicken production. Additionally, chicken made up 45.99% of total retail meat consumption in the United States, further cementing the degree to which development is accelerating in the poultry industry. The chicken products resulting from this acceleration have the potential to stimulate economic growth by diversifying exports, generating employment opportunities, increasing foreign exchange earnings, and enhancing food security (Wen, Li, Sun, He, & Tsai, 2019).

Chicken composition review

Dietary choices play a fundamental part in human health and well-being, with consumers generally preferring lean meat with lower fat content and higher protein content. Poultry meat is a significant source of high-quality animal protein and fat. Regardless, chicken meat is relatively low in fat and cholesterol compared to other meats and foods. The quality of meat and the fatty acid profile will depend on the poultry cuts,

for example, breast and leg muscles are predominantly influenced by the components in their feed (Kumar & Rani, 2014).

The collagen content of ground chicken is low compared to other types of meat like pork or beef muscle, this makes chicken more suitable for different processing methods, including high temperatures and high pressures. Alterations in collagen solubility because of varying heating temperatures have an impact on the products texture, protein solubility, and the ability to retain water. On the other hand, ground chicken texture is affected by high temperatures. These changes in texture are altered by changes in myofibrillar proteins, and connective tissue of the ground chicken. Heating has been shown to lead to the tenderization of connective tissue through the conversion of collagen into gelatin, while at the same time, causing the meat fibers to become tougher through the coagulation of myofibrillar proteins (Murphy & Marks, 2000).

Principal pathogens in ground chicken

In the United States, approximately 9.4 million cases of foodborne illnesses occur annually due to the consumption of contaminated food products. A significant portion of these illnesses is attributed to the consumption of contaminated poultry meat. Poultry meat is recognized as a significant transporter of multiple bacteria, including *Salmonella spp.*, *Campylobacter spp.*, *Escherichia coli spp.*, and *Clostridium spp*. However, non-typhoidal *Salmonella* and *Campylobacter* are two of the most persistent and primary pathogens that continue to raise substantial food safety concerns in poultry processing (Thames & Theradiyil Sukumaran, 2020). According to USDA Agricultural Marketing Service (AMS), cooked poultry is sampled and tested for the following indicator

microorganisms: standard plate count (mesophilic aerobic plate counts), total coliforms, generic *E. coli*, and *Staphylococcus (S.) aureus*, with critical limits of 1,000 cfu/g, 50 cfu/g, 10 cfu/g, and 10 cfu/g, respectively. *Salmonella* and *L. monocytogenes* are also tested with zero tolerance indicator. Additionally, over the years, bacteria such as *E. coli* have proven to be a strong threat due to their ability to acquire, carry and transfer resistance genes to other pathogens either in the intestinal tract of humans/animals or the environment. With the constant use of antibiotics and drugs in poultry meat processing, there has been an increasing concern about *E. coli* potentially transfer of antibiotic resistance genes to other bacteria, including potential pathogens, particularly as recent studies shown that strains of *E.coli* present in poultry meat are becoming more resistant to frontline drugs used for human treatments, including third-generation cephalosporins, fluoroquinolones, and even trimethoprim-sulfamethoxazole (Mor-Mur & Yuste, 2010).

Table 2.2. AMS cooked chicken critical limits (AMS, 2022)

Indicator Microorganism	**Critical Limit**
Standard Plate count	1,000 cfu/g
Total coliforms	50 cfu/g
Generic *E. coli*	10 cfu/g
S. aureus	10 cfu/g
Salmonella	0 cfu/g
L. monocytogenes	0 cfu/g

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CHAPTER THREE

INTERACTIONS BETWEEN HIGH-PRESSURE PROCESSING AND NATURAL ANTIMICROBIALS IN GROUND WHITE-MEAT CHICKEN

Abstract

Comminuted poultry products pose multiple challenges regarding shelf stability and safety, many of which stem from the peculiarities of the processes involved in making them. Grinding increases surface area, while mixing not only exposes more of that surface area to air, potentially aiding in the formation of oxidative byproducts, it also can distribute any localized microbial contaminants, potentially leading to shelf life and safety issues for entire batches. Processors have, quite expectedly, attempted to mitigate these concerns using a variety of techniques. One of the most common is the use of natural antimicrobials like essential oils, buffered vinegar and citrus juices. Another option that has seen more limited use is High Pressure Processing (HPP). Although it can eliminate spoilage, HPP can also have undesirable effects on the texture, color and oxidation rate, making some processors hesitant to adopt it. To address these issues, this chapter examines the effects of two different HPP treatments on ground white-meat chicken with or without one of two common antimicrobials, (Buffered concentrated vinegar with rosemary essential oil (V+REO) and a lemon juice-vinegar blend). Samples were inoculated with a non-pathogenic E. coli strain and individually packaged in polyethylene bags. Refrigerated samples with or without antimicrobial additives were then processed at pressures of either 300 MPa or 600 MPa, each with a three-minute hold time. Likewise, a control treatment without pressure or inoculum was analyzed.

Measurements of color were taken prior to processing, within 4 hours of processing, and after a 24-hour refrigerated storage. *E.coli* and *pseudomonas spp* counts were also performed at the 24-hour mark, along with pH, texture profile analysis (TPA) of raw samples and TPA and yield of cooked samples. All samples at 600 MPa showed greater than 5 log reductions in E. coli, while non-HPP samples saw more modest reductions in samples which had been treated with antimicrobials. All treatments had an absence of pseudomonas, proving that the interaction of V+REO and HPP present complex bacterial inactivation. Higher HPP treatment results in changes in the color of ground chicken, primarily evidenced by an increase in whiteness. Meanwhile, samples treated with 600 MPa led to a remarkable increase of lipid oxidation (21% compared to treatments without HPP). Similarly, we observed that the treatments with addition of V + REO and vinegar had a reduction of lipid oxidation greater than 6% with respect to the control without addition of antimicrobial solution.

Introduction

Non-thermal processing technologies have drawn the interest of food industry due to their advantageous effects on protein functionality without affecting the heat-sensitive nature of meat proteins. Among these techniques, HPP has emerged as an effective and environmentally friendly method that demonstrate their positive effect on disinfection and destruction of the cell membrane of microorganisms (>400 MPa) and the inactivation of enzymes. Additionally, appropriate HPP parameters (100-300 MPa), can modify meat proteins in a way that enhances their functionality (Xue et al., 2017). In this research, commonly used natural antimicrobials were also investigated to determine any

synergistic effects of high-pressure on pathogens without negatively affecting texture and quality parameters. The use of naturally occurring compounds serves as an alternative for synthetic food additives to prevent the proliferation of undesirable microorganisms, thereby enhancing food safety (Batiha et al., 2021). The main purpose of HPP technology is to improve meat quality and throughout this investigation, poultry has been the main subject. Food processors know that poultry is highly perishable, with short shelf-life, limited from 4 to 15 days under refrigeration, depending on the environment, transportation, type of packaging used, poultry carcass quality, slaughtering process, and environmental microbiota (Argyri, Papadopoulou, Sourri, Chorianopoulos, & Tassou, 2019). Even though many studies have measured and analyzed the effect of high-pressure processing on the spoilage microbiota of red meat (beef and pork), only limited research has been done on raw chicken meat. Unlike red meats, which often suffer color changes during HPP that are significant enough to erode consumer confidence, the effect of pressure is not as noticeable for chicken meat as for beef or pork as the normal color of raw chicken is slightly pink to white or yellow (result of the low content of myoglobin) (Bak, Bolumar, Karlsson, Lindahl, & Orlien, 2019). This advantage makes the investigation of high-pressure processing as an alternative for poultry producers very appealing. Therefore, the objective of the present chapter was to evaluate the inactivation of E. coli on ground chicken applying High-Pressure processing in combination of two natural antimicrobials, buffered concentrated vinegar with rosemary oil (V+REO) and a lemon-vinegar blend. In addition, the effect of this method on select physicochemical and

quality parameters was also evaluated, with the purpose of optimizing the process for obtaining a viable option for food processors.

Materials and Methods

Preparation of E. coli inoculum.

A non-pathogenic *E. coli* strain (modified to be *Ampicillum* resistant and produce endogenous Green Fluorescent Protein) was used for the preparation of the inoculum. Initially, a loopful of the reference culture was transferred to a 9 ml sterile TSB (Tryptic Soy Broth) tube and incubated for 24-48 hours at 35°C. Following the incubation period, the TSB tube containing the culture was agitated and poured into a 1-liter sterile TSB flask, which was then placed in a 35-37 °C incubator for at least 24 hours. Subsequently, the culture from the incubated flasks was evenly distributed into four centrifuge bottles of similar weights to maintain balance within the centrifuge. The capped bottles were then centrifuged in an Avanti J-26S XPI centrifuge at 4 °C and 5000 RPM for 15 minutes. Simultaneously, peptone sterile water (0.1%) was weighed, representing 10% of the overall cultural weight. After the centrifugation process, the supernatant was discarded, and sterile water was added to each of the four centrifuge tubes, followed by suspension of the pellet. The final inoculum level used in this experiment was 6.5 Log cfu/g (LibreTexts, 2017).

Raw Chicken Handling and Antimicrobials collection.

Two different antimicrobials buffered concentrated vinegar with rosemary oil (V+REO) and a lemon and vinegar blend, and the chicken samples were donated from

Fieldale Farms Corporation (Fieldale, 2023). Approximately 12 kg of chicken breast meat was received and frozen for later use in the experiments. Two days prior to each replication, 4 kg of chicken was placed in rigid containers and allowed to thaw at least 48 hours at refrigeration temperature (40 F° or below). The thawed chicken was then ground, separated into 1 kg sub-samples and placed in individual plastic bags. All four sub-samples were prepared as below, vacuum sealed and stomached for 15 seconds. Four sub-samples were prepared following the description below.

Sub-sample 1: 1% inoculum, 1% buffered with rosemary oil and vinegar.

Sub-sample 2: 1% inoculum, 1% Lemon and vinegar blend.

Sub-sample 3: 1% inoculum, 1% sterile water.

Sub-sample 4: 2% sterile water.

Sample Packaging and High-Pressure processing.

Samples were prepared by placing 100 g of fabricated sub-samples into individual vacuum plastic bags, and subsequently vacuum packaged using a commercial chamber sealer (VacMaster, VP215, Kansas City, MO). To facilitate HPP processing, samples were classified according to the same HPP treatment and placed in an insulated container with sufficient frozen cooling packs for transport to the processing facility. Samples were processed under two pressure conditions: 300 MPa and 600 MPa for 3 minutes. After processing, the samples were stored under refrigeration conditions.

Analytical Methods

Analytical tests were performed the day after chicken samples were processed with high pressure, except for color determination analysis. Three pouches of each

treatment were evaluated in triplicate each for color, pH, yield, TBARS, TPA (texture analysis), cohesiveness, and microbial enumeration.

Color Determination

Vacuum-packaged samples were analyzed before and after High-Pressure Processing through each transparent bag, avoiding the edges and labels. The color of the ground chicken samples was measured using a handheld colorimeter (KONICA MINOLTA), which quantified the color samples in the CIELAB color scale as L*, a*, and b* values. The instrument was calibrated by a white reference (L*= 93.45, a*= -1.83, b*= 0.55 for the white instrument standard). These scale values were used to calculate the total color difference (ΔE*) between before and after high-pressure processing.

pH

Three grams (3g) of each treatment were placed in Fisher brand sample bags with wire closure, and fifteen ml (15 ml) of distilled water was added and then homogenized. The pH was measured using a digital VWR® pHenomenal® pH/mV/°C meter with a glass electrode. pH analysis was performed at 27 °C.

Texture Analysis

Texture analysis was performed in raw and cooked not inoculated but high-pressurized ground chicken. Four cylindrical patties were formed with 25 g of each treatment. Two different textural tests were performed using a TA.XTplusC Texture Analyzer (Stable Micro Systems). Differences in treatments were evaluated through Warner-Bratzler Shear Force (WBSF), and the other test was subjected to Texture Profile Analysis (TPA) (The Lab Depot, 2023).

Yield

Four cylindrical patties (25 g) were formed, placed in vacuum bags, then the bags were sealed, and vacuum packaged. A circulating water bath was prepared at 165°C using an immersion circulator. The packaged samples were placed into the water bath and cooked for 60 minutes. At the end of cooking, the packages were removed from the water bath, and the bags were dried prior to removal of the cooked patties. Finally, each patty was weighed and transferred to the refrigerator for 2 hours to reach uniform temperature and evaporate any surface moisture.

Thiobarbituric reactive substances (TBARS)

TBARS was determined in duplicate for each replication as described by Oxford Biomedical Research (Oxford Biomedical Research, 2010) with modifications. Five grams (5g) of ground chicken was placed in a 50 ml conical-bottom disposable plastic tube (Avantor®, VWR). Ten ml of deionized water was added to the same tube containing chicken and homogenized using a Kinematica ShearSTAR 2500 Homogenizer for 10 seconds. One ml was taken from the 50 ml tube and placed in a 15 ml test tube. Three ml of Thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution was added to the sample and mixed using a vortex for 1 minute and then heated at 90°C for 15 minutes. The sample was cooled and mixed by vortex for 1 minute. The sample was centrifuged (Eppendorf 5804R, Germany) at 14000 rpm for 10 minutes. The supernatant was filtered using 0.45 µm GHP Membrane Acrodisc and 2 ml of the filtered liquid was placed in another tube with 4 ml TBA solution. The sample was added to a 96-well microplate (0.25 ml) to measure the absorbance at 532 nm. TBARS values were obtained

from a standard curve (Figure 3.1), prepared using known concentrations from 0 to 3 mg/ml of a mixture of 1,1,3,3−tetrathoxypropane solution (TEP) and deionized water solutions with 4 ml of TBA solution. The tubes used for the standard curve were placed in a 90°C water bath and measured the absorbance at 532 nm.

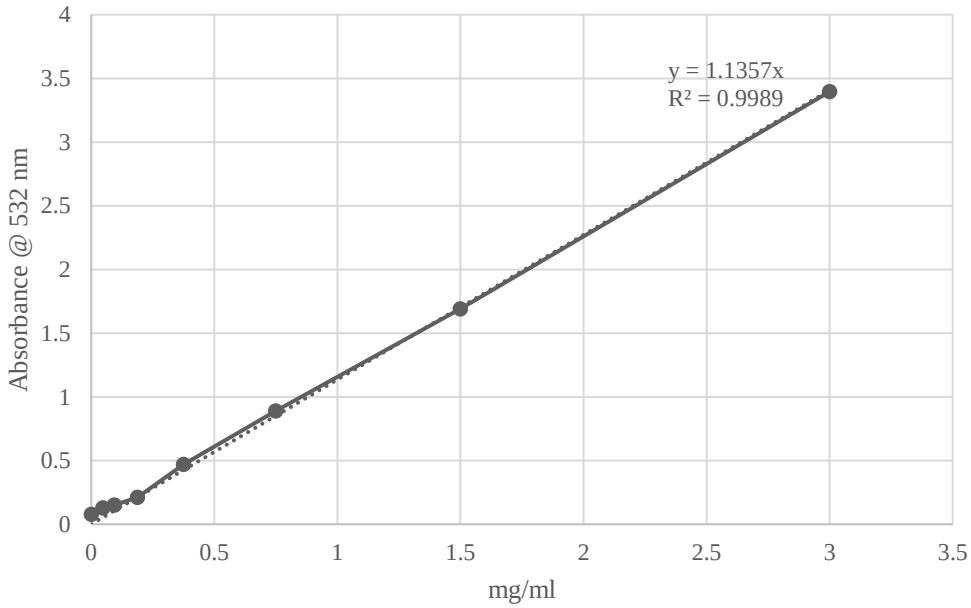


Figure 3.1 Standard curve used for TBARS analysis.

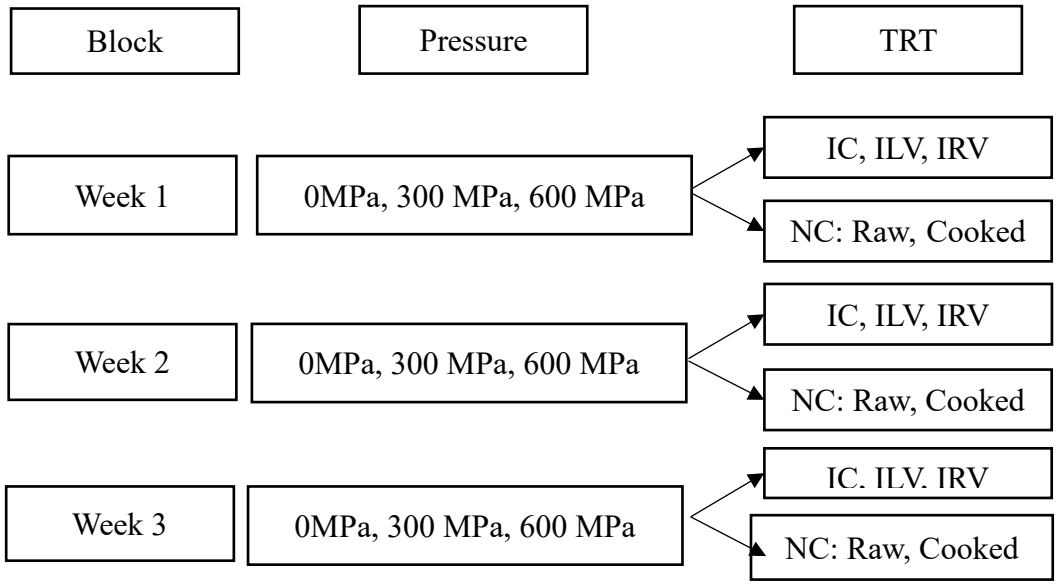
Microbial Enumeration

To estimate the number of viable cells, 10 g of inoculated chicken were weighed and placed in Fisher brand sample bags with wire closure. 90 ml of peptone water was added to the bags, and the mixture was homogenized for 15 seconds with the hand. Using established techniques (FDA Bacteriological Analytical Manual) six dilution tubes were prepared using 9 ml sterile peptone blanks. Each dilution was plated onto Aerobic Plate Count (APC) agar (with 0.5% *Ampicillin* final concentration) and Pseudomonas Agar.

Finally, the plates were incubated at 37 °C. To enumerate the *E.coli* populations, the inoculum was diluted from 10^2 to 10^8 and plated in APC agar.

Statistical Analyses

A Randomized Complete Block Design (RCBD) was used to analyze the data. The study consisted of four treatments, three pressure conditions and three replications, making in total 36 experimental units, as outlined in table 3.10.1. A 3x2 factorial arrangement was used for the texture analysis to perceive if the relationship between pressure (0, 300, 600 MPa) and if the meat was cooked or raw which was determined to be significantly different ($p \leq 0.05$). Additionally, a 3x3 factorial arrangement was used for the rest of the analysis to perceive if the relationship between pressure (0, 300, 600 MPa) and antimicrobials (Lemon-vinegar blend, Vinegar-Rosemary oil antimicrobial solution, without antimicrobial solution) which was determined to be significantly different ($p \leq 0.05$). Statistical analysis was completed using the JMP Pro 14 Statistical Software (SAS Institute, Inc, Cary, NC). Least Squares Means Student's t-test ($p < 0.05$) was used to separate means.



Abbreviations: NC: Inoculated control; **IC:** Inoculated control; **ILV:** Inoculated sample with Lemon/vinegar antimicrobial solution; **IRV:** Inoculated sample with Rosemary oil/ vinegar antimicrobial solution.

Figure 3. 1. Description of experimental design with respective blocks, pressures, and natural antimicrobials solutions.

Results and Discussion

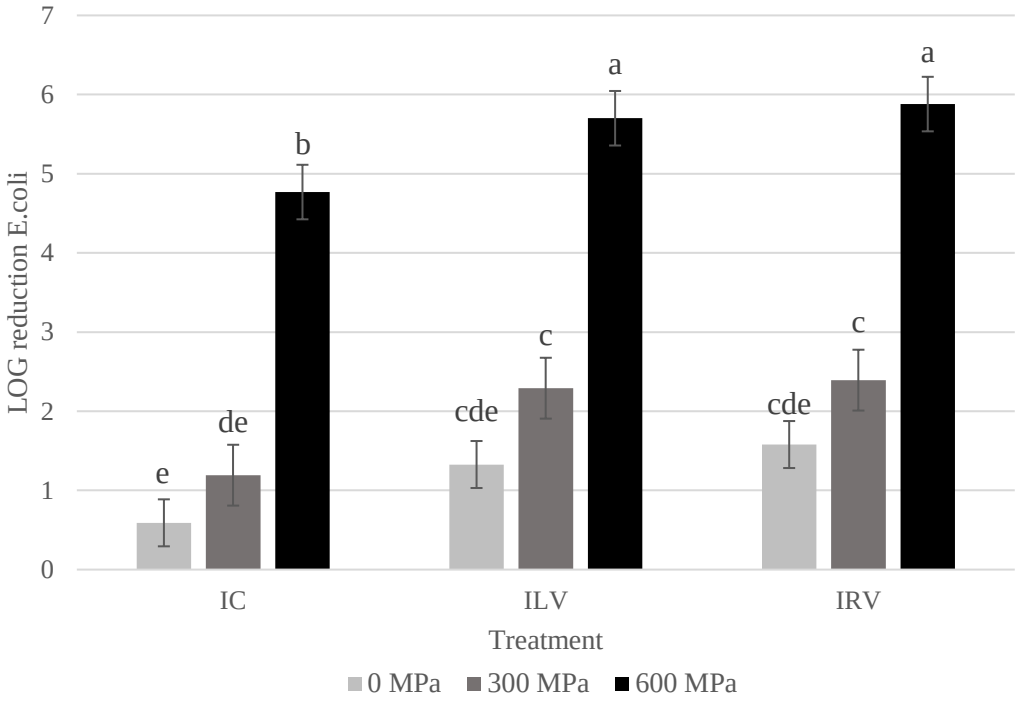
Microbiology reduction

The effect of high-pressure processing on *E. coli* growth (log CFU/g) of ground chicken is shown in Fig. 3.3 and table 3.1. When considering the initial *E. coli* cell count (6.52 Log CFU/g), it is evident that microbial growth of *E. coli* was slightly reduced in chicken samples processed at 300 MPa (1 log) compared to the inoculated control without high pressure processing. However, a significantly greater reduction in non-pathogenic *E. coli* occurred under pressures of 600 MPa (4-5 log) compared to the control at 0 MPa. Several studies report cell count reductions exceeding 8 log (CFU/g)

after treatment with 400-600 MPa. Pressures lower or equal to 300 MPa at ambient temperature, however, result in a reduction of cell count less than 2 log reduction, having a limited impact on *E. coli* reduction, which, in terms of food safety, is insufficient for ground chicken samples to be considered as safe to eat. HPP triggers numerous chemical reactions inside bacterial cells. Over the past years, diverse research has revealed various targets in *E. coli* that are sensitive to pressure, contributing to sub lethal injury and cell death. Initially, these chemical reactions were based on the observed synergistic activity of pressure with pediocin or nicin, which results in the permeabilization of the outer membrane of gram-negative bacteria. The effect of pressure on gram-negative bacteria includes changes in the composition and barrier properties of both outer and cytoplasmic membranes, ribosome assembly and functionality, protein folding, and oxidative stress resulting from metabolic imbalance or the release of iron from denatured proteins (Gänzle & Liu, 2015).

The efficacy of microorganism inactivation under HPP depends on factors such as microbial species and strain type, physiological condition, pH, media composition and high-pressure process parameters. Generally, gram-negative bacteria and cells in the exponential growth phase are more susceptible, while spore-forming bacteria exhibit greater resistance. A lower pH plays a fundamental role in amplifying the bacteriostatic impact of high pressure (Malinowska-Pa & Kołodziejska, 2015). This research demonstrates that the addition of two antimicrobial solutions, lowering the pH in conjunction with high pressure, yields more satisfactory results in the logarithmic reduction of *E. coli*. Statistical results indicate that the most effective treatment was

ground chicken processed at 600 MPa with rosemary oil and vinegar antimicrobial solution. This treatment significantly differs from the inoculated control without this antimicrobial solution, achieving a major effect on the logarithmic reduction (6 log). No significant difference is observed between IRV and ILV treatments, suggesting both antimicrobial solutions have a similar effect on chicken samples, regardless of pressure. Plant extracts and essential oils derived from plants offer potential alternatives to enhance significantly the shelf life and safety of food products. The antimicrobial effect of certain plant materials and acid fruit extracts result from key bioactive compounds, including phenolics, terpenes, aliphatic alcohols, aldehydes, acids, and isoflavonoids. Aziz & Karboune, (2016) found that rosemary essential oil exhibited antimicrobial effects against both gram negative (*Escherichia coli, Klebsiella penumoniae*) and Gram positive (*Bacillus subtilis, Staphylococcus aureus*) bacteria. This is attributed to the presence of phenolic diterpenes, like carnosic, carnosol, rosmanol, rosmadial as well as phenolic acids like rosmarinic and caffeic acids. Fruit extracts, oils and natural antimicrobials offer antioxidant activity that plays a crucial role in interacting with the outer membrane of bacteria, altering permeability for cations such as H^+ and K^+. Plant substances can impact microbial cells through various antimicrobial mechanisms, including targeting the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material, and oxidizing unsaturated fatty acids (Aziz & Karboune, 2016).



Abbreviations:
IC: Inoculated control
ILV: Inoculated sample with lemon/vinegar antimicrobial solution
IRV: Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Figure 3. 2. Microbial inactivation of *E. coli* in ground white chicken under three pressure conditions (0 MPa, 300 MPa, 600 MPa) and with two natural antimicrobial agents. Lettering indicates significant differences between the treatments using Student t-test ($P < 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 1. Microbial inactivation of *E. coli* in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Initial number (Log CFU/ml)	Pressure (MPa)	Treatments		
		IC[1]	ILV[2]	IRV[3]
6.5	0	$0.590^{e} \pm 0.168$	$1.354^{cde} \pm 0.881$	$1.432^{cde} \pm 1.039$
	300	$1.196^{de} \pm 1.198$	$1.977^{c} \pm 0.800$	$1.602^{c} \pm 0.899$
	600	$4.881^{b} \pm 1.454$	$5.701^{a} \pm 1.049$	$5.944^{a} \pm 1.791$

[1]**IC:** Inoculated control
[2]**ILV:** Inoculated sample with lemon/vinegar antimicrobial solution
[3]**IRV:** Inoculated sample with Rosemary oil/vinegar antimicrobial solution

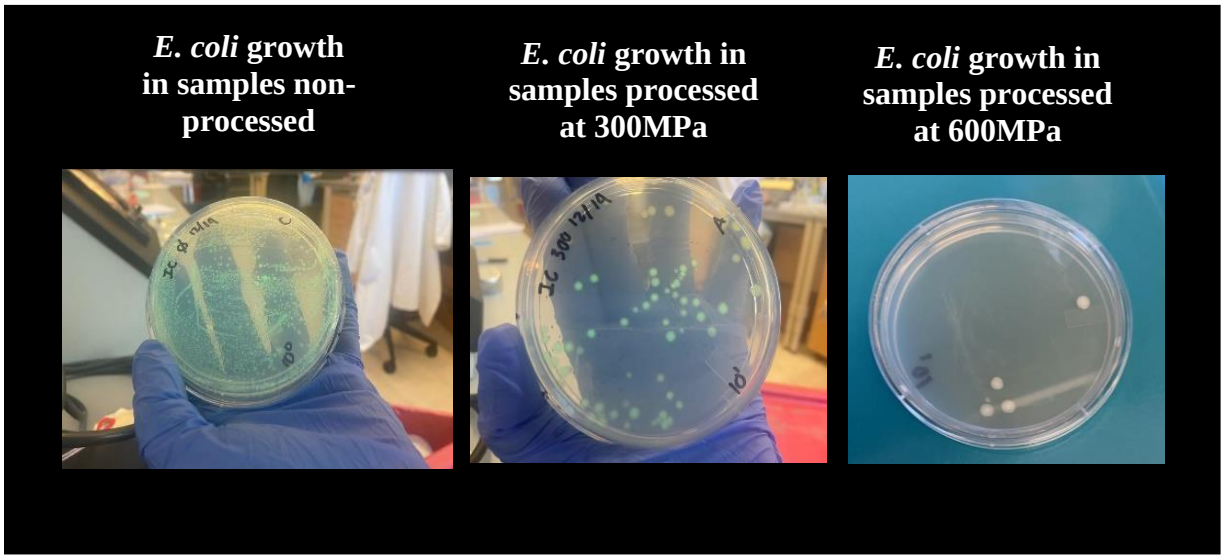
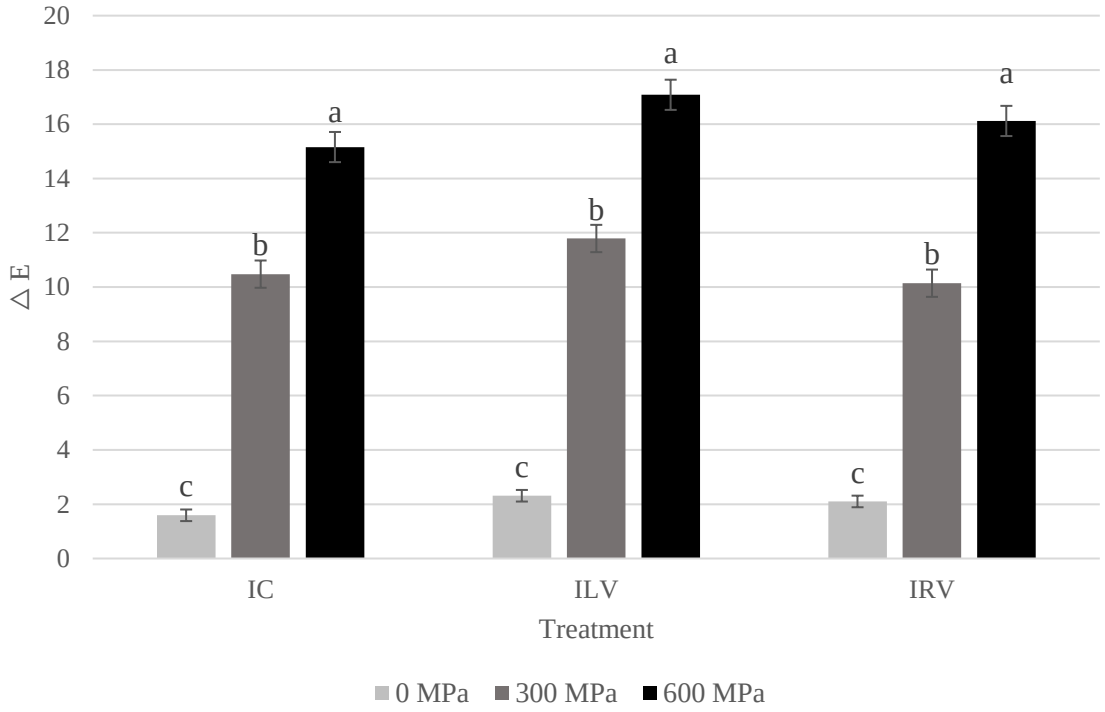


Figure 3. 3. Non-pathogenic *E. coli* growth in ground chicken samples processed at 3 different types of pressures (non-processed, 300 MPa and 600 MPa).

Color Difference

The color difference parameter (ΔE) values for ground chicken are presented in Figure 3.5 and table 3.2. Regarding ΔE values, only the pressure condition had a significant effect ($P<0.05$). This analysis, which considers the evolution of the three-color parameters (L*, a*, and b*), indicates that an increase in pressure intensity corresponds to an increase in color difference (ΔE). Many studies suggest that a rise of 10 units of ΔE produce a significant alteration in the appearance of meat color. In this study, such changes were observed at pressures around 300 MPa, solely attributable to the pressure level and no other conditions. Additionally, the applied pressure level had significantly different effects on each other, emphasizing substantial total color difference between pressurized and not-treated chicken meat. For fresh meat, a* is the most important color parameter, which defined the red-green spectrum with a range of −60 (green) to +60 (red) (Yang et al., 2022). The increase in pressure at 300 MPa and 600 MPa resulted in heightened discoloration of ground chicken samples which was evidenced by the decrease in a* values. Higher pressure introduces the potential disruption in enzymatic systems responsible for the formation of myoglobin pigments, particularly the met myoglobin form (Jung, Ghoul, & De Lamballerie-Anton, 2003). Moreover, L* values were high. L* value represents the light-dark spectrum, where 0 is black and 100 is white, which is related to the browning level of the samples. It was observed that when chicken samples were processed with high pressure (300 and 600 MPa) the L* value also increased. These alterations in the L* parameter was caused by the oxidation of ferrous

myoglobin to metmyoglobin, signifying the denaturation of globular proteins (Park et al., 2021).



Abbreviations:
IC: Inoculated control
ILV: Inoculated sample with lemon/vinegar antimicrobial solution
IRV: Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Figure 3. 4. Color difference (ΔE) in ground white chicken under three pressure conditions (0 MPa, 300 MPa, 600 MPa) and with two natural antimicrobial agents. Lettering indicates significant differences between the treatments using Student t-test ($P< 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 2. Color difference (ΔE) in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Pressure (MPa)	Treatments		
	IC[1]	ILV[2]	IRV[3]
0	$1.884^{c} \pm 0.369$	$2.312^{c} \pm 1.879$	$2.102^{c} \pm 1.214$
300	$10.476^{b} \pm 0.444$	$11.789^{b} \pm 1.580$	$10.142^{b} \pm 1.452$
600	$15.158^{a} \pm 0.667$	$17.084^{a} \pm 0.632$	$16.120^{a} \pm 2.445$

[1]**IC:** Inoculated control
[2]**ILV:** Inoculated sample with lemon/vinegar antimicrobial solution
[3]**IRV:** Inoculated sample with Rosemary oil/vinegar antimicrobial solution

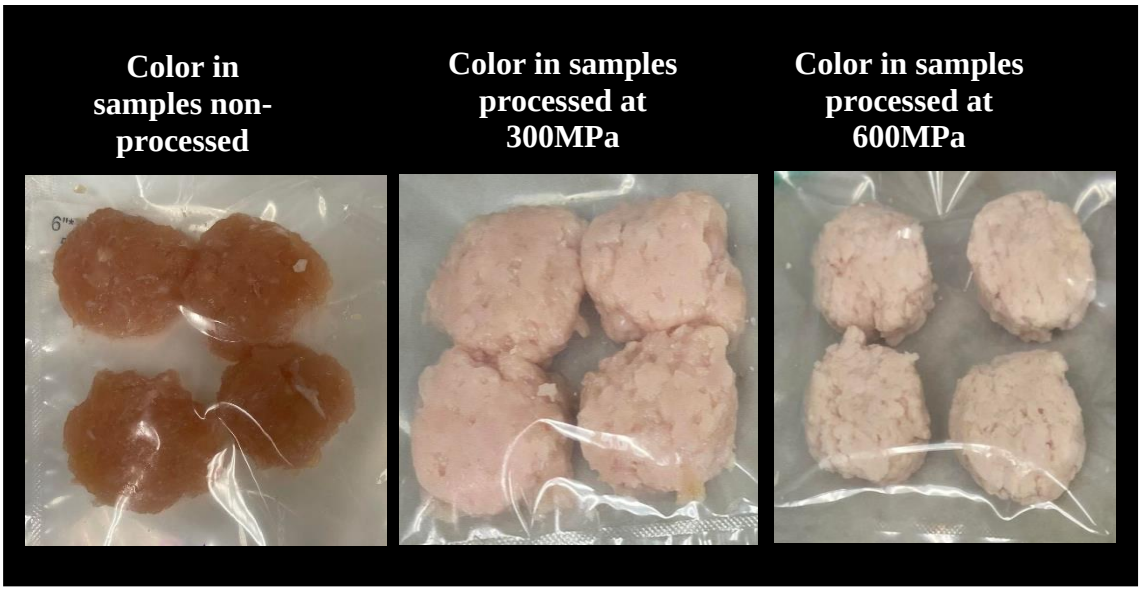
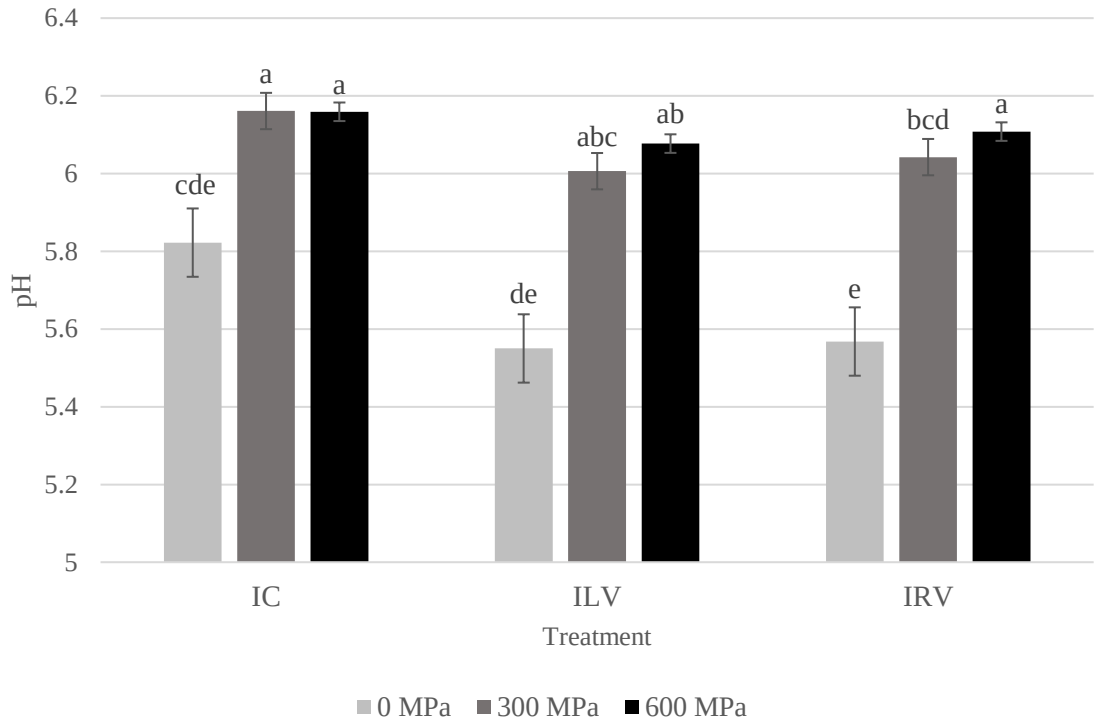


Figure 3. 5. Color difference in ground chicken samples processed at 3 different types of pressures (non-processed, 300 MPa and 600 MPa)

pH

Figure 3.7 and table 3.3 illustrate the influence of three different pressures and antimicrobial treatments on pH value. Although most of the treatments did not change ($P>0.05$), a clear and significant direct relationship was observed between pressure and pH. Within each treatment, whether with or without antimicrobial solutions, there was a notable and significant increase ($P<0.05$) in the pH of chicken samples after each pressure application, without pressure and the ones high pressurized. This pattern aligns with findings from previous studies that reported a similar pH increase, suggestion that pressure induced conformational changes, linked to protein denaturation, may reduce the exposure of acidic groups, resulting in an increase in the chicken samples pH (Evrendilek, 2022). Interestingly, the addition of antimicrobial solutions had an impact on decreasing pH values. Among all processed and non-processed samples, the ones that were treated with rosemary and vinegar solution showed the highest decrease in pH values. However, only the inoculated control processed at 600 MPa showed a significant difference compared to the pH results of IRV treatment. Gao, Zhuang, Yeh, Bowker, & Zhang (2019) observed a significant decrease in pH values in ground chicken patties processed with cols plasma and rosemary extract. They established a correlation between the pH reduction and the presence of carnosic acid and rosmarinic acid in the extract. Initially, high-pressure treatment did not show any significant effect on pH of cooked chicken nuggets. However, after 30 days of chilled storage, pH was significantly lower ($P< 0.05$) in control samples as compared to pressure treated samples (Table 1). High-pressure treatment of meat products generally produces a small increase in pH due to a decrease in acidic groups (Angsupanich and Ledward, 1998).

Making a relationship with the analysis of yield performed previously. Under high-pressure processing, it is possible to achieve a high yield even at low pH (samples no pressurized). HPP can influence the functionality of proteins in a way that enhances their water-holding capacity and structural integrity, counteracting the typical effects of low pH.



Abbreviations:
IC: Inoculated control
ILV: Inoculated sample with lemon/vinegar antimicrobial solution
IRV: Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Figure 3. 6. pH parameter in ground white chicken under three pressure conditions (0 MPa, 300 MPa, 600 MPa) and with two natural antimicrobial agents. Lettering indicates significant differences between the treatments using Student t-test ($P < 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 3. pH parameter in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

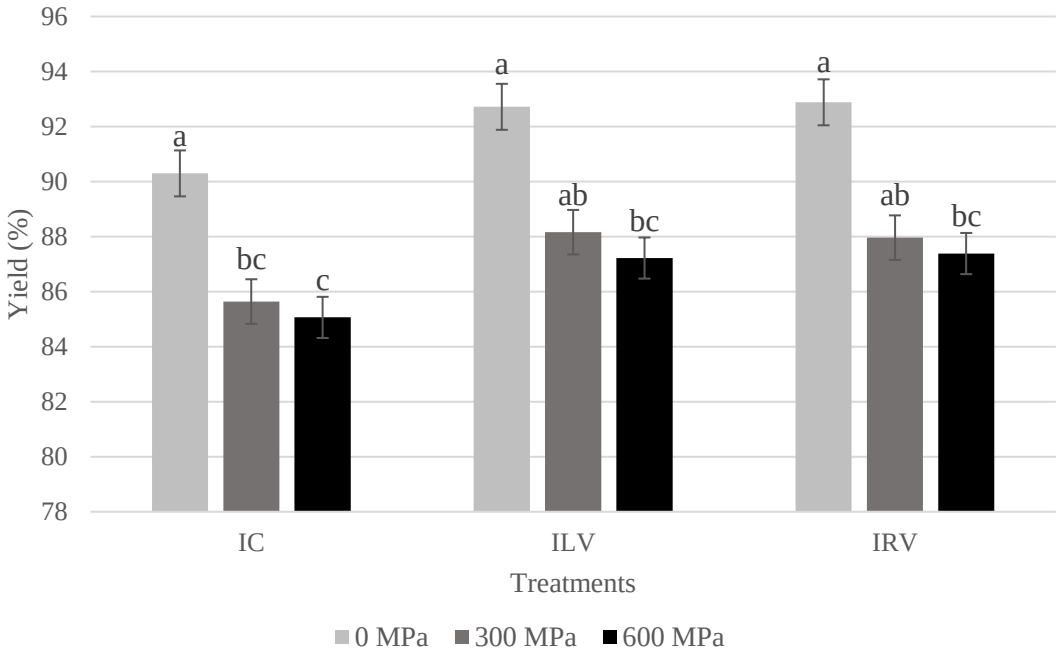
Pressure (MPa)	Treatments		
	IC[1]	ILV[2]	IRV[3]
0	$5.822^{cde} \pm 0.340$	$5.555^{de} \pm 0.017$	$5.567^{e} \pm 0.121$
300	$6.161^{a} \pm 0.018$	$6.006^{abc} \pm 0.024$	$6.042^{bcd} \pm 0.304$
600	$6.159^{a} \pm 0.010$	$6.077^{ab} \pm 0.031$	$6.108^{a} \pm 0.026$

[1]**IC:** Inoculated control
[2]**ILV:** Inoculated sample with lemon/vinegar antimicrobial solution
[3]**IRV:** Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Yield (%)

Figure 3.8 and Table 3.4 illustrate the impact of three different pressures and antimicrobial treatments on cooking yield (%). No significant effect (P>0.05) on cooking yield was observed between the inoculated control and the treatments with added antimicrobial solutions across the three pressures conditions. In contrast, only the inoculated control showed a significant difference (P<0.05) between the non-pressurized samples and those subjected to pressure, suggesting and inverse relationship between pressure increase and cooking yield. However, above 300 MPa, there was no significant difference, indicating that between 300 and 600, the yield loss in chicken meat was considered substantial. This finding is consistent with existing literature, particularly studies on meat treated with high-pressure processing within the range of 200 to 300 MPa range, suggesting no consistent trend for protein denaturation and cook loss above 200 MPa. Usually, adverse effects are observed in various types of meat when processed at

pressures exceeding 200 MPa. This is potentially attributed to protein denaturation, leading to alterations in protein solubility values and changes in the ability of the proteins to bind water. High-pressure processing tends to increase cooking loss and decrease water holding capacity (WHC). The diminished water holding capacity contributes to purge loss from meat, representing a notable reduction in chicken patties weight (Warner, 2017).



Abbreviations:
IC: Inoculated control
ILV: Inoculated sample with lemon/vinegar antimicrobial solution
IRV: Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Figure 3. 7. Yield (%) parameter in ground white chicken under three pressure conditions (0 MPa, 300 MPa, 600 MPa) and with two natural antimicrobial agents. Lettering indicates significant differences between the treatments using Student t-test ($P<0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 4. Yield (%) parameter. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Pressure (MPa)	Treatments		
	IC[1]	ILV[2]	IRV[3]
0	$90.296^{a} \pm 2.866$	$92.716^{a} \pm 0.072$	$92.880^{a} \pm 0.072$
300	$85.642^{bc} \pm 2.866$	$88.160^{ab} \pm 2.866$	$87.962^{ab} \pm 2.866$
600	$85.062^{c} \pm 1.126$	$87.220^{bc} \pm 1.126$	$87.384^{bc} \pm 1.126$

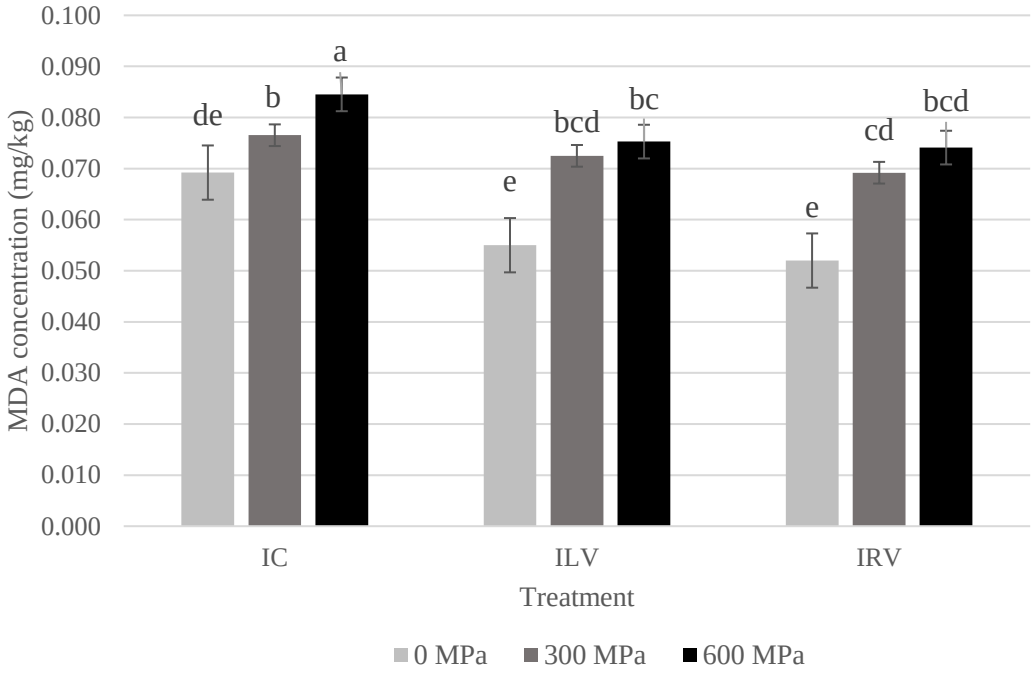
[1]**IC:** Inoculated control
[2]**ILV:** Inoculated sample with lemon/vinegar antimicrobial solution
[3]**IRV:** Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Thiobarbituric reactive substances (TBARS)

Figure 3.9 and Table 3.5 show the effect of different pressures and antimicrobial treatments on the TBARS value of ground chicken samples, represented as Malondialdehyde (MDA) concentration. MDA is a prominent aldehyde produced during secondary lipid oxidation and serves as a common marker for oxidation. The presence of oxidized lipids in certain food products has a directly proportional relationship with the rise in Thiobarbituric Acid Reactive Substances (TBARS) (Dong et al., 2020). It is evident that an increase in pressure results in a significant elevation in MDA concentration. Samples treated with 600 MPa led to a remarkable increase in lipid oxidation of 21% compared to treatments without high pressure. Similar findings were reported by Kai et al. (2020) in their research on high-pressure-processed chicken breast. They established a proportional relationship between the pressure increase and TBARS value, attributing it to the denaturation of pigments proteins such as myoglobin and oxymyoglobin. This denaturation could potentially release Fe^{3+} and Fe^{2+} in reduced state,

known to catalyze fat oxidation. However, the addition of both natural antimicrobials demonstrated significant efficacy in reducing lipid oxidation compared to the control at 600 MPa without the addition of antimicrobials. The ILV treatment at 600 MPa showed an 11.8% reduction in MDA concentration compared to the inoculated control, while the IRV treatment processed at 600 MPa showed a reduction of 9.16%. All treatments with addition of antimicrobial solutions presented an improvement in the reduction of thiobarbituric acid-reactive substances compared to the inoculated control, regardless of the pressure type or the specific of antimicrobial solutions used. A comparison among the treatments reveals that samples not subjected to high pressure but treated with lemon/vinegar antimicrobial solution exhibited a significant 5.23% reduction in lipid oxidation compared to the inoculated control, signifying a notable decrease. Similarly, samples treated with rosemary oil/vinegar antimicrobial solution displayed a comparable effect, resulting in a 5% reduction in lipid oxidation compared to the inoculated control. The antioxidant properties of citrus products stem from their rich flavonoid content, particularly glycosylated flavonones and polymethoxyflavones (Ahmad, Gokulakrishnan, Giriprasad, & Yatoo, 2013). These compounds can scavenge free radicals and chelate metals, reducing the rate of oxidation. The antioxidant activity of rosemary is attributed to the presence of phenolic diterpenes such as carnosic acid along with phenolic acids. Numerous studies have demonstrated the efficacy of rosemary extracts and oils in reducing the levels of thiobarbituric acid reactive substances and hexanal values (Aziz & Karboune, 2016). Carnosic acid acts as a chemical quencher of Reactive Oxygen Species

(ROS), undergoing consumption and oxidation into various derivatives upon reacting with ROS (Gao, Zhuang, Yeh, Bowker, & Zhang, 2019).



Abbreviations:
IC: Inoculated control
ILV: Inoculated sample with lemon/vinegar antimicrobial solution
IRV: Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Figure 3. 8. TBARS analysis in ground white chicken under three pressure conditions (0 MPa, 300 MPa, 600 MPa) and two natural antimicrobial agents. Lettering indicates significant differences between the treatments using Student t- ($P<0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 5. TBARS analysis in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Pressure (MPa)	Treatments		
	IC[1]	ILV[2]	IRV[3]
0	$0.069^{de} \pm 0.001$	$0.055^{f} \pm 0.002$	$0.051^{f} \pm 0.003$
300	$0.077^{b} \pm 0.001$	$0.072^{bcd} \pm 0.001$	$0.069^{cde} \pm 0.005$
600	$0.082^{a} \pm 0.002$	$0.075^{bc} \pm 0.0008$	$0.073^{bc} \pm 0.001$

[1]**IC:** Inoculated control
[2]**ILV:** Inoculated sample with lemon/vinegar antimicrobial solution
[3]**IRV:** Inoculated sample with Rosemary oil/vinegar antimicrobial solution

Texture Analysis

The textural profile assessed in this study included cohesiveness and shear force. Both analyses revealed a significant difference (P<0.05) between raw and cooked chicken meat. For raw and cooked chicken meat, it is evident that an increase in pressure leads to a corresponding increase in shear force. Among the raw samples analyzed for shear force, no significant difference (P>0.05) was observed between non-pressurized samples and those processed at 300 MPa and 600 MPa. However, in the case of cooked samples, a notable increase in the force required to cut the chicken patties was observed with the increasing pressure. The textural properties of meat undergo changes due to pressure-induced modifications in proteins, which differ from alterations caused by heat. While heat induces the shrinkage of connective tissue, such as collagen, and the breakage of hydrogen bonds, high-pressure processing changes the muscle texture by rupturing hydrophobic and electrostatic interactions. Both processing mechanisms have an impact in the increase of the force applied along the surface of the cooked chicken patties. In

general, pressures lower than 200 MPa can tenderize meat; however, pressures higher than that lead to an increase in hardness, resulting in higher shear force values (Radovčić et al., 2019).

Shear Force.

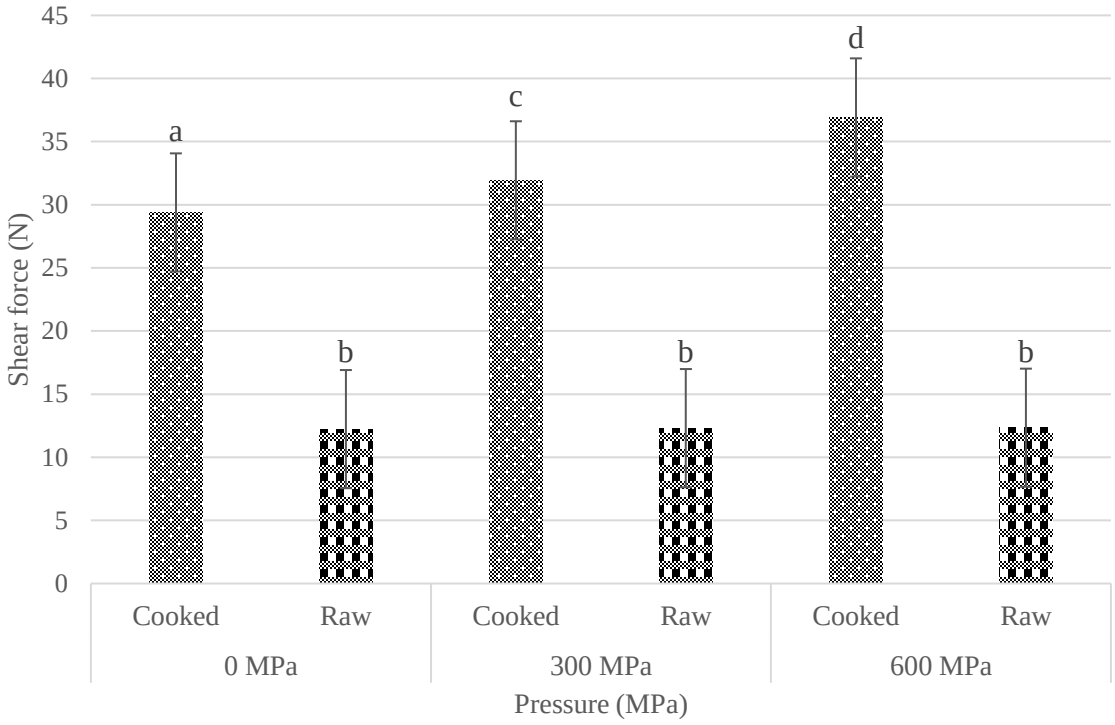


Figure 3. 9. Effect of pressures (0 MPa, 300 MPa, 600MPa) and two types of condition (raw and cooked) on shear force texture analysis in ground chicken. Lettering indicates significant differences between the treatments using Student t-test ($P<0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 6. Shear force results in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Pressure (MPa)	Treatments	
	Cooked	Raw
0	$29.390^{a} \pm 0.548$	$12.230^{b} \pm 0.053$
300	$31.934^{c} \pm 2.615$	$12.308^{b} \pm 0.067$
600	$36.916^{d} \pm 2.603$	$12.340^{b} \pm 0.023$

Cohesiveness

No significant differences (P>0.05) in cohesiveness values were observed between pressure for the cooked and raw samples except for the raw treatment pressurized at 600 MPa. Table 3.7 shows that cohesiveness increases with the application of high pressure. Cohesiveness reflects the degree of tightness of muscle tissue bonding (Şayin Sert & Coşkun, 2022). Various studies have established that cohesiveness significantly increases at 300 MPa, but at pressures higher than 500 MPa, the increase has a reversible behavior, starting to reduce the cohesiveness degree in meat (Radovčić et al., 2019). In minimal heat treatment and high-pressure processing, proteins are given sufficient time to unfold and interact with each other, facilitating the formation of a stronger gel and inducing myofibrillar protein denaturation, ultimately resulting in higher cohesiveness (Iheagwara, Okonkwo, Ofoedu, Shorstkii, & Okpala, 2021).

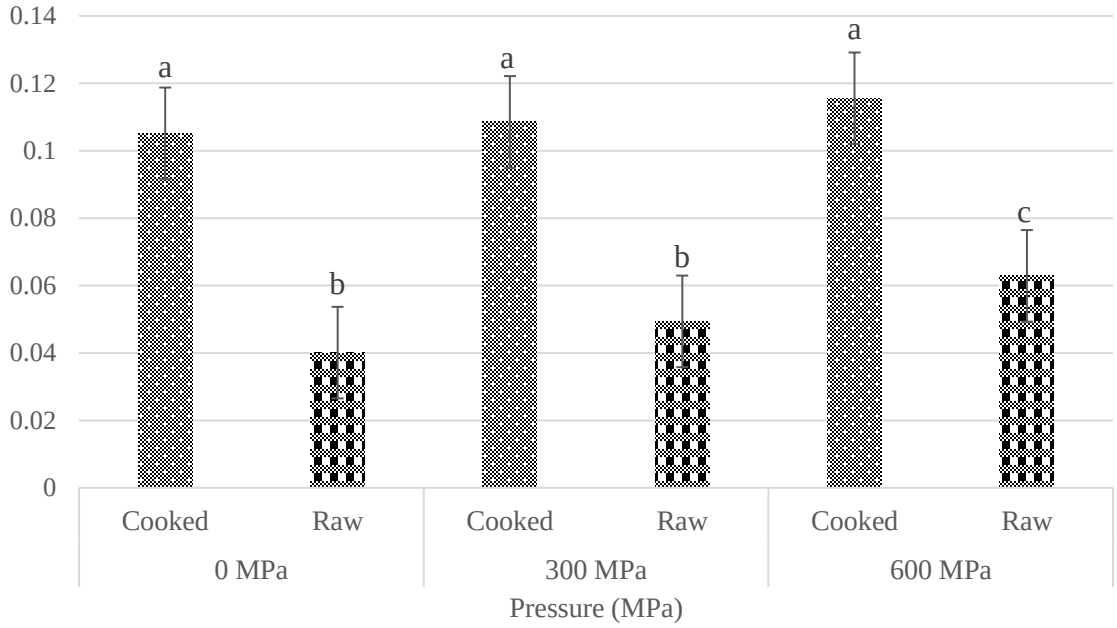


Figure 3. 10. Effect of pressures (0 MPa, 300 MPa. 600MPa) and two types of condition (raw and cooked) on cohesiveness texture analysis in ground chicken. Lettering indicates significant differences between the treatments using Student t-test least test ($P<0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 3. 7. Cohesiveness results in ground chicken. The mean ± SE illustrates the relationship between pressure and natural antimicrobials. Lettering denotes significant differences between treatments based on student t-test (P< 0.05). Average data from 3 independent replications.

Pressure (MPa)	Treatments	
	Cooked	Raw
0	$0.105^{a} \pm 0.527$	$0.040^{b} \pm 0.046$
300	$0.108^{a} \pm 0.022$	$0.049^{b} \pm 0.043$
600	$0.115^{a} \pm 0.002$	$0.062^{c} \pm 0.031$

Conclusion

These results suggest that High-Pressure Processing can be used to improve the safety of ground chicken. Given the results in microbiology analysis using 600 MPa process can cause *E. coli* reductions greater than 5 Log. This logarithm reduction is enough to be considered as safe to eat, even when the ground chicken has not been cooked. This reduction effect was magnified with the addition of natural antimicrobials, such as rosemary essential oil and concentrated buffered vinegar, which provides additional lethality to the process, particularly at lower pressures. Additionally, the use of natural antimicrobials showed a positive effect in the reduction of lipid oxidation that can be a significant issue for quality of the ground chicken. While these results are promising, further study is needed to optimize the effects of high-pressure processing on color difference and texture, particularly as these changes are readily apparent and could have a negative effect on consumer acceptance of the product.

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CHAPTER FOUR

THE EFFECTS OF HIGH-PRESSURE PROCESSING ON THE TEXTURAL AND SENSORY CHARACTERISTICS OF GROUND POULTRY

Abstract

High pressure processing is a non-thermal processing technique capable of reducing or eliminating pathogens while preserving desirable sensory characteristics in a wide range of products. Despite this, the technology has seen limited use in muscle foods due to potentially undesirable organoleptic changes. In this study, ground chicken samples (either with or without natural antimicrobial solutions, including buffered concentrated vinegar with rosemary oil (V+REO) and a lemon juice-vinegar blend) were processed using HPP technology at pressures of 600 MPa and 300 MPa. Subsequently, they were analyzed to identify any textural and sensory differences. Textural analysis revealed a notable increase in the hardness of uncooked products, resulting in a 27% higher shear force value than non HPP samples, while cooked products were much more similar. Interestingly, cohesiveness had no significant difference between non-processed and processed samples, either cooked or uncooked. Sensory evaluation was conducted on small patties which were formed from processed and unprocessed samples before cooking via sous vide. Immediately prior to serving, each sample received a surface sear, and panelists were asked to evaluate each chicken patty treatment using a 9-point hedonic scale and rank the samples based on overall preference to assess each attribute, including juiciness, cohesiveness, flavor, mouthfeel, color and other characteristics. The preference

analysis revealed that among the panelist, 23% favored the sample processed at 300 MPa without the addition of any natural antimicrobial, showing a significant preference over samples sample processed at 600 MPa. On the other hand, the samples processed at 600 MPa, regardless of the addition of natural antimicrobials, were the least preferred overall and did not show a significant difference between each other. Ther lowest rating on the 9-point hedonic scale were consistently given to the sample processed at 600 MPa, ranging between 4 and 5 (indicating “Dislike slightly” and “Neither like or dislike”) across all attributes, including juiciness, cohesiveness, tenderness, mouthfeel, and overall texture. Conversely, the highest ratings were assigned to samples processed at 300 MPa, with panelist grading them between 6 and 7 on the hedonic scale (indicating “Like slightly” and “Like moderately”). Panelist noted that these samples exhibited greater juiciness, cohesiveness, and flavor compared to the other samples.

Introduction

High-pressure processing offers several benefits, including maintaining flavor, color, and thermosensitive vitamins. However, it can also impact important qualities like color and texture, potentially affecting consumer acceptance. High-pressure treatment can enhance the functional properties of muscle proteins by increasing moisture-protein interactions, improving water holding capacity, which is crucial in meat processing. Nonetheless, this improvement affects the color, potentially reducing their appeal. Exists a lot of variability in meat properties due to pressurization level, time, and temperature and while HPP have an excellent effect in reducing bacterial spoilage and extending

shelf-life, it also has a negative impact in some quality characteristics (Radovčić et al., 2019). Due to increasing demand for chicken meat, the poultry industry has had to seek new methods to increase production and industrialization without affecting the quality of the meat (Umaña, 2015). Until recently, the predominant factor guiding chicken meat selection was its price. However, with cultural changes and access to more information, quality has become the primary determinant of purchase decisions. Quality includes various aspects including food sourcing, safety, nutritional value and sensory attributes (Damaziak et al., 2019). Various factors are known to potentially affect the sensory characteristics of chicken meat, with tenderness being one of the most affected attributes. The major poultry quality characteristics are appearance, texture, juiciness, flavor and functionality. Appearance and texture have been the most influencing factors on consumer's initial selection of poultry meat products, while juiciness and flavor are attributes more dependent on the preparation than the product itself (Fletcher, 2002). The concept of texture includes properties related to the structural elements of food, detectable through the senses and factors influencing how meat is perceived in the mouth. Texture often refers to the smoothness or fineness of muscle tissue. Juiciness and tenderness, as major referents of textural description, have been among the most difficult characteristics to define and measure in cooked chicken breast meat products (Surmacka & Weiss Torgeson, 1965). This problem has been attacked by many food technologists, who searched for new techniques to better understand tenderness and juiciness. That is why diverse methods have been discovered. High pressure treatment is relatively a new method used for meat tenderization. Numerous studies have investigated the

tenderization of meat during aging, which is attributed to structural changes in myofibrils induced by high pressure. HPP can tenderize meat when applied before rigor mortis, but its beneficial effects are not measurable on post-rigor meat at low temperature. Actually, numerous research has demonstrated that HPP by itself may lead to meat hardening or toughening. Typically, low pressure (<300MPa) can tenderize meat before rigor mortis starts, while achieving post rigor tenderization with high hydrostatic pressure requires higher temperatures between 104 °F and 140 °F (Sun & Holley, 2010). As observed in the previous chapter and as will be further highlighted in the upcoming one, this research assessed various methods for determining texture differences in cooked chicken patties. Warner-Bratzler method is commonly used in the food industry for quality control and extensively utilized in research as a mechanical method for texture evaluation. Lyon & Lyon (1991) a classification of textures relating the attribute of meat tenderness to Warner-Bratzler mechanical texture measurements. They found that values below 3.62 Kg represent a very tender texture, 3.62-6.61 Kg moderately tender, 6.62-9.60 Kg slightly tender to slightly tough, 9.61-12,60 Kg moderately tough, and values greater than 12.60 Kg for meat classified by consumer as very tough. On the other hand, affective sensory analysis allows to determine the perception of meat texture and other related attributes such as tenderness, and even firmness (Umaña, 2015). This chapter has as an objective to explore the sensory attributes of cooked high-pressurized chicken patties incorporating natural antimicrobials through a sensory study involving untrained panelist, while comparing results with mechanical results (TPA analysis) obtained in the previous chapter.

Material and Methodology

Preparation of Samples for High-Pressure Processing.

Approximately 15 kg of boneless, skinless chicken breasts were obtained from The Best Dressed Chicken Company located in Ward, South Carolina. The chicken was divided in 1kg bags and frozen until sample preparation. Two days before starting the experiment, the chicken was placed in rigid containers and allowed to thaw for at least 48 hours at refrigeration temperatures. The thawed chicken breasts were ground, weight and distributed in polyethylene laminate vacuum bags (1 kg of ground chicken per bag). Three sub-samples were prepared following the description below:

Sub-sample 1: 1% of distilled water per kilogram of chicken

Sub-sample 2: 1% of buffered concentrated vinegar with rosemary oil per kilogram of chicken

Sub-sample 3: 1% Lemon and vinegar blend per kilogram of chicken

The sub-samples were sealed, vacuum packed and hand stomached for 15 seconds. Samples were processed under two pressure conditions: 300 MPa and 600 MPa for 3 minutes. After processing, the samples were stored under refrigeration conditions. In total, nine treatments were chosen to capture a comprehensive range of textural characteristics across various processing methods.

Cooking of Samples

To replicate a standard consumer experience without introducing any flavor bias, each treatment was seasoned with 10 grams of salt per kilogram of raw ground chicken and thoroughly mixed. Following the same methodology from the previous chapter,

cylindrical patties were formed with 25 g of each treatment. Four cylindrical patties were placed in polyethylene pouches, sealed and vacuum packaged. The samples were held for up to 48 hours at refrigeration temperature until the day of sensory analysis. Sous vide processing parameters were verified to meet the minimum time and temperature combinations for chicken by the USDA (FSIS, 2021) prior to beginning the study. A circulating water bath was prepared at 160°C using an immersion circulator. The packaged samples were placed into the water bath and cooked for 60 minutes. At the end of cooking, the packages were removed from the water bath. The cooked chicken patties were then seared using a handheld surface broiler (Searzall, Booker and Dax, New York, NY, USA) for 3 minutes per side immediately prior to serving to the consumer to provide surface color, aroma, and texture.

Panel Makeup and Details

An untrained panel consisting of sixty-three adults, divided across three separate days, evaluated the samples via hedonic scoring and preference ranking for each of the nine treatments. Participants who were younger than 18 years older or who had any food allergies were excluded. Demographic data, such as age and country of origin were collected from panelists but are not presented here. Additionally, participants were asked about their frequency of chicken consumption and their preferred method of preparing and consuming chicken. Each treatment was coded following AMSA procedure as showed table 4.1 (Wheeler, Papadopoulos, & Miller, 2016).

Table 4. 1. Treatment description, with specification of coding corresponding to each pressure and natural antimicrobial condition.

Treatment description		
Code	**Pressure condition**	**Natural Antimicrobial Condition**
291	0 MP	Without addition of natural antimicrobial
645	0 MP	Rosemary and vinegar antimicrobial solution (RV)
139	0 MP	Lemon and vinegar antimicrobial solution (LV)
841	300 MPa	Without addition of natural antimicrobial
297	300 MPa	Rosemary and vinegar antimicrobial solution (RV)
783	300 MPa	Lemon and vinegar antimicrobial solution (LV)
574	600 MPa	Without addition of natural antimicrobial
161	600 MPa	Rosemary and vinegar antimicrobial solution (RV)
392	600 MPa	Lemon and vinegar antimicrobial solution (LV)

Hedonic Panel

A hedonic sensory panel was conducted to evaluate consumer preferences for both high-pressure processing and natural antimicrobials addition. Panelists used a 9-point hedonic scale, where 1 is “dislike extremely” and 9 is “like extremely, to rate various attributes of the samples, which were presented as patties to the panelists. Drinking water was provided to cleanse the palate between samples. The panelists provided scores for juiciness, tenderness, cohesiveness, overall texture, flavor and color.

To prevent any bias in the order of presentation, the samples were arranged in a balanced manner following the Latin Square design with n=9, ensuring an equitable presentation pattern. The specific presentation is illustrated in table 4.2.

Table 4. 2. Williams Design Latin Square

1st Sample	**2nd Sample**	**3rd Sample**	**4th Sample**	**5th Sample**	**6th Sample**	**7th Sample**	**8th Sample**	**9th Sample**
1	2	9	3	8	4	7	5	6
2	3	1	4	9	5	8	6	7
3	4	2	5	1	6	9	7	8
4	5	3	6	2	7	1	8	9
5	6	4	7	3	8	2	9	1
6	7	5	8	4	9	3	1	2
7	8	6	9	5	1	4	2	3
8	9	7	1	6	2	5	3	4
9	1	8	2	7	3	6	4	5
6	5	7	4	8	3	9	2	1

Ranked Preference Panel

A consumer preference test was conducted with 63 panelists following the hedonic scale scoring. All nine treatments were simultaneously presented to the panelist, enabling comparison and ranking of the samples. Similarly to the hedonic panelist, the presentation sequence of the samples followed a Williams Design Latin Square (Table 4.2). The panelist ranked the products on a scale from 1 (“least preferred”) to 9 (“most preferred”).

Statistical Analysis

The hedonic scores were analyzed using JMP Pro 14 Statistical Software (SAS Institute Inc., Cary, NC). Least Squares Means Student’s t-test ($p < 0.05$) was used to discriminate among the means. For the ranked preference scores, a Friedman’s

nonparametric chi squared test was conducted in JMP. Additionally, a comparison t-test was conducted to determine if there were any significant differences between the treatments at the $P \leq 0.05$ level.

Results and Discussion

Hedonic Consumer sensory panel

Juiciness

With the exception of flavor, each attribute presented at least one sample with significant differences compared to the others, in terms of pressures and natural antimicrobials. Figure 4.1 and table 4.3 show the hedonic scores assigned to the attribute of juiciness. Comparisons among pressures and treatments revealed that the sample with the higher score, ranging from slightly-like to moderately, was the one treated at 300 MPa without any addition of natural antimicrobial solutions. However, this did not show a significant difference ($P>0.05$) compared to the other treatments with the addition of rosemary-vinegar and lemon-vinegar antimicrobial solutions. On the other hand, the least favored sample was the one processed at 600 MPa, regardless of the presence or absence of antimicrobial solutions, and these were significantly different compared to the control and the samples processed at 300 MPa ($P<0.05$). Previous research reported an increase in juiciness with the application of 300 MPa pressure. It was observed that high-pressure treatment tends to decrease juiciness with increasing pressure (Kruk et al., 2011). Although any significant difference was observed between the control and the samples with natural antimicrobials in each pressure, is important to notice that many authors

have observed the effect of pH on juiciness and shrinkage. Higher pH in meat leads to increased juiciness and products (Surmacka & Weiss Torgeson, 1965).

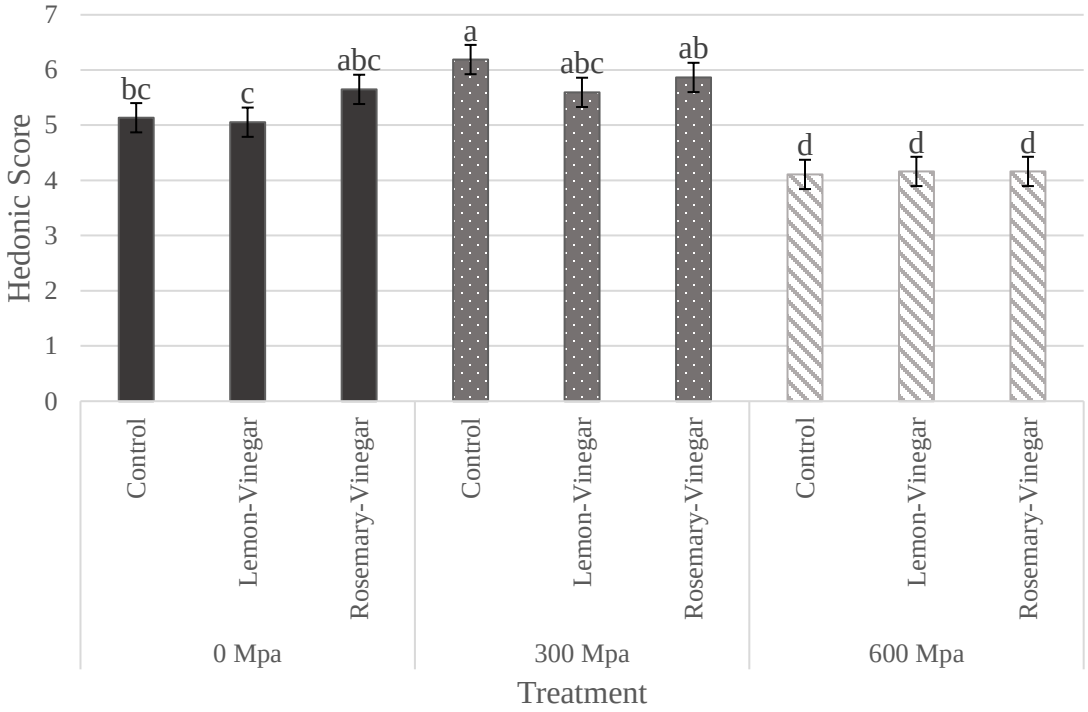

Figure 4. 1. Hedonic Scores for Juiciness. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 4. 3. Hedonic Scores for Juiciness (raw data). The mean ± SE illustrates the correlation effect of pressure and treatment. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Average data from 3 independent replications.

Treatments	Pressure		
	0MPa	300 MPa	600MPa
Control	5.135 ± 1.902^{bc}	6.189 ± 1.663^{a}	4.108 ± 1.744^{d}
Lemon-Vinegar	5.054 ± 1.884^{c}	5.594 ± 1.755^{abc}	4.162 ± 1.907^{d}
Rosemary-Vinegar	5.648 ± 1.751^{abc}	5.864 ± 1.960^{ab}	4.162 ± 2.075^{d}

Cohesiveness

Figure 4.2 and table 4.4 show the effect of high-pressure treatment and natural antimicrobials on attribute cohesiveness. Cohesiveness is defined as the ability of an object to remain together in a singular mass. Although cohesiveness results presented in the previous chapter related with cooked high pressurized chicken did not show significant difference, panelist could feel a significant decrease in cohesiveness when pressure increased ($P<0.05$) (Table 4.4). Cohesiveness attribute related with high pressure has a non-linear or unpredictable behavior. Many authors, including Macfarlane (1985), Suzuki (2006) and Sun and Holley (2010), have reported that pressure treatment improves cohesion between meat particles when the pressure is lower than 400 MPa and time of processing does not exceed 5 minutes. Contrary, higher pressures (>500MPa) dramatically reduced cohesiveness and increase hardness. The rise in hardness observed in chicken samples treated with high pressure before or after thermal treatment was referred to muscle compaction and was attributed to alterations in muscle resulting from modifications in meat protein conformation, protein denaturation, aggregation, and

gelation (Akhtar & Abrha, 2022). Even when in both types of analysis samples were cooked via sous vide, the weak correlation observed in the behavior of cohesiveness in mechanical analysis of texture and sensory study could stem from the heterogenous nature of chicken muscle and the differing approaches used by TPA and sensory evaluations in measuring texture (Bland et al., 2018)

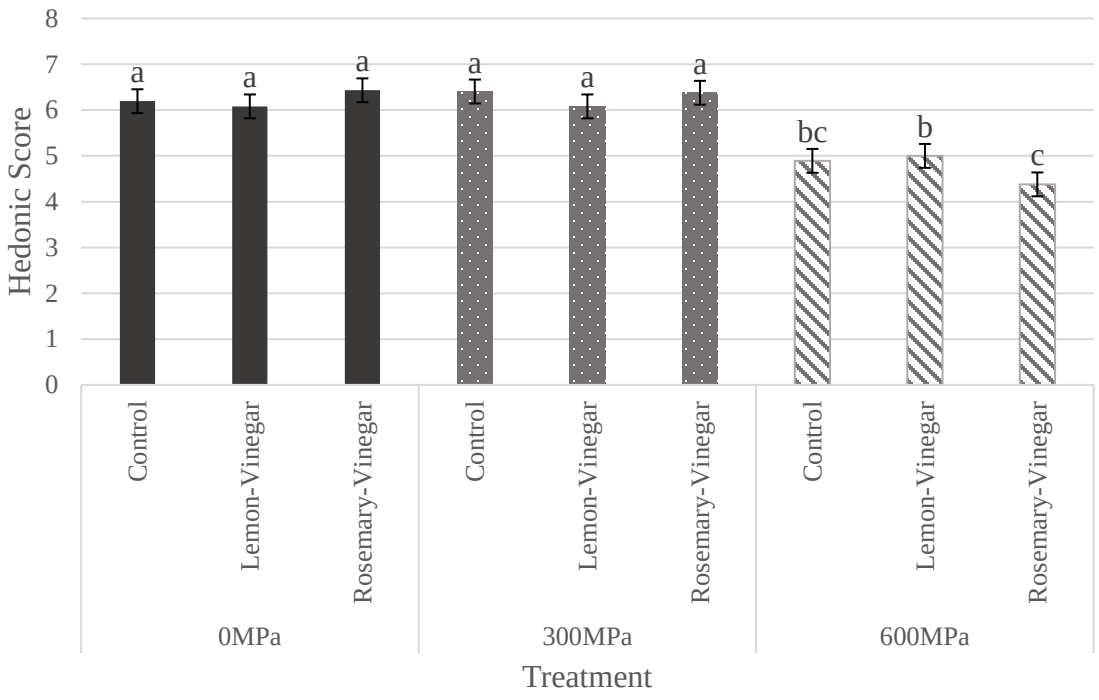

Figure 4. 2. Hedonic Scores for Cohesiveness. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 4. 4. Hedonic Scores for Cohesiveness (raw data). The mean ± SE illustrates the correlation effect of pressure and treatment. Lettering indicates significant differences between the treatments using Student t-test least test (α = 0.05). Average data from 3 independent replications.

Treatments	Pressure		
	0MPa	300 MPa	600MPa
Control	6.194±1.582[a]	6.405±1.189[a]	4.892±1.559[bc]
Lemon-Vinegar	6.081±1.210[a]	6.081±1.441[a]	5.000±1.632[b]
Rosemary-Vinegar	6.432±1.214[a]	6.378±1.533[a]	4.378±1.962[c]

Tenderness

Table 4.5 and figure 4.3 show the effect of high-pressure processing and natural antimicrobials on tenderness attribute. It can be observed that there is not a significant difference between non-pressurized samples and those treated at 300 MPa (P>0.05). However, a decrease in tenderness is observed in chicken patties treated at 600 MPa. The tenderness of poultry meat is influenced by two major factors: the maturity of connective tissues and the contractile state of myofibrillar proteins. The maturity of connective tissue entails the chemical bonding of collagen within the muscle. The age of the chicken used in this experiment was 6 weeks old, which can have a negative influence on tenderness. With aging, collagen cross-linking increases, resulting in tougher meat from older animals (Fletcher, 2002), Typically, low pressure (<300MPa) can tenderize meat before rigor mortis starts, while achieving post rigor tenderization with high hydrostatic pressure requires higher temperatures between 104 °F and 140 °F (Sun & Holley, 2010). However, with pressures surpassing 400 MPa, the density of myofibrils progressively rose, leading to a rebound in hardness, resulting in a reduction of tenderness. Various studies consider that the densest muscle structure and highest hardness were achieved at

500 MPa. This outcome could be attributed to the pressure's tendency to densify the muscle fiber network structure, resulting in a more compact and tougher muscle structure (Zhang et al., 2023). High-pressure treatment increases myofibril fragmentation, similar to the effects of aging, where myosin filaments dissociate and actinin is released. Pressures exceeding 300 MPa, the myofibrillar cross sections undergo transformation, assuming unrecognizable changes. The pressure recommended to increase tenderness in chicken are between 150-300 MPa for 5-10 minutes (Akhtar & Abrha, 2022). Although sous vide is an excellent alternative to improve products texture, especially maintaining the juiciness and tenderness of the product, prevention of lipid oxidation, enhancement of sensory attributes and reduction of protein denaturation, this method did not show any improvement in meat processed at 600 MPa (Hasani et al., 2022).

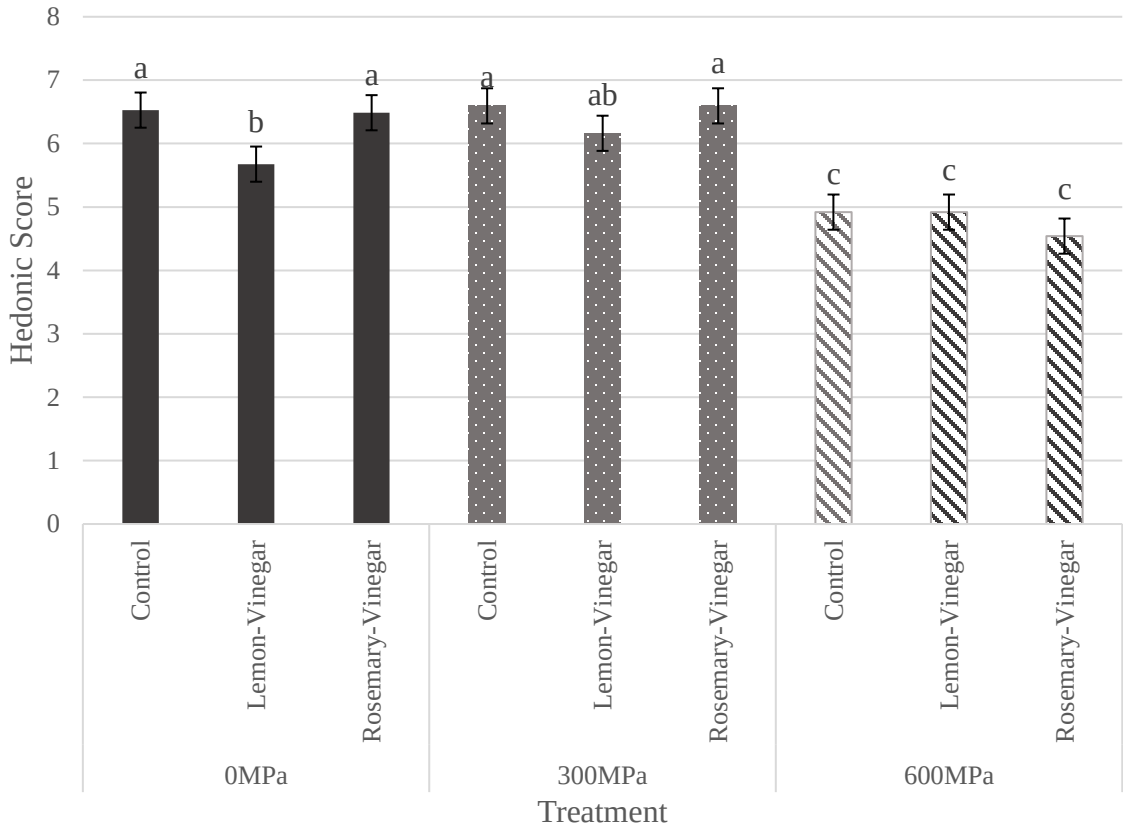

Figure 4. 3. Hedonic Scores for Tenderness. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 4. 5. Hedonic Scores for Tenderness (raw data). The mean ± SE illustrates the correlation effect of pressure and treatment. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Average data from 3 independent replications.

	Pressure		
Treatments	0MPa	300 MPa	600MPa
Control	6.527±1.594^{a}	6.594±1.279^{a}	4.918±1.587^{c}
Lemon-Vinegar	5.675±1.780^{b}	6.162±1.572^{ab}	4.918±1.977^{c}
Rosemary-Vinegar	6.486±1.609^{a}	6.594±1.461^{a}	4.540±2.008^{c}

Overall Texture

Figure 4.4 and table 4.6 showed the effect of high-pressure processing and natural antimicrobials on overall texture attribute. As a summary of textural parameters, figure 4.4 showed a similar behavior compared to the attributes discussed before. The samples processed at 600 MPa were the only ones that showed significant difference compared to the non-processed samples and the ones processed at 300 MPa. Additionally, the addition of natural antimicrobial did not show any effect on texture, regardless of the pressure. Texture plays a pivotal role in determining consumer's satisfaction with poultry meat products. During high-pressure treatment, the texture of meat from various animal species undergoes alterations, leading to a decrease in protein volume. Factors such as the solubilization of peptide bonds and amino acid-branched chains, the volume of internal cavities, and the constitutive volume of atoms collectively influence the protein's volume in a solution. When an object is subjected to high pressure, those interior cavities are compressed, resulting in a reduction in the overall volume of the proteins (Akhtar & Abrha, 2022). Although, the effects of high pressure on the texture of food products are undeniable. Different research has indicated that the changes in the chemical composition and sensorial attributes of food products are less than the changes observed in those treated with thermal treatments (Sánchez et al., 2012). Sensory techniques have been employed since the early stages of scientific inquiry into meat palatability and remain widely used today. They provide the benefit of replicating typical eating conditions, making them a reliable way to collect data. In this sensory study, panelist expressed less favorable acceptance of high-pressure processed chicken patties, noting that the texture

seemed unusual, particularly in the samples processed at 600 MPa (Alexander, Clark, & Howe, 1933).

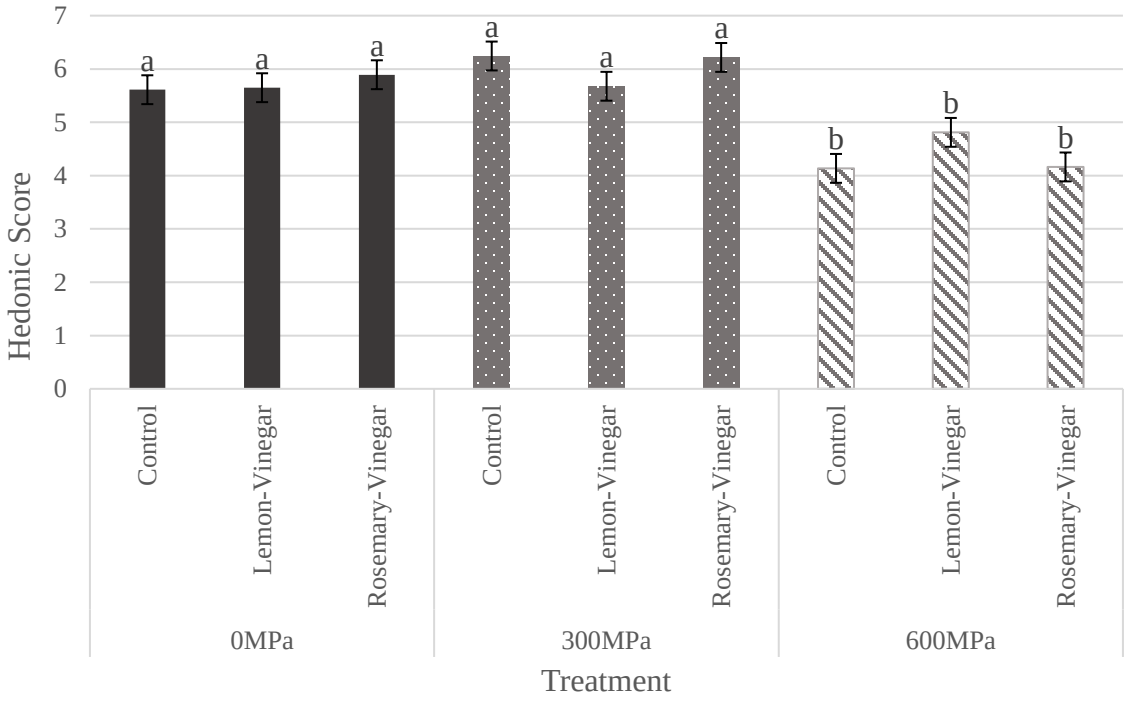

Figure 4. 4. Hedonic Scores for Overall Texture. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 4. 6. Hedonic Scores for Overall Texture (raw data). The mean ± SE illustrates the correlation effect of pressure and treatment. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Average data from 3 independent replications.

Treatments	Pressure		
	0MPa	300 MPa	600MPa
Control	5.611±1.840[a]	6.243±1.516[a]	4.135±1.493[b]
Lemon-Vinegar	5.648±1.531[a]	5.675±1.886[a]	4.810±1.941[b]
Rosemary-Vinegar	5.891±1.897[a]	6.216±1.635[a]	4.162±1.907[b]

Color

Figure 4.5 and table 4.7 showed the effect of high-pressure processing and natural antimicrobials on the color of chicken samples. When comparing the results of this sensory analysis with the physical color analysis previously presented, a similar pattern can be observed, particularly evident among the samples processed at 600 MPa. Panelists were able to notice significant differences between samples processed at 600 MPa compared to the non-processed control and those pressurized at 300 MPa, resulting in lower scores on the hedonic scale. High- pressure processing can significantly modify the typical attributes of fresh meat, altering the quality criteria. Studies suggest that high-pressure processing results in significant alterations in the color of fresh meat. High-pressure treatments disrupt electrostatic connections and stimulate processes involving the exchange of sulfhydryl-disulfide bonds, leading to the dissociation and unfolding of

protein structures, which are responsible for imparting color to chicken meat (Khalid et al., 2023).

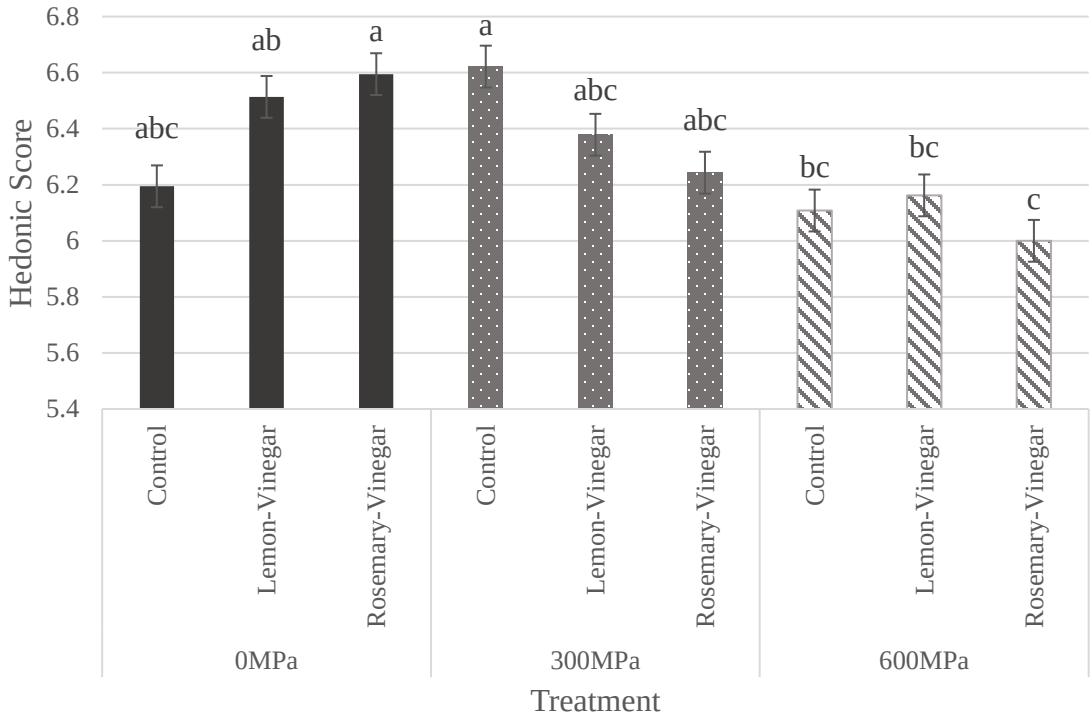

Figure 4. 5. Hedonic Scores for Color. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Error bars represent ±1 SE. Average data from 3 independent replications.

Table 4. 7. Hedonic Scores for Overall Texture (raw data). The mean ± SE illustrates the correlation effect of pressure and treatment. Lettering indicates significant differences between the treatments using Student t-test least test ($\alpha = 0.05$). Average data from 3 independent replications.

Treatments	Pressure		
	0MPa	300 MPa	600MPa
Control	6.194 ± 1.801^{abc}	6.622 ± 1.298^{a}	6.108 ± 1.264^{bc}
Lemon-Vinegar	6.514 ± 1.193^{ab}	6.378 ± 1.569^{abc}	6.162 ± 1.118^{bc}
Rosemary-Vinegar	6.595 ± 1.442^{a}	6.243 ± 1.534^{abc}	6.000 ± 1.354^{c}

Ranked Preference Consumer Panel

A ranked preference panel, consisting of 63 panelists, was tasked with ranking the nine treatments based on overall texture and their level of acceptance, with 9 indicating the most preferred sample and 1 the least preferred. As shown in table 4.8, the sample treated with 300 MPa and without the addition of any antimicrobial was the most preferred. Although there was a significant difference observed among the natural antimicrobial conditions ($P<0.05$), consumers expressed a similar preference for the samples treated with 300 MPa, noting that they were "juicier" and "tender". As revealed by the hedonic panel, the samples treated at 600 MPa were consistently the least preferred, irrespective of the natural antimicrobial condition. Feedback indicated that these samples were perceived as "dryer" and "less cohesive" by the panelist. Even though, in this study sous vide technique was used to cook the samples and considering that this is an effective approach to maintaining juiciness and achieving higher water holding capacity, it did not have a major effect on 600 MPa pressurized chicken. Hayes and co-workers (2014) reported a low rating for juiciness on beef patties treated at 400 MPa, despite their higher water holding capacity. Comparisons with other research indicate that optimal high-pressure parameters (100-300 MPa) can prevent excessive protein denaturation. Nonetheless, higher pressures may alter meat proteins to enhance their functionalities, including texture, rheology, and water-binding properties. These pressures lead to the internal exposure of hydrophobic and sulfhydryl groups induced by high pressure, increased hydrogen bonding, some unfolding of α-helices, and formation of β-sheets in myosin (Xue et al., 2017).

Table 4. 8. Average Mean Position from Ranked Preference Consumer Panel

Pressure	Natural Antimicrobial Condition	Average [A]
300 MPa	Without addition of natural antimicrobial	8.0[A]
300 MPa	Lemon and vinegar antimicrobial solution (LV)	7.0[B]
300 MPa	Rosemary and vinegar antimicrobial solution (RV)	6.0[C]
0 MPa	Without addition of natural antimicrobial	5.0[D]
0 MPa	Rosemary and vinegar antimicrobial solution (RV)	3.0[E]
0 MPa	Lemon and vinegar antimicrobial solution (LV)	2.1[F]
600 MPa	Lemon and vinegar antimicrobial solution (LV)	2.0[F]
600 MPa	Without addition of natural antimicrobial	1.5[G]
600 MPa	Rosemary and vinegar antimicrobial solution (RV)	1.0[G]

A: Means reported using a 9 point preference scale where 1= least preferred sample and 9= most preferred sample

Conclusion

High-pressure processing (600MPa) notably altered the expected sensory attributes like texture and color, affecting quality standards. Chicken patties subjected to the highest pressure, regardless of natural antimicrobial addition, exhibited reduced juiciness, tenderness, and cohesiveness. Panelists expressed dissatisfaction with these samples, stating they were least preferred due to being overly chewy and lacking cohesion, making them difficult to chew. In contrast, samples processed at 300 MPa had the highest score in most of the texture categories examined, even though in most cases, their instrumental texture attributes did not differ significantly from those of the non-pressurized samples. Considering the beneficial impact observed in terms of microbiology and TBARS analysis with the use of buffered concentrated vinegar with rosemary oil (V+REO) and a lemon juice-vinegar blend, it’s reassuring to note that

consumers did not detect significant differences among samples, regardless of the pressure applied.

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CHAPTER FIVE

OVERALL CONCLUSIONS

This research investigated the potential use of high-pressure processing and natural antimicrobials on ground white chicken. The study focused on three pressure conditions: 300 MPa, 600 MPa and samples without any process. Additionally, the effect on lipid oxidation rate and pathogens growth of two natural antimicrobials: buffered concentrated vinegar with rosemary oil (V+REO) and a lemon-vinegar blend, was studied. Significant differences ($P \leq 0.05$) in logarithm reduction of *E. coli* were found in samples treated at 600 MPa compared with the samples without treatment and processed at 300 MPa. A 600 MPa process can cause E. coli reductions greater than 5 Log, but A 300 MPa process can only slightly reduce the microbial load (1 Log- 1.7 Log reductions). In this research, it was observed that a combination of rosemary essential oil and concentrated buffered vinegar provides additional lethality to the process, particularly at lower pressures, but the effect is modest. Higher HPP treatment results in changes in the color of ground chicken, primarily evidenced by an increase in whiteness. Meanwhile, samples treated with 600 MPa led to a remarkable increase in lipid oxidation (21% compared to treatments without HPP). Similarly, it was observed that treatments with the addition of V + REO and vinegar had a reduction of lipid oxidation greater than 6% concerning the control without the addition of an antimicrobial solution. Cooked ground poultry which had previously been subjected to HPP shows only minor changes to cohesiveness and toughness in the TPA and cohesiveness mechanical analysis. However, the sensory study showed different results. Consumers were able to differentiate between the samples

processed at 600 MPa and the other two pressure conditions and rated these samples with lower score in the hedonic scale. This study concluded that consumers preferred the samples processed at 300 MPa, claiming that their texture was more tender and juicier. By combining the insights from microbial and physicochemical analyses of high pressurized ground chicken with sensory panel studies, producers and researchers can develop strategies to enhance food safety, quality, and consumer satisfaction by improving other properties inherent in ground chicken that can minimize the unsatisfactory effect of HPP on color and texture of this meat product. This holistic approach will help ensure that products not only meet safety standards but also align with consumer preferences, guiding industry practices towards innovation.